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EDITORIAL

Explaining the increased mortality in type 1 diabetes

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Abstract

Despite large improvements in the management of glucose levels and in the treatment of cardiovascular risk factors, the mortality rate in individuals with type 1 diabetes (T1D) is still high. Recently, Lind *et al* found that T1D individuals with glycated hemoglobin levels of 6.9% or lower had a risk of death from any cause or from cardiovascular causes that is twice as high as the risk for matched controls. T1D is a chronic disease with an early onset (*e.g.*, pediatric age) and thus in order to establish a clear correlation between death rate and the glycometabolic control, the whole history of glycemic control should be considered; particularly in the early years of diabetes. The switch from a normo- to hyperglycemic milieu in an individual with T1D in the pediatric age, represents a stressful event that may impact outcomes and death rate many years later. In this paper we will discuss the aforementioned issues, and offer our view on these findings, paying a particular attention to the several alterations occurring in the earliest phases of T1D and to the many factors that may be associated with the chronic history of T1D. This may help us to better understand the recently published death rate data and to develop future innovative and effective preventive strategies.

Key words: Type 1 diabetes; Hyperglycemia; Death rates; Adolescence; Autonomic neuropathy; Children; Endothelial dysfunction; Exercise; Metabolic memory

Core tip: Despite large improvements in the management of glucose levels and in the treatment of cardiovascular risk factors, the mortality rate in individuals with type 1 diabetes (T1D) is still high. A better understanding of the several different alterations occurring in the earliest phases of T1D and of the many factors that may be associated with a chronic history of T1D may help us to develop future innovative and effective preventive strategies.

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INTRODUCTION

Whether mortality in type 1 diabetes mellitus (T1D) is improved by intensive glycemic therapy has not been clarified yet. A number of studies have recently been published claiming that mortality rate is still higher than in age-matched controls without diabetes, despite improvements in management of glucose levels and treatment of cardiovascular risk factors[1-3]. Lind *et al*[1] reported data on current life expectancy for adults with T1D in a population-based sample using Swedish national registries of adults with and without diabetes. The Authors found that individuals with T1D and glycated hemoglobin (HbA1c) level of 6.9% or lower had a risk of death from any cause or from cardiovascular causes that was twice as high as the risk for matched controls. The multivariable-adjusted hazard ratios for death from any cause according to the HbA1c level for individuals with T1D as compared with controls are reported in Table 1.

Livingstone *et al*[2] report data on current life expectancy for adults with T1D in a population-based sample using Scottish national registries of adults with and without diabetes. At the age of 20 years, women and men with T1D could expect to live 12.9 years (95%CI: 11.7-14.1) and 11.1 years (95%CI: 10.1-12.1), respectively, less than aged-matched adults without it. Finally, Orchard *et al*[3] report survival data on the selective cohort of North Americans with T1D who participated in the Diabetes Control and Complications Trial (DCCT)[4] and its observational follow-up study, Epidemiology of Diabetes Interventions and Complications (EDIC)[5]. They found that 27 years after entry into the trial, 6.5 years of initial intensive diabetes therapy was associated with a modestly lower all-cause mortality rate when compared with conventional therapy. Few editorials accompanied[6] or commented[7] these data, without suggesting any conclusive hypothesis about the reason why this happens. T1D is known to be associated with an increased risk of premature mortality among the affected individuals, as documented by a recent systematic review on this topic by Morgan *et al*[8]. Authors identified thirteen relevant publications with mortality data, describing 23 independent studies. Standardized mortality ratios varied markedly ($P < 0.0001$). The increased mortality in childhood/adolescent-diagnosed with T1D was apparent across countries worldwide. Excesses were less marked in more recent studies and in countries with lower infant mortality and higher health expenditure. Given that good metabolic control has been shown to be effective reducing micro-vascular and macrovascular complication rates, one should expect that also the mortality rate might be reduced, but this is not the case[4,5]. Therefore, we would like to propose our appraisal to these important findings.

THE IMPORTANCE OF CHILDHOOD YEARS OF T1D

All studies reporting mortality rates in T1D refer to adult individuals, most of the time with a diabetes which occurred in childhood. For instance all individuals studied in the Lind paper were at least 18-year-old at the moment of enrollment, with a mean age at baseline of 35.8 years and mean diabetes duration of 20.4 years[1]. This implies that the average age for the onset of diabetes was 15.4 years, during their adolescence. The study does not provide any information at all with respect to HbA1c levels between diabetes onset and the time of data collection (on average 40 to 50 years after T1D diagnosis). From previous studies, (*e.g.*, DCCT and EDIC), we know how "metabolic memory" provides an important footprinting to future long-term complications[4,5,9]. We can thus argue that "metabolic memory" may partially be accounted for the higher death rate observed in individuals with T1D, whose onset was during childhood or adolescence. This aforementioned aspect reinforces the important of obtaining an optimal glycometabolic control in the first years of T1D. This is an important issue, since T1D incidence rate increases from birth, and peaks between the ages 10-14 years[10], with an even increased incidence especially marked in the youngest children (0-4 years)[11], making T1D the second most frequent chronic disease of childhood, after asthma. Further emphasis should be pointed to vascular complications, which start at the onset of the disease[12], although the consequences become clinically evident later in adulthood[13,14]. Once again, we may speculate that the reported excess mortality in adult individuals showing HbA1C < 6.9% in the study by Lind *et al*[1], may be the residual effect of previous cardiovascular insults started in infancy, childhood or adolescence. It is still unclear and partially unexplained why cardiovascular complications start so early in the disease history of T1D; indeed we can only speculate that a chronic state of mild hyperglycemia might be the culprit of cardiovascular morbidity, and thereby of excess death, as unaccounted in the Swedish observational trial[1].

Table 1 Adjusted hazard ratios for death from any cause and death from cardiovascular disease among individuals with type 1 diabetes *vs* control according to the glycated hemoglobin

	Hazard ratios	
Mean HbA1c	Death from any cause	Death from cardiovascular disease
≤ 6.9%	2.36 (95%CI: 1.97-2.83)	2.92 (95%CI: 2.07-4.13)
7.0%-7.8%	2.38 (95%CI: 2.02-2.80)	3.39 (95%CI: 2.49-4.61)
7.9%-8.7%	3.11 (95%CI: 2.66-3.62)	4.44 (95%CI: 3.32-5.96)
8.8%-9.6%	3.65 (95%CI: 3.11-4.30)	5.35 (95%CI: 3.94-7.26)
≥ 9.7%	8.51 (95%CI: 7.24-10.01)	10.46 (95%CI: 7.62-14.37)

Adapted from Lind *et al*[1]. HbA1c: Glycated hemoglobin.

Indeed, according the A1c-Derived Average Glucose study group, a HbA1c value of 6.9% indicate an average glucose level as high as 151 mg/dL (8.4 mmol/L)[15].

Additionally, the HbA1c measurement has some limitations itself. It has been shown to be unreliable in several different clinical scenarios, such as anemia or hemolysis, in presence of implanted mechanical heart valves, hypothyroidism, or during the use of medications such as erythropoietin[16]. Moreover, there is a recognized biological variability in the glycation process of the hemoglobin molecule in response to hyperglycemia. This is the result of a different glycation rate in "high glycating" *vs* "low glycating" subjects, where the same mean blood glucose was associated with an HbA1c level of 9.6% *vs* 7.6%, respectively[17]. To summarize, besides the issues related to the use of HbA1c, multiple factors contributed to the augmented risk of death in T1D individuals despite a good metabolic control. Indeed, several challenges are offered by the constantly evolving age-appropriate care needed by diabetic individuals transitioning from infancy to adulthood[18].

ENDOTHELIAL DYSFUNCTION AND EARLY ATHEROSCLEROSIS

Several different systems show altered homeostasis early along the course of diabetes[19-21]. Among them, the endothelium is definitely one of the most important and earlier targeted organs. Evidence suggests that impairment in nitric oxide-mediated smooth muscle vasodilation is an early pathophysiologic process and underlies the onset endothelial dysfunction, a key event for the development of atherosclerosis[22]. Among factors that may worsen endothelial function in individuals with T1D, we should mention: a long disease duration[23], a severely altered glycemic control[24], high low density lipoprotein cholesterol levels[25], high levels od advanced glycated end products[26], and altered mitochondrial dynamics[27]. Our group recently observed a high prevalence of endothelial dysfunction (76.7%) in adolescents with T1D for a mean duration of 9 years, particularly in those individuals with impaired glycometabolic control, subclinical signs of autonomic neuropathy and sedentary lifestyle. We did not observe any correlations between endothelial dysfunction and diabetes duration or individuals' age. A HbA1c below 7.5% (58 mmol/mol) and regular physical activity of at least 4 h per week, were indeed associated with better endothelial function. Atherosclerosis, the late event of endothelial dysfunction, is frequently linked to the likelihood of death from cardiovascular origin, especially in individuals with T1D. Compared to non-diabetic subjects, individuals with T1D show an increased risk up to 10-fold to develop atherosclerotic plaques, starting since childhood and adolescence[28]. Furthermore, intima media thickness measurement of the carotid artery is considered another valid surrogate marker for cardiovascular risk allowing assessment of atherosclerotic changes at a very early stage[29]. Finally, Paroni *et al*[30] showed that hyperhomocysteinemia in individuals with T1D may further increase the risk of endothelial dysfunction.

CARDIOVASCULAR AUTONOMIC NEUROPATHY

Cardiac autonomic neuropathy is an often overlooked and common complication of diabetes mellitus and by itself it is associated with increased cardiovascular morbidity and mortality[31], together with cardiac abnormalities typical of individuals with diabetes[32,33]. Data demonstrate the dual (vagal and sympathetic) control of heart rate and the dominant role of respiration in the genesis of heart rate and blood pressure fluctuations, suggesting that reduced vagal control of the sinoatrial node and impaired vascular regulation are the two main pathophysiological alterations[34]. Few years ago, our group investigated the autonomic performance of 93 children and adolescents with uncomplicated well-controlled T1D compared to age-matched controls. We found a significant increase in arterial blood pressure, a blunted baroreceptor reflex, and an increase of the low-frequency component of systolic arterial pressure variability. These findings entail the simultaneous impairment of the capability of the vagal system to influence the heart function, together with an increased sympathetic vasomotor regulation[21]. A follow-up study conducted 1-year later showed further impairment of the neuro vegetative performance, thereby suggesting early progression of the autonomic disturbance[21]. Interestingly, a small weekly increase in exercise in these same individuals can greatly help to improve cardiac autonomic neuropathy.

INFLAMMATION AND OXIDATIVE STRESS

In the last fifteen years several groups worked in the direction of uncovering the association between the increased cardiovascular risk in individuals with T1D and inflammation. Schaumberg *et al*[35] measured levels of inflammatory biomarkers at baseline and after a 3-year follow-up in a random sample of 385 participants of the DCCT cohort. Results were controversial and emphasized the extremely complex interaction between

inflammation, T1D and insulin therapy. Some of the inflammation indexes were high in both intensive and conventional insulin treatment groups; others were higher in the intensive insulin therapy group, others in the conventional one. What seemed to be linked to increased inflammation status in individuals using intensive insulin therapy was the weight gain they showed[35], underlining the need for a more effective weight control in individuals with T1D. Indeed, a recent study by Valerio *et al*[36] found that T1D adolescents, particularly females, showed a considerable occurrence of abdominal adiposity and metabolic syndrome. That is why pediatric diabetologists need to make every effort to achieve normal weight and better health outcomes in their young T1D patients. Davì *et al*[37] found that newly T1D diagnosed individuals showed significantly augmented lipid peroxidation and platelet activation, paralleled by a higher degree of systemic inflammation. This data strongly support the idea of a significantly noxious effect of even the earliest form of damage triggered by the disease. The biochemical picture depicted is suggestive of a true acute inflammatory response accompanying the disease in its very earliest, hence pediatric, phase[37]. The SEARCH Case-Control Study showed that young individuals with T1D, when compared to healthy controls, were characterized by excess inflammation despite good glycemic control[38]. Interestingly, Folli *et al*[39] demonstrated that persistent cellular changes of anti-oxidative machinery and of aerobic/anaerobic glycolysis are present in individuals with T1D (with or without end-stage renal disease), and these abnormalities may play a key role in the pathogenesis of hyperglycemia-related vascular complications. Restoration of euglycemia and removal of uremia with kidney pancreas transplant can correct these abnormalities. Some of these identified pathways may become potential therapeutic targets for a new generation of drugs[39].

HYPOGLYCEMIC EVENTS

Another possible explanation for the increased death rate in individuals with T1D despite good glycemic control may be hypoglycemia. The T1D Exchange Registry seems to confirm this hypothesis[40]. Elderly individuals and children younger than 5 years seem to be the two populations at greater risk[41,42]. A value of HbA1c in the low range ("good" metabolic control) may not only be associated with well controlled glucose control but with recurrent episodes of hyper/hypoglycemic oscillations. We may speculate that, in the study by Lind *et al*[1], one of the factors potentially explaining the persistence of a sizeable mortality hazard ratio in individuals with low HbA1c could be a high rate of hypoglycemic events in those individuals.

HOW TO IMPROVE OUTCOMES

T1D is a complex disease whose management may be extremely awkward and demanding[43]. Diet and exercise, in combination with a correct insulin therapy, play a pivotal role in obtaining and maintaining the best glycemic control possible. In the evaluation of a subgroup of individuals from the treatment group of the DCCT cohort, Delahanty *et al*[44] established the relation between diet and glycemic control beyond the sole intensive insulin therapy. A higher content of total and saturated fat, associated with a lower carbohydrate intake are linked to worse glycemic control, thereby further increasing the cardiovascular risk[44]. Adequate fibers intake, usually lower than suggested, is also recommended. Indeed, fibers offer a beneficial dietary profile: (1) by reducing or at least delaying the overall glucose absorption; (2) by blunting post-prandial glycemic peaks, and finally (3) by impacting low-density lipoproteins by enhancing biliary acid secretion. For the aforementioned reasons, a proper nutritional education is a crucial part of diabetes management and needs to be promoted in all pediatric individuals with T1D and their families[45]. Interestingly, recent study reported that children with T1D show less healthy food habits than same age healthy subjects[46].

Routine physical exercise is known to have beneficial effects on the cardiovascular system in the general population, and even more in individuals with T1D[47]. For this reason, we should strongly encourage individuals with T1D to participate in regular physical activity since childhood. One hour of moderate, aerobic exercise every day is currently recommended. Lucini *et al*[48] recently found the favorable effects of moderate increase (10%) in spontaneous exercise load in adolescents with T1D. Similarly, in children with T1D (mean age 11 years old), 60 min per day of exercise improves endothelial dysfunction, a well-known risk factor for cardiovascular diseases[49]. Moreover, in the recent years, technology has helped to reduce the impact of T1D especially in children[50]. Continuous glucose monitoring has emerged as one of the most significant innovation in the management of children with T1D. The combination of continuous glucose monitoring and insulin pumps, provides better glycemic control with less hypoglycemic episodes[51,52]. The ultimate technological advance of such automated insulin administration systems, currently under development, is the completely automated glycemic management, the closed-loop system also known as "external artificial pancreas"[53].

Finally, we would like to highlight recent stem cell-based trials, for which expectations in the scientific community and among individuals with T1D are high[54]. One of the most promising is cord blood stem cells that have been demonstrated to became a powerful tool not only for regenerative medicine but for autoimmune (*e.g.*, T1D) and inflammatory diseases as well[55,56]. Recently, a novel hematopoietic stem cell-based strategy has been tested in individuals with new-onset T1D, suggesting that remission of the disease is possible by combining hematopoietic stem cell transplantation and immunosuppression; however safer hematopoietic stem cell-based therapeutic options are required[57].

MORTALITY IN INDIVIDUALS WITH TYPE 2 DIABETES

As the prevalence of type 2 diabetes (T2D) continues to increase worldwide, diabetes-related morbidity and mortality increase as well. There is scarce evidence on the effect of HbA1c reduction on mortality rate in T2D individuals. Recently a study by Skriver *et al*[58] in a large cohort (n = 11205) of Danish individuals with T2D, showed that HbA1c variability was associated with mortality irrespective of the magnitude of absolute change in HbA1c. An increased mortality was observed even in those individuals with a HbA1c $\leqslant$ 8% if presenting a higher HbA1c variability[58]. However, in T2D individuals many factors other than glycometabolic control may contribute to increase the mortality rate (*e.g.*, hypertension, obesity, dyslipidemia, elevated uric acid and insulin resistance). Therefore, an early diagnosis and a prompt management of T2D comorbidities is required[59-62].

CONCLUSION

In conclusion, the recent findings describing an increased mortality in individuals with T1D as compared to age-matched population, even in the presence of on-target HbA1c, are important. Whenever the outcomes of a chronic disease like T1D are being studied, it is important to acquire data from the onset. Indeed, any events in the early phase may affect its future course and especially its final outcome (*i.e.*, death rates). A better understanding of the several alterations occurring in the earliest phases of T1D and of the factors that may be associated with the chronic history of T1D may help us to develop future innovative and effective preventive strategies.

REFERENCES

1 **Lind M**, Svensson AM, Kosiborod M, Gudbjörnsdottir S, Pivodic A, Wedel H, Dahlqvist S, Clements M, Rosengren A. Glycemic control and excess mortality in type 1 diabetes. *N Engl J Med* 2014; **371**: 1972-1982 [PMID: 25409370 DOI: 10.1056/NEJMc1415677]

2 **Livingstone SJ**, Levin D, Looker HC, Lindsay RS, Wild SH, Joss N, Leese G, Leslie P, McCrimmon RJ, Metcalfe W, McKnight JA, Morris AD, Pearson DW, Petrie JR, Philip S, Sattar NA, Traynor JP, Colhoun HM. Estimated life expectancy in a Scottish cohort with type 1 diabetes, 2008-2010. *JAMA* 2015; **313**: 37-44 [PMID: 25562264 DOI: 10.1001/jama.2014.16425]

3 **Orchard TJ**, Nathan DM, Zinman B, Cleary P, Brillon D, Backlund JY, Lachin JM. Association between 7 years of intensive treatment of type 1 diabetes and long-term mortality. *JAMA* 2015; **313**: 45-53 [PMID: 25562265 DOI: 10.1001/jama.2014.16107]

4 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]

5 **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]

6 **Katz M**, Laffel L. Mortality in type 1 diabetes in the current era: two steps forward, one step backward. *JAMA* 2015; **313**: 35-36 [PMID: 25562263 DOI: 10.1001/jama.2014.16327]

7 **Snell-Bergeon JK**, Maahs DM. Diabetes: Elevated risk of mortality in type 1 diabetes mellitus. *Nat Rev Endocrinol* 2015; **11**: 136-138 [PMID: 25583696 DOI: 10.1038/nrendo.2014.245]

8 **Morgan E**, Cardwell CR, Black CJ, McCance DR, Patterson CC. Excess mortality in Type 1 diabetes diagnosed in childhood and adolescence: a systematic review of population-based cohorts. *Acta Diabetol* 2015; Epub ahead of print [PMID: 25585594]

9 **Ceriello A**, Ihnat MA, Thorpe JE. Clinical review 2: The «metabolic memory»: is more than just tight glucose control necessary to prevent diabetic complications? *J Clin Endocrinol Metab* 2009; **94**: 410-415 [PMID: 19066300 DOI: 10.1210/jc.2008-1824]

10 **Maahs DM**, West NA, Lawrence JM, Mayer-Davis EJ. Epidemiology of type 1 diabetes. *Endocrinol Metab Clin North Am* 2010; **39**: 481-497 [PMID: 20723815 DOI: 10.1016/j.ecl.2010.05.011]

11 Variation and trends in incidence of childhood diabetes in Europe. EURODIAB ACE Study Group. *Lancet* 2000; **355**: 873-876 [PMID: 10752702]

12 **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]

13 **Hamilton J**, Brown M, Silver R, Daneman D. Early onset of severe diabetes mellitus-related microvascular complications. *J Pediatr* 2004; **144**: 281-283 [PMID: 14760278]

14 **Kavey RE**, Allada V, Daniels SR, Hayman LL, McCrindle BW, Newburger JW, Parekh RS, Steinberger J. Cardiovascular risk reduction in high-risk pediatric patients: a scientific statement from the American Heart Association Expert Panel on Population and Prevention Science; the Councils on Cardiovascular Disease in the Young, Epidemiology and Prevention, Nutrition, Physical Activity and Metabolism, High Blood Pressure Research, Cardiovascular Nursing, and the Kidney in Heart Disease; and the Interdisciplinary Working Group on Quality of Care and Outcomes Research: endorsed by the American Academy of Pediatrics. *Circulation* 2006; **114**: 2710-2738 [PMID: 17130340]

15 **Nathan DM**, Kuenen J, Borg R, Zheng H, Schoenfeld D, Heine RJ. Translating the A1C assay into estimated average glucose values. *Diabetes Care* 2008; **31**: 1473-1478 [PMID: 18540046 DOI: 10.2337/dc08-0545]

16 **Saudek CD**, Derr RL, Kalyani RR. Assessing glycemia in diabetes using self-monitoring blood glucose and hemoglobin A1c. *JAMA* 2006; **295**: 1688-1697 [PMID: 16609091]

17 **Hempe JM**, Soros AA, Chalew SA. Estimated average glucose and self-monitored mean blood glucose are discordant estimates of glycemic control. *Diabetes Care* 2010; **33**: 1449-1451 [PMID: 20357368 DOI: 10.2337/dc09-1498]

18 **Hood KK**, Beavers DP, Yi-Frazier J, Bell R, Dabelea D, Mckeown RE, Lawrence JM. Psychosocial burden and glycemic control during the first 6 years of diabetes: results from the SEARCH for Diabetes in Youth study. *J Adolesc Health* 2014; **55**: 498-504 [PMID: 24815959 DOI: 10.1016/j.jadohealth.2014.03.011]

19 **Scaramuzza AE**, Redaelli F, Giani E, Macedoni M, Giudici V, Gazzarri A, Bosetti A, De Angelis L, Zuccotti GV. Adolescents and young adults with type 1 diabetes display a high prevalence of endothelial dysfunction. *Acta Paediatr* 2015; **104**: 192-197 [PMID: 25424745 DOI: 10.1111/apa.12877]

20 **Scaramuzza AE**, Morelli M, Rizzi M, Borgonovo S, De Palma A, Mameli C, Giani E, Beretta S, Zuccotti GV. Impaired diffusing capacity for carbon monoxide in children with type 1 diabetes: is this the first sign of long-term complications? *Acta Diabetol* 2012; **49**: 159-164 [PMID: 22105342 DOI: 10.1007/s00592-011-0353-2]

21 **Lucini D**, Zuccotti G, Malacarne M, Scaramuzza A, Riboni S, Palombo C, Pagani M. Early progression of the autonomic dysfunction observed in pediatric type 1 diabetes mellitus. *Hypertension* 2009; **54**: 987-994 [PMID: 19805636 DOI: 10.1161/HYPERTENSIONAHA.109.140103]

22 **Flammer AJ**, Anderson T, Celermajer DS, Creager MA, Deanfield J, Ganz P, Hamburg NM, Lüscher TF, Shechter M, Taddei S, Vita JA, Lerman A. The assessment of endothelial function: from research into

clinical practice. *Circulation* 2012; **126**: 753-767 [PMID: 22869857 DOI: 10.1161/CIRCULATIONAHA.112.093245]
23 **Järvisalo MJ**, Lehtimäki T, Raitakari OT. Determinants of arterial nitrate-mediated dilatation in children: role of oxidized low-density lipoprotein, endothelial function, and carotid intima-media thickness. *Circulation* 2004; **109**: 2885-2889 [PMID: 15159289]
24 **Clarkson P**, Celermajer DS, Donald AE, Sampson M, Sorensen KE, Adams M, Yue DK, Betteridge DJ, Deanfield JE. Impaired vascular reactivity in insulin-dependent diabetes mellitus is related to disease duration and low density lipoprotein cholesterol levels. *J Am Coll Cardiol* 1996; **28**: 573-579 [PMID: 8772741]
25 **Cé GV**, Rohde LE, da Silva AM, Puñales MK, de Castro AC, Bertoluci MC. Endothelial dysfunction is related to poor glycemic control in adolescents with type 1 diabetes under 5 years of disease: evidence of metabolic memory. *J Clin Endocrinol Metab* 2011; **96**: 1493-1499 [PMID: 21346068 DOI: 10.1210/jc.2010-2363]
26 **Kostolanská J**, Jakus V, Barák L. HbA1c and serum levels of advanced glycation and oxidation protein products in poorly and well controlled children and adolescents with type 1 diabetes mellitus. *J Pediatr Endocrinol Metab* 2009; **22**: 433-442 [PMID: 19618662]
27 **Shenouda SM**, Widlansky ME, Chen K, Xu G, Holbrook M, Tabit CE, Hamburg NM, Frame AA, Caiano TL, Kluge MA, Duess MA, Levit A, Kim B, Hartman ML, Joseph L, Shirihai OS, Vita JA. Altered mitochondrial dynamics contributes to endothelial dysfunction in diabetes mellitus. *Circulation* 2011; **124**: 444-453 [PMID: 21747057 DOI: 10.1161/CIRCULATIONAHA.110.014506]
28 **Heier M**, Margeirsdottir HD, Torjesen PA, Seljeflot I, Stensæth KH, Gaarder M, Brunborg C, Hanssen KF, Dahl-Jørgensen K. The advanced glycation end product methylglyoxal-derived hydroimidazolone-1 and early signs of atherosclerosis in childhood diabetes. *Diab Vasc Dis Res* 2015; **12**: 139-145 [PMID: 25616705 DOI: 10.1177/1479164114560910]
29 **Dalla Pozza R**, Ehringer-Schetitska D, Fritsch P, Jokinen E, Petropoulos A, Oberhoffer R. Intima media thickness measurement in children: A statement from the Association for European Paediatric Cardiology (AEPC) Working Group on Cardiovascular Prevention endorsed by the Association for European Paediatric Cardiology. *Atherosclerosis* 2015; **238**: 380-387 [PMID: 25555270 DOI: 10.1016/j.atherosclerosis.2014.12.029]
30 **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]
31 **Dimitropoulos G**, Tahrani AA, Stevens MJ. Cardiac autonomic neuropathy in patients with diabetes mellitus. *World J Diabetes* 2014; **5**: 17-39 [PMID: 24567799 DOI: 10.4239/wjd.v5.i1.17]
32 **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]
33 **Del Carro U**, Fiorina P, Amadio S, De Toni Franceschini L, Petrelli A, Menini S, Martinelli Boneschi F, Ferrari S, Pugliese G, Maffi P, Comi G, Secchi A. Evaluation of polyneuropathy markers in type 1 diabetic kidney transplant patients and effects of islet transplantation: neurophysiological and skin biopsy longitudinal analysis. *Diabetes Care* 2007; **30**: 3063-3069 [PMID: 17804685]
34 **Mésangeau D**, Laude D, Elghozi JL. Early detection of cardiovascular autonomic neuropathy in diabetic pigs using blood pressure and heart rate variability. *Cardiovasc Res* 2000; **45**: 889-899 [PMID: 10728415]
35 **Schaumberg DA**, Glynn RJ, Jenkins AJ, Lyons TJ, Rifai N, Manson JE, Ridker PM, Nathan DM. Effect of intensive glycemic control on levels of markers of inflammation in type 1 diabetes mellitus in the diabetes control and complications trial. *Circulation* 2005; **111**: 2446-2453 [PMID: 15867184]
36 **Valerio G**, Iafusco D, Zucchini S, Maffeis C. Abdominal adiposity and cardiovascular risk factors in adolescents with type 1 diabetes. *Diabetes Res Clin Pract* 2012; **97**: 99-104 [PMID: 22336634]
37 **Davì G**, Chiarelli F, Santilli F, Pomilio M, Vigneri S, Falco A, Basili S, Ciabattoni G, Patrono C. Enhanced lipid peroxidation and platelet activation in the early phase of type 1 diabetes mellitus: role of interleukin-6 and disease duration. *Circulation* 2003; **107**: 3199-3203 [PMID: 12810609]
38 **Snell-Bergeon JK**, West NA, Mayer-Davis EJ, Liese AD, Marcovina SM, D'Agostino RB, Hamman RF, Dabelea D. Inflammatory markers are increased in youth with type 1 diabetes: the SEARCH Case-Control study. *J Clin Endocrinol Metab* 2010; **95**: 2868-2876 [PMID: 20371668 DOI: 10.1210/jc.2009-1993]
39 **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 DOI: 10.1371/journal.pone.0009923]
40 **Weinstock RS**, Xing D, Maahs DM, Michels A, Rickels MR, Peters AL, Bergenstal RM, Harris B, Dubose SN, Miller KM, Beck RW. Severe hypoglycemia and diabetic ketoacidosis in adults with type 1 diabetes: results from the T1D Exchange clinic registry. *J Clin Endocrinol Metab* 2013; **98**: 3411-3419 [PMID: 23760624 DOI: 10.1210/jc.2013-1589]
41 **Soo Yeon S**, Nesto RW. Implications of intensive glycemic control on cardiovascular disease: early reports from the ACCORD and ADVANCE Trials. *Rev Cardiovasc Med* 2008; **9**: 1-4 [PMID: 18418304 DOI: 10.1056/NEJMe0804182]
42 **Perantie DC**, Lim A, Wu J, Weaver P, Warren SL, Sadler M, White NH, Hershey T. Effects of prior hypoglycemia and hyperglycemia on cognition in children with type 1 diabetes mellitus. *Pediatr Diabetes* 2008; **9**: 87-95 [PMID: 18208449 DOI: 10.1111/j.1399-5448.2007.00274.x]
43 **Scaramuzza AE**, Ludvigsson J, Zuccotti GV. The insulin, love and care project. *Austin J Pediatr* 2014; **1**: 2
44 **Delahanty LM**, Nathan DM, Lachin JM, Hu FB, Cleary PA, Ziegler GK, Wylie-Rosett J, Wexler DJ. Association of diet with glycated hemoglobin during intensive treatment of type 1 diabetes in the Diabetes Control and Complications Trial. *Am J Clin Nutr* 2009; **89**: 518-524 [PMID: 19106241 DOI: 10.3945/ajcn.2008.26498]
45 **Smart CE**, Annan F, Bruno LP, Higgins LA, Acerini CL ; International Society for Pediatric and Adolescent Diabetes. ISPAD Clinical Practice Consensus Guidelines 2014. Nutritional management in children and adolescents with diabetes. *Pediatr Diabetes* 2014; **15** Suppl 20: 135-153 [PMID: 25182313 DOI: 10.1111/pedi.12175]
46 **Nansel TR**, Lipsky LM, Liu A, Laffel LM, Mehta SN. Contextual factors are associated with diet quality in youth with type 1 diabetes mellitus. *J Acad Nutr Diet* 2014; **114**: 1223-1229 [PMID: 24651028 DOI: 10.1016/j.jand.2014.01.012]
47 **Wolfsdorf JI**. Children with diabetes benefit from exercise. *Arch Dis Child* 2005; **90**: 1215-1217 [PMID: 16301546]
48 **Lucini D**, Zuccotti GV, Scaramuzza A, Malacarne M, Gervasi F, Pagani M. Exercise might improve cardiovascular autonomic regulation in adolescents with type 1 diabetes. *Acta Diabetol* 2013; **50**: 341-349 [PMID: 22941280 DOI: 10.1007/s00592-012-0416]
49 **Trigona B**, Aggoun Y, Maggio A, Martin XE, Marchand LM, Beghetti M, Farpour-Lambert NJ. Preclinical noninvasive markers of atherosclerosis in children and adolescents with type 1 diabetes are influenced by physical activity. *J Pediatr* 2010; **157**: 533-539 [PMID: 20826281 DOI: 10.1016/j.jpeds.2010.04.023]
50 **Scaramuzza AE**, Zuccotti GV. Modern clinical management helps reducing the impact of type 1 diabetes in children. *Pharmacol Res* 2015; **98**: 16-21 [PMID: 25779986 DOI: 10.1016/j.phrs.2015.03.001]
51 **Mameli C**, Scaramuzza AE, Ho J, Cardona-Hernandez R, Suarez-Ortega L, Zuccotti GV. A 7-year follow-up retrospective, international, multicenter study of insulin pump therapy in children and adolescents with type 1 diabetes. *Acta Diabetol* 2014; **51**: 205-210 [PMID: 23681558 DOI: 10.1007/s00592-013-0481-y]
52 **DiMeglio LA**, Pottorff TM, Boyd SR, France L, Fineberg N, Eugster EA. A randomized, controlled study of insulin pump therapy in diabetic preschoolers. *J Pediatr* 2004; **145**: 380-384

[PMID: 15343195]
53 **Russell SJ**, El-Khatib FH, Sinha M, Magyar KL, McKeon K, Goergen LG, Balliro C, Hillard MA, Nathan DM, Damiano ER. Outpatient glycemic control with a bionic pancreas in type 1 diabetes. *N Engl J Med* 2014; **371**: 313-325 [PMID: 24931572 DOI: 10.1056/NEJMoa1314474]
54 **Ben Nasr M**, D'Addio F, Usuelli V, Tezza S, Abdi R, Fiorina P. The rise, fall, and resurgence of immunotherapy in type 1 diabetes. *Pharmacol Res* 2015; **98**: 31-38 [PMID: 25107501 DOI: 10.1016/j.phrs.2014.07.004]
55 **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]
56 **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]
57 **D'Addio F**, Valderrama Vasquez A, Ben Nasr M, Franek E, Zhu D, Li L, Ning G, Snarski E, Fiorina P. Autologous nonmyeloablative hematopoietic stem cell transplantation in new-onset type 1 diabetes: a multicenter analysis. *Diabetes* 2014; **63**: 3041-3046 [PMID: 24947362 DOI: 10.2337/db14-0295]
58 **Skriver MV**, Sandbæk A, Kristensen JK, Støvring H. Relationship of HbA1c variability, absolute changes in HbA1c, and all-cause mortality in type 2 diabetes: a Danish population-based prospective observational study. *BMJ Open Diabetes Res Care* 2015; **3**: e000060 [PMID: 25664182 DOI: 10.1136/bmjdrc-2014-000060]
59 **Twito O**, Frankel M, Nabriski D. Impact of glucose level on morbidity and mortality in elderly with diabetes and pre-diabetes. *World J Diabetes* 2015; **6**: 345-351 [PMID: 25789117 DOI: 10.4239/wjd.v6.i2.345]
60 **Price HI**, Agnew MD, Gamble JM. Comparative cardiovascular morbidity and mortality in patients taking different insulin regimens for type 2 diabetes: a systematic review. *BMJ Open* 2015; **5**: e006341 [PMID: 25762229 DOI: 10.1136/bmjopen-2014-006341]
61 **Mayor S**. People with diabetes have one third higher mortality risk than general population, audit shows. *BMJ* 2015; **350**: h529 [PMID: 25636825 DOI: 10.1136/bmj.h529]
62 **Khunti K**, Davies M, Majeed A, Thorsted BL, Wolden ML, Paul SK. Hypoglycemia and risk of cardiovascular disease and all-cause mortality in insulin-treated people with type 1 and type 2 diabetes: a cohort study. *Diabetes Care* 2015; **38**: 316-322 [PMID: 25492401 DOI: 10.2337/dc14-0920]

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REVIEW

Metabolic syndrome: A review of the role of vitamin D in mediating susceptibility and outcome

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Abstract

Despite the well-recognised role of vitamin D in a wide range of physiological processes, hypovitaminosis is common worldwide (prevalence 30%-50%) presumably arising from inadequate exposure to ultraviolet radiation and insufficient consumption. While generally not at the very low levels associated with rickets, hypovitaminosis D has been implicated in various very different, patho-physiological processes. These include putative effects on the pathogenesis of neoplastic change, inflammatory and demyelinating conditions, cardiovascular disease (CVD) and diabetes. This review focuses on the association between hypovitaminosis D and the metabolic syndrome as well as its component characteristics which are central obesity, glucose homeostasis, insulin resistance, hypertension and atherogenic dyslipidaemia. We also consider the effects of hypovitaminosis D on outcomes associated with the metabolic syndrome such as CVD, diabetes and non-alcoholic fatty liver disease. We structure this review into 3 distinct sections; the metabolic syndrome, vitamin D biochemistry and the putative association between hypovitaminosis D, the metabolic syndrome and cardiovascular risk.

Key words: Vitamin D; Hypovitaminosis D; Metabolic syndrome; Type 2 diabetes mellitus; Insulin resistance; Cardiovascular disease; Atherogenic dyslipidaemia; Hypertension; Non-alcoholic fatty liver disease

Core tip: The metabolic syndrome is common, affecting about 40% of Americans. It is defined by combinations of risk factors for cardiovascular disease (CVD) including insulin resistance and abdominal obesity. Research implicates hypovitaminosis D in the causation and phenotype of the syndrome and we present relevant data. While hypovitaminosis appears a risk factor for components of the syndrome and its outcome, the mechanism is unclear. The risks associated with varying

levels of hypovitaminosis and the benefits of vitamin replacement are unknown. However, unravelling the association between hypovitaminosis and the syndrome is warranted as even a modest decrease in CVD risk would confer substantial benefits.

Strange RC, Shipman KE, Ramachandran S. Metabolic syndrome: A review of the role of vitamin D in mediating susceptibility and outcome. *World J Diabetes* 2015; 6(7): 896-911 Available from: URL: http://www.wjgnet.com/1948-9358/full/v6/i7/896.htm DOI: http://dx.doi.org/10.4239/wjd.v6.i7.896

INTRODUCTION

Much research over the last 30 years has shown that the pleiotrophic actions of 1, 25 dihydroxy-vitamin D [1,25(OH)$_2$D] are central to cell, organ and organism homeostasis. Thus, along with its historic functions as a mediator of calcium and bone metabolism, 1,25(OH)$_2$D has effects on a wide range of physiological processes. It is perhaps surprising, given its perceived importance to public health, to find that hypovitaminosis D is common worldwide (prevalence 30%-50%). This deficiency presumably arises from failure to firstly, ensure adequate exposure to ultraviolet radiation (UVR) because of skin cancer fears and secondly, consume food with sufficient levels of the vitamin. Vitamin D status is identified by low serum levels of biologically inactive 25-hydroxylated vitamin D [25(OH)D]. While generally not at the very low levels associated with rickets, hypovitaminosis D has been implicated in various very different, patho-physiological processes. These include a putative effect on the development of neoplastic, inflammatory, demyelinating, cardiovascular and diabetic conditions. While the impact of hypovitaminosis D on health remains unclear, accumulating data indicates it confers increased disease risk and in some cases worse outcome.

In the context of this review, the finding that hypo-vitaminosis D is associated with impaired glucose homeostasis is of particular interest. A meta-analysis of 28 studies demonstrated that higher serum 25(OH)D levels were associated with a 55% reduction in diabetes, a 51% decreased risk of the metabolic syndrome and a 33% lower risk of cardiovascular disease (CVD)[1]. Further, treatment with vitamin D supplements over 2 mo improved fasting glucose levels and insulin resistance homeostasis model assessment for insulin resistance (HOMA-IR) in 100 patients with type 2 diabetes[2]. It is suggested that the mechanism for this latter finding involves improved sensitivity of target tissues such as the liver, muscle and bone to insulin as well as enhanced beta cell function. Given that many risk factors for CVD are clustered in the highly prevalent metabolic syndrome, which is characterised by insulin resistance and abdominal obesity, it is reasonable to speculate a significant role for the vitamin in the development of the syndrome and its sequelae of diabetes and CVD.

In this review we focus on the association between hypovitaminosis D and the metabolic syndrome and how this may contribute to increased CVD risk. We present 3 sections describing firstly, the metabolic syndrome, secondly, vitamin D biochemistry and thirdly, the putative association between hypovitaminosis D, the syndrome and CVD risk.

METABOLIC SYNDROME; HOW IT WAS IDENTIFIED

The relationship between sensitivity to insulin, obesity and glucose homeostasis was first observed by the Swedish physician Eskil Kylin[3]. He described a syndrome comprising hyperglycaemia, hypertension and hyperuricaemia and suggested insulin resistance as a possible causative factor[3]. Subsequently Himsworth *et al*[4] laid the foundations for the classification of type 1 and 2 diabetes by showing that while some patients were insulin sensitive (younger, normal weight and blood pressure) others are insulin insensitive (older, more obese, hypertensive and atherosclerotic). Vague, in studies on gender-related obesity patterns described android obesity (now termed central obesity and linked with diabetes and atherosclerosis) and suggested a hormonal aetiology with over-activity of the pituitary-adrenal axis playing a key role[5].

Such observations were brought together by Reaven[6] in his Banting Lecture to the American Diabetes Association in 1988. He termed the combination of hypertension, dyslipidaemia and glucose intolerance as syndrome X and proposed that this mix of phenotypes provided a pathophysiological basis for atherosclerosis. Obesity, was also seen as a further essential component and following a number of iterations (dyslipidaemic hypertension, deadly quartet, insulin resistance, hazardous waist), the combination of phenotypes is now termed the metabolic syndrome[7] with the International Classification of Disease code of 277[7,8].

Classification of the metabolic syndrome

Various groups including the World Health Organisation[9], European Group for the Study of Insulin Resistance[7], American Association of Clinical Endocrinologists[10], National Cholesterol Education Program - Adult Treatment Panel Ⅲ[11] and, more recently, the International Diabetes Federation (IDF)[12,13] have provided definitions of the metabolic syndrome (Table 1). While all are based on the characteristics presented by Reaven[6], there are various inclusion thresholds. A form of consensus was arrived at in 2009[14] with the IDF, National Heart, Lung and Blood Institute, American Heart Association, World Heart Federation, International Atherosclerosis and the International Association for the Study of Obesity agreeing on threshold levels that were similar to those originally proposed by the IDF. Guidelines for classifying metabolic syndrome in children over 10 years of age were also issued[15] and population and gender specific

Table 1 Thresholds defining the metabolic syndrome issued by individual organisations

	WHO 1998 (Alberti 1998)	EGIR (Balkau 1999)	NCEP/ATP Ⅲ 2001 (NCEP 2002)	AACE (2003) (Einhorn 2003)	IDF consensus 2005 (Zimmet 2005)	IDF consensus (10 to < 16 yr) (Zimmet 2007)
Definition	IGT, IFG, T2DM or lowered insulin sensitivity Plus 2 of the following	Plasma insulin > 75th percentile Plus 2 of the following	3 of the following	IGT or IFG plus any of the following based on clinical judgement	See below	
Europoid waist circumference (cm)	W:H > 0.90 M W:H > 0.85 F or BMI > 30 kg/m^2	⩾ 94 M ⩾ 80 F	⩾ 102 M ⩾ 88 F	BMI ⩾ 25 kg/m^2	⩾ 94 M ⩾ 80 F or BMI > 30 kg/m^2 Plus 2 of the following	> 90th percentile Plus 2 of the following
Triglyceride [mg/dL (mmol/L)]	> 150 (1.7)	> 150 (1.7)	⩾ 150 (1.7)	> 150 (1.7)	> 150 (1.7)	⩾ 150 (1.7)
HDL [mg/dL (mmol/L)]	< 35 (0.91) M < 39 (1.01) F	< 39 (0.91)	< 40 (1.03) M < 50 (1.29) F	< 40 (1.03) M < 50 (1.29) F	< 40 (1.03) M < 50 (1.29) F	< 40 (1.03)
BP (mmHg)	⩾ 140/90	⩾ 140/90 or on treatment	⩾ 130/85	⩾ 130/85	SBP ⩾ 130 or DBP ⩾ 85 or on treatment	SBP ⩾ 130 and/or DBP ⩾ 85
Glucose [mg/dL (mmol/L)]	IGT, IFG or T2DM	IGT or IFG (but not diabetes)	⩾ 100 (5.6) (Grundy) or diabetes	IGT or IFG (but not diabetes)	⩾ 100 (5.6)	⩾ 100 (5.6) or known T2DM
Others	Microalbuminuria ACR > 30 mg/g			Other features of IR[1]		

[1]Includes polycystic ovary syndrome, family history or ethnic group susceptible to type 2 diabetes, sedentary lifestyle and advancing age. ACR: Albumin creatinine ration; BMI: Body mass index; DBP: Diastolic blood pressure; F: Female; IFG: Impaired fasting glucose; IGT: Impaired glucose tolerance; IR: Insulin resistance; SBP: Systolic blood pressure; M: Male; T2DM: Type 2 diabetes mellitus; W:H: Waist to hip ratio; WHO: World Health Organization; HDL: High density lipoprotein; IDF: International Diabetes Federation; EGIR: European Group for the Study of Insulin Resistance; NCEP: National Cholesterol Education Program; AACE: American Association of Clinical Endocrinologists; BP: Blood pressure; IR: Insulin resistance.

waist circumference thresholds were published to define central obesity[13]. The prevalence for the metabolic syndrome varies between countries. Based on the IDF classification a 40% prevalence in the United States has been reported[16].

Is there a clinical value in identifying the metabolic syndrome: It is not surprising, given the presence of known risk factors, to find that the metabolic syndrome confers an approximately two-fold increased relative risk of CVD[17]. However, it is important to determine whether this impact is the effect of the metabolic syndrome (added risk due to a clustering of risk factors) or just the sum of its defining phenotypes. Studies using different CVD endpoints indicate the latter is the case. For example, Eddy *et al*[18] used data from NHANES Ⅲ (third national health and nutrition survey) to simulate a population matching that of the United States, estimated its metabolic syndrome prevalence (using the various definitions) and associated this with CVD. While the number of individuals identified by the various metabolic syndrome classifications differed, they reported that fasting glucose levels > 110 mg/dL (6.1 mmol/L) were a better predictor of CVD than the presence of the metabolic syndrome classified by any of the definitions[18]. Further, using change in atheroma volume as an endpoint, Bayturan *et al*[19] reviewed 3459 patients enrolled in 7 trials that used intravascular ultrasonography to measure plaque progression. While the metabolic syndrome was significantly associated [odds ratio (OR) = 1.29, 95%CI: 1.09-1.53] with increased atheroma volume, the relationship was not significant (OR = 1.04, 95%CI: 0.79-1.37) when adjusted for its individual components; serum triglycerides ⩾ 150 mg/dL (1.7 mmol/L), body mass index (BMI) ⩾ 30 kg/m^2, high density lipoprotein cholesterol (HDL-C) < 40 mg/dL (1.0 mmol/L) in men or < 50 mg/dL (1.3 mmol/L) in women, blood pressure ⩾ 135/85 mmHg or treatment of hypertension[19]. In this multifactorial model, only serum triglyceride concentrations (⩾ 150 mg/dL) remained significantly associated with plaque progression[19].

These findings (and others) question the clinical value of identifying the metabolic syndrome in patients. Indeed, the identification is dependent on the thresholds of each of the contributing factors. Thus, for example, if age-related thresholds were used there would be a marked change in the numbers of affected individuals. While in theory its identification does not appear to add anything to prognosis in an individual patient, we and others[20] argue that it has clinical value. As the metabolic syndrome is based on related and modifiable CVD risk factors, its identification encourages a holistic approach rather than a focus on the individual aspects (glycaemia, dyslipidaemia, weight reduction and blood pressure management) of the patients' condition. It therefore, has value in encouraging the clinician to address CVD risk using a multifactorial approach. It is also arguably useful in a research setting when considering the role of possible risk factors.

We also believe it is important to consider the

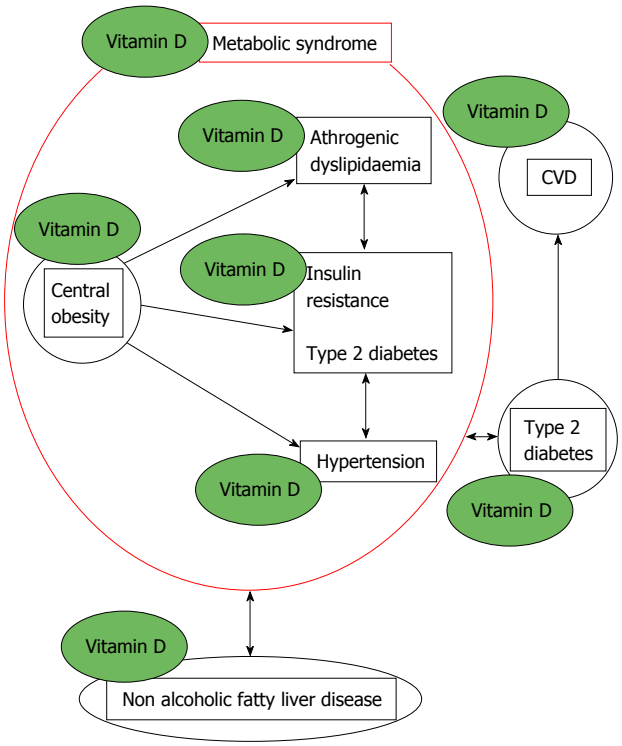

Figure 1 Simplified illustrations of the component risk factors of the metabolic syndrome, the complex relationships between them and the outcomes leading to increased morbidity and mortality. We also identify the areas that may be affected by hypovitaminosis D which are covered in this review. CVD: Cardiovascular disease.

metabolic syndrome as a heterogeneous entity. Indeed, in patients with the syndrome, we have shown that following treatment with statins and fibrates, outcomes can vary considerably indicating the presence of subgroups both known (gender, baseline lipids, and concurrent therapy) and unknown[21-24].

Metabolic syndrome - putative pathway to CVD: While it is accepted that central obesity and insulin resistance are core drivers of the metabolic syndrome, the timescale and inter-relationships between these and other factors that lead to an individual being classified with the syndrome and the consequent increased risk of CVD remain unclear[25-27]. Clearly, while obesity and insulin resistance are common in adults worldwide they are rare in childhood indicating that environmental factors interacting with a genetic predisposition drive the development of the syndrome from birth through childhood to its identification in adulthood. Once an individual develops the metabolic syndrome, the combination of risk factors leads to an increased risk of CVD (Figure 1).

Obesity is a recognised risk factor associated with mortality, this probably due to the link between obesity and risk of developing diabetes, hypertension, atherogenic dyslipidaemia and CVD[28]. However, the National Health and Nutrition Examination Survey (NHANES) indicated that individuals with a BMI between 30 and 35 kg/m^2 demonstrated only a modest increase in mortality compared to those with BMI 18.5-25 kg/m^2[27,29]. These findings suggest the presence of a subgroup of obese individuals who are not at high risk of metabolic disturbances or increased mortality. Their presence may be a reason for the relatively modest increase in overall mortality in obese subjects. It has been speculated that the link between obesity and CVD may be *via* insulin resistance[27]. Individuals with high insulin sensitivity and not fulfilling the ATP Ⅲ metabolic syndrome criteria are considered to be a "metabolically healthy obese" group[29].

The concept that not all obesity is bad in the context of developing CVD is interesting. Abdominal obesity, visceral as opposed to subcutaneous fat, appears to be critical in the development of insulin resistance[30]. Abdominal adipose tissue was initially considered an inert storage depot for triglycerides (glycerol and fatty acids). The current view however, is that it is also an active endocrine organ. Intra-abdominal obesity, a classifying characteristic of the metabolic syndrome promotes insulin resistance (the reverse of insulin sensitivity), perhaps by secreting metabolically active substances (adipokines) and making available an increased quantity of free fatty acids[30,31].

Insulin resistance, the other key factor in the metabolic syndrome, is defined as a condition where greater than normal levels of the peptide are needed to clear a glucose load (and effect its other metabolic actions). Thus, for a given blood glucose level the amount of insulin secreted is high. Impairment of sensitivity appears to be a contributing factor to all of the features of the metabolic syndrome in addition to having a direct causative role in the pathogenesis of type 2 diabetes. It can be considered a pre-diabetic state in non-diabetic patients, conferring a 5 fold increased risk of developing diabetes[32]. Insulin resistance has also been demonstrated to be associated with hypertension, atherogenic dyslipidaemia and higher amounts of the atherogenic small dense low density lipoprotein cholesterol (LDL-C), features associated with the metabolic syndrome[20,33].

Thus, in addition to weight reduction measures, reducing insulin resistance, a feature that may be an intermediate factor linking obesity with morbidity and mortality, must be addressed in patients with the metabolic syndrome. Apart from abdominal obesity there are other factors that may modify insulin resistance. Physical fitness (as measured by aerobic capacity) has been seen to increase insulin sensitivity[34].

VITAMIN D BIOCHEMISTRY

Vitamin D, in addition to its role in calcium and bone metabolism, has pleiotrophic effects in many cell types in many life forms. These include a potential role in the actions of insulin and development of obesity (Figure 1). Thus, not surprisingly hypovitaminosis D has been linked with hypertension, atherogenic dyslipidaemia and increased CVD risk (Figure 1). An association has

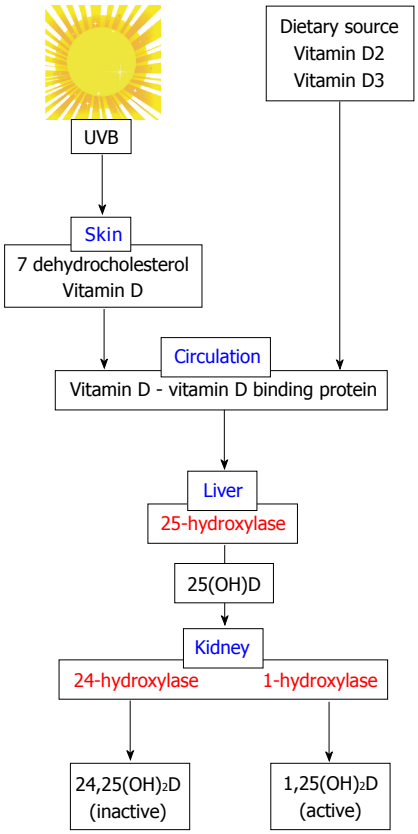

Figure 2 Simplified synthetic pathway leading to the formation of the active metabolite 1,25(OH)$_2$D. UVB: Ultraviolet B.

also been noted with non-alcoholic fatty liver disease independent of the features classifying the metabolic syndrome. Hypovitaminosis D can be addressed by both lifestyle measures and supplementation; hence, it is important to understand the relationship between vitamin D and the metabolic syndrome at both mechanistic and epidemiological levels.

Vitamin D synthesis

Bioactive vitamin D, 1,25(OH)$_2$D is synthesised in a pathway involving different organs and intermediates (Figure 2). Some inactive chemicals are produced that may have a regulatory role but will not be considered further. The first step in the pathway is the photochemical production of cholecalciferol in the skin from 7-dehydrocholesterol. Thus, production of bioactive 1,25(OH)D can only be initiated in skin *via* a photochemical process. Accordingly, animals have to eat foods containing the vitamin or be exposed to sunlight to allow its photosynthesis in skin.

Cholecalciferol is produced in the stratum basale and stratum spinosum layers of skin following reaction of 7-dehydrocholesterol with ultraviolet B (UVB) (270-300 nm). It is noteworthy that the concentration of 7-dehydrocholesterol falls with increasing age resulting in reduced capacity to synthesise vitamin D$_3$. This effect is marked; for example, the skin of a 70-year-old subject has approximately 25% of the 7-dehydrocholesterol compared with that of a young adult[35]. Cholecalciferol (and ergocalciferol) is carried in blood to the liver and hydroxylated at position 25 to form 25(OH)D. The final step in the pathway is hydroxylation of circulating 25(OH)D at the 1 position to form biologically active 1,25(OH)$_2$D. This occurs in the kidney, and other tissues, and is followed by its release into blood bound to vitamin D binding protein and transported to target organs.

How vitamin D works

Systemic or locally produced 1,25(OH)$_2$D binds to the vitamin D receptor (VDR), a nuclear receptor that dimerises with the retinoid X receptor and, in turn, becomes a regulator of transcription[36]. Dimerisation allows interaction with the vitamin D response element on target genes initiating transcription[37]. The VDR is a member of the steroid receptor superfamily and is responsible for regulating transcription in many responsive genes. Indeed, more than 200 genes, including those that regulate cell differentiation and proliferation as well as multiple metabolic systems, are targets for vitamin D.

Skin pigmentation, UVR and vitamin D

Vitamin D photosynthesis is long-established among animals implying a key role in metabolism. Phytoplankton in the sea have synthesised vitamin D for more than 500 million years and land vertebrates for more than 350 million years. Further, the sophisticated biochemical systems used by humans to balance the harmful and beneficial effects of sunlight demonstrate the evolutionary pressures on these processes. Protection from UVR has been provided by the development of a sunscreen; eumelanin. Eumelanin absorbs UVR, reducing its penetration and, thereby, formation of potentially harmful free radicals (reactive oxygen species) in the skin. The migration of humans from Africa to environments of often low and highly seasonal UVR placed pressure on the original constitutive, dark-skinned phenotype[38]. Thus vitamin D$_3$ synthetic ability, following movement into higher latitudes, was enabled by polymorphic change in genes that determine skin pigmentation, such as melanocortin 1 receptor, with the resulting development of partially depigmented phenotypes capable of tanning. Thus, the present range of skin pigmentation results from a requirement to promote cutaneous UVR-induced vitamin D$_3$ synthesis (depigmented phenotype) and simultaneously prevent UVR-induced damage (pigmented phenotype)[38].

Studying the relationship between UVR exposure, vitamin D status, skin type and disease risk is complicated by historical and recent population movements resulting in many people living under solar regimes very different to those in which their ancestors developed mechanisms to balance sunlight's harmful and beneficial effects. The health penalties of these movements are still under assessment though the potentially serious consequences of chronically low exposure are now being recognised. This of course, does not mitigate the need to ensure that the risks associated with inappropriate and excessive UVR exposure in terms of skin and other

cancers continues to be emphasised.

Environmental factors affecting exposure to UVR

The amount of UVR reaching earth varies with the angle at which radiation passes through the atmosphere (solar zenith angle), its path length through air, the presence of clouds and pollution in the lower atmosphere[39,40]. Consequently, place and time of day and season are important. Outside of tropical latitudes, ensuring a year-round, adequate level of vitamin D synthesis is problematic because large solar zenith angles and long path lengths result in increased absorption and scattering of UVR. During the year the availability of vitamin D_3-inducing UVB wavelengths varies with latitude and outside the tropics there is little or no UVB in sunlight except at high altitudes for much of the year. For example, the equator sees only about a 20% variation while 50° N (circle of latitude that crosses the English Channel, Belgium, Czech Republic, Russia, Mongolia and Canada) sees around 250% variation. Indeed, between November-February, people living at latitude 50° N and higher receive no effective vitamin D_3-inducing UVB and can effect no vitamin synthesis[39,40]. This latitude effect is compounded by dark skin pigmentation; the higher the eumelanin content the lower the vitamin D_3 production. Thus, for many individuals there is insufficient UVB over the year to allow adequate vitamin D synthesis and therefore a need to consume vitamin D_3-rich foods such as oily fish. An additional problem in ensuring adequate vitamin D status, particularly away from the equator, is presented by modern urban lifestyles. Exposure to UVR is limited by clothing, shade-seeking behaviour, often because of skin cancer fears, and occupations that result in 80%-90% of work time being spent indoors.

Assessing vitamin D status and defining hypovitaminosis

Exposure to sunlight or dietary intake of vitamin D increases the serum concentration of 25(OH)D making this a ready indicator of body vitamin D status. Establishing a link between chronic hypovitaminosis and disease risk clearly requires definition of a normal serum concentration of 25(OH)D. The serum 25(OH)D concentrations that identify hypovitaminosis D are not fully defined though the following ranges have been suggested; deficiency: $\leqslant$ 12 ng/mL (30 nmol/L), insufficiency: 12-20 ng/mL and satisfactory status: $\geqslant$ 20 ng/mL (50 nmol/L). However, given the well-recognised seasonal variation in vitamin synthesis, particularly in northerly latitudes, any reference range needs to be considered in the context of season. Recently Tandeter described an Individual Mean Annual vitamin D level termed the "IMAD level" and a recovery formula "RF" that may be used to calculate a mean that encompasses values from four seasons[41].

Relationship between season and vitamin D status

Understanding the temporal relationship between seasons, solar radiation and vitamin D photosynthesis is important if epidemiological approaches are used to establish associations between these variables, disease risk and outcome. Furthermore, the impact of relative acute or chronic hypovitaminosis on the relationship between seasons and disease pathogenesis is unclear. For example, if chronic hypovitaminosis D was pathological, a visible consequence might take some time to be clinically evident and therefore not easily associated with the seasons[42].

Surprisingly given the potential impact of vitamin D on public health, there is little data on the relationship between seasons, serum vitamin concentrations and lag time between firstly, solar radiation and building up of adequate levels of the vitamin and secondly, which chronic patterns of hypovitaminosis have most impact on the pathogenesis of particular diseases[42]. Thus, while the causal link between skin exposure to solar UVR and serum vitamin D cyclicity is recognised, neither the mathematical relationship between the peaks and troughs of serum 25(OH)D concentrations during the year nor how (or if) particular patterns affect disease risk have been well defined. Kasahara *et al*[42] also provide a model describing the seasonality of serum 25(OH)D concentrations in the United States that could be extrapolated to other studies[41]. They argued that in the temperate northern hemisphere, serum 25(OH)D concentrations vary during the year because production is determined by the area of skin exposed to UVR and the intensity of the radiation. Thus, serum vitamin concentrations demonstrate maximum levels in late summer and lowest in late winter. This presumably reflects significant photosynthesis and gradual accumulation of vitamin D during the early spring months and a gradual use of reserves in months immediately after photosynthesis ceases when there is little sunlight. Thus, serum vitamin D concentrations demonstrate a seasonal lag pattern that is influenced by how much atmosphere sunlight must pass through before reaching the human body.

ASSOCIATION BETWEEN HYPOVITAMINOSIS D, THE METABOLIC SYNDROME AND CVD RISK

Importance of vitamin D: Population studies using mortality/morbidity as outcome

Many studies suggest that low serum vitamin D concentrations, even when above those associated with rickets, are deleterious. A variety of criteria have been used as clinical endpoints. For example, Schöttker *et al*[43] studied the association between serum 25(OH)D concentrations and mortality in a meta-analysis of data from eight prospective cohort studies involving 26018 men and women aged 50-79 years from Europe and the United States. The outcome measures were all-cause, cardiovascular, and cancer mortality. As expected, 25(OH)D concentrations were higher in summer and in

men. During follow-up a total of 6695 study participants died; 2624 of these subjects died of CVDs and 2227 of cancer. Despite levels of 25(OH)D strongly varying with country, gender and season, the association between 25(OH)D concentration and all-cause and cause-specific mortality was consistent[43]. The lowest 25(OH)D quintile was associated with increased all-cause mortality, cardiovascular mortality and cancer mortality (in those with a history of cancer)[43]. The inverse association across quintiles was consistent across countries, genders, season and age groups despite 25(OH)D cut-off values varying according to these characteristics[43].

Associations between UVR exposure and disease risk and outcome have been reported for a wide range of pathologies, although in most cases conflicting data have also presented. Corresponding studies using serum 25(OH)D also show conflicting data. For example, we have presented data indicating that UVR may influence disease risk by a vitamin D mediated mechanism in the pathogenesis of prostate cancer[44,45] and multiple sclerosis[46] though we emphasise that these associations remain unproven and any mechanistic basis is uncertain[47].

Metabolic syndrome and seasons

Clearly, any suggestion that risk of the metabolic syndrome is partly determined by vitamin D status would be helped by evidence that the incidence of the syndrome, and/or its component phenotypes, is linked with availability of the vitamin and/or the seasons. Some evidence supporting this view is available. Kamezaki *et al*[48] reported such links in 1202 Japanese males (44 ± 10 years) who were assessed in summer and winter in 2008 for the metabolic syndrome defined using the criteria proposed by the NCEP, the IDF and the Japanese Society of Internal Medicine (JSIM). The prevalence rates of NCEP, IDF, and JSIM defined metabolic syndrome in winter were 3.8%, 15.1% and 12.4% and in summer, 3.2%, 10.7% and 8.4% respectively[48]. Blood pressure changes were most significantly correlated with this seasonal variation in metabolic syndrome prevalence[48].

However, inconsistent results regarding the putative association of key components of the metabolic syndrome with season have been reported including more insulin resistance and higher triglyceride concentrations during the summer in some, winter in others and some showing no significant seasonal variation. Taiwanese subjects described by Chen *et al*[49] were studied in winter (January and February) and summer (July and August) in 2002. They found higher levels of fasting insulin, HOMA-insulin resistance and triglycerides, but lower levels of HDL-C in summer compared with winter. The prevalence of metabolic syndrome in summer was higher than in winter; difference of 7.7% in both genders (P = 0.0092 in men, P = 0.0037 in women). After controlling for BMI and other risk profiles, summer was independently and positively associated with fasting insulin and insulin resistance regardless of metabolic syndrome[49].

A further interesting association between the metabolic syndrome and season is the report by Rintamäki *et al*[50] showing a significant association between seasonal changes in mood and behaviour and the metabolic syndrome. Individuals with the syndrome had greater seasonal changes in mood and behaviour.

Metabolic syndrome and vitamin D status: Observational studies

Considerable research has focussed on associations between vitamin D levels and the prevalence of the metabolic syndrome and its component features. Many studies demonstrate an inverse relationship between serum 25(OH)D and diabetes, metabolic syndrome, insulin resistance and beta cell function[51,52]. The NHANES data confirmed the inverse relationship between 25(OH)D levels and diabetes and insulin resistance in the non-Hispanic white and Mexican American, but not in the non-Hispanic black populations[53,54].

A meta-analysis of 28 studies (between 1990 and 2009) including 99745 participants (age range: 40.5-74.5 years) by Parker *et al*[1] investigated the effects of vitamin D on the risk of CVD, diabetes and the metabolic syndrome[1]. Higher levels of vitamin D were seen to be associated with reduction of all the outcomes studied among middle aged and elderly individuals. The 28 studies reported 33 ORs when considering the association between 25(OH)D and cardiometabolic outcomes; 29 of these ORs suggested an inverse relationship with 3 indicating an opposite effect with 1 analysis remaining non-significant[1]. The pooled OR was 0.57 (95%CI: 0.48-0.57). Prevalence of the metabolic syndrome was the outcome in 8 of the studies; all these showing a significant association between high 25(OH)D levels and reduced metabolic syndrome prevalence (OR = 0.49, 95%CI: 0.38-0.64).

Ju *et al*[55] studied the relationship between serum 25(OH)D levels and metabolic syndrome in the general adult population using a dose-response meta-analysis based on studies reporting risk ratios for metabolic syndrome in categories of serum 25(OH)D concentrations. The pooled OR for the metabolic syndrome per 25 nmol/L (10 ng/mL) increment in the 25(OH)D concentration was 0.87 (95%CI: 0.83-0.92), based on 16 cross-sectional studies and 1.00 (95%CI: 0.98-1.02) for 2 cohort and nested case-control studies[55]. The dose-response meta-analysis showed a generally linear, inverse relationship between 25(OH)D levels and the metabolic syndrome in the cross-sectional studies [probability (P) value for linear trend < 0.001]. They concluded that vitamin D status was associated with metabolic syndrome risk in cross-sectional but not longitudinal studies[55].

Song *et al*[56] reported a cross-sectional study comprising 778 Korean adults. Metabolic syndrome was defined according to the American Heart Association/National Heart, Lung, and Blood Institute criteria and the Korean Society for the Study of Obesity. The overall prevalence of the metabolic syndrome was 18.9%[56]. After multiple adjustments, compared with the highest

quartile serum 25(OH)D level group (19.9-55.9 ng/mL), the OR for metabolic syndrome in the lowest level group (4.2-9.7 ng/mL) was 2.44 (95%CI:1.32-4.48). The intermediate quartiles (9.8-14.1 ng/mL) and (14.3-19.8 ng/mL) had ORs of 2.20 (95%CI: 1.24-3.90) and 1.81 (95%CI: 1.02-3.20) respectively when compared to the highest quartile. Among the components of metabolic syndrome, the adjusted ORs for elevated blood pressure and high triglycerides in the lowest 25(OH)D level were 1.81 (95%CI: 1.15-2.85) and 2.74 (95%CI: 1.64-4.57) respectively[56].

Thus, it is clear from these observational surveys that a relationship may exist between 25(OH)D levels and glucose homeostasis, metabolic syndrome and type 2 diabetes. These population studies do not hint as causation as 25(OH)D status and other established risk factors were not measured at or prior to diagnosis. Thus, prospective studies are required that take into account other confounding factors such as serial weight measurements, physical activity and family history.

Metabolic syndrome and vitamin D status: Prospective studies

A number of prospective studies have also presented data that support the proposal that low serum 25(OH)D concentrations are associated with increased risk of the development of the metabolic syndrome. For example, Gagnon *et al*[57] studied 4164 adults (mean age 50 years; 58% women; 92% Europids). Over the following 5 years, 528 incident cases (12.7%) of the metabolic syndrome were identified[57]. Compared with the reference category [highest quintile 25(OH)D $\geqslant$ 34 ng/mL], the metabolic syndrome risk was significantly higher in people with 25(OH)D in the first (< 18 ng/mL) and second (18-23 ng/mL) quintiles [OR = 1.41 (95%CI: 1.02-1.95) and 1.74 (95%CI: 1.28-2.37) respectively][57]. Serum 25(OH)D was inversely associated with waist circumference ($P < 0.001$), triglycerides ($P < 0.01$), fasting glucose ($P < 0.01$), and HOMA-IR ($P < 0.001$) but not with 2-h plasma glucose ($P = 0.29$), HDL-C ($P = 0.70$), or blood pressure ($P = 0.46$)[57].

More recently Kayaniyil *et al*[58] examined the prospective association of 25(OH)D with the metabolic syndrome in a multi-ethnic cohort of non-diabetic adults with pre-existing risk factors in Ontario, Canada. Of 654 participants enrolled at baseline, 489 attended a 3 year follow-up visit. Multivariate logistic regression analyses indicated a decreased risk of the metabolic syndrome at follow-up per standard deviation increase in baseline 25(OH)D after adjustment for sociodemographics, season, baseline and change in supplement use, physical activity and insulin resistance (OR = 0.63, 95%CI: 0.44-0.90)[58].

Associations between the defining components of the metabolic syndrome and vitamin D status: Observational, prospective and interventional studies

The observational and prospective studies previously described demonstrate associations between 25(OH)D concentrations and the metabolic syndrome, but were not designed to explore mechanistic aspects. We now review the effect that 25(OH)D levels may have on the defining characteristics of the metabolic syndrome; abdominal adiposity, insulin resistance (and beta cell function), hypertension and atherogenic dyslipidaemia.

Karatas *et al*[59] investigated the association between 25(OH)D levels and all components of the metabolic syndrome in 287 Turkish subjects. Of these, 214 participants were either obese (BMI $\geqslant$ 30 kg/m^2) or overweight (BMI: 25-29.9 kg/m^2). Metabolic syndrome was classified using IDF criteria. Multiple logistic regression analyses were carried out with metabolic syndrome, abdominal obesity, low HDL-C, hypertriglyceridaemia and hypertension as the dependent variable and with 25(OH)D as a continuous independent variable in one set of analyses and 25(OH)D levels stratified as deficiency (< 20 ng/mL), insufficiency (20-29.9 ng/mL) and sufficient (reference level) groups as a factorised independent variable in further analyses. The analyses were corrected for age, gender and season. Hypovitaminosis was significantly more common in the overweight/obese individuals with and without the metabolic syndrome[59]. There was a significant inverse relationship between triglyceride levels and serum 25(OH)D concentration. No significant associations between 25(OH)D and HDL-C, hypertension and insulin resistance were observed.

Obesity has been associated with hypovitaminosis D, perhaps *via* multiple mechanisms[60,61]. The nature of this association was investigated by a bi-directional genetic study that suggested higher BMI resulted in lower 25(OH)D levels but with the reverse effect being small[62]. They concluded that weight reducing interventions would be expected to reduce the prevalence of hypovitaminosis D[62]. In contrast Salehpour *et al*[63] carried out a 12 wk study following cholecalciferol supplementation and showed a significant decrease in body fat mass in both healthy and obese women compared to the placebo arm[63]. These conflicting findings make it essential that both interventions (weight reduction and vitamin D replacement) are studied in detail with suitably designed trials. Other studies investigating mechanisms, unlike Vimaleswaran *et al*[62], have indicated a bi-directional association between obesity and hypovitaminosis D. It has been seen from animal studies that vitamin D may play a part in adipogenesis and energy metabolism. The VDR is expressed in adipose tissue pre-maturation[64] and in early adipogenesis[65]. The presence of a role in adipogenesis is also suggested by adipocyte atrophy seen in VDR knockout mice[66].

The relationship between volume of adipose tissue and vitamin D status, at least as reflected in serum 25(OH)D concentrations, is unclear. Vitamin D is sequestered in adipose tissue and it has been speculated that obesity, by increasing the volume of distribution of available adiposity, will lead to lower serum vitamin D levels[67,68]. This view is contradicted by Pramyothin *et al*[69] who measured vitamin D levels in the subcutaneous

abdominal fat of 17 patients undergoing gastric bypass. Vitamin measurements were made at surgery and over a 12 mo follow-up period[69]. It was found that vitamin D levels in adipose tissue varied considerably and no significant change in serum 25(OH)D was noted during follow-up despite intake of supplements (> 2500 U/d).

There has been speculation that behaviour traits associated with obesity, such as reduced outdoor exercise levels, could be associated with decreased exposure and reduced vitamin D synthesis. Results from studies investigating this possible association have varied[70,71]. Thus, although a clear association is evident between adiposity and vitamin D levels the nature of this association has yet to be determined. It is important to establish this relationship as central adiposity is a key driver in the development of the metabolic syndrome.

Dysfunction of insulin secretion by pancreatic beta cells and insulin resistance are considered to be causative drivers in the aetiology of type 2 diabetes[26]. Insulin secretion may be affected by lipotoxicity, due to increased free fatty acids, and glucotoxicity, due to elevated serum glucose and lipid accumulation within the beta cells[72]. We have seen that insulin resistance is a core component of the metabolic syndrome. Contrasting findings are evident in observational studies investigating the relationship between 25(OH)D levels and insulin sensitivity. Chiu *et al*[52], in Californian students of mixed ethnicity, and Kamycheva *et al*[73], in a study of patients with hyperparathyroidism, [patients grouped by the median 25(OH)D concentration] noted a positive correlation between insulin sensitivity and 25(OH)D levels. However, there have been other studies which have not shown the above association, these having been carried out in patient groups characterised by obesity[74], non-diabetic status[75] and the metabolic syndrome[76]. A prospective study of 524 non-diabetic individuals by Forouhi *et al*[77] showed an inverse association between 25(OH)D levels and the risk of insulin resistance and elevated blood sugars[77]. However, the Mini-Finland Health Survey did not demonstrate a significant correlation between 25(OH)D quartiles and the onset of diabetes when the analysis was corrected for BMI and activity[78].

Vitamin D supplementation has been seen to alter insulin sensitivity in non-diabetic patients, but not in patients diagnosed with type 2 diabetes[79,80]. Pittas *et al*[81] demonstrated that, when compared to placebo, vitamin D had a positive effect on insulin resistance and glycaemic control (non-primary outcome) in a randomised controlled study of patients with impaired fasting glucose[81]. A complex mechanism is suggested by the SURAYA trial of obese south Asian women as insulin resistance was seen to improve only when supplementation elevated the 25(OH)D concentration above 80 nmol/L this perhaps indicates either a dose response or threshold effect[82].

Given the association between 25(OH)D levels and obesity it is expected that there would be a similar relationship with the lipid concentrations; however, study results have varied. A large study in Norway, both longitudinal (n = 2159) and cross sectional (n = 10105) demonstrated that higher levels of cholesterol, HDL-C and LDL-C and lower levels of triglyceride were associated with reduced 25(OH)D concentrations[83]. A survey of 108711 patients who had multiple 25(OH)D and lipid profiles measured revealed a similar relationship between 25(OH)D and cholesterol and LDL-C levels[84]. Further, optimal levels of 25(OH)D were associated with higher HDL-C[84]. More confusion has arisen as vitamin D supplementation following their cross sectional survey in patients with hypovitaminosis did not led to consistent changes in the lipid profile[84]. Jorde *et al*[85] reviewed the findings of 22 cross sectional and 10 placebo controlled double blind randomised controlled trials and concluded that, while the cross sectional studies demonstrated a uniform inverse relationship between 25(OH)D and triglyceride levels, the intervention studies with vitamin D supplementation have led to varied results. They concluded that these intervention studies were not adequately designed to specifically investigate the relationship between 25(OH)D and lipids and speculated that the relationship between 25(OH)D and lipids could be either direct or *via* changes in parathyroid hormone and/or calcium concentrations[85].

Many studies using mouse and human hepatoma cell lines[86,87] and VDR knockout mice[88] have been carried out to understand the observed associations between 25(OH)D and lipid concentrations. Some have examined the effect of VDR on bile acid synthesis, and cholesterol levels, once again with inconsistent results[89].

Hypertension, one of the defining components of the metabolic syndrome, has been reported to display a seasonal and geographical variability raising the possibility of sun exposure having a role[90]. Even before this observation Resnick *et al*[91] in 1986 suggested that vitamin D metabolites were associated with hypertension potentially *via* the renin-angiotensin system[91]. Both animal and cross-sectional human studies have suggested vitamin D to be an inhibitor of the renin-angiotensin system in VDR knockout[92] and 1α-hydroxylase knockout[93] mice with significantly raised renin activity and plasma angiotensin 2 concentrations. The effects were reversed in the 1α-hydroxylase knockout mice by administration of $1,25(OH)_2D$[93]. Vascular smooth muscle and endothelial cells express VDR and the 1α-hydroxylase enzyme indicating that vitamin D may influence endothelial function which could lead to arterial stiffness and hypertension, in addition to plaque formation[94]. The change in endothelial function could be due to either a direct effect or *via* improved blood pressure.

Most of the surveys such as NHANES Ⅲ[95], the German National Health Interview and Examination Survey[96] and the 1958 British Birth Cohort[97] investigating the relationship between vitamin D and hypertension have pointed to an inverse association. However, there have been studies that have not shown this

association[98,99]. Once again the mixed findings could have been due to confounding variables common in multifactorial pathology. Similarly prospective studies too have not been consistent with regards to outcome[100,101]. Further, interventional trials have also resulted in varied results[102,103]. A meta-analysis of 11 interventional trials showed a modest reduction in diastolic blood pressure (3.1 mmHg), but this was not accompanied by any significant change in systolic blood pressure[104]. It was evident that most of the studies were not designed to investigate the association in question. Although observational studies have suggested endothelial dysfunction in individuals with hypovitaminosis D[105,106] results following vitamin D supplements have been missed. While some intervention trials have shown a beneficial effect on endothelial function[107,108] others have not[109,110]. Thus, it is clear that although most studies indicate an association between vitamin D status and blood pressure the findings from observational, prospective and interventional studies have not been unanimous.

We have seen that much of the work presented above, with the exemption of Karatas *et al*[59], has focussed on individual associations between hypovitaminosis D, vitamin D supplementation and components of the metabolic syndrome. As evident from Figure 1 these factors are inter-related and it is essential that future studies take this into account.

Benefits in mortality, CVD and onset of type 2 diabetes observed following vitamin D supplements

As we have seen previously there is considerable evidence that hypovitaminosis D is associated with increased CVD risk although the mechanisms still remain largely unclear. It is essential to determine if this increase in risk can be reversed by supplements. Many questions remain that can only be answered by long term intervention studies. It is important to estimate benefit in the overall study group as well as subgroups based on age, gender, ethnicity, CVD risk, vitamin D levels and other baseline characteristics. Further, benefit associated with different replacement dosage must be evaluated. To this date no large intervention trial fulfilling the above criteria has reported findings.

Vacek *et al*[111] in 2012 carried out an observational retrospective study of 10889 patients seen in a secondary care cardiology setting. Hypovitaminosis (< 30 ng/mL) was diagnosed in 70.3% of this cohort. Vitamin D supplements were taken by 31.6% of the vitamin D deficient patients and 21.3% of patients with normal values and the association between treatment and all-cause mortality studied. Hypovitaminosis D was significantly associated with mortality in patients not on vitamin D replacement (OR = 3.72, 95%CI: 2.563-5.396)[111]. In contrast hypovitaminosis was not significantly associated with mortality in patients on supplements (OR = 1.46, 95%CI: 0.760-2.799). This analysis was not carried out with CVD mortality and morbidity as an outcome measure. It must be noted that this study was in a selected population and was retrospective and observational. Gotsman studied the impact of vitamin D supplements on mortality in 3069 patients with heart failure[112]. Supplementation was associated with significantly reduced mortality (HR = 0.68, 95%CI: 0.54-0.85). However, no convincing data exists regarding the benefits in mortality that may be related to vitamin D supplements in a healthy population.

There are very few studies examining CVD risk reduction following vitamin D supplementation. A systematic review of 17 prospective and randomised trials using vitamin D and/or calcium supplements showed vitamin D supplements, at approximately 1000 IU/d, caused a 10% relative risk reduction that was not significant when compared to placebo[113]. When the analysis was restricted to the 5 prospective studies of patients receiving vitamin D a reduction in CVD related mortality was observed. It must be noted that 4 of these studies consisted of patients receiving dialysis, a high risk group. Interestingly calcium supplementation did not appear to influence any of the outcome measures.

No large randomised control trial has been carried out with onset of metabolic syndrome/diabetes as the primary outcome. The RECORD study where patients were randomised to receive 800 IU/d of vitamin D recorded onset of type 2 diabetes as a secondary outcome (primary outcome was fracture rate)[114]. A non-significant 33% relative risk reduction was seen. Similarly, onset of diabetes was the monitored outcome in the Womens Health Initiative Calcium/Vitamin D Trial with 33951 women randomised to either 400 IU/d of vitamin D or placebo for 7 years and no significant benefit was observed[115]. Most other studies have included smaller patient numbers and have not demonstrated reduced incidence of type 2 diabetes or the metabolic syndrome. Further, no convincing evidence exists that supplementation reduces the progression from the metabolic syndrome to type 2 diabetes.

Mixed results have been observed when insulin sensitivity has been determined following treatment with vitamin D. Mitri *et al*[116] demonstrated a significant improvement in insulin secretion in 92 individuals at high risk of developing diabetes following randomisation to either 2000 IU/d of vitamin D supplements or placebo. Nagpal *et al*[117] determined the effect vitamin D supplementation (3 doses of 120000 IU) had on insulin sensitivity compared to placebo in 71 healthy male volunteers with central obesity. Insulin sensitivity was seen to improve in the treatment arm[117]. However, there have been other trials demonstrating no improvement in insulin sensitivity. Luo *et al*[118] treated 21 Chinese patients with type 2 diabetes and hypovitaminosis D ($\leqslant$ 50 nmol/L) with 2000 IU/d of vitamin D for a 3 mo period. No changes were observed in any of the metabolic syndrome parameters, HbA1c or in insulin requirements[118]. George *et al*[119] published a systematic review of 15 trials assessing the effects of vitamin D supplementation compared to placebo on fasting glucose,

glycaemic control and insulin resistance. When all the studies were combined no significant improvement in outcomes was observed. When the analyses were restricted, to patients with diabetes or impaired glucose tolerance, significant but small improvements were observed in both fasting glucose and insulin sensitivity, but no changes seen in HbA1c[119].

All the studies described above leave an impression that vitamin D supplementation could potentially be beneficial. However, current evidence does not allow us to identify patient groups that would benefit maximally.

Vitamin D and type 2 diabetes

We have focussed this review on hypovitaminosis D in the metabolic syndrome and its defining components as well as CVD. We have described that hypovitaminosis D appears to be related to the metabolic syndrome, potentially a pre-diabetic state and its component characteristics such as obesity and insulin resistance. Thus, we would expect there to be a relationship between vitamin D levels and type 2 diabetes. We have also described current evidence as to the effects of vitamin D supplementation on diabetes control. In addition to actions that may be mediated *via* obesity and insulin resistance which we have described above, hypovitaminosis D appears to have a direct effect on glycaemic control. It has been suggested that vitamin D could have a role in ensuring calcium influx into cells which may be essential to the actions of insulin in skeletal muscle and adipocytes[120]. There have been hints that elevated parathyroid hormone levels may blunt the actions of insulin[121]. Although outside the boundaries of this review, an association between type 1 diabetes and hypovitaminosis D also suggests at a direct action of vitamin D on insulin action that may also be relevant to type 2 diabetes[122].

Vitamin D and non-alcoholic fatty liver disease

Individuals with the metabolic syndrome of long duration are considered to be at greater risk of developing hepatic steatosis[123]. A two or three hit hypothesis has been proposed[124]. The first hit is considered to be the damage caused by fatty infiltration associated with insulin resistance and obesity[124]. The second and third hits are thought to be due to hepatic injury resulting from mechanisms linked to oxidative stress and impaired cellular regeneration[124]. Hepatic fatty infiltration could progress through non-alcoholic steatohepatitis and liver fibrosis to liver cirrhosis. Management of this spectrum has focused on improving the metabolic syndrome phenotype with weight reduction and management of dyslipidaemia and hyperglycaemia[125].

As hypovitaminosis D is related to the metabolic syndrome we would expect an association with non-alcoholic fatty liver disease. A review of 6800 patients on the NHANES Ⅲ database showed that those with an elevated serum alanine transaminase activity were seen to have lower vitamin D concentrations compared to matched controls with normal enzyme levels, the analysis being corrected for the metabolic syndrome[126]. This association (independent of age, gender, triglycerides and insulin resistance) was also observed by Barchetta *et al*[127] in a study of 262 patients. Further, vitamin D levels were lower in patients with non-alcoholic fatty liver disease diagnosed by liver biopsy[128]. Hypovitaminosis D has been associated with altered regulation of inflammatory and anti-oxidant pathways in addition to influencing the metabolic syndrome phenotype; all the hits postulated in the aetiology of steatosis[129]. At present there is no conclusive evidence that vitamin D supplementation could lead to clinical improvement of hepatic steatosis. Interestingly treatment with agents such as ursodeoxycholic acid, which increases vitamin D concentrations, has shown some improvement in non-alcoholic steatohepatitis with alanine transaminase levels used as the outcome[130]. However, ursodeoxycholic acid may possess direct anti-inflammatory anti-oxidant properties which may be significant confounding factors.

CONCLUSION

It is clear that hypovitaminosis D has extra-skeletal effects that impact on the development of various pathologies including those that make up a large majority of morbidity and mortality; cancer, CVD and diabetes. In this review we have focussed on the association between hypovitaminosis D and the metabolic syndrome. Recently there has been a significant increase in the number of individuals with the metabolic syndrome. Indeed, as much as 40% of the United States population suffers from the condition comprising some or all of a cluster of CVD risk factors. Although the metabolic syndrome does not confer additional risk compared to the component risk factors we believe it helpful to the clinician and researcher to classify patients because it encourages a holistic approach to CVD risk reduction and study of the inter-relationships between the different relevant factors respectively.

There is considerable confusion surrounding the association between vitamin D and the metabolic syndrome, its component factors, CVD and mortality. Although studies have not been unanimous in their findings we are left with the impression that hypovitaminosis D is probably associated with all the above outcomes. However, the nature of this relationship in subgroups (*e.g.*, gender, age groups, ethnicity, *etc*.) is not clear. The risk associated with varying levels of vitamin D has not been estimated. Mechanisms that lead to increased prevalence of the components of the metabolic syndrome and its associated risk have not been worked out. Even more confusing is whether there is any benefit in vitamin D replacement therapy as trials have been contradictory.

However, there appears to be sufficient evidence to make the unravelling of the association between hypovitaminosis D and the metabolic syndrome a priority. Today both conditions are of high prevalence. This suggests that even if a modest decrease in CVD risk is

observed following vitamin D replacement it will translate to substantial overall benefits. Due to the modest price of supplements and relative safety, the cost benefits could be in favour of vitamin D replacement.

What is required are well designed studies, both prospective and intervention. In addition to estimating overall benefit, they must be sufficiently powered to study subgroups as well as risk and benefits at varying serum vitamin D concentrations as well as replacement regimes. It is only following the availability of this data that clear recommendations can be made with regards vitamin D replacement in patients with the components of the metabolic syndrome.

REFERENCES

1 **Parker J**, Hashmi O, Dutton D, Mavrodaris A, Stranges S, Kandala NB, Clarke A, Franco OH. Levels of vitamin D and cardiometabolic disorders: systematic review and meta-analysis. *Maturitas* 2010; **65**: 225-236 [PMID: 20031348 DOI: 10.1016/j.maturitas.2009.12.013]

2 **Talaei A**, Mohamadi M, Adgi Z. The effect of vitamin D on insulin resistance in patients with type 2 diabetes. *Diabetol Metab Syndr* 2013; **5**: 8 [PMID: 23443033 DOI: 10.1186/1758-5996-5-8]

3 **Kylin E**. Studien uber das hypertonie-hyperglykamie-hyperuri-kamiesyndrom. *Zentrablfinnere Med Leipz* 1923; **81**: 105-127

4 **Himsworth HP**, Kerr RB. Insulin-sensitive and insulin-insensitive types of diabetes mellitus. *Clin Sci* 1939; **4**: 119-152

5 **Vague J**. The degree of masculine differentiation of obesities: a factor determining predisposition to diabetes, atherosclerosis, gout, and uric calculous disease. *Am J Clin Nutr* 1956; **4**: 20-34 [PMID: 13282851]

6 **Reaven GM**. Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes* 1988; **37**: 1595-1607 [PMID: 3056758 DOI: 10.2337/diab.37.12.1595]

7 **Balkau B**, Charles MA. Comment on the provisional report from the WHO consultation. European Group for the Study of Insulin Resistance (EGIR). *Diabet Med* 1999; **16**: 442-443 [PMID: 10342346 DOI: 10.1046/j.1464-5491.1999.00059.x]

8 **International Statistical Classification of Diseases and Related Health Problems 10th Revision**. ICD-10 World Health Organisation. 2010. Available from: URL: http://apps.who.int/classifications/icd10/browse/2010/en

9 **Alberti KG**, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med* 1998; **15**: 539-553 [PMID: 9686693 DOI: 10.1002/(SICI)1096-9136(199807)15:7<539::AID-DIA668>3.0.CO;2-S]

10 **Einhorn D**, Reaven GM, Cobin RH, Ford E, Ganda OP, Handelsman Y, Hellman R, Jellinger PS, Kendall D, Krauss RM, Neufeld ND, Petak SM, Rodbard HW, Seibel JA, Smith DA, Wilson PW. American College of Endocrinology position statement on the insulin resistance syndrome. *Endocr Pract* 2003; **9**: 237-252 [PMID: 12924350]

11 **National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III)**. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation* 2002; **106**: 3143-3421 [PMID: 12485966]

12 **Alberti KG**, Zimmet P, Shaw J. The metabolic syndrome--a new worldwide definition. *Lancet* 2005; **366**: 1059-1062 [PMID: 16182882 DOI: 10.1016/S0140-6736(05)67402-8]

13 **Zimmet P,** Alberti KG, Serrano Ríos M. A New International Diabetes Federation (IDF) Worldwide Definition of the Metabolic Syndrome: the Rationale and the Results. *Rev Esp Cardiol* (Engl Ed) 2005; **58**: 1371-1375 [PMID: 16371194]

14 **Alberti KG**, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, Fruchart JC, James WP, Loria CM, Smith SC. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation* 2009; **120**: 1640-1645 [PMID: 19805654 DOI: 10.1161/CIRCULATIONAHA.109.192644]

15 **Zimmet P**, Alberti KG, Kaufman F, Tajima N, Silink M, Arslanian S, Wong G, Bennett P, Shaw J, Caprio S. The metabolic syndrome in children and adolescents - an IDF consensus report. *Pediatr Diabetes* 2007; **8**: 299-306 [PMID: 17850473 DOI: 10.1111/j.1399-5448.2007.00271.x]

16 **Ford ES**. Prevalence of the metabolic syndrome defined by the International Diabetes Federation among adults in the U.S. *Diabetes Care* 2005; **28**: 2745-2749 [PMID: 16249550]

17 **Gami AS**, Witt BJ, Howard DE, Erwin PJ, Gami LA, Somers VK, Montori VM. Metabolic syndrome and risk of incident cardiovascular events and death: a systematic review and meta-analysis of longitudinal studies. *J Am Coll Cardiol* 2007; **49**: 403-414 [PMID: 17258085 DOI: 10.1016/j.jacc.2006.09.032]

18 **Eddy DM**, Schlessinger L, Heikes K. The metabolic syndrome and cardiovascular risk: implications for clinical practice. *Int J Obes* (Lond) 2008; **32** Suppl 2: S5-10 [PMID: 18469838 DOI: 10.1038/ijo.2008.28]

19 **Bayturan O**, Tuzcu EM, Lavoie A, Hu T, Wolski K, Schoenhagen P, Kapadia S, Nissen SE, Nicholls SJ. The metabolic syndrome, its component risk factors, and progression of coronary atherosclerosis. *Arch Intern Med* 2010; **170**: 478-484 [PMID: 20212186 DOI: 10.1001/archinternmed.2009.551]

20 **Eckel RH**, Alberti KG, Grundy SM, Zimmet PZ. The metabolic syndrome. *Lancet* 2010; **375**: 181-183 [PMID: 20109902 DOI: 10.1016/S0140-6736(09)61794-3]

21 **Shetty C**, Balasubramani M, Capps N, Milles J, Ramachandran S. Paradoxical HDL-C reduction during rosiglitazone and fibrate treatment. *Diabet Med* 2007; **24**: 94-97 [PMID: 17227331 DOI: 10.1111/j.1464-5491.2007.02029.x]

22 **Ramachandran S**, Saraf S, Shetty C, Capps N, Bailey C. Paradoxical decrease in HDL-cholesterol and apolipoprotein A1 with simvastatin and atorvastatin in a patient with type 2 diabetes. *Ann Clin Biochem* 2011; **48**: 75-78 [PMID: 21115572 DOI: 10.1258/acb.2010.010081]

23 **Abbas A**, Saraf S, Ramachandran S, Raju J, Ramachandran S. Fibrates and estimated glomerular filtration rate: observations from an outpatient clinic setting and clinical implications. *Postgrad Med J* 2012; **88**: 503-506 [PMID: 22460858 DOI: 10.1136/postgradmedj-2011-130594]

24 **Ramachandran S**, Abbas A, Saraf S, Raju J, Jewkes C, Jones AF. Significant increase in high-density lipoprotein cholesterol with fibrates is associated with low pretreatment high-density lipoprotein cholesterol: findings from an outpatient clinic setting. *Metab Syndr Relat Disord* 2012; **10**: 189-194 [PMID: 22283634 DOI: 10.1089/met.2011.0112]

25 **Reilly MP**, Rader DJ. The metabolic syndrome: more than the sum of its parts? *Circulation* 2003; **108**: 1546-1551 [PMID: 14517150 DOI: 10.1161/01.CIR.0000088846.10655.E0]

26 **Gallagher EJ**, Leroith D, Karnieli E. The metabolic syndrome--from insulin resistance to obesity and diabetes. *Med Clin North Am* 2011; **95**: 855-873 [PMID: 21855696 DOI: 10.1016/j.mcna.2011.06.001]

27 **Reaven GM**. Insulin resistance: the link between obesity and cardiovascular disease. *Med Clin North Am* 2011; **95**: 875-892 [PMID: 21855697 DOI: 10.1016/j.mcna.2011.06.002]

28 **Chrostowska M**, Szyndler A, Hoffmann M, Narkiewicz K. Impact of obesity on cardiovascular health. *Best Pract Res Clin Endocrinol Metab* 2013; **27**: 147-156 [PMID: 23731877 DOI: 10.1016/j.beem.2013.01.004]

29 **Durward CM**, Hartman TJ, Nickols-Richardson SM. All-cause mortality risk of metabolically healthy obese individuals in NHANES III. *J Obes* 2012; **2012**: 460321 [PMID: 23304462 DOI: 10.1155/2012/460321]

30 **Patel P**, Abate N. Body fat distribution and insulin resistance. *Nutrients* 2013; **5**: 2019-2027 [PMID: 23739143 DOI: 10.3390/nu5062019]

31 **Snel M**, Jonker JT, Schoones J, Lamb H, de Roos A, Pijl H, Smit JW, Meinders AE, Jazet IM. Ectopic fat and insulin resistance: pathophysiology and effect of diet and lifestyle interventions. *Int J Endocrinol* 2012; **2012**: 983814 [PMID: 22675355 DOI: 10.1155/2012/983814]

32 **Stern MP**, Williams K, González-Villalpando C, Hunt KJ, Haffner SM. Does the metabolic syndrome improve identification of individuals at risk of type 2 diabetes and/or cardiovascular disease? *Diabetes Care* 2004; **27**: 2676-2681 [PMID: 15505004 DOI: 10.2337/diacare.27.11.2676]

33 **Lada AT**, Rudel LL. Associations of low density lipoprotein particle composition with atherogenicity. *Curr Opin Lipidol* 2004; **15**: 19-24 [PMID: 15166804 DOI: 10.1097/00041433-200402000-00005]

34 **Hawley JA**, Lessard SJ. Exercise training-induced improvements in insulin action. *Acta Physiol* (Oxf) 2008; **192**: 127-135 [PMID: 18171435 DOI: 10.1111/j.1748-1716.2007.01783.x]

35 **Holick MF**, Chen TC. Vitamin D deficiency: a worldwide problem with health consequences. *Am J Clin Nutr* 2008; **87**: 1080S-1086S [PMID: 18400738]

36 **Ramagopalan SV**, Heger A, Berlanga AJ, Maugeri NJ, Lincoln MR, Burrell A, Handunnetthi L, Handel AE, Disanto G, Orton SM, Watson CT, Morahan JM, Giovannoni G, Ponting CP, Ebers GC, Knight JC. A ChIP-seq defined genome-wide map of vitamin D receptor binding: associations with disease and evolution. *Genome Res* 2010; **20**: 1352-1360 [PMID: 20736230 DOI: 10.1101/gr.107920.110]

37 **Whitfield GK**, Hsieh JC, Jurutka PW, Selznick SH, Haussler CA, MacDonald PN, Haussler MR. Genomic actions of 1,25-dihydroxyvitamin D3. *J Nutr* 1995; **125**: 1690S-1694S [PMID: 7782928]

38 **Jablonski NG**. The evolution of human skin colouration and its relevance to health in the modern world. *J R Coll Physicians Edinb* 2012; **42**: 58-63 [PMID: 22441067 DOI: 10.4997/JRCPE.2012.114]

39 **Diffey BL**. Human exposure to solar ultraviolet radiation. *J Cosmet Dermatol* 2002; **1**: 124-130 [PMID: 17147711 DOI: 10.1046/j.1473-2165.2002.00060.x]

40 **Jablonski NG**, Chaplin G. Human skin pigmentation, migration and disease susceptibility. *Philos Trans R Soc Lond B Biol Sci* 2012; **367**: 785-792 [PMID: 22312045 DOI: 10.1098/rstb.2011.0308]

41 **Tandeter H**. Challenging the present definition of "normal" vitamin D levels obtained by a single blood test. Can we develop a formula to predict vitamin D levels in the 4 seasons from a single season's measure? *Med Hypotheses* 2014; **83**: 224-226 [PMID: 24854576 DOI: 10.1016/j.mehy.2014.04.025]

42 **Kasahara AK**, Singh RJ, Noymer A. Vitamin D (25OHD) Serum Seasonality in the United States. *PLoS One* 2013; **8**: e65785 [PMID: 23805188]

43 **Schöttker B**, Jorde R, Peasey A, Thorand B, Jansen EH, Groot Ld, Streppel M, Gardiner J, Ordóñez-Mena JM, Perna L, Wilsgaard T, Rathmann W, Feskens E, Kampman E, Siganos G, Njølstad I, Mathiesen EB, Kubínová R, Pająk A, Topor-Madry R, Tamosiunas A, Hughes M, Kee F, Bobak M, Trichopoulou A, Boffetta P, Brenner H. Vitamin D and mortality: meta-analysis of individual participant data from a large consortium of cohort studies from Europe and the United States. *BMJ* 2014; **348**: g3656 [PMID: 24938302 DOI: 10.1136/bmj.g3656]

44 **Rukin NJ**, Zeegers MP, Ramachandran S, Luscombe CJ, Liu S, Saxby M, Lear J, Strange RC. A comparison of sunlight exposure in men with prostate cancer and basal cell carcinoma. *Br J Cancer* 2007; **96**: 523-528 [PMID: 17262085]

45 **Moon SJ**, Fryer AA, Strange RC. Ultraviolet radiation, vitamin D and risk of prostate cancer and other diseases. *Photochem Photobiol* 2005; **81**: 1252-1260 [PMID: 16022557]

46 **Strange RC**, Ramachandran S, Zeegers MP, Emes RD, Abraham R, Raveendran V, Boggild M, Gilford J, Hawkins CP. The Multiple Sclerosis Severity Score: associations with MC1R single nucleotide polymorphisms and host response to ultraviolet radiation. *Mult Scler* 2010; **16**: 1109-1116 [PMID: 20670983 DOI: 10.1177/1352458510373784]

47 **Rukin NJ**, Luscombe CJ, Strange RC. Vitamin D: new insights into its broader role in disease pathogenesis. *Retinoids* 2005; **21**: 1-5

48 **Kamezaki F**, Sonoda S, Tomotsune Y, Yunaka H, Otsuji Y. Seasonal variation in metabolic syndrome prevalence. *Hypertens Res* 2010; **33**: 568-572 [PMID: 20300109 DOI: 10.1038/hr.2010.32]

49 **Chen SH**, Chuang SY, Lin KC, Tsai ST, Chou P. Community-based study on summer-winter difference in insulin resistance in Kin-Chen, Kinmen, Taiwan. *J Chin Med Assoc* 2008; **71**: 619-627 [PMID: 19114326 DOI: 10.1016/S1726-4901(09)70004-8]

50 **Rintamäki R**, Grimaldi S, Englund A, Haukka J, Partonen T, Reunanen A, Aromaa A, Lönnqvist J. Seasonal changes in mood and behavior are linked to metabolic syndrome. *PLoS One* 2008; **3**: e1482 [PMID: 18213390 DOI: 10.1371/journal.pone.0001482]

51 **Baynes KC**, Boucher BJ, Feskens EJ, Kromhout D. Vitamin D, glucose tolerance and insulinaemia in elderly men. *Diabetologia* 1997; **40**: 344-347 [PMID: 9084975 DOI: 10.1007/s001250050685]

52 **Chiu KC**, Chu A, Go VL, Saad MF. Hypovitaminosis D is associated with insulin resistance and beta cell dysfunction. *Am J Clin Nutr* 2004; **79**: 820-825 [PMID: 15113720]

53 **Scragg R**, Sowers M, Bell C. Serum 25-hydroxyvitamin D, diabetes, and ethnicity in the Third National Health and Nutrition Examination Survey. *Diabetes Care* 2004; **27**: 2813-2818 [PMID: 15562190 DOI: 10.2337/diacare.27.12.2813]

54 **Ford ES**, Ajani UA, McGuire LC, Liu S. Concentrations of serum vitamin D and the metabolic syndrome among U.S. adults. *Diabetes Care* 2005; **28**: 1228-1230 [PMID: 15855599 DOI: 10.2337/diacare.28.5.1228]

55 **Ju SY**, Jeong HS, Kim do H. Blood vitamin D status and metabolic syndrome in the general adult population: a dose-response meta-analysis. *J Clin Endocrinol Metab* 2014; **99**: 1053-1063 [PMID: 24423309 DOI: 10.1210/jc.2013-3577]

56 **Song HR**, Park CH. Low serum vitamin D level is associated with high risk of metabolic syndrome in post-menopausal women. *J Endocrinol Invest* 2013; **36**: 791-796 [PMID: 24317304 DOI: 10.1007/BF03346758]

57 **Gagnon C**, Lu ZX, Magliano DJ, Dunstan DW, Shaw JE, Zimmet PZ, Sikaris K, Ebeling PR, Daly RM. Low serum 25-hydroxyvitamin D is associated with increased risk of the development of the metabolic syndrome at five years: results from a national, population-based prospective study (The Australian Diabetes, Obesity and Lifestyle Study: AusDiab). *J Clin Endocrinol Metab* 2012; **97**: 1953-1961 [PMID: 22442263 DOI: 10.1210/jc.2011-3187]

58 **Kayaniyil S**, Harris SB, Retnakaran R, Vieth R, Knight JA, Gerstein HC, Perkins BA, Zinman B, Hanley AJ. Prospective association of 25(OH)D with metabolic syndrome. *Clin Endocrinol* (Oxf) 2014; **80**: 502-507 [PMID: 23452164 DOI: 10.1111/cen.12190]

59 **Karatas S**, Hekimsoy Z, Dinc G, Onur E, Ozmen B. Vitamin D levels in overweight/obese adults with and without metabolic syndrome. *J Endocrinol Metab* 2013; **3**: 47-56 [DOI: 10.4021/jem166e]

60 **Parikh SJ**, Edelman M, Uwaifo GI, Freedman RJ, Semega-Janneh M, Reynolds J, Yanovski JA. The relationship between obesity and serum 1,25-dihydroxy vitamin D concentrations in healthy adults. *J Clin Endocrinol Metab* 2004; **89**: 1196-1199 [PMID: 15001609 DOI: 10.1210/jc.2003-031398]

61 **Lagunova Z**, Porojnicu AC, Lindberg F, Hexeberg S, Moan J. The dependency of vitamin D status on body mass index, gender, age and season. *Anticancer Res* 2009; **29**: 3713-3720 [PMID: 19667169]

62 **Vimaleswaran KS**, Berry DJ, Lu C, Tikkanen E, Pilz S, Hiraki LT, Cooper JD, Dastani Z, Li R, Houston DK, Wood AR, Michaëlsson K, Vandenput L, Zgaga L, Yerges-Armstrong LM, McCarthy MI, Dupuis J, Kaakinen M, Kleber ME, Jameson K, Arden N, Raitakari

O, Viikari J, Lohman KK, Ferrucci L, Melhus H, Ingelsson E, Byberg L, Lind L, Lorentzon M, Salomaa V, Campbell H, Dunlop M, Mitchell BD, Herzig KH, Pouta A, Hartikainen AL, Streeten EA, Theodoratou E, Jula A, Wareham NJ, Ohlsson C, Frayling TM, Kritchevsky SB, Spector TD, Richards JB, Lehtimäki T, Ouwehand WH, Kraft P, Cooper C, März W, Power C, Loos RJ, Wang TJ, Järvelin MR, Whittaker JC, Hingorani AD, Hyppönen E. Causal relationship between obesity and vitamin D status: bi-directional Mendelian randomization analysis of multiple cohorts. *PLoS Med* 2013; **10**: e1001383 [PMID: 23393431 DOI: 10.1371/journal.pmed.1001383]

63 **Salehpour A**, Hosseinpanah F, Shidfar F, Vafa M, Razaghi M, Dehghani S, Hoshiarrad A, Gohari M. A 12-week double-blind randomized clinical trial of vitamin D3 supplementation on body fat mass in healthy overweight and obese women. *Nutr J* 2012; **11**: 78 [PMID: 22998754 DOI: 10.1186/1475-2891-11-78]

64 **Sato M**, Hiragun A. Demonstration of l alpha,25-dihydroxyvitamin D3 receptor-like molecule in ST 13 and 3T3 L1 preadipocytes and its inhibitory effects on preadipocyte differentiation. *J Cell Physiol* 1988; **135**: 545-550 [PMID: 2840436 DOI: 10.1002/jcp.1041350326]

65 **Blumberg JM**, Tzameli I, Astapova I, Lam FS, Flier JS, Hollenberg AN. Complex role of the vitamin D receptor and its ligand in adipogenesis in 3T3-L1 cells. *J Biol Chem* 2006; **281**: 11205-11213 [PMID: 16467308 DOI: 10.1074/jbc.M510343200]

66 **Guzey M**, Jukic D, Arlotti J, Acquafondata M, Dhir R, Getzenberg RH. Increased apoptosis of periprostatic adipose tissue in VDR null mice. *J Cell Biochem* 2004; **93**: 133-141 [PMID: 15352170 DOI: 10.1002/jcb.20172]

67 **Blum M**, Dolnikowski G, Seyoum E, Harris SS, Booth SL, Peterson J, Saltzman E, Dawson-Hughes B. Vitamin D(3) in fat tissue. *Endocrine* 2008; **33**: 90-94 [PMID: 18338271 DOI: 10.1007/s12020-008-9051-4]

68 **Wortsman J**, Matsuoka LY, Chen TC, Lu Z, Holick MF. Decreased bioavailability of vitamin D in obesity. *Am J Clin Nutr* 2000; **72**: 690-693 [PMID: 10966885]

69 **Pramyothin P**, Biancuzzo RM, Lu Z, Hess DT, Apovian CM, Holick MF. Vitamin D in adipose tissue and serum 25-hydroxyvitamin D after roux-en-Y gastric bypass. *Obesity* (Silver Spring) 2011; **19**: 2228-2234 [PMID: 21701564 DOI: 10.1038/oby.2011.170]

70 **Kull M**, Kallikorm R, Lember M. Body mass index determines sunbathing habits: implications on vitamin D levels. *Intern Med J* 2009; **39**: 256-258 [PMID: 19402866 DOI: 10.1111/j.1445-5994.2009.01900.x]

71 **Harris SS**, Dawson-Hughes B. Reduced sun exposure does not explain the inverse association of 25-hydroxyvitamin D with percent body fat in older adults. *J Clin Endocrinol Metab* 2007; **92**: 3155-3157 [PMID: 17535990 DOI: 10.1210/jc.2007-0722]

72 **Karunakaran U**, Kim HJ, Kim JY, Lee IK. Guards and culprits in the endoplasmic reticulum: glucolipotoxicity and β-cell failure in type II diabetes. *Exp Diabetes Res* 2012; **2012**: 639762 [PMID: 21977023 DOI: 10.1155/2012/639762]

73 **Kamycheva E**, Jorde R, Figenschau Y, Haug E. Insulin sensitivity in subjects with secondary hyperparathyroidism and the effect of a low serum 25-hydroxyvitamin D level on insulin sensitivity. *J Endocrinol Invest* 2007; **30**: 126-132 [PMID: 17392602 DOI: 10.1007/BF03347410]

74 **Manco M**, Calvani M, Nanni G, Greco AV, Iaconelli A, Gasbarrini G, Castagneto M, Mingrone G. Low 25-hydroxyvitamin D does not affect insulin sensitivity in obesity after bariatric surgery. *Obes Res* 2005; **13**: 1692-1700 [PMID: 16286516]

75 **Muscogiuri G**, Sorice GP, Prioletta A, Policola C, Della Casa S, Pontecorvi A, Giaccari A. 25-Hydroxyvitamin D concentration correlates with insulin-sensitivity and BMI in obesity. *Obesity* (Silver Spring) 2010; **18**: 1906-1910 [PMID: 20150902 DOI: 10.1038/oby.2010.11]

76 **Gulseth HL**, Gjelstad IM, Tierney AC, Lovegrove JA, Defoort C, Blaak EE, Lopez-Miranda J, Kiec-Wilk B, Risérus U, Roche HM, Drevon CA, Birkeland KI. Serum vitamin D concentration does not predict insulin action or secretion in European subjects with the metabolic syndrome. *Diabetes Care* 2010; **33**: 923-925 [PMID: 20067973 DOI: 10.2337/dc09-1692]

77 **Forouhi NG**, Luan J, Cooper A, Boucher BJ, Wareham NJ. Baseline serum 25-hydroxy vitamin d is predictive of future glycemic status and insulin resistance: the Medical Research Council Ely Prospective Study 1990-2000. *Diabetes* 2008; **57**: 2619-2625 [PMID: 18591391 DOI: 10.2337/db08-0593]

78 **Mattila C**, Knekt P, Männistö S, Rissanen H, Laaksonen MA, Montonen J, Reunanen A. Serum 25-hydroxyvitamin D concentration and subsequent risk of type 2 diabetes. *Diabetes Care* 2007; **30**: 2569-2570 [PMID: 17626891]

79 **Sugden JA**, Davies JI, Witham MD, Morris AD, Struthers AD. Vitamin D improves endothelial function in patients with Type 2 diabetes mellitus and low vitamin D levels. *Diabet Med* 2008; **25**: 320-325 [PMID: 18279409 DOI: 10.1111/j.1464-5491.2007.02360.x]

80 **Jorde R**, Figenschau Y. Supplementation with cholecalciferol does not improve glycaemic control in diabetic subjects with normal serum 25-hydroxyvitamin D levels. *Eur J Nutr* 2009; **48**: 349-354 [PMID: 19370371 DOI: 10.1007/s00394-009-0020-3]

81 **Pittas AG**, Harris SS, Stark PC, Dawson-Hughes B. The effects of calcium and vitamin D supplementation on blood glucose and markers of inflammation in nondiabetic adults. *Diabetes Care* 2007; **30**: 980-986 [PMID: 17277040]

82 **von Hurst PR**, Stonehouse W, Coad J. Vitamin D supplementation reduces insulin resistance in South Asian women living in New Zealand who are insulin resistant and vitamin D deficient - a randomised, placebo-controlled trial. *Br J Nutr* 2010; **103**: 549-555 [PMID: 19781131 DOI: 10.1017/S0007114509992017]

83 **Jorde R**, Figenschau Y, Hutchinson M, Emaus N, Grimnes G. High serum 25-hydroxyvitamin D concentrations are associated with a favorable serum lipid profile. *Eur J Clin Nutr* 2010; **64**: 1457-1464 [PMID: 20823896 DOI: 10.1038/ejcn.2010.176]

84 **Ponda MP**, Huang X, Odeh MA, Breslow JL, Kaufman HW. Vitamin D may not improve lipid levels: a serial clinical laboratory data study. *Circulation* 2012; **126**: 270-277 [PMID: 22718799 DOI: 10.1161/CIRCULATIONAHA.111.077875]

85 **Jorde R**, Grimnes G. Vitamin D and metabolic health with special reference to the effect of vitamin D on serum lipids. *Prog Lipid Res* 2011; **50**: 303-312 [PMID: 21640757 DOI: 10.1016/j.plipres.2011.05.001]

86 **Jiang W**, Miyamoto T, Kakizawa T, Nishio SI, Oiwa A, Takeda T, Suzuki S, Hashizume K. Inhibition of LXRalpha signaling by vitamin D receptor: possible role of VDR in bile acid synthesis. *Biochem Biophys Res Commun* 2006; **351**: 176-184 [PMID: 17054913]

87 **Chow EC**, Magomedova L, Quach HP, Patel R, Durk MR, Fan J, Maeng HJ, Irondi K, Anakk S, Moore DD, Cummins CL, Pang KS. Vitamin D receptor activation down-regulates the small heterodimer partner and increases CYP7A1 to lower cholesterol. *Gastroenterology* 2014; **146**: 1048-1059 [PMID: 24365583 DOI: 10.1053/j.gastro.2013.12.027]

88 **Schmidt DR**, Holmstrom SR, Fon Tacer K, Bookout AL, Kliewer SA, Mangelsdorf DJ. Regulation of bile acid synthesis by fat-soluble vitamins A and D. *J Biol Chem* 2010; **285**: 14486-14494 [PMID: 20233723 DOI: 10.1074/jbc.M110.116004]

89 **Li T**, Chiang JY. Nuclear receptors in bile acid metabolism. *Drug Metab Rev* 2013; **45**: 145-155 [PMID: 23330546 DOI: 10.3109/03602532.2012.740048]

90 **Rostand SG**. Ultraviolet light may contribute to geographic and racial blood pressure differences. *Hypertension* 1997; **30**: 150-156 [PMID: 9260973]

91 **Resnick LM**, Müller FB, Laragh JH. Calcium-regulating hormones in essential hypertension. Relation to plasma renin activity and sodium metabolism. *Ann Intern Med* 1986; **105**: 649-654 [PMID: 3532893 DOI: 10.7326/0003-4819-105-5-649]

92 **Bouillon R**, Carmeliet G, Verlinden L, van Etten E, Verstuyf A, Luderer HF, Lieben L, Mathieu C, Demay M. Vitamin D and human health: lessons from vitamin D receptor null mice. *Endocr Rev* 2008; **29**: 726-776 [PMID: 18694980 DOI: 10.1210/er.2008-0004]

93 **Zhou C**, Lu F, Cao K, Xu D, Goltzman D, Miao D. Calcium-independent and 1,25(OH)2D3-dependent regulation of the renin-angiotensin system in 1alpha-hydroxylase knockout mice. *Kidney Int* 2008; **74**: 170-179 [PMID: 18385669 DOI: 10.1038/ki.2008.101]

94 **Somjen D**, Weisman Y, Kohen F, Gayer B, Limor R, Sharon O, Jaccard N, Knoll E, Stern N. 25-hydroxyvitamin D3-1alpha-hydroxylase is expressed in human vascular smooth muscle cells and is upregulated by parathyroid hormone and estrogenic compounds. *Circulation* 2005; **111**: 1666-1671 [PMID: 15795327 DOI: 10.1161/01.CIR.0000160353.27927.70]

95 **Scragg R**, Sowers M, Bell C. Serum 25-hydroxyvitamin D, ethnicity, and blood pressure in the Third National Health and Nutrition Examination Survey. *Am J Hypertens* 2007; **20**: 713-719 [PMID: 17586404 DOI: 10.1016/j.amjhyper.2007.01.017]

96 **Hintzpeter B**, Mensink GB, Thierfelder W, Müller MJ, Scheidt-Nave C. Vitamin D status and health correlates among German adults. *Eur J Clin Nutr* 2008; **62**: 1079-1089 [PMID: 17538533 DOI: 10.1038/sj.ejcn.1602825]

97 **Hyppönen E**, Boucher BJ, Berry DJ, Power C. 25-hydroxyvitamin D, IGF-1, and metabolic syndrome at 45 years of age: a cross-sectional study in the 1958 British Birth Cohort. *Diabetes* 2008; **57**: 298-305 [PMID: 18003755 DOI: 10.2337/db07-1122]

98 **Reis JP**, von Mühlen D, Kritz-Silverstein D, Wingard DL, Barrett-Connor E. Vitamin D, parathyroid hormone levels, and the prevalence of metabolic syndrome in community-dwelling older adults. *Diabetes Care* 2007; **30**: 1549-1555 [PMID: 17351276 DOI: 10.2337/dc06-2438]

99 **Snijder MB**, Lips P, Seidell JC, Visser M, Deeg DJ, Dekker JM, van Dam RM. Vitamin D status and parathyroid hormone levels in relation to blood pressure: a population-based study in older men and women. *J Intern Med* 2007; **261**: 558-565 [PMID: 17547711 DOI: 10.1111/j.1365-2796.2007.01778.x]

100 **Griffin FC**, Gadegbeku CA, Sowers MR. Vitamin D and subsequent systolic hypertension among women. *Am J Hypertens* 2011; **24**: 316-321 [PMID: 21088670 DOI: 10.1038/ajh.2010.226]

101 **Jorde R**, Figenschau Y, Emaus N, Hutchinson M, Grimnes G. Serum 25-hydroxyvitamin D levels are strongly related to systolic blood pressure but do not predict future hypertension. *Hypertension* 2010; **55**: 792-798 [PMID: 20065152 DOI: 10.1161/HYPERTENSIONAHA.109.143990]

102 **Pfeifer M**, Begerow B, Minne HW, Nachtigall D, Hansen C. Effects of a short-term vitamin D(3) and calcium supplementation on blood pressure and parathyroid hormone levels in elderly women. *J Clin Endocrinol Metab* 2001; **86**: 1633-1637 [PMID: 11297596]

103 **Scragg R**, Wishart J, Stewart A, Ofanoa M, Kerse N, Dyall L, Lawes CM. No effect of ultraviolet radiation on blood pressure and other cardiovascular risk factors. *J Hypertens* 2011; **29**: 1749-1756 [PMID: 21720260 DOI: 10.1097/HJH.0b013e328349666d]

104 **Witham MD**, Nadir MA, Struthers AD. Effect of vitamin D on blood pressure: a systematic review and meta-analysis. *J Hypertens* 2009; **27**: 1948-1954 [PMID: 19587609 DOI: 10.1097/HJH.0b013e32832f075b]

105 **Yiu YF**, Chan YH, Yiu KH, Siu CW, Li SW, Wong LY, Lee SW, Tam S, Wong EW, Cheung BM, Tse HF. Vitamin D deficiency is associated with depletion of circulating endothelial progenitor cells and endothelial dysfunction in patients with type 2 diabetes. *J Clin Endocrinol Metab* 2011; **96**: E830-E835 [PMID: 21325459 DOI: 10.1210/jc.2010-2212]

106 **Jablonski KL**, Chonchol M, Pierce GL, Walker AE, Seals DR. 25-Hydroxyvitamin D deficiency is associated with inflammation-linked vascular endothelial dysfunction in middle-aged and older adults. *Hypertension* 2011; **57**: 63-69 [PMID: 21115878 DOI: 10.1161/HYPERTENSIONAHA.110.160929]

107 **Harris RA**, Pedersen-White J, Guo DH, Stallmann-Jorgensen IS, Keeton D, Huang Y, Shah Y, Zhu H, Dong Y. Vitamin D3 supplementation for 16 weeks improves flow-mediated dilation in overweight African-American adults. *Am J Hypertens* 2011; **24**: 557-562 [PMID: 21311504 DOI: 10.1038/ajh.2011.12]

108 **Witham MD**, Dove FJ, Sugden JA, Doney AS, Struthers AD. The effect of vitamin D replacement on markers of vascular health in stroke patients - a randomised controlled trial. *Nutr Metab Cardiovasc Dis* 2012; **22**: 864-870 [PMID: 21194910 DOI: 10.1016/j.numecd.2010.11.001]

109 **Gepner AD**, Ramamurthy R, Krueger DC, Korcarz CE, Binkley N, Stein JH. A prospective randomized controlled trial of the effects of vitamin D supplementation on cardiovascular disease risk. *PLoS One* 2012; **7**: e36617 [PMID: 22586483 DOI: 10.1371/journal.pone.0036617]

110 **Witham MD**, Dove FJ, Dryburgh M, Sugden JA, Morris AD, Struthers AD. The effect of different doses of vitamin D(3) on markers of vascular health in patients with type 2 diabetes: a randomised controlled trial. *Diabetologia* 2010; **53**: 2112-2119 [PMID: 20596692 DOI: 10.1007/s00125-010-1838-1]

111 **Vacek JL**, Vanga SR, Good M, Lai SM, Lakkireddy D, Howard PA. Vitamin D deficiency and supplementation and relation to cardiovascular health. *Am J Cardiol* 2012; **109**: 359-363 [PMID: 22071212 DOI: 10.1016/j.amjcard.2011.09.020]

112 **Gotsman I**, Shauer A, Zwas DR, Hellman Y, Keren A, Lotan C, Admon D. Vitamin D deficiency is a predictor of reduced survival in patients with heart failure; vitamin D supplementation improves outcome. *Eur J Heart Fail* 2012; **14**: 357-366 [PMID: 22308011 DOI: 10.1093/eurjhf/hfr175]

113 **Wang L**, Manson JE, Song Y, Sesso HD. Systematic review: Vitamin D and calcium supplementation in prevention of cardiovascular events. *Ann Intern Med* 2010; **152**: 315-323 [PMID: 20194238 DOI: 10.7326/0003-4819-152-5-201003020-00010]

114 **Avenell A**, Cook JA, MacLennan GS, McPherson GC. Vitamin D supplementation and type 2 diabetes: a substudy of a randomised placebo-controlled trial in older people (RECORD trial, ISRCTN 51647438). *Age Ageing* 2009; **38**: 606-609 [PMID: 19617604 DOI: 10.1093/ageing/afp109]

115 **de Boer IH**, Tinker LF, Connelly S, Curb JD, Howard BV, Kestenbaum B, Larson JC, Manson JE, Margolis KL, Siscovick DS, Weiss NS. Calcium plus vitamin D supplementation and the risk of incident diabetes in the Women's Health Initiative. *Diabetes Care* 2008; **31**: 701-707 [PMID: 18235052 DOI: 10.2337/dc07-1829]

116 **Mitri J**, Dawson-Hughes B, Hu FB, Pittas AG. Effects of vitamin D and calcium supplementation on pancreatic β cell function, insulin sensitivity, and glycemia in adults at high risk of diabetes: the Calcium and Vitamin D for Diabetes Mellitus (CaDDM) randomized controlled trial. *Am J Clin Nutr* 2011; **94**: 486-494 [PMID: 21715514 DOI: 10.3945/ajcn.111.011684]

117 **Nagpal J**, Pande JN, Bhartia A. A double-blind, randomized, placebo-controlled trial of the short-term effect of vitamin D3 supplementation on insulin sensitivity in apparently healthy, middle-aged, centrally obese men. *Diabet Med* 2009; **26**: 19-27 [PMID: 19125756 DOI: 10.1111/j.1464-5491.2008.02636.x]

118 **Luo C**, Wong J, Brown M, Hooper M, Molyneaux L, Yue DK. Hypovitaminosis D in Chinese type 2 diabetes: lack of impact on clinical metabolic status and biomarkers of cellular inflammation. *Diab Vasc Dis Res* 2009; **6**: 194-199 [PMID: 20368211 DOI: 10.1177/1479164109337974]

119 **George PS**, Pearson ER, Witham MD. Effect of vitamin D supplementation on glycaemic control and insulin resistance: a systematic review and meta-analysis. *Diabet Med* 2012; **29**: e142-e150 [PMID: 22486204 DOI: 10.1111/j.1464-5491.2012.03672.x]

120 **Ojuka EO**. Role of calcium and AMP kinase in the regulation of mitochondrial biogenesis and GLUT4 levels in muscle. *Proc Nutr Soc* 2004; **63**: 275-278 [PMID: 15294043 DOI: 10.1079/PNS2004339]

121 **McCarty MF**, Thomas CA. PTH excess may promote weight gain by impeding catecholamine-induced lipolysis-implications for the impact of calcium, vitamin D, and alcohol on body weight. *Med Hypotheses* 2003; **61**: 535-542 [PMID: 14592784 DOI: 10.1016/S0306-9877(03)00227-5]

122 Vitamin D supplement in early childhood and risk for Type I (insulin-dependent) diabetes mellitus. The EURODIAB Substudy

2 Study Group. *Diabetologia* 1999; **42**: 51-54 [PMID: 10027578 DOI: 10.1007/s001250051112]

123 **Marchesini G**, Brizi M, Bianchi G, Tomassetti S, Bugianesi E, Lenzi M, McCullough AJ, Natale S, Forlani G, Melchionda N. Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. *Diabetes* 2001; **50**: 1844-1850 [PMID: 11473047]

124 **Dowman JK**, Tomlinson JW, Newsome PN. Pathogenesis of non-alcoholic fatty liver disease. *QJM* 2010; **103**: 71-83 [PMID: 19914930 DOI: 10.1093/qjmed/hcp158]

125 **Chalasani N**, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, Charlton M, Sanyal AJ. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology. *Gastroenterology* 2012; **142**: 1592-1609 [PMID: 22656328 DOI: 10.1053/j.gastro.2012.04.001]

126 **Liangpunsakul S**, Chalasani N. Serum vitamin D concentrations and unexplained elevation in ALT among US adults. *Dig Dis Sci* 2011; **56**: 2124-2129 [PMID: 21503677 DOI: 10.1007/s10620-011-1707-x]

127 **Barchetta I**, Angelico F, Del Ben M, Baroni MG, Pozzilli P, Morini S, Cavallo MG. Strong association between non alcoholic fatty liver disease (NAFLD) and low 25(OH) vitamin D levels in an adult population with normal serum liver enzymes. *BMC Med* 2011; **9**: 85 [PMID: 21749681 DOI: 10.1186/1741-7015-9-85]

128 **Targher G**, Bertolini L, Scala L, Cigolini M, Zenari L, Falezza G, Arcaro G. Associations between serum 25-hydroxyvitamin D3 concentrations and liver histology in patients with non-alcoholic fatty liver disease. *Nutr Metab Cardiovasc Dis* 2007; **17**: 517-524 [PMID: 16928437]

129 **Kwok RM**, Torres DM, Harrison SA. Vitamin D and nonalcoholic fatty liver disease (NAFLD): is it more than just an association? *Hepatology* 2013; **58**: 1166-1174 [PMID: 23504808 DOI: 10.1002/hep.26390]

130 **Ratziu V**, de Ledinghen V, Oberti F, Mathurin P, Wartelle-Bladou C, Renou C, Sogni P, Maynard M, Larrey D, Serfaty L, Bonnefont-Rousselot D, Bastard JP, Rivière M, Spénard J. A randomized controlled trial of high-dose ursodesoxycholic acid for nonalcoholic steatohepatitis. *J Hepatol* 2011; **54**: 1011-1019 [PMID: 21145828 DOI: 10.1016/j.jhep.2010.08.030]

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REVIEW

Mechanisms of hypoglycemia unawareness and implications in diabetic patients

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Abstract

Hypoglycemia unawareness (HU) is defined at the onset of neuroglycopenia before the appearance of autonomic warning symptoms. It is a major limitation to achieving tight diabetes and reduced quality of life. HU occurs in approximately 40% of people with type 1 diabetes mellitus (T1DM) and with less frequency in T2DM. Though the aetiology of HU is multifactorial, possible mechanisms include chronic exposure to low blood glucose, antecedent hypoglycaemia, recurrent severe hypoglycaemia and the failure of counter-regulatory hormones. Clinically it manifests as the inability to recognise impeding hypoglycaemia by symptoms, but the mechanisms and mediators remain largely unknown. Prevention and management of HU is complex, and can only be achieved by a multifactorial intervention of clinical care and structured patient education by the diabetes team. Less know regarding the impact of medications on the development or recognition of this condition in patients with diabetes. Several medications are thought to worsen or promote HU, whereas others may have an attenuating effect on the problem. This article reviews recent advances in how the brain senses and responds to hypoglycaemia, novel mechanisms by which people with insulin-treated diabetes develop HU and impaired counter-regulatory responses. The consequences that HU has on the person with diabetes and their family are also described. Finally, it examines the evidence for prevention and treatment of HU, and summarizes the effects of medications that may influence it.

Key words: Hypoglycemia unawareness; Impaired awareness of hypoglycemia; Hypoglycemia associated autonomic failure; Diabetes mellitus; Counter-regulation

Core tip: This review describes novel mechanisms by which people with insulin-treated diabetes develop hypoglycemia unawareness (HU), the consequences that HU has on the person with diabetes and their family, the evidence for prevention and treatment of HU, and the effects of medications that may influence it.

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INTRODUCTION

Hypoglycemia is usually defined as a plasma glucose level < 70 mg/dL (3.9 mmol/L)[1]. Since the brain is permanently dependent on glucose, strong counter-regulatory mechanisms exists to quickly increase glucose levels to protect the human body from the negative consequences of hypoglycemia. Counter-regulatory response to hypoglycemia (Figure 1) includes inhibition of the endogenous insulin secretion and stimulation of glucagon, catecholamines (norepinephrine, epinephrine), cortisol and growth hormone secretion, which all together stimulate hepatic glucose production and cut down glucose utilization in peripheral tissues, increasing in this way plasma glucose levels. As glycaemia comes down, the activation of the autonomic nervous system leads to neurogenic symptoms (palpitations, sweating, hunger, anxiety, tremors, *etc.*), which allows the perception of hypoglycaemia (hypoglycaemia awareness) (Figure 2).

Hypoglycemia unawareness (HU) is defined as the onset of neuroglycopenia before the appearance of autonomic warning symptoms[2] or as the failure to sense a significant fall in blood glucose below normal levels[3]. In patients with type 1 (T1DM) or type 2 diabetes mellitus (T2DM), recurrent hypoglycemia has been shown to reduce the glucose level that precipitates the counter-regulatory response necessary to restore euglycemia during a subsequent episode of hypoglycemia[4,5].

HU was observed in 40% T1DM patients[6] and less frequently in T2DM patients with low C-peptide levels. The presence of HU increases the risk of severe hypoglycaemia (six-fold for T1DM[7] and 17-fold for T2DM[8]). HU is more common in individuals with longer duration of diabetes, history of recent and/or recurrent hypoglycaemic events, patients with intensive glycemic therapy and in advanced age[9].

Presently, the major risk factors for the development of HU are duration of the disease and improved metabolic control. The severity of HU was associated with longer diabetes duration and with a history of frequent low glycemic levels[6], whereas aging and the blood glucose decreasing rate using professional continuous glucose monitoring systems (CGMS), which falls from near blood glucose level, were risk of severe HU[10]. Data from Pittsburgh Epidemiology of Diabetes Complications[11] showed that diabetes duration, HbA1c and intensive insulin therapy predicted HU in men, whereas severity and frequency of hypoglycemia, QTc interval and hypertension predicted HU in women. Thus, women are more likely to have HU, which unlike in men, is also marginally related to hypertension, QTc interval and hypoglycemia. On the other hand, in patients with T1DM, HU was 3.4-fold more common among patients homozygous for Gly16 than among patients with other variants of the Arg16Gly polymorphism, so that T1DM patients who carry two alleles of the Gly16 variant of ADRB2 are at increased risk of developing HU[12]. Finally, in both T1 and T2DM patients with impaired HU, hypoglycemia-induced electroencephalogram changes, such increased theta band amplitude, were not affected by antecedent of hypoglycemia[13].

This article reviews recent advances in how the brain senses and responds to hypoglycemia, novel mechanisms by which people with insulin-treated diabetes develop HU and impaired counter-regulatory responses. The consequences that HU had on the person with diabetes and their family is also described. Finally, it examines the evidence for prevention and management of HU, and summarizes the effects of medications that may influence it.

MECHANISMS OF HU

Aberrant glucose counter-regulation (as a result of a failure in the reduction of insulin production and an increase in glucagon release), and HU (as the result of an attenuated increase in sympathoadrenal activity) are the components of hypoglycemia-associated autonomic failure (HAAF) in diabetics patients. HAAF is most often caused by recent/recurrent iatrogenic hypoglycemia, and indeed HAAF is maintained by recurrent hypoglycemia[14,15] (Figure 3).

Diverse causes of HAAF and HU in diabetes[16]

Catecholamines: Previous hypoglycemia leads to a blunted catecholamine response to a following episode of hypoglycemia. These has been demonstrated in several studies; for example Ramanathan *et al*[17] showed that intravenous infusion of adrenergic blockers on one day of a hypoglycemia prevent the counter-regulatory failure in the response on the next day of hypoglycemia. This study implicates that HAAF needs a previous hypoglycemia (with its sympathoadrenal responses). If we use this hypothesis to think in a possible pharmacologic treatment, we can concluded that blocking the action of catecholamines we can limit the development of HAAF and protect against subsequent hypoglycemias; but unfortunately, blocking the action of catecholamines in periphery we would tend to an increase in the severity of hypoglycemia. We would need to develop a selective adrenergic receptor modulators that favourably change central nervous system response without modify the beneficial peripheral effects of the sympathoadrenal response.

Sleep: Sleep is a peripheral mediator of HAAF linked with catecholamine response. Patients with T1DM, while they are sleeping, they have a significantly decreased epinephrine response to hypoglycemia[18], and also a

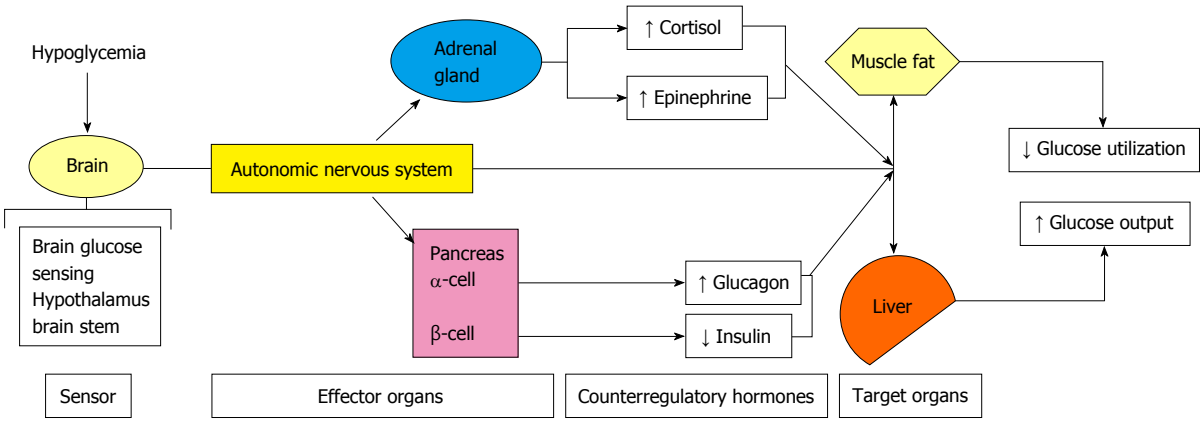

Figure 1 Counter-regulatory response to hypoglycemia.

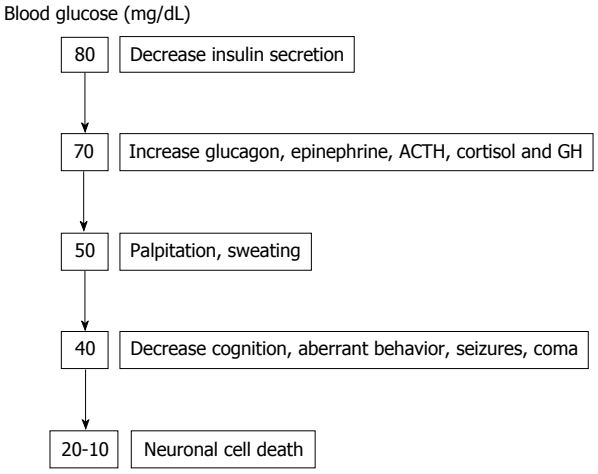

Figure 2 Symptoms and signs associated with progressive hypoglycemia. ACTH: Adrenocorticotropic hormone; GH: Growth hormone.

reduced awakening from sleep during hypoglycemia[19]. So, because of the HU and the impaired adrenomedullary response, we can explain some of the overnight deaths of healthy young people with T1DM.

Cortisol: Hypoglycemia is associated with an elevation in systemic corticosteroids, and this has been proposed to feedback to the hypothalamus contributing to HAAF[20-22]. However it remains controversial if the endogenous hypercortisolemia is of sufficient magnitude to blunt de counter-regulatory response to hypoglycemia[23,24]. It have been shown that corticotrophin releasing hormone agonist impair the counter-regulatory response to a subsequent hypoglycemia, suggesting a possible role in HAAF[25].

Opioids: Preclinical and clinical studies with opioids demonstrated a rise in endogenous opioids during hypoglycemia, for example naloxone (an opioid receptor blocker), increased the sympathoadrenal response to hypoglycemia, and when is infused during previous hypoglycemia, it prevent HAAF[26,27]. Hence there is a potential therapeutic function for opioid receptor blockade to protect against HAAF.

Exercise: The inability to reduced circulating insulin during exercise, lead T1DM patients, at an increased risk for hypoglycemia during or after exercise. In addition to, during exercise the opioid beta endorphin is released to activate the sympathoadrenal response. In a recent study, healthy individuals who exercised and elevated endorphin levels, they had reduced catecholamine response during hypoglycemia in the next day[28], suggesting that endogenous opioids, again, play a role in HAAF, and that blocking their action may protect against exercise-autonomic failure.

Recurrent hypoglycemia and HU

Clinically HAAF can be viewed as both, maladaptative or adaptative response[29]. At one end, patients with T1DM and HU make tests of cognitive function during hypoglycemia better than patients with HU. Additionally, the time necessary for complete cognitive recovery after restoration of normoglycemia is faster in patients who have HU[30]. HAAF in humans may be similar than in rats; rats with recurrent moderate hypoglycemia had less brain cell death[31] and less mortality during or following marked hypoglycemia than those without recurrent hypoglycemia. On the other hand, HAAF is without doubt a maladaptive response if we consider that defective glucose counter-regulation and HU rise the risk of severe hypoglycemia with its morbidity and potential mortality[32].

Although it is well established that recurrent hypoglycemia leads to HU, the mechanism responsible for this are unknown. Several current mechanistic hypotheses are discussed below.

The brain glucose transport or glucose metabolism hypothesis: Several studies have identified specific brain regions that exhibit decrease glucose uptake. In diabetic patients with and without HU, the effects of acute moderate hypoglycemia and the condition of HU on regional brain uptake of the labeled glucose analog [(18)F]fluorodeoxyglucose (FDG) using positron emission tomography were examined[33,34]. In the group with hypoglycemia awareness, there was an increase

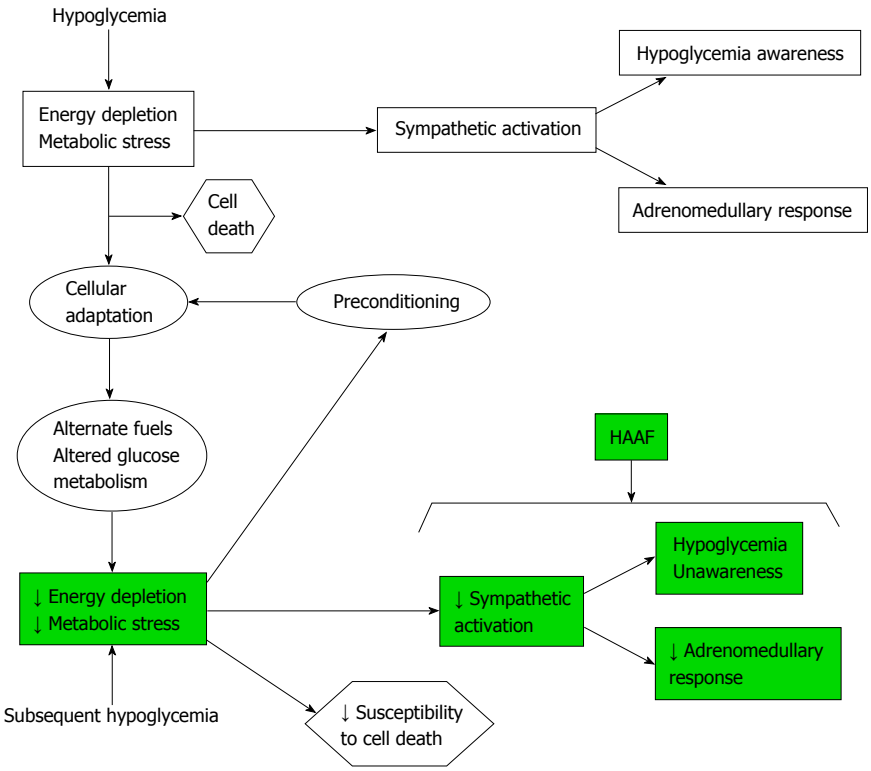

Figure 3 Recurrent hypoglycemia leads to cellular adaptation and hypoglycemia-associated autonomic failure. HAAF: Hypoglycemia-associated autonomic failure.

in the normalized FDG uptake in a subthalamic brain region[33], in left amygdale and in bilateral ventral striatum[34] in response to hypoglycemia; whereas in the group with HU the uptake in these brain regions fell significantly[33,34]. Reduced responses in these brain regions in HU, suggest habituation of higher behavioral responses to hypoglycemia as a basis for unawareness, and demonstrated a change in its metabolic function associated with the failure to trigger a counter-regulatory response. On the other hand, in subjects with T1DM and HU a positive correlation was observed between thalamic response and epinephrine response to hypoglycemia, suggesting that this brain region may be involved in the coordination of the counter-regulatory response to hypoglycemia[35]. During recurrent hypoglycemia, cerebral blood flow reduced significantly in the thalamus and hypothalamus of T1DM subjects, compared to healthy controls[36], suggesting that there is reduced neuronal activation in these brain regions that participate in glucose sensing and/or coordination of counter-regulation response in subjects with T1DM that likely contributes to the development of HU.

It has been hypothesized that recurrent hypoglycemia leads to HU through an alteration in the glucose transport or metabolism. Altered glucose transport or metabolism as a cause of HU is less substantiated in humans. Subjects with T1DM and HU had significantly higher brain glucose concentrations compared to that in controls under the same conditions[37]. These date suggest that changes in brain glucose transport or metabolism may occur as a result of recurrent hypoglycemia.

The brain glycogen supercompensation hypothesis: It has been hypothesized that increased brain glycogen contributes to the development of HU and impaired sympathoadrenal responses by providing energy for the brain during periods of systemic hypoglycemia. Experimental studies and in humans have shown that after one or more episodes of hypoglycemia, increased glycogen content in the brain[38,39]. Subsequent studies indicated lower glycogen content in brain of humans with T1DM, implying that glycogen supercompensation does not contribute to the development of HU[40]. The most important question to resolve is whether changes to brain glucose levels, physiologically or pharmacologically induced, may provide people who suffer from recurrent hypoglycemia a therapeutic benefit to preserve both the sympathoadrenal response and HU.

The brain fuel hypothesis: When there is a decrease in the supply of glucose from the periphery, the brain may be able to keep your metabolic processes by increasing uptake of alternative carbon fuels such as lactate or ketones. Plasma lactate concentrations are approximately tenfold higher than those of acetate, making it a primary candidate as an alternative brain fuel during hypoglycemia. On the other hand, increased of blood-brain barrier monocarboxylic acid (MCA) transport and metabolism among T1DM individuals with HU may be a mechanism to supply the brain with non-glucose fuels during episodes of acute hypoglycemia and may contribute to the maintenance of brain energetic during hypoglycemia and to the syndrome of HU, independent

of diabetes[41]. Finally, in T1DM patients with HU, upregulation of the MCA transporter promotes increased brain lactate uptake[42].

The brain neuronal communication hypothesis: Neuronal communication relies on the release of classical neurotransmitters, such as Gamma-Aminobutyric Acid (GABA), a potent inhibitory neurotransmitter. GABA levels in ventromedial hypothalamus (VMH) interstitial fluid are decreased during acute hypoglycemia[43]. Recurrent hypoglycemia leads to a significant increase in VMH GABA concentrations[44], that fail to decrease normally during subsequent hypoglycemia, and which correlates with the reduced glucagon and epinephrine responses[45]. These data suggest that recurrent hypoglycemia results in increased VMH GABA inhibitory tone, and that altered GABA tone may be an important common mediator in the development of HAAF, especially in diabetic patients.

CONSEQUENCES OF HU

Consequences of HU on morbidity, mortality, and cardiovascular outcomes

People who have HU have a much greater risk of severe hypoglycemia, up to six fold, with its attendant morbidity[46,47]. HU may result in many serious forms of morbidity including seizure, coma, fractures and joint dislocation and cardiac arrhythmias, and is occasionally fatal.

Severe episodes of hypoglycemia or HU requiring the assistance of another have been shown to be associated with an increased risk of mortality in both the Action to Control Cardiovascular Risk in Diabetes (ACCORD)[48] and the Action in Diabetes and Vascular Disease[49] studies. On the other hand, *post hoc* analysis of the ACCORD study cohort, to examine the relationship between frequent and unrecognized hypoglycemia and mortality, 10096 ACCORD study participants were included. In this study, recognized and unrecognized hypoglycemia was more common in the intensive group than in the standard group; and in the intensive group, a small but statistically significant inverse relationship was identified between the number of hypoglycemic episodes and the risk of death among participants[50]. This latter finding does not mean that we should change our clinical practice and include frequent episodes of hypoglycemia in the targets of T2DM patients and cardiovascular risk factors. Instead, we must strive to achieve optimal glycemic control in our patients, without episodes of hypoglycemia.

Consequences of HU on adults with T1DM

Several prospective studies as the Diabetes Control and Complications Trial[51] and the Stockholm Diabetes Intervention Study[52] suggests that cognitive function does not deteriorate in patients with T1DM who suffer recurrent hypoglycemia, at least less than 10 years of these studies.

Gold *et al*[53] to compare the degree of cognitive dysfunction experienced by T1DM patients who had normal awareness of the onset of hypoglycemia with patients who had history of impaired awareness of hypoglycemia, found that T1DM patients with HU exhibited more profound cognitive dysfunction during acute hypoglycemia which persisted for longer following blood glucose recovery. Intellectual activity is likely to be affected and cause sub-optimal performance during this recovery period. Recent investigations with advanced imaging techniques have demonstrated that adults with T1DM appear to call upon a greater volume of the brain to perform a working memory task during hypoglycemia[54]. These findings suggest that adults with T1DM must recruit more regions to preserve cognitive function during hypoglycemia than adults without the disease.

Evidence of clinical audit in T1DM patients with intensive insulin therapy with HU showed that these patients had less adhesion to changes in insulin regimens to compare them with patients with hypoglycemia awareness, despite the observed increase in clinical contacts[55]. Neuroimaging studies have shown that patients with HU showed a reduced activation in appetitive motivational networks associated with integrated behavioral responses to hypoglycemia[34]. This may suggest that in some patients with HU behavioral strategies are more important than educational strategies; however treatment of HU will require a combination of both strategies, behavioral and educational, along with the use of technology, such as therapy with continuous insulin pump and online glucose monitoring[56].

Consequences of HU in children and adolescents with T1DM

A significant proportion of children and adolescents with T1DM have HU. Screening for HU is an important component of routine diabetes care and can identify patients at increased risk of severe hypoglycemic events[57]. The youngest patients are most vulnerable to the adverse consequences of hypoglycemia. Ongoing maturation of the central nervous system puts these children at greater risk for cognitive deficits as a consequence of HU[58]. HU is a significant problem for children and adolescents with T1DM and the major risk factor for development of hypoglycemia[57]. Those children with T1DM diagnosed before age of 6, who suffer repeated and severe episodes of hypoglycemia may have more increased range of cognitive dysfunction, brain abnormalities[59], structural brain changes[60], lower mental abilities latter on in life, and behavior problems than those who do not have HU until latter[61,62].

Consequences of HU on subjects with T2DM

HU is less common in T2DM patients. Two retrospective surveys of subjects with insulin-treated T2DM showed that only 8% and 9.8% respectively had HU estimated by a validated scoring system[8,46]. However, in the patients with HU the incidence of severe hypoglycemia was nine-fold and 17-fold higher respectively than

those with normal hypoglycemia awareness[8,46]. In several studies, using continuous monitoring system, asymptomatic hypoglycemia was detected in 47%[63] and 56%[64] of subjects with T2DM, treated with different treatment regimes. These findings suggest that HU may be more prevalent in T2DM than is appreciated.

Severe hypoglycemia, due to HU, was associated in T2DM patients with cardiovascular and neurological complications[1,48]. In patients with T2DM and coronary artery disease, severe hypoglycemia was associated with ischemic electrocardiogram changes and chest pain, and may account for sudden mortality[65,66]. In a retrospective study in T2DM subjects, the patients who experienced outpatient severe hypoglycemia were also shown to have a 79% higher odds ratio of experiencing acute cardiovascular events than patients without severe hypoglycemia[67]; and a case-control study in patients with T2DM showed a 65% increase in the odds of myocardial infarction with severe hypoglycemia within the previous two weeks; the risk of myocardial infarction persisted elevated for up to six months following a hypoglycemic event[68].

Behavioral changes, cognitive impairment, seizures, coma and a mortality rate estimated at between 4.9% and 9% are well-known neurological complications of severe and prolonged hypoglycemia secondary to HU[69-71]. Severe hypoglycemia secondary to HU can cause neuronal cell death and may damage regions of the brain that oversea memory, especially in older people with T2DM[72].

Finally, a frequently problem in T2DM is nocturnal hypoglycemia. Undetected nocturnal hypoglycemia often contributes to HU. Nocturnal hypoglycemia has been associated with cardiac arrhythmias resulting in sudden death[73].

Consequences of HU on the elderly

Patients in the older age-groups are especially vulnerable to HU. Aging modifies the cognitive, symptomatic, and counter-regulatory hormonal responses to hypoglycemia[74]. Older adults with diabetes are at much higher risk for the geriatric syndrome, which includes falls, incontinence, frailty, cognitive impairment and depressive symptoms[75]. In the elderly subjects, episodes of severe hypoglycemia are more likely to be followed by changes in the blood brain circulation which may further increase the risk of neurological damage in this population[76,77]. In older patients with T2DM, Whitmer *et al*[72] found a significant association between the number of severe hypoglycemic episodes and dementia; with $\geqslant$ 3 episodes almost doubling the risk more episodes of severe hypoglycemia secondary to HU had increasing likelihoods of being subsequently diagnosed with dementia. Another authors also found an association between severe hypoglycemia and cognitive impairment in these patients[78]. These reports suggest that severe hypoglycemia and HU in older people with diabetes may be associated with cognitive decline[79].

Consequences of HU during pregnancy

Pregnancy is associated with a high risk of severe hypoglycemia in diabetic subjects. History of HU has been documented as risk factors of severe hypoglycemia during pregnancy[80-82]. Reduced sympathoadrenal responses during hypoglycemia may contribute to defective glucose counter-regulation and HU[83,84]. In pregnant woman severe hypoglycemia episodes and HU occur three to five times more frequently in first trimester than third trimester when compared with the incidence in the year preceding the pregnancy[80,81,85] and may lead to severe morbidity and even death[86].

Consequences of HU on quality of life and social impact

Hypoglycemia and HU are associated with significant reductions in quality of life measures in both T1DM and T2DM patients[87-89]. The wellbeing of patients may be affected both from the effects of hypoglycemia and from fear of recurrence[89,90]. A positive association was found between severity and/or frequency of hypoglycemic events and greater fear of hypoglycemic episodes[71]. As a result fear of hypoglycemia makes the patients to promote compensatory behaviors in a way to have less episodes of hypoglycemia such as decreased insulin doses resulting in negative glycemic control, and an increased risk of serious health consequences[91]. Patients with recurrent hypoglycemia and HU were more likely to have a lower quality of life in several parameters including depression and anxiety[89,92,93], increased pain and limitations in mobility and usual activities[89], and decline in the quantity and quality of sleep[94]. On the other hand, young adults with T1DM reported the presence of interpersonal conflict, and difficulty talking about issues related to hypoglycemia with significant others[95], that may carry over to their work life, where hypoglycemia has been linked to reduced productivity[88].

Despite that many countries require documentation that severe hypoglycemia and HU is not occurring before persons with diabetes are permitted to have a license to operate a motor vehicle; HU has not consistently been associated with an increased risk of car collisions[96-98].

Consequences of HU on family members

In the subjects with diabetes, HU can have a profound impact on the lives of their family members, and are often reliant on immediate relatives or partners to detect and treat hypoglycemia episodes. A recent study based in-depth interviews with 24 adult family members of persons with T1DM and HU, showed that family members restricted their own lives in order to help the person with HU to detect and treat hypoglycemia[99]. In this study, some family members of people with HU, report that they are afraid of their partners, during episodes of hypoglycemia because of their aggressive behavior and their personality changes, making it difficult managing their treatment. The study showed that family

members of patients with HU restricted their own lives in order to help the person with HU to detect and treat hypoglycemia, and felt anxious about the safety of the person with HU; which sometimes leads family members to neglect their own health, leading to resentment over time[100]. On the other hand, personality changes during hypoglycemia events of the person with diabetes, such as aggression, also caused, in some family members, physical fear of your partner or relative, and made treatment difficult. Family members emphasized that there is an unmet need for information and emotional support for caregivers, and the researchers suggest that proactive support for the families of patients with diabetes and HU should be considered and provided by healthcare professionals[99].

Psychological consequences of HU

The psychological consequences of HU include subsequent fear to hypoglycemia, and secondary poor treatment compliance, increased anxiety and decreased levels of satisfaction and happiness. Fear of hypoglycemia will be a barrier to achieving good glycemic control. The hypoglycemia fear survey (HFS) used to measure behaviors (HFS-B) and worries (HFS-W) related to hypoglycemia in adults with T1DM, such as maintaining higher blood glucose levels than recommended, and limiting exercise or physical activity, or concerns may have about hypoglycemic episodes, such as nocturnal episodes; have been shown to be significantly higher in women than in men and among patients who have experienced severe hypoglycemia in the past compared with those that have not[100]. If patients experience repeated severe hypoglycemic events, both the patient's and the physician's subsequent treatment policy are affected. In one study that reviewed hospital records and examined daily insulin doses and HbA1c levels before and after and episode of severe hypoglycemia in patients with insulin-dependent diabetes, it was found that, in 69% of these cases, either the physician or patient or both decreased the daily insulin dose. Furthermore, physicians decreased the insulin dose in a third of patients in whom the cause of hypoglycemia was preventable and due to a cause other than erroneous administration of excess insulin[101].

Economic consequences of HU

The economic consequences of severe hypoglycemia events and HU in patients with diabetes are higher than that of a mild episode and have been examined in a number of studies in Europe and United States[102-105]. Reported costs of a severe hypoglycemic event varied from approximately $80 to $5000, depending on the requirement for resources including hospitalization, emergency services, healthcare professionals and diagnostic test.

A United Kingdom study estimated the total cost of emergency treatments of 244 episodes of severe hypoglycemia in 160 patients with T1DM and T2DM over the course of one year. The total cost was approximately £92078 (£400 per episode)[102]. On the other hand, in a Swedish study the total cost (direct and indirect) of severe hypoglycemia in T2DM patients was between $12.90 and $14.10 for one month period[90].

An analysis of several United States studies, the estimated annual total cost attributable to severe hypoglycemia was between $1400 and $1500[106]. In this analysis the estimated work days lost per hypoglycemic event was between 0.22 and 6.60 d[103]. A recent study estimated that in patients with diabetes who experienced severe hypoglycemia, the lost of productivity ranged from $15.26 to $93.47 per severe hypoglycemic event, representing 8.3-15.9 h of lost work time per month[106]. Among the patients who experimented a severe hypoglycemic event at work, 18.3% missed work for a mean duration of 9.9 h, whereas the patients who had severe hypoglycemic event outside working hours, 22.7% arrived late for work or missed a full day[104]. If the hypoglycemia has occurred during the night, the number of working hours lost increased to 14.7 h[104].

PREVENTION AND MANAGEMENT OF HU

Prevention of HU

Prevention of HU is an important part of modern day intensive diabetes therapy. To prevent HU, the goal is the complete avoidance of hypoglycemia, which is very difficult to achieve[105]. Blood glucose monitoring, individualized targets and educational programs are important in the bid to prevent and manage HU.

Blood glucose monitoring: CGMS, that can detect hypoglycemia, represents an important technological advance on the methods used for self-monitoring of blood glucose, and they are welcome to both patients and clinicians[106]. The ability of CGMS systems is to advise patients when glucose levels fall too low or rise too high, and has the potential to reduce de duration of hypoglycemia and hyperglycemia events[107,108]. Also, CGMS can be used for objective detection of patients with HU[109]. In adult patients with long-standing T1DM, a fasting level of C peptide of $\leqslant$ 0.6 ng/mL, and a HbA1c $\leqslant$ 9%, hypoglycemic episodes with a duration more than 90 minutes detected by CGMS, identified patients who had HU with an 88% specificity and 75% sensitivity[109]. On the other hand, the epinephrine response to hypoglycemia in adolescents patients with T1DM with HU was greater after the use of real-time CGMS with low glucose alarms than with standard medical therapy alone[110]. This suggests that real-time CGMS is a useful clinical tool to improve HU in adolescents with T1DM[110]. Choudhary *et al*[111] assessed the effect of CGMS on the frequency of severe hypoglycemia episodes, using the Gold scoring method[46] in 35 people with T1DM who have HU, *via* retrospective audit. A significant decline was observed in the mean rate of severe hypoglycemia (8.1 to 0.6 events per year) and also in HbA1c level

(8.1% to 7.6%), between its initiation and the end of the 1-year follow-up period; while the mean Gold score did not change significantly[111]. These results support previous reports that CGMS can lower the incidence of severe hypoglycemia in patients with T1DM and HU, with no impact on the severity of HU over a 1-year period. A randomized cross-over study to assess the effects of CGMS use on glycemic levels and quality of life in patients with T1DM and HU, using the change in the Gold scoring as one of the secondary endpoints, is currently in progress and the results will not be available until 2015[112].

The impact of closed-loop CGMS, which link CGM technology with insulin pumps, whereby insulin infusion is programmed to stop automatically when glucose levels drop below a pre-determined glycemic threshold, on reducing the incidence of hypoglycemia events appears to be limited and so their usefulness in improving HU is debatable[16].

Individualized targets: In diabetic patients with HU blood glucose targets should be relaxed but not abandoned. Appropriate targeting of plasma glucose may help patients and practitioners achieve HbA1c goals, reduce excessive self-testing and minimize the occurrence of severe hypoglycemic events[113]. Glycemic goals should be individualized with some degree of safety particularly for patients with long duration of diabetes, patients who have a high risk of HU and severe hypoglycemia development, and/or subjects with multiple co-morbidities[114,115]. Basically, an HbA1c goal of less than 7% remains recommended, but is there a safe range for HbA1c? In patients with T1DM undertaking insulin therapy, the rates of severe hypoglycemia were increased among those with HbA1c < 6% and therefore it was suggested that using current therapy, an HbA1c of between 6%-7% represents the best compromise between the risk of severe hypoglycemia and that of developing microvascular complications[116].

Educational programs: The central objective of a hypoglycemia-reversal program is to prevent any period of hypoglycemia for at least four weeks. In diabetic patients with HU an appropriate educational program includes an emphasis on regular snacks at right times, warnings to take special care at periods of greater risk such as before lunch, moderation in alcohol intake and about the danger of delayed hypoglycemia after heavy alcohol intake or prolonged exercise. Diabetes self-management education can have physical and psychosocial benefits, and results in behavior changes with positive influence in outcome. A self-awareness intervention of 8 sessions, each lasting 3 h, was designed to determine whether there are psychosocial and physical benefits of self-awareness intervention in 29 adults with T1DM and HU. Post-intervention the participants detected more cues of euglycemia and hypoglycemia and experienced significant increases in integration and metabolic control[117].

In a randomized, prospective multi-centre trial, the effect of a specific training program for patients with hypoglycemia problem was compared with a control group receiving a standardized education program aiming of at avoidance of hypoglycemia by optimization of insulin therapy[118]. Compared to control group, the specific training program demonstrates additional benefits in terms of improving HU, reducing mild hypoglycemia, and detecting ant treating low blood glucose[118]. In the Dose Adjustment for Normal Eating-Hypoglycemia Awareness Restoration study, a 6-wk pilot intervention using motivational interviews and cognitive behavioral techniques around hypoglycemia, in 23 people with HU; support the importance of educational programs to improve HU. One year after the intervention HU had improved, mean rates of severe hypoglycemia fell from 3 to 0 per person per year, and worry and behavior around hypoglycemia improved[119]. In a sub-study of HypoCOMPaSS trial aimed to assess the restoration of impaired hypoglycemia awareness and defective hypoglycemia counter-regulation by an educational strategy targeted at hypoglycemia avoidance, in 18 adults patients with T1DM; following the 6-mo intervention the mean glucose concentration at which participants first experienced symptoms of hypoglycemia significantly increased from baseline (from 2.6 to 3.1 mg/dL), and counter-regulatory responses to hypoglycemia were also enhanced[120].

Jointly, the results of these three studies suggest that interventions that include education around hypoglycemia avoidance may help to decrease HU.

Treatment of HU

The treatment options for the management of HU are listed in Table 1.

Optimizing insulin treatment: It is important that in patients with a history of recurrent hypoglycemia and HU, the time of episodes be identified and the treatment regimen be adjusted accordingly[121]. Compared with regular insulin, rapid-acting insulin analogs have a more rapid onset of action, higher peak action, and shorter duration of action, which more closely approximates endogenous mealtime insulin response, allowing more flexibility in the time of meals and exercise, and, consequently, a lower risk of severe hypoglycemic events[122]. Similarly, long-acting insulin analogs exhibit a more consistent, longer, and flatter action profile than NPH insulin, and demonstrate a lower risk of hypoglycemia, particularly nocturnal[123,124]. In diabetic patients with HU substitution of regular insulin with rapid-acting insulin analogs (aspart, lispro or glulisine) reduces frequency of daytime hypoglycemia; and substitution of long-acting insulin analogues (detemir or glargine) for intermediate-acting insulin (NPH or premix) reduces the frequency of nocturnal and day time hypoglycemia[121,125]. Compared with insulin glargine, the newest basal analog insulin degludec offers a more constant time-action profile, a long duration of action, and a lower risk of

Table 1 Treatment options for the management of hypoglycemia unawareness and mechanisms of action

Treatments options	Mechanism of action
Optimizing insulin treatment	Avoidance of hypoglycemia
Pharmacological therapy	
β2-adrenergic agents	Enhancement of adrenaline effect
Methylxanthine derivates (caffeine, theophylline)	Central nervous system stimulation
Serotonin reuptake inhibitors (fluoxetine, sertraline, paroxetine)	Unknown. It has been hypothesized that the effect could be mediated by an atypical presentation of serotonin syndrome that will lead to autonomic dysfunction
KATP channel modulators	Modulation of hypoglycemia sensing
Other treatments	
Islet cell transplantation	Improving metabolic control
Fructose	Modulation of hypoglycemia sensing

hypoglycemia[126,127]. While clinical experience with insulin degludec is limited, a meta-analysis evaluating 5 clinical trials of 3372 subjects with T2DM demonstrated a 17% lower rate of overall hypoglycemia and a 32% lower rate of nocturnal hypoglycemia with insulin degludec, compared with insulin glargine[128]. These characteristics may facilitate the achievement of glycemic control with insulin degludec with fewer hypoglycemic events in patients with HU.

An alternative approach is to use continuous subcutaneous insulin infusion (CSII). A study was designed by Giménez *et al*[129] to evaluate the effect of CSII on hypoglycemia awareness and on glucose profile in a cohort of T1DM subjects in which 95% had established HU and had experienced two or more episodes of severe hypoglycemia in the preceding two years, for a 24-mo period. Severe hypoglycemic episodes fell from 1.25 per subject-year to 0.05 after 24 mo, an improvement in all the aspects of quality of life, and an improved symptomatic response to experimentally-induced hypoglycemia was observed[130]. Previous studies[130-132] have also shown a reduction in hypoglycemia with CSII, particularly when a short-acting insulin analogue is used[2,133]. The decrease is partly due to better pharmacokinetic delivery of insulin and a 15%-20% reduction in insulin requirements compared with multiple doses of insulin[134]. Substitution of CSII for NPH insulin in patients with T1DM, especially at bedtime, resulted in a lower frequency of hypoglycemic episodes, and improved counter-regulatory and symptomatic responses during subsequent acute hypoglycemia[135]. On the other hand, administration of bolus doses of glucagon at times of impeding hypoglycemia during CSII lowered the frequency of hypoglycemia[136].

Pharmacological therapy: β-adrenergic antagonists or β-blockers alter the effects of epinephrine and could have potential effects on glucose homeostasis and the hypoglycemic counter-regulatory system. The more troubling concern regarding β-blockers is their potential effect on HU and blunting of the return to euglycemic levels after hypoglycemia has occurred, through the suppression of all adrenergically mediated symptoms of hypoglycemia. In patients with T1DM without HU, adrenergic symptoms did occur at lower glucose levels when subjects were treated with β-blockers[137]. Cardioselective β-blockers cause less alteration in the perception of hypoglycemia and may have an effect on correction of hypoglycemia than do their noncardioselective counterparts[138]. These agents should not be avoided in patients with diabetes but should be used with the same caution as when any new medication is added to a patient's therapeutic regime.

It has been suggested that people with HU may have reduced β-adrenergic sensitivity, and this can be reversed by strict avoidance of hypoglycemia[139]. In T1DM patients, the use of β-adrenergic agonist terbutaline was associated with statistically significant higher glucose levels compared to control subjects during the first half and second half of the night, and with reduction of nocturnal hypoglycemic episodes (22 in the control group *vs* 1 in the group of terbutaline). β-adrenergic agonist had therefore been suggested as possible therapeutic options for HU, at the cost of inducing morning hyperglycemia. One of the concerns about using β-adrenergic agonist for the treatment of HU was associated with reduced β_2 sensitivity observed *in vitro*. A recent study from De Galan *et al*[140] showed that sensitivity to β_2-adrenergic receptor agonist stimulation is preserved in T1DM patients with HU. No long-term clinical trials to evaluate the usefulness of β-adrenergic agonist in the prevention of HU have been reported.

Several studies have evaluated the effects of the methylxantines derivatives caffeine and theophylline on HU and the counter-regulatory response to hypoglycemia. Both have been shown to augment symptom intensity and improve counter-regulatory responses in patients with T1DM with and without HU[2,141]. Using functional magnetic imaging, caffeine can restore regional brain activation normally lost during acute hypoglycemia[142]. In another trial designed to assess the impact of caffeine on the frequency and perception of hypoglycemia over a 3-mo period; patients receiving caffeine (200 mg/twice-daily) had statistically significant more symptomatic hypoglycemia episodes and more intense warning symptoms than patients receiving placebo[143]. These results suggest that modest amounts of caffeine enhance the sensitivity of hypoglycemia warning symptoms in patients with T1DM without increasing the incidence of severe hypoglycemia. de Galan *et al*[144] planned one

study to evaluate the impact of theophylline on the response to hypoglycemia in 15 patients with T1DM who had a history of HU and 15 matched healthy control subjects. When compared with placebo, theophylline (2.8 mg/kg) improves de counter-regulatory response to a perception of hypoglycemia in the group with T1DM with HU[144]. Although modest doses of caffeine and theophylline may be effective at reducing HU in patients with T1DM at a low cost and without significant toxicity, larger doses may carry risk, and large trials are needed to determine efficacy, toxicity and dose-response curves.

The development of HU was associated with the use of selective serotonin reuptake inhibitors (SSRIs) in three patients with T1DM treated with different SSRIs (fluoxetine, sertraline and paroxetine) for depression and who were previously able to recognize and treat hypoglycemia symptoms[145]. HU occurred in all three patients within weeks of starting SSRI therapy. HU reversed after discontinuation of SSRI therapy[145]. The mechanism by which SSRIs might be associated with HU is unknown, but it has been hypothesized that the effect could be mediated by an atypical presentation of serotonin syndrome that will lead to autonomic dysfunction[146]. These observations suggest that in some patients, treatment with SSRIs may alter the perception of hypoglycemia, and should be used with caution in diabetic subjects with HU.

Infusion of the opioid-receptor antagonist naloxone increases the plasma epinephrine response to hypoglycemia and, when administered during hypoglycemia prevents attenuation of the plasma epinephrine response to subsequent hypoglycemia in humans[26,27].

Administration of a selective Kir6.2/SUR-1 K_{ATP}-channel agonist increases the epinephrine response to hypoglycemia in rats[147]. However, systemic administration of the nonselective K_{ATP}-channel agonist diazoxide suppresses the glucagon response and has no effect on the epinephrine response to hypoglycemia in nondiabetic humans[148]. These results suggest that K_{ATP}-channel modulators are not effective in humans, possibly due to inability to cross blood-brain barrier.

Other treatments: Islet cell transplantation (ICTx) prevents severe hypoglycemia[149], and restores some counter-regulatory hormone secretion[150]. In a retrospective study conducted in 31 T1DM recipients of ICTx, HU was assessed using the Clark hypoglycemic score (minimum = 0; maximum = 7; no hypoglycemia = 0; HU $\geqslant$ 4)[151] twice. A reduction in the proportion of patients with HU was observed post-ICTx (pre *vs* post-ICTx: 87% *vs* 13%) and a significant increase in glucose threshold that resulted in symptoms (pre *vs* post-ICTx: 41.4 mg/dL *vs* 58.4 mg/dL)[152]. These results were sustained even after the patient's stratification based in islet function, graft dysfunction and graft failure[152]. These results suggests that improved metabolic control achieved with ICTx can restore hypoglycemia awareness in patients with T1DM, persisting even after islet graft failure.

Fructose infusion amplifies epinephrine and glucagon responses and increases glucose production during hypoglycemia in humans[153]. Fructose is a promising treatment but has not been tested in clinical trials.

CONCLUSION

HU is a complex, difficult-to-study phenomenon that carries with it great risk to patients. HU is common in people with T1DM and is observed with less frequency in insulin-treated T2DM. Exposure to antecedent hypoglycemia, especially repeated episodes, is an important factor in the pathogenesis of HU. Although enormous advances have been made in our knowledge of the mechanisms of HU, further research is needed to elucidate the pathophysiology of counter-regulatory impairment and HU, and enable the development of more targeted strategies that support glucose counter-regulation and consequently reduce hypoglycemia. Numerous research studies have begun to uncover the mechanisms by which the central nervous system responds and adapts to hypoglycemia. Understanding these mechanisms will lead to better management and therapies that reduce the risk for hypoglycemia. Studies aiming to improve or even reverse HU have met with variable success and a number of research groups are considering new candidate pathways to develop a therapy. Therefore, until effective measures are developed to reverse HU, part of the role of the healthcare professional should be to educate people with diabetes on the risks associated with HU and should discuss hypoglycemia prevention strategies with their patients, so that they can have a better chance of achieving their glucose controls goals while avoiding the morbidity and mortality associated with hypoglycemia.

REFERENCES

1 **Desouza CV**, Bolli GB, Fonseca V. Hypoglycemia, diabetes, and cardiovascular events. *Diabetes Care* 2010; **33**: 1389-1394 [PMID: 20508232 DOI: 10.2337/dc09-2082]

2 **de Galan BE**, Schouwenberg BJ, Tack CJ, Smits P. Pathophysiology and management of recurrent hypoglycaemia and hypoglycaemia unawareness in diabetes. *Neth J Med* 2006; **64**: 269-279 [PMID: 16990690]

3 **Moghissi E**, Ismail-Beigi F, Devine RC. Hypoglycemia: minimizing its impact in type 2 diabetes. *Endocr Pract* 2013; **19**: 526-535 [PMID: 23425655 DOI: 10.4158/EP13005.RA]

4 **Briscoe VJ**, Davis SN. Hypoglycemia in type 1 and type 2 diabetes: physiology, pathophysiology, and management. *Clinical Diabetes* 2006; **24**: 115-121 [DOI: 10.2337/diaclin.24.3.115]

5 **Vignesh JP**, Mohan V. Hypoglycaemia unawareness. *J Assoc Physicians India* 2004; **52**: 727-732 [PMID: 15839452]

6 **Czyzewska K**, Czerniawska E, Szadkowska A. Prevalence of hypoglycemia unawareness in patients with type 1 diabetes. *Pediatr Diabet* 2012; **13** Suppl 17: 77 [DOI: 10.1111/j.1399-5448.2012.00919.x]

7 **Geddes J**, Schopman JE, Zammitt NN, Frier BM. Prevalence of impaired awareness of hypoglycaemia in adults with Type 1 diabetes. *Diabet Med* 2008; **25**: 501-504 [PMID: 18387080 DOI: 10.1111/j.1464-5491.2008.02413.x]

8 **Schopman JE**, Geddes J, Frier BM. Prevalence of impaired awareness of hypoglycaemia and frequency of hypoglycaemia in

insulin-treated type 2 diabetes. *Diabetes Res Clin Pract* 2010; **87**: 64-68 [PMID: 19939489 DOI: 10.1016/j.diabres.2009.10.013]
9 **Cryer PE**. The barrier of hypoglycemia in diabetes. *Diabetes* 2008; **57**: 3169-3176 [PMID: 19033403 DOI: 10.2337/db08-1084]
10 **Miura J**, Kajiura M, Hoshina S, Kobayashi H, Uchigata Y. The investigation of risk factor for the hypoglycemia unawareness in patients with type 1 diabetes using CGMS. *Diabetes* 2012; **61**: A554
11 **Pambianco GL**, Costacou T, Orchard TJ. Does hypoglycemia unawareness (HU) differ by gender in type 1 diabetes (T1D)? *Diabetes* 2009; **58** Suppl 1: A544
12 **Schouwenberg BJ**, Veldman BA, Spiering W, Coenen MJ, Franke B, Tack CJ, de Galan BE, Smits P. The Arg16Gly variant of the beta2-adrenergic receptor predisposes to hypoglycemia unawareness in type 1 diabetes mellitus. *Pharmacogenet Genomics* 2008; **18**: 369-372 [PMID: 18334922 DOI: 10.1097/FPC.obo13e3282f70481]
13 **Sejling AS**, Kjaer TW, Pedersen-Bjergaard U, Remvig LS, Larsen A, Nielsen MN, Tarnow L, Thorsteinsson B, Juhl CB. The effect of recurrent hypoglycaemia on cerebral electrical activity in patients with type 1 diabetes and hypoglycaemia unawareness. *Diabetes* 2013; **62**: A104
14 **Dagogo-Jack S**, Rattarasarn C, Cryer PE. Reversal of hypoglycemia unawareness, but not defective glucose counterregulation, in IDDM. *Diabetes* 1994; **43**: 1426-1434 [PMID: 7958494 DOI: 10.2337/diab.43.12.1426]
15 **Fanelli C**, Pampanelli S, Epifano L, Rambotti AM, Di Vincenzo A, Modarelli F, Ciofetta M, Lepore M, Annibale B, Torlone E. Long-term recovery from unawareness, deficient counterregulation and lack of cognitive dysfunction during hypoglycaemia, following institution of rational, intensive insulin therapy in IDDM. *Diabetologia* 1994; **37**: 1265-1276 [PMID: 7895957 DOI: 10.1007/BF00399801]
16 **Reno CM**, Litvin M, Clark AL, Fisher SJ. Defective counterregulation and hypoglycemia unawareness in diabetes: mechanisms and emerging treatments. *Endocrinol Metab Clin North Am* 2013; **42**: 15-38 [PMID: 23391237 DOI: 10.1016/j.ecl.2012.11.005]
17 **Ramanathan R**, Cryer PE. Adrenergic mediation of hypoglycemia-associated autonomic failure. *Diabetes* 2011; **60**: 602-606 [PMID: 21270270 DOI: 10.2337/db10-1374]
18 **Jones TW**, Porter P, Sherwin RS, Davis EA, O'Leary P, Frazer F, Byrne G, Stick S, Tamborlane WV. Decreased epinephrine responses to hypoglycemia during sleep. *N Engl J Med* 1998; **338**: 1657-1662 [PMID: 9614256 DOI: 10.1056/NEJM199806043382303]
19 **Banarer S**, Cryer PE. Sleep-related hypoglycemia-associated autonomic failure in type 1 diabetes: reduced awakening from sleep during hypoglycemia. *Diabetes* 2003; **52**: 1195-1203 [PMID: 12716752 DOI: 10.2337/diabetes.52.5.1195]
20 **McGregor VP**, Banarer S, Cryer PE. Elevated endogenous cortisol reduces autonomic neuroendocrine and symptom responses to subsequent hypoglycemia. *Am J Physiol Endocrinol Metab* 2002; **282**: E770-E777 [PMID: 11882496 DOI: 10.1152/ajpendo.oo47.2001]
21 **Davis SN**, Shavers C, Davis B, Costa F. Prevention of an increase in plasma cortisol during hypoglycemia preserves subsequent counterregulatory responses. *J Clin Invest* 1997; **100**: 429-438 [PMID: 9218521 DOI: 10.1172/JCI119550]
22 **Davis SN**, Shavers C, Costa F, Mosqueda-Garcia R. Role of cortisol in the pathogenesis of deficient counterregulation after antecedent hypoglycemia in normal humans. *J Clin Invest* 1996; **98**: 680-691 [PMID: 8698859 DOI: 10.1172/JCI118839]
23 **Raju B**, McGregor VP, Cryer PE. Cortisol elevations comparable to those that occur during hypoglycemia do not cause hypoglycemia-associated autonomic failure. *Diabetes* 2003; **52**: 2083-2089 [PMID: 12882926 DOI: 10.2337/diabetes.52.8.2083]
24 **Goldberg PA**, Weiss R, McCrimmon RJ, Hintz EV, Dziura JD, Sherwin RS. Antecedent hypercortisolemia is not primarily responsible for generating hypoglycemia-associated autonomic failure. *Diabetes* 2006; **55**: 1121-1126 [PMID: 16567537 DOI: 10.2337/diabetes.55.04.06.db05-1169]
25 **McCrimmon RJ**, Song Z, Cheng H, McNay EC, Weikart-Yeckel C, Fan X, Routh VH, Sherwin RS. Corticotrophin-releasing factor receptors within the ventromedial hypothalamus regulate hypoglycemia-induced hormonal counterregulation. *J Clin Invest* 2006; **116**: 1723-1730 [PMID: 16741581 DOI: 10.1172/JCI27775]
26 **Caprio S**, Gerety G, Tamborlane WV, Jones T, Diamond M, Jacob R, Sherwin RS. Opiate blockade enhances hypoglycemic counterregulation in normal and insulin-dependent diabetic subjects. *Am J Physiol* 1991; **260**: E852-E858 [PMID: 2058661]
27 **Vele S**, Milman S, Shamoon H, Gabriely I. Opioid receptor blockade improves hypoglycemia-associated autonomic failure in type 1 diabetes mellitus. *J Clin Endocrinol Metab* 2011; **96**: 3424-3431 [PMID: 21917871 DOI: 10.1210/jc.2011-1723]
28 **Milman S**, Leu J, Shamoon H, Vele S, Gabriely I. Magnitude of exercise-induced β-endorphin response is associated with subsequent development of altered hypoglycemia counterregulation. *J Clin Endocrinol Metab* 2012; **97**: 623-631 [PMID: 22170706 DOI: 10.1210/jc.2011-1391]
29 **Seaquist ER**, Anderson J, Childs B, Cryer P, Dagogo-Jack S, Fish L, Heller SR, Rodriguez H, Rosenzweig J, Vigersky R. Hypoglycemia and diabetes: a report of a workgroup of the American Diabetes Association and the Endocrine Society. *Diabetes Care* 2013; **36**: 1384-1395 [PMID: 23589542 DOI: 10.2337/dc12-2480]
30 **Zammitt NN**, Warren RE, Deary IJ, Frier BM. Delayed recovery of cognitive function following hypoglycemia in adults with type 1 diabetes: effect of impaired awareness of hypoglycemia. *Diabetes* 2008; **57**: 732-736 [PMID: 18039813 DOI: 10.2337/db07-0695]
31 **Puente EC**, Silverstein J, Bree AJ, Musikantow DR, Wozniak DF, Maloney S, Daphna-Iken D, Fisher SJ. Recurrent moderate hypoglycemia ameliorates brain damage and cognitive dysfunction induced by severe hypoglycemia. *Diabetes* 2010; **59**: 1055-1062 [PMID: 20086229 DOI: 10.2337/db09-1495]
32 **Cryer PE**. Death during intensive glycemic therapy of diabetes: mechanisms and implications. *Am J Med* 2011; **124**: 993-996 [PMID: 22017775 DOI: 10.1016/j.amjmed.20]
33 **Cranston I**, Reed LJ, Marsden PK, Amiel SA. Changes in regional brain (18)F-fluorodeoxyglucose uptake at hypoglycemia in type 1 diabetic men associated with hypoglycemia unawareness and counter-regulatory failure. *Diabetes* 2001; **50**: 2329-2336 [PMID: 11574416 DOI: 10.2337/diabetes.50.10.2329]
34 **Dunn JT**, Cranston I, Marsden PK, Amiel SA, Reed LJ. Attenuation of amydgala and frontal cortical responses to low blood glucose concentration in asymptomatic hypoglycemia in type 1 diabetes: a new player in hypoglycemia unawareness? *Diabetes* 2007; **56**: 2766-2773 [PMID: 17660265 DOI: 10.2337/db07-0666]
35 **Mangia S**, Tesfaye N, De Martino F, Kumar AF, Kollasch P, Moheet AA, Eberly LE, Seaquist ER. Hypoglycemia-induced increases in thalamic cerebral blood flow are blunted in subjects with type 1 diabetes and hypoglycemia unawareness. *J Cereb Blood Flow Metab* 2012; **32**: 2084-2090 [PMID: 22892724 DOI: 10.1038/jcbfm.2012.117]
36 **Tesfaye N**, Nangia S, De Martino F, Kumar A, Moheet A, Iverson E, Eberly LE, Seaquist ER. Hypoglycemia-induced increases in cerebral blood flow (CBF) are blunted in subjects with type 1 diabetes (TID) and hypoglycemia unawareness (HU). *Diabetes* 2011; **60**: A79-A80
37 **Criego AB**, Tkac I, Kumar A, Thomas W, Gruetter R, Seaquist ER. Brain glucose concentrations in patients with type 1 diabetes and hypoglycemia unawareness. *J Neurosci Res* 2005; **79**: 42-47 [PMID: 15578722 DOI: 10.1002/jnr.20296]
38 **Oz G**, Kumar A, Rao JP, Kodl CT, Chow L, Eberly LE, Seaquist ER. Human brain glycogen metabolism during and after hypoglycemia. *Diabetes* 2009; **58**: 1978-1985 [PMID: 19502412 DOI: 10.2337/db09-0226]
39 **Canada SE**, Weaver SA, Sharpe SN, Pederson BA. Brain glycogen supercompensation in the mouse after recovery from insulin-induced hypoglycemia. *J Neurosci Res* 2011; **89**: 585-591 [PMID: 21259334]
40 **Öz G**, Tesfaye N, Kumar A, Deelchand DK, Eberly LE, Seaquist ER. Brain glycogen content and metabolism in subjects with type 1 diabetes and hypoglycemia unawareness. *J Cereb Blood*

Flow Metab 2012; **32**: 256-263 [PMID: 21971353 DOI: 10.1038/jcbfm.2011.138]
41 **Gulanski BI**, De Feyter HM, Page KA, Belfort-DeAguiar R, Mason GF, Rothman DL, Sherwin RS. Increased brain transport and metabolism of acetate in hypoglycemia unawareness. *J Clin Endocrinol Metab* 2013; **98**: 3811-3820 [PMID: 23796565 DOI: 10.1210/jc.2013-1701]
42 **De Feyter HM**, Mason GF, Shulman GI, Rothman DL, Petersen KF. Increased brain lactate concentrations without increased lactate oxidation during hypoglycemia in type 1 diabetic individuals. *Diabetes* 2013; **62**: 3075-3080 [PMID: 23715622 DOI: 10.2337/db13-0313]
43 **Moheet A**, Emir UE, Terpstra M, Kumar A, Eberly LE, Seaquist ER, Öz G. Initial experience with seven tesla magnetic resonance spectroscopy of hypothalamic GABA during hyperinsulinemic euglycemia and hypoglycemia in healthy humans. *Magn Reson Med* 2014; **71**: 12-18 [PMID: 23423963 DOI: 10.1002/mrm.24663]
44 **Chan O**, Cheng H, Herzog R, Czyzyk D, Zhu W, Wang A, McCrimmon RJ, Seashore MR, Sherwin RS. Increased GABAergic tone in the ventromedial hypothalamus contributes to suppression of counterregulatory responses after antecedent hypoglycemia. *Diabetes* 2008; **57**: 1363-1370 [PMID: 18375441 DOI: 10.2337/db07-1559]
45 **Chan O**, Paranjape S, Czyzyk D, Horblitt A, Zhu W, Ding Y, Fan X, Seashore M, Sherwin R. Increased GABAergic output in the ventromedial hypothalamus contributes to impaired hypoglycemic counterregulation in diabetic rats. *Diabetes* 2011; **60**: 1582-1589 [PMID: 21411513 DOI: 10.2337/db10-1579]
46 **Gold AE**, MacLeod KM, Frier BM. Frequency of severe hypoglycemia in patients with type I diabetes with impaired awareness of hypoglycemia. *Diabetes Care* 1994; **17**: 697-703 [PMID: 7924780 DOI: 10.2337/diacare.17.7.697]
47 **Choudhary P**, Geddes J, Freeman JV, Emery CJ, Heller SR, Frier BM. Frequency of biochemical hypoglycaemia in adults with Type 1 diabetes with and without impaired awareness of hypoglycaemia: no identifiable differences using continuous glucose monitoring. *Diabet Med* 2010; **27**: 666-672 [PMID: 20546285 DOI: 10.1111/j.1464-5491.2010.03006.x]
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 **Zoungas S**, Patel A, Chalmers J, de Galan BE, Li Q, Billot L, Woodward M, Ninomiya T, Neal B, MacMahon S, Grobbee DE, Kengne AP, Marre M, Heller S. Severe hypoglycemia and risks of vascular events and death. *N Engl J Med* 2010; **363**: 1410-1418 [PMID: 20925543 DOI: 10.1056/NEJMoa1003795]
50 **Seaquist ER**, Miller ME, Bonds DE, Feinglos M, Goff DC, Peterson K, Senior P. The impact of frequent and unrecognized hypoglycemia on mortality in the ACCORD study. *Diabetes Care* 2012; **35**: 409-414 [PMID: 22179956 DOI: 10.2337/dc11-0996]
51 **Jacobson AM**, Musen G, Ryan CM, Silvers N, Cleary P, Waberski B, Burwood A, Weinger K, Bayless M, Dahms W, Harth J. Long-term effect of diabetes and its treatment on cognitive function. *N Engl J Med* 2007; **356**: 1842-1852 [PMID: 17476010 DOI: 10.1056/NEJMoa066397]
52 **Reichard P**, Pihl M. Mortality and treatment side-effects during long-term intensified conventional insulin treatment in the Stockholm Diabetes Intervention Study. *Diabetes* 1994; **43**: 313-317 [PMID: 8288056 DOI: 10.2337/diab.43.2.313]
53 **Gold AE**, MacLeod KM, Deary IJ, Frier BM. Hypoglycemia-induced cognitive dysfunction in diabetes mellitus: effect of hypoglycemia unawareness. *Physiol Behav* 1995; **58**: 501-511 [PMID: 8587958 DOI: 10.1016/0031-9384(95)00085-W]
54 **Bolo NR**, Musen G, Jacobson AM, Weinger K, McCartney RL, Flores V, Renshaw PF, Simonson DC. Brain activation during working memory is altered in patients with type 1 diabetes during hypoglycemia. *Diabetes* 2011; **60**: 3256-3264 [PMID: 21984582 DOI: 10.2337/db11-0506]
55 **Smith CB**, Choudhary P, Pernet A, Hopkins D, Amiel SA. Hypoglycemia unawareness is associated with reduced adherence to therapeutic decisions in patients with type 1 diabetes: evidence from a clinical audit. *Diabetes Care* 2009; **32**: 1196-1198 [PMID: 19389817 DOI: 10.2337/dc08-2259]
56 **Graveling AJ**, Frier BM. Hypoglycemia unawareness is associated with reduced adherence to therapeutic decisions in patients with type 1 diabetes: evidence from a clinical audit: response to Smith et al. *Diabetes Care* 2010; **33**: e15; author reply e16 [PMID: 20040665 DOI: 10.2337/dc09-1847]
57 **Ly TT**, Gallego PH, Davis EA, Jones TW. Impaired awareness of hypoglycemia in a population-based sample of children and adolescents with type 1 diabetes. *Diabetes Care* 2009; **32**: 1802-1806 [PMID: 19587370 DOI: 10.2337/dc09-0541]
58 **Hannonen R**, Tupola S, Ahonen T, Riikonen R. Neurocognitive functioning in children with type-1 diabetes with and without episodes of severe hypoglycaemia. *Dev Med Child Neurol* 2003; **45**: 262-268 [PMID: 12647928 DOI: 10.1111/j.1469-8749.2003.tb00341.x]
59 **Northam EA**, Anderson PJ, Jacobs R, Hughes M, Warne GL, Werther GA. Neuropsychological profiles of children with type 1 diabetes 6 years after disease onset. *Diabetes Care* 2001; **24**: 1541-1546 [PMID: 11522696 DOI: 10.2337/diacare.24.9.1541]
60 **Ho MS**, Weller NJ, Ives FJ, Carne CL, Murray K, Vanden Driesen RI, Nguyen TP, Robins PD, Bulsara M, Davis EA, Jones TW. Prevalence of structural central nervous system abnormalities in early-onset type 1 diabetes mellitus. *J Pediatr* 2008; **153**: 385-390 [PMID: 18534238 DOI: 10.1016/j.jpeds.2008.03.005]
61 **Golden MP**, Ingersoll GM, Brack CJ, Russell BA, Wright JC, Huberty TJ. Longitudinal relationship of asymptomatic hypoglycemia to cognitive function in IDDM. *Diabetes Care* 1989; **12**: 89-93 [PMID: 2702906 DOI: 10.2337/diacare.12.2.89]
62 **Perantie DC**, Lim A, Wu J, Weaver P, Warren SL, Sadler M, White NH, Hershey T. Effects of prior hypoglycemia and hyperglycemia on cognition in children with type 1 diabetes mellitus. *Pediatr Diabetes* 2008; **9**: 87-95 [PMID: 18208449 DOI: 10.1111/j.1399-5448.2007.00274.x]
63 **Chico A**, Vidal-Ríos P, Subirà M, Novials A. The continuous glucose monitoring system is useful for detecting unrecognized hypoglycemias in patients with type 1 and type 2 diabetes but is not better than frequent capillary glucose measurements for improving metabolic control. *Diabetes Care* 2003; **26**: 1153-1157 [PMID: 12663589 DOI: 10.2337/diacare.26.4.1153]
64 **Hay LC**, Wilmshurst EG, Fulcher G. Unrecognized hypo- and hyperglycemia in well-controlled patients with type 2 diabetes mellitus: the results of continuous glucose monitoring. *Diabetes Technol Ther* 2003; **5**: 19-26 [PMID: 12725703 DOI: 10.1089/152091503763816427]
65 **Desouza C**, Salazar H, Cheong B, Murgo J, Fonseca V. Association of hypoglycemia and cardiac ischemia: a study based on continuous monitoring. *Diabetes Care* 2003; **26**: 1485-1489 [PMID: 12716809 DOI: 10.2337/diacare.26.5.1485]
66 **Tanenberg RJ**, Newton CA, Drake AJ. Confirmation of hypoglycemia in the "dead-in-bed" syndrome, as captured by a retrospective continuous glucose monitoring system. *Endocr Pract* 2010; **16**: 244-248 [PMID: 19833577 DOI: 10.4158/EP09260.CR]
67 **Johnston SS**, Conner C, Aagren M, Smith DM, Bouchard J, Brett J. Evidence linking hypoglycemic events to an increased risk of acute cardiovascular events in patients with type 2 diabetes. *Diabetes Care* 2011; **34**: 1164-1170 [PMID: 21421802 DOI: 10.2337/dc10-1915]
68 **Miller DR**, Fincke G, Lafrance JP, Palnati M, Shao Q, Zhang Q, Fonseca V, Riddle M, Vijan S, Christiansen CI. Hypoglycaemia and risk of myocardial infarction in US veterans with diabetes. *Diabetologia* 2009; **52** (suppl 1): S63
69 **Holstein A**, Egberts EH. Risk of hypoglycaemia with oral antidiabetic agents in patients with Type 2 diabetes. *Exp Clin Endocrinol Diabetes* 2003; **111**: 405-414 [PMID: 14614647 DOI: 10.1055/s-2003-44287]
70 **Amiel SA**, Dixon T, Mann R, Jameson K. Hypoglycaemia in Type

2 diabetes. *Diabet Med* 2008; **25**: 245-254 [PMID: 18215172 DOI: 10.1111/j.1464-5491.2007.02341.x]
71 **Marrett E**, Radican L, Davies MJ, Zhang Q. Assessment of severity and frequency of self-reported hypoglycemia on quality of life in patients with type 2 diabetes treated with oral antihyperglycemic agents: A survey study. *BMC Res Notes* 2011; **4**: 251 [PMID: 21777428 DOI: 10.1186/1756-0500-4-251]
72 **Whitmer RA**, Karter AJ, Yaffe K, Quesenberry CP, Selby JV. Hypoglycemic episodes and risk of dementia in older patients with type 2 diabetes mellitus. *JAMA* 2009; **301**: 1565-1572 [PMID: 19366776 DOI: 10.1001/jama.2009.460]
73 **Allen KV**, Frier BM. Nocturnal hypoglycemia: clinical manifestations and therapeutic strategies toward prevention. *Endocr Pract* 2003; **9**: 530-543 [PMID: 14715482 DOI: 10.4158/EP.9.6.530]
74 **Alagiakrishnan K**, Mereu L. Approach to managing hypoglycemia in elderly patients with diabetes. *Postgrad Med* 2010; **122**: 129-137 [PMID: 20463422]
75 **Bruce DG**, Casey GP, Grange V, Clarnette RC, Almeida OP, Foster JK, Ives FJ, Davis TM. Cognitive impairment, physical disability and depressive symptoms in older diabetic patients: the Fremantle Cognition in Diabetes Study. *Diabetes Res Clin Pract* 2003; **61**: 59-67 [PMID: 12849924 DOI: 10.1016/S0168-8227(03)00084-6]
76 **Bree AJ**, Puente EC, Daphna-Iken D, Fisher SJ. Diabetes increases brain damage caused by severe hypoglycemia. *Am J Physiol Endocrinol Metab* 2009; **297**: E194-E201 [PMID: 19435850 DOI: 10.1152/ajpendo.91041.2008]
77 **Abbaszadeh Ahranjani S**, Tabatabaei-Malazy O, Pajouhi M. Diabetes in old age, a review. *Iranian J Diabetes and Lipid Disorders* 2009; **8**: 113-128
78 **Aung PP**, Strachan MW, Frier BM, Butcher I, Deary IJ, Price JF. Severe hypoglycaemia and late-life cognitive ability in older people with Type 2 diabetes: the Edinburgh Type 2 Diabetes Study. *Diabet Med* 2012; **29**: 328-336 [PMID: 22023662 DOI: 10.1111/j.1464-5491.2011.03505.x]
79 **Budnitz DS**, Lovegrove MC, Shehab N, Richards CL. Emergency hospitalizations for adverse drug events in older Americans. *N Engl J Med* 2011; **365**: 2002-2012 [PMID: 22111719 DOI: 10.1056/NEJMsa1103053]
80 **Evers IM**, ter Braak EW, de Valk HW, van Der Schoot B, Janssen N, Visser GH. Risk indicators predictive for severe hypoglycemia during the first trimester of type 1 diabetic pregnancy. *Diabetes Care* 2002; **25**: 554-559 [PMID: 11874946 DOI: 10.2337/diacare.25.3.554]
81 **Nielsen LR**, Pedersen-Bjergaard U, Thorsteinsson B, Johansen M, Damm P, Mathiesen ER. Hypoglycemia in pregnant women with type 1 diabetes: predictors and role of metabolic control. *Diabetes Care* 2008; **31**: 9-14 [PMID: 17909091 DOI: 10.2337/dc07-1066]
82 **Robertson H**, Pearson DW, Gold AE. Severe hypoglycaemia during pregnancy in women with Type 1 diabetes is common and planning pregnancy does not decrease the risk. *Diabet Med* 2009; **26**: 824-826 [PMID: 19709154 DOI: 10.1111/j.1464-5491.2009.02769.x]
83 **Rossi G**, Lapaczewski P, Diamond MP, Jacob RJ, Shulman GI, Sherwin RS. Inhibitory effect of pregnancy on counterregulatory hormone responses to hypoglycemia in awake rat. *Diabetes* 1993; **42**: 1440-1445 [PMID: 8375583 DOI: 10.2337/diab.42.10.1440]
84 **Rosenn BM**, Miodovnik M, Khoury JC, Siddiqi TA. Counterregulatory hormonal responses to hypoglycemia during pregnancy. *Obstet Gynecol* 1996; **87**: 568-574 [PMID: 8602310 DOI: 10.1016/0029-7844(95)00495-5]
85 **Ringholm L**, Pedersen-Bjergaard U, Thorsteinsson B, Damm P, Mathiesen ER. Hypoglycaemia during pregnancy in women with Type 1 diabetes. *Diabet Med* 2012; **29**: 558-566 [PMID: 22313112 DOI: 10.1111/j.1464-5491.2012.03604.x]
86 **Heller S**, Damm P, Mersebach H, Skjøth TV, Kaaja R, Hod M, Durán-García S, McCance D, Mathiesen ER. Hypoglycemia in type 1 diabetic pregnancy: role of preconception insulin aspart treatment in a randomized study. *Diabetes Care* 2010; **33**: 473-477 [PMID: 20007944 DOI: 10.2337/dc09-1605]
87 **Barendse S**, Singh H, Frier BM, Speight J. The impact of hypoglycaemia on quality of life and related patient-reported outcomes in Type 2 diabetes: a narrative review. *Diabet Med* 2012; **29**: 293-302 [PMID: 21838763 DOI: 10.1111/j.1464-5491.2011.03416.x]
88 **Davis RE**, Morrissey M, Peters JR, Wittrup-Jensen K, Kennedy-Martin T, Currie CJ. Impact of hypoglycaemia on quality of life and productivity in type 1 and type 2 diabetes. *Curr Med Res Opin* 2005; **21**: 1477-1483 [PMID: 16197667 DOI: 10.1185/030079905X61929]
89 **Williams SA**, Pollack MF, Dibonaventura M. Effects of hypoglycemia on health-related quality of life, treatment satisfaction and healthcare resource utilization in patients with type 2 diabetes mellitus. *Diabetes Res Clin Pract* 2011; **91**: 363-370 [PMID: 21251725 DOI: 10.1016/j.diabres.2010.12.027]
90 **Lundkvist J**, Berne C, Bolinder B, Jönsson L. The economic and quality of life impact of hypoglycemia. *Eur J Health Econ* 2005; **6**: 197-202 [PMID: 15761775 DOI: 10.1007/s10198-005-0276-3]
91 **Fidler C**, Elmelund Christensen T, Gillard S. Hypoglycemia: an overview of fear of hypoglycemia, quality-of-life, and impact on costs. *J Med Econ* 2011; **14**: 646-655 [PMID: 21854191 DOI: 10.3111/13696998.2011.610852]
92 **Gold AE**, Deary IJ, Frier BM. Hypoglycaemia and non-cognitive aspects of psychological function in insulin-dependent (type 1) diabetes mellitus (IDDM). *Diabet Med* 1997; **14**: 111-118 [PMID: 9047087 DOI: 10.1002/(SICI)1096-9136(199702)14:2<111::AID-DIA309>3.0.CO;2-S]
93 **Strachan MW**, Deary IJ, Ewing FM, Frier BM. Recovery of cognitive function and mood after severe hypoglycemia in adults with insulin-treated diabetes. *Diabetes Care* 2000; **23**: 305-312 [PMID: 10868856 DOI: 10.2337/diacare.23.3.305]
94 **King P**, Kong MF, Parkin H, Macdonald IA, Tattersall RB. Well-being, cerebral function, and physical fatigue after nocturnal hypoglycemia in IDDM. *Diabetes Care* 1998; **21**: 341-345 [PMID: 9540013 DOI: 10.2337/diacare.21.3.341]
95 **Ritholz MD**, Jacobson AM. Living with hypoglycemia. *J Gen Intern Med* 1998; **13**: 799-804 [PMID: 9844077 DOI: 10.1046/j.1525-1497.1998.00243.x]
96 **Eadington DW**, Frier BM. Type 1 diabetes and driving experience: an eight-year cohort study. *Diabet Med* 1989; **6**: 137-141 [PMID: 2522855 DOI: 10.1111/j.1464-5491.1989.tb02101.x]
97 **Lave LB**, Songer TJ, LaPorte RE. Should persons with diabetes be licensed to drive trucks?--Risk management. *Risk Anal* 1993; **13**: 327-334 [PMID: 8341808 DOI: 10.1111/j.1539-6924.1993.tb01084.x]
98 **Cox DJ**, Kovatchev B, Vandecar K, Gonder-Frederick L, Ritterband L, Clarke W. Hypoglycemia preceding fatal car collisions. *Diabetes Care* 2006; **29**: 467-468 [PMID: 16443915 DOI: 10.2337/diacare.29.02.06.dc05-1836]
99 **Lawton J**, Rankin D, Elliott J, Heller SR, Rogers HA, De Zoysa N, Amiel S. Experiences, views, and support needs of family members of people with hypoglycemia unawareness: interview study. *Diabetes Care* 2014; **37**: 109-115 [PMID: 23990515 DOI: 10.2337/dc13-1154]
100 **Gonder-Frederick LA**, Schmidt KM, Vajda KA, Greear ML, Singh H, Shepard JA, Cox DJ. Psychometric properties of the hypoglycemia fear survey-ii for adults with type 1 diabetes. *Diabetes Care* 2011; **34**: 801-806 [PMID: 21346182 DOI: 10.2337/dc10-1343]
101 **Tupola S**, Rajantie J, Akerblom HK. Experience of severe hypoglycaemia may influence both patient's and physician's subsequent treatment policy of insulin-dependent diabetes mellitus. *Eur J Pediatr* 1998; **157**: 625-627 [PMID: 9727844 DOI: 10.1007/s004310050899]
102 **Leese GP**, Wang J, Broomhall J, Kelly P, Marsden A, Morrison W, Frier BM, Morris AD. Frequency of severe hypoglycemia requiring emergency treatment in type 1 and type 2 diabetes: a population-based study of health service resource use. *Diabetes Care* 2003; **26**: 1176-1180 [PMID: 12663593 DOI: 10.2337/diacare.26.4.1176]
103 **Zhang Y**, Wieffer H, Modha R, Balar B, Pollack M, Krishnarajah G. The burden of hypoglycemia in type 2 diabetes: a systematic review of patient and economic perspectives. *J Clin Outcomes*

Manage 2010; **17**: 547-557
104 **Brod M**, Christensen T, Thomsen TL, Bushnell DM. The impact of non-severe hypoglycemic events on work productivity and diabetes management. *Value Health* 2011; **14**: 665-671 [PMID: 21839404 DOI: 10.1016/j.jval.2011.02.001]
105 **Fanelli CG**, Epifano L, Rambotti AM, Pampanelli S, Di Vincenzo A, Modarelli F, Lepore M, Annibale B, Ciofetta M, Bottini P. Meticulous prevention of hypoglycemia normalizes the glycemic thresholds and magnitude of most of neuroendocrine responses to, symptoms of, and cognitive function during hypoglycemia in intensively treated patients with short-term IDDM. *Diabetes* 1993; **42**: 1683-1689 [PMID: 8405713 DOI: 10.2337/diabetes.42.11.1683]
106 **Klonoff DC**. Continuous glucose monitoring: roadmap for 21st century diabetes therapy. *Diabetes Care* 2005; **28**: 1231-1239 [PMID: 15855600 DOI: 10.2337/diacare.28.5.1231]
107 **Bode B**, Gross K, Rikalo N, Schwartz S, Wahl T, Page C, Gross T, Mastrototaro J. Alarms based on real-time sensor glucose values alert patients to hypo- and hyperglycemia: the guardian continuous monitoring system. *Diabetes Technol Ther* 2004; **6**: 105-113 [PMID: 15117576 DOI: 10.1089/152091504773731285]
108 **Garg S**, Zisser H, Schwartz S, Bailey T, Kaplan R, Ellis S, Jovanovic L. Improvement in glycemic excursions with a transcutaneous, real-time continuous glucose sensor: a randomized controlled trial. *Diabetes Care* 2006; **29**: 44-50 [PMID: 16373894 DOI: 10.2337/diacare.29.01.06.dc05-1686]
109 **Streja D**. Can continuous glucose monitoring provide objective documentation of hypoglycemia unawareness? *Endocr Pract* 2005; **11**: 83-90 [PMID: 15901522 DOI: 10.4158/EP.11.2.83]
110 **Ly TT**, Hewitt J, Davey RJ, Lim EM, Davis EA, Jones TW. Improving epinephrine responses in hypoglycemia unawareness with real-time continuous glucose monitoring in adolescents with type 1 diabetes. *Diabetes Care* 2011; **34**: 50-52 [PMID: 20929999 DOI: 10.2337/dc10-1042]
111 **Choudhary P**, Ramasamy S, Green L, Gallen G, Pender S, Brackenridge A, Amiel SA, Pickup JC. Real-time continuous glucose monitoring significantly reduces severe hypoglycemia in hypoglycemia-unaware patients with type 1 diabetes. *Diabetes Care* 2013; **36**: 4160-4162 [PMID: 24103902 DOI: 10.2337/dc13-0939]
112 **The Clinical Trials gov**. The effect of RT-CGM on glycemia and Qol in patients with T1DM and IHA (IN CONTROL). [Updated 2013 Mar 6]. Available from: URL: https://clinicaltrials.gov/ct2/show/NCT01787903
113 **Schrot RJ**. Targeting plasma glucose: preprandial versus postprandial. *Clin Diabetes* 2004; **22**: 169-172 [DOI: 10.2337/diaclin.22.4.169]
114 **Cryer PE**, Axelrod L, Grossman AB, Heller SR, Montori VM, Seaquist ER, Service FJ. Evaluation and management of adult hypoglycemic disorders: an Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab* 2009; **94**: 709-728 [PMID: 19088155 DOI: 10.1210/jc.2008-1410]
115 **Qaseem A**, Vijan S, Snow V, Cross JT, Weiss KB, Owens DK. Glycemic control and type 2 diabetes mellitus: the optimal hemoglobin A1c targets. A guidance statement from the American College of Physicians. *Ann Intern Med* 2007; **147**: 417-422 [PMID: 17876024 DOI: 10.7326/0003-4819-147-6-200709180-00012]
116 **Pampanelli S**, Fanelli C, Lalli C, Ciofetta M, Sindaco PD, Lepore M, Modarelli F, Rambotti AM, Epifano L, Di Vincenzo A, Bartocci L, Annibale B, Brunetti P, Bolli GB. Long-term intensive insulin therapy in IDDM: effects on HbA1c, risk for severe and mild hypoglycaemia, status of counterregulation and awareness of hypoglycaemia. *Diabetologia* 1996; **39**: 677-686 [PMID: 8781763 DOI: 10.1007/BF00418539]
117 **Hamdan M**, Brunet P, Wagner J. [Metastatic jejunal perforation of cancer of the larynx]. *J Chir* (Paris) 2008; **128**: 332 [PMID: 1894709]
118 **Hermanns N**, Kulzer B, Kubiak T, Krichbaum M, Haak T. The effect of an education programme (HyPOS) to treat hypoglycaemia problems in patients with type 1 diabetes. *Diabetes Metab Res Rev* 2007; **23**: 528-538 [PMID: 17245692 DOI: 10.1002/dmrr.710]
119 **de Zoysa N**, Rogers H, Stadler M, Gianfrancesco C, Beveridge S, Britneff E, Choudhary P, Elliott J, Heller S, Amiel SA. A psychoeducational program to restore hypoglycemia awareness: the DAFNE-HART pilot study. *Diabetes Care* 2014; **37**: 863-866 [PMID: 24319119 DOI: 10.2337/dc13-1245]
120 **Leelarathna L**, Little SA, Walkinshaw E, Tan HK, Lubina-Solomon A, Kumareswaran K, Lane AP, Chadwick T, Marshall SM, Speight J, Flanagan D, Heller SR, Shaw JA, Evans ML. Restoration of self-awareness of hypoglycemia in adults with long-standing type 1 diabetes: hyperinsulinemic-hypoglycemic clamp substudy results from the HypoCOMPaSS trial. *Diabetes Care* 2013; **36**: 4063-4070 [PMID: 24130355 DOI: 10.2337/dc13-1004]
121 **Cryer PE**, Davis SN, Shamoon H. Hypoglycemia in diabetes. *Diabetes Care* 2003; **26**: 1902-1912 [PMID: 12766131 DOI: 10.2337/diacare.26.6.1902]
122 **Hirsch IB**. Insulin analogues. *N Engl J Med* 2005; **352**: 174-183 [PMID: 15647580 DOI: 10.1056/NEJMra040832]
123 **Yki-Järvinen H**, Dressler A, Ziemen M. Less nocturnal hypoglycemia and better post-dinner glucose control with bedtime insulin glargine compared with bedtime NPH insulin during insulin combination therapy in type 2 diabetes. HOE 901/3002 Study Group. *Diabetes Care* 2000; **23**: 1130-1136 [PMID: 10937510 DOI: 10.2337/diacare.23.8.1130]
124 **Rosenstock J**, Schwartz SL, Clark CM, Park GD, Donley DW, Edwards MB. Basal insulin therapy in type 2 diabetes: 28-week comparison of insulin glargine (HOE 901) and NPH insulin. *Diabetes Care* 2001; **24**: 631-636 [PMID: 11315821 DOI: 10.2337/diacare.24.4.631]
125 **Mensing C**, Boucher J, Cypress M, Weinger K, Mulcahy K, Barta P, Hosey G, Kopher W, Lasichak A, Lamb B, Mangan M, Norman J, Tanja J, Yauk L, Wisdom K, Adams C. National standards for diabetes self-management education. *Diabetes Care* 2006; **29** Suppl 1: S78-S85 [PMID: 16373939]
126 **Garber AJ**, King AB, Del Prato S, Sreenan S, Balci MK, Muñoz-Torres M, Rosenstock J, Endahl LA, Francisco AM, Hollander P. Insulin degludec, an ultra-longacting basal insulin, versus insulin glargine in basal-bolus treatment with mealtime insulin aspart in type 2 diabetes (BEGIN Basal-Bolus Type 2): a phase 3, randomised, open-label, treat-to-target non-inferiority trial. *Lancet* 2012; **379**: 1498-1507 [PMID: 22521072 DOI: 10.1016/S0140-6736(12)60205-0]
127 **Heise T**, Nosek L, Bøttcher SG, Hastrup H, Haahr H. Ultra-long-acting insulin degludec has a flat and stable glucose-lowering effect in type 2 diabetes. *Diabetes Obes Metab* 2012; **14**: 944-950 [PMID: 22726241 DOI: 10.1111/j.1463-1326.2012.01638.x]
128 **Ratner RE**, Gough SC, Mathieu C, Del Prato S, Bode B, Mersebach H, Endahl L, Zinman B. Hypoglycaemia risk with insulin degludec compared with insulin glargine in type 2 and type 1 diabetes: a pre-planned meta-analysis of phase 3 trials. *Diabetes Obes Metab* 2013; **15**: 175-184 [PMID: 23130654 DOI: 10.1111/dom.12032]
129 **Giménez M**, Lara M, Conget I. Sustained efficacy of continuous subcutaneous insulin infusion in type 1 diabetes subjects with recurrent non-severe and severe hypoglycemia and hypoglycemia unawareness: a pilot study. *Diabetes Technol Ther* 2010; **12**: 517-521 [PMID: 20597825 DOI: 10.1089/dia.2010.0028]
130 **Chantelau E**, Spraul M, Mühlhauser I, Gause R, Berger M. Long-term safety, efficacy and side-effects of continuous subcutaneous insulin infusion treatment for type 1 (insulin-dependent) diabetes mellitus: a one centre experience. *Diabetologia* 1989; **32**: 421-426 [PMID: 2509271 DOI: 10.1007/BF00271261]
131 **Bendtson I**, Kverneland A, Pramming S, Binderboland C. Incidence of nocturnal hypoglycaemia in insulin-dependent diabetic patients on intensive therapy. *Acta Med Scand* 1988; **223**: 543-548 [DOI: 10.1111/j.0954-6820.1988.tb17693.x]
132 **Fanelli CG**, Porcellati F, Pampanelli S, Bolli GB. Insulin therapy and hypoglycaemia: the size of the problem. *Diabetes Metab Res Rev* 2004; **20** Suppl 2: S32-S42 [PMID: 15551297 DOI: 10.1002/dmrr.514]
133 **Skyler JS**, Ponder S, Kruger DF, Matheson D, Parkin CG. Is

there a place for insulin pump therapy in your practice? *Clinical Diabetes* 2007; **25**: 50-56 [DOI: 10.2337/diaclin.25.2.50]

134 **Bode BW**, Steed RD, Davidson PC. Reduction in severe hypoglycemia with long-term continuous subcutaneous insulin infusion in type I diabetes. *Diabetes Care* 1996; **19**: 324-327 [PMID: 8729154 DOI: 10.2337/diacare.19.4.324]

135 **Kanc K**, Janssen MM, Keulen ET, Jacobs MA, Popp-Snijders C, Snoek FJ, Heine RJ. Substitution of night-time continuous subcutaneous insulin infusion therapy for bedtime NPH insulin in a multiple injection regimen improves counterregulatory hormonal responses and warning symptoms of hypoglycaemia in IDDM. *Diabetologia* 1998; **41**: 322-329 [PMID: 9541173 DOI: 10.1007/s001250050910]

136 **Castle JR**, Engle JM, El Youssef J, Massoud RG, Yuen KC, Kagan R, Ward WK. Novel use of glucagon in a closed-loop system for prevention of hypoglycemia in type 1 diabetes. *Diabetes Care* 2010; **33**: 1282-1287 [PMID: 20332355 DOI: 10.2337/dc09-2254]

137 **Hirsch IB**, Boyle PJ, Craft S, Cryer PE. Higher glycemic thresholds for symptoms during beta-adrenergic blockade in IDDM. *Diabetes* 1991; **40**: 1177-1186 [PMID: 1657673 DOI: 10.2337/diab.40.9.1177]

138 **White JR**, Campbell RK. Dangerous and common drug interactions in patients with diabetes mellitus. *Endocrinol Metab Clin North Am* 2000; **29**: 789-802 [PMID: 11149162 DOI: 10.1016/S0889-8529(05)70164-X]

139 **Fritsche A**, Stumvoll M, Häring HU, Gerich JE. Reversal of hypoglycemia unawareness in a long-term type 1 diabetic patient by improvement of beta-adrenergic sensitivity after prevention of hypoglycemia. *J Clin Endocrinol Metab* 2000; **85**: 523-525 [PMID: 10690848 DOI: 10.1210/jcem.85.2.6353]

140 **De Galan BE**, De Mol P, Wennekes L, Schouwenberg BJ, Smits P. Preserved sensitivity to beta2-adrenergic receptor agonists in patients with type 1 diabetes mellitus and hypoglycemia unawareness. *J Clin Endocrinol Metab* 2006; **91**: 2878-2881 [PMID: 16705070 DOI: 10.1210/jc.2006-0528]

141 **Kerr D**, Sherwin RS, Pavalkis F, Fayad PB, Sikorski L, Rife F, Tamborlane WV, During MJ. Effect of caffeine on the recognition of and responses to hypoglycemia in humans. *Ann Intern Med* 1993; **119**: 799-804 [PMID: 8379601 DOI: 10.7326/0003-4819-119-8-199310150-00005]

142 **Rosenthal MJ**, Smith D, Yaguez L, Giampietro V, Kerr D, Bullmore E, Brammer M, Williams SC, Amiel SA. Caffeine restores regional brain activation in acute hypoglycaemia in healthy volunteers. *Diabet Med* 2007; **24**: 720-727 [PMID: 17509073 DOI: 10.1111/j.1464-5491.2007.02131.x]

143 **Watson JM**, Jenkins EJ, Hamilton P, Lunt MJ, Kerr D. Influence of caffeine on the frequency and perception of hypoglycemia in free-living patients with type 1 diabetes. *Diabetes Care* 2000; **23**: 455-459 [PMID: 10857934 DOI: 10.2337/diacare.23.4.455]

144 **de Galan BE**, Tack CJ, Lenders JW, Pasman JW, Elving LD, Russel FG, Lutterman JA, Smits P. Theophylline improves hypoglycemia unawareness in type 1 diabetes. *Diabetes* 2002; **51**: 790-796 [PMID: 11872681 DOI: 10.2337/diabetes.51.3.790]

145 **Kerr D**, Everett J. Coffee, diabetes and insulin sensitivity. *Diabetologia* 2005; **48**: 1418 [PMID: 15933860 DOI: 10.1007/s00125-005-1790-7]

146 **Sawka AM**, Burgart V, Zimmerman D. Loss of awareness of hypoglycemia temporally associated with selective serotonin reuptake inhibitors. *Diabetes Care* 2001; **24**: 1845-1846 [PMID: 11574457 DOI: 10.2337/diacare.24.10.1845]

147 **Fan X**, Ding Y, Cheng H, Gram DX, Sherwin RS, McCrimmon RJ. Amplified hormonal counterregulatory responses to hypoglycemia in rats after systemic delivery of a SUR-1-selective K(+) channel opener? *Diabetes* 2008; **57**: 3327-3334 [PMID: 18776135 DOI: 10.2337/db08-0793]

148 **Raju B**, Cryer PE. Loss of the decrement in intraislet insulin plausibly explains loss of the glucagon response to hypoglycemia in insulin-deficient diabetes: documentation of the intraislet insulin hypothesis in humans. *Diabetes* 2005; **54**: 757-764 [PMID: 15734853 DOI: 10.2337/diabetes.54.3.757]

149 **Froud T**, Ricordi C, Baidal DA, Hafiz MM, Ponte G, Cure P, Pileggi A, Poggioli R, Ichii H, Khan A, Ferreira JV, Pugliese A, Esquenazi VV, Kenyon NS, Alejandro R. Islet transplantation in type 1 diabetes mellitus using cultured islets and steroid-free immunosuppression: Miami experience. *Am J Transplant* 2005; **5**: 2037-2046 [PMID: 15996257 DOI: 10.1111/j.1600-6143.2005.00957.x]

150 **Rickels MR**, Schutta MH, Mueller R, Kapoor S, Markmann JF, Naji A, Teff KL. Glycemic thresholds for activation of counterregulatory hormone and symptom responses in islet transplant recipients. *J Clin Endocrinol Metab* 2007; **92**: 873-879 [PMID: 17192287 DOI: 10.1210/jc.2006-2426]

151 **Clarke WL**, Cox DJ, Gonder-Frederick LA, Julian D, Schlundt D, Polonsky W. Reduced awareness of hypoglycemia in adults with IDDM. A prospective study of hypoglycemic frequency and associated symptoms. *Diabetes Care* 1995; **18**: 517-522 [PMID: 7497862 DOI: 10.2337/diacare.18.4.517]

152 **Leitão CB**, Tharavanij T, Cure P, Pileggi A, Baidal DA, Ricordi C, Alejandro R. Restoration of hypoglycemia awareness after islet transplantation. *Diabetes Care* 2008; **31**: 2113-2115 [PMID: 18697903 DOI: 10.2337/dc08-0741]

153 **Gabriely I**, Hawkins M, Vilcu C, Rossetti L, Shamoon H. Fructose amplifies counterregulatory responses to hypoglycemia in humans. *Diabetes* 2002; **51**: 893-900 [PMID: 11916904 DOI: 10.2337/diabetes.51.4.893]

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REVIEW

Relationship between diabetes and periodontal infection

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Abstract

Periodontal disease is a high prevalent disease. In the United States 47.2% of adults ⩾ 30 years old have been diagnosed with some type of periodontitis. Longitudinal studies have demonstrated a two-way relationship between diabetes and periodontitis, with more severe periodontal tissue destruction in diabetic patients and poorer glycemic control in diabetic subjects with periodontal disease. Periodontal treatment can be successful in diabetic patients. Short term effects of periodontal treatment are similar in diabetic patients and healthy population but, more recurrence of periodontal disease can be expected in no well controlled diabetic individuals. However, effects of periodontitis and its treatment on diabetes metabolic control are not clearly defined and results of the studies remain controversial.

Key words: Diabetes; Diabetes mellitus; Periodontitis; Periodontal disease; Periodontal treatment; Scaling and root planning; Non surgical periodontal treatment; Antibiotic; Glycosylated hemoglobin; C-reactive protein

Core tip: Longitudinal studies have demonstrated a two-way relationship between diabetes and periodontitis, with more severe periodontal tissue destruction in diabetic patients and poorer glycemic control in diabetic subjects with periodontal disease. Periodontal treatment can be successful in diabetic patients, but more recurrence of periodontal disease can be expected in non well controlled diabetic individuals. However, effects of periodontitis and its treatment on diabetes metabolic control are not clearly defined and results of the studies remain controversial. Recommendations for future investigations are included in this review.

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PERIODONTAL DISEASE

What is periodontal disease?

Periodontal disease is the destruction of the tissues that support the tooth by accumulation and maturation of oral bacteria on teeth.

Periodontal diseases include two major entities, gingivitis and periodontitis. Gingivitis is characterized by reversible inflammation of periodontal tissues whereas periodontitis also presents destruction of tooth supporting structures, and may lead to tooth loss. Exiting evidence indicates that gingival inflammation (gingivitis) is required for periodontitis, however some gingivitis never transform to periodontitis[1,2]. This is because bacterial plaque accumulation is necessary for the onset of both entities but individual susceptibility is required to develop periodontitis[2,3].

The currently used classification of periodontal diseases was introduced by the 1999 International Workshop for a Classification of Periodontal Diseases and Conditions[4]. Since the current classification has been used only in the last years, a substantial part of the existing literature on the prevalence and extent of periodontal diseases in various populations is still based on earlier classification systems.

Due to its high prevalence in current populations, it has become a public health priority. Epidemiologic studies have determined that about 50% of the population suffer from gingivitis and approximately 14% show periodontitis[5]. This percentage was higher in a recent study on United States population, which showed that 47.2% of adults $\geqslant$ 30 years old had periodontitis. Prevalence of periodontitis increased with age up to the point that 70.1% of adults $\geqslant$ 65 years old were affected by periodontal disease[6]. Men exhibit worse periodontal status than women [(56.4% *vs* 38.4%), as well as those with limited education (66.9%) and income (65.4%)]. These factors, together with cigarette smoking are increased risk factors for periodontal progression[7].

Etiology and pathogenesis of periodontal disease

Microorganisms in combination with individual host susceptibility and environmental factors are the main etiologic factors of periodontal diseases.

Plaque accumulation on teeth produces gingivitis, but the degree of inflammation and destruction of the alveolar bone that supports teeth depend on the host susceptibility[8].

Oral bacteria can damage periodontal tissues through the action of matrix-degrading enzymes and molecules that affect host cells. The transition from gingivitis to periodontitis involves the spreading of the inflammatory front to deeper areas in the connective tissue. However the reason why this happens is not well established. One etiopathogenic mechanism could involve the presence of bacteria or their products, such as lipopolysaccharides, in the periodontal connective tissue. They may induce an immune response with production of interleukins and tumor necrosis factor (TNF), which play an important role in the regulation of inflammatory processes. This inflammation stimulates the production of secondary mediators, which amplify the inflammatory response. Simultaneously, the presence of these cytokines reduces the ability to repair damaged tissue by cells such as fibroblasts, and finally, bacterial products and this inflammatory cascade stimulate osteoclastogenesis, leading to alveolar bone destruction[9,10] (Figure 1).

Several studies have shown how gingival inflammation can be modulated by a number of conditions. Systemic diseases, steroid hormones variations, nutritional deficiency, the intake of drugs, diabetes, tobacco smoking and other conditions have comprehensive and profound effects on the host, resulting in an increased response to bacterial plaque accumulation[10].

The high prevalence of *Helicobacter pylori* (*H. pylori*) among the microorganisms isolated from the oral environment induce to think that it may have an effect in the development of periodontal disease. Umeda *et al*[11] determined that periodontal patients showed a higher level of *H. pylori* than healthy subjects but, there was no significant correlation between the presence of *H. pylori* and the severity of periodontitis[12]. The addition of periodontal treatment to eradication therapy may reduce *H. pylori* recurrence compared with eradication therapy alone in periodontal patients suffering from gastric diseases associated with *H. pylori*[13].

Clinical manifestation of periodontal disease

Clinical signs of gingival inflammation (gingivitis) involve enlarged gingival contours due to edema or fibrosis, color transition to a red and/or bluish red hue, elevated sulcular temperature, bleeding upon probing and, increased gingival exudates (Figure 2).

Periodontitis clinical features include clinical attachment loss (CAL), alveolar bone loss (BL), periodontal pocketing and gingival inflammation. In addition, enlargement or recession of the gingiva; increase tooth mobility, drifting, and even tooth exfoliation may occur (Figure 3)[14].

Diagnosis of periodontal disease

Clinical evaluation includes periodontal probing (Figure 4) to evaluate: (1) Probing depth: the distance a periodontal probe penetrates into a periodontal pocket measured from the gingival margin to its bottom; (2) Clinical attachment level: The distance from the cemento-enamel junction to the bottom of the periodontal pocket; (3) Bleeding on probing. Bleeding after probing to the base of the periodontal pocket has been

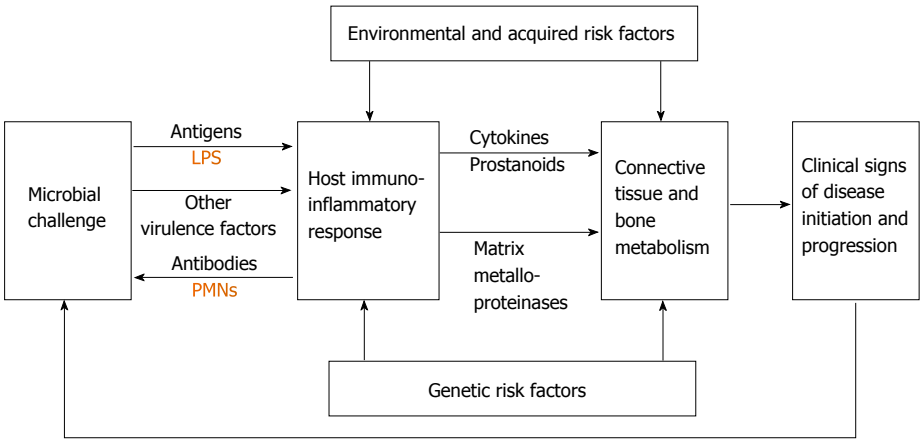

Figure 1 Etiology and pathogenesis of periodontal diseases. Adapted from: Page RC, Kornman KS. The pathogenesis of human periodontitis: an introduction. *Periodontol* 2000 1997; **14**: 9-11.

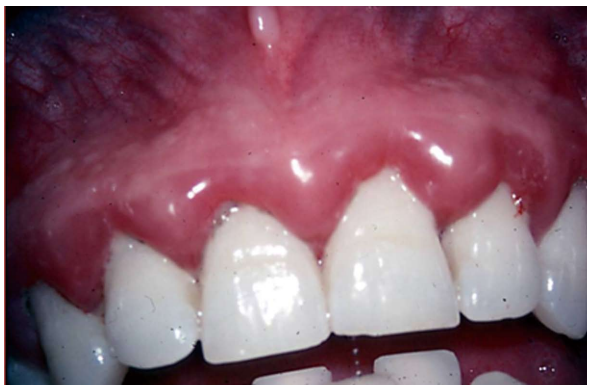

Figure 2 Clinical features of plaque-induced gingivitis associated with systemic diseases (diabetes mellitus-associates gingivitis).

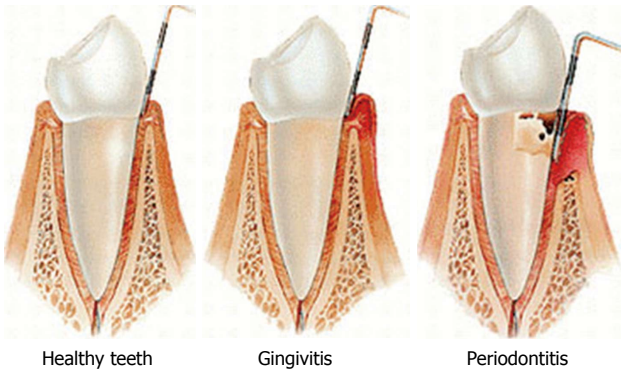

Figure 4 Clinical diagnosis of periodontitis.

a common way to identify presence of subgingival inflammation; and (4) Tooth mobility and furcations. The movement of a tooth in its socket resulting from an applied force can be classified into three categories. Furcation involvement is defined as BL affecting the base of the root trunk of a tooth where two or more roots meet.

Radiographic evaluation will show if alveolar bone that support tooth roots is lost. In a healthy situation alveolar bone will remain 1-2 mm below the crown of the teeth. If bone is located further from the crown, it means that loss has occurred (Figure 5).

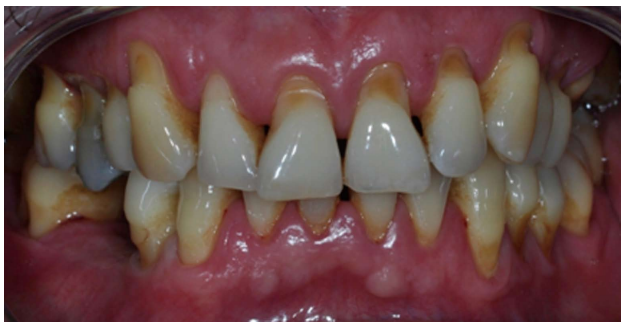

Figure 3 Clinical features of chronic periodontitis in diabetic subject.

Classification of periodontal disease

In 1999, the American Academy of Periodontology organized an international symposium with the aim of reaching a consensus regarding the classification of periodontal diseases and disorders, resulting in eight categories: gingival diseases, chronic periodontitis, aggressive periodontitis, periodontitis as manifestation of systemic diseases, necrotizing periodontal diseases, periodontal abscesses, periodontitis associated with endodontic lesions and, developmental or acquired deformities and conditions[4,15,16].

It is possible to include in this classification additional subcategories such as "diabetes mellitus-associated chronic periodontitis" and "diabetes mellitus-associated aggressive periodontitis" under the category of periodontitis as manifestation of systemic diseases.

INTERRELATIONSHIP BETWEEN PERIODONTITIS AND DIABETES

Investigations have demonstrated associations between periodontitis and various systemic diseases[17,18] such as cardiovascular disorders[19,20], respiratory diseases[21,22], osteoporosis[23,24], immunodeficiencies[25] and also diabetes

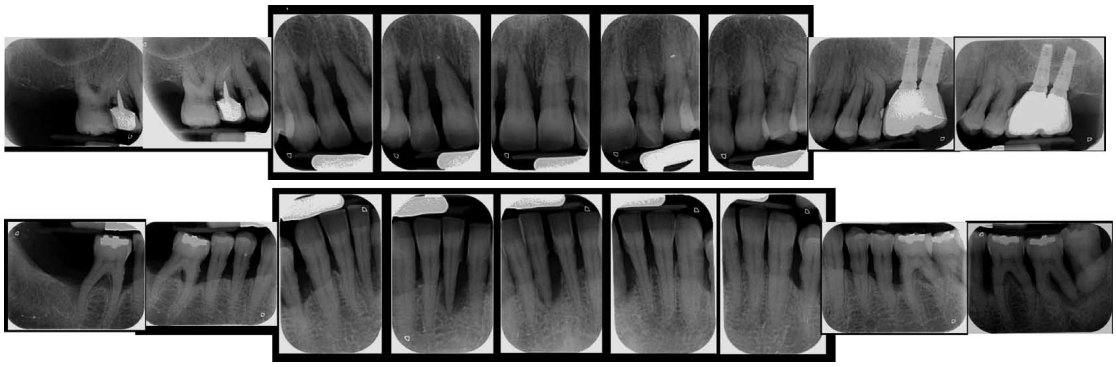

Figure 5 Radiographic diagnosis of periodontitis.

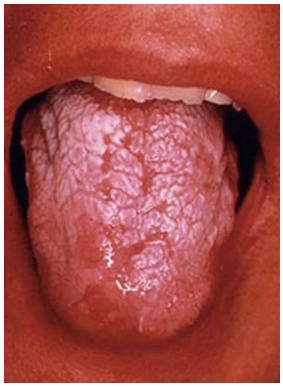

Figure 6 Clinical features of acute pseudomembranous candidiasis.

mellitus[26].

As already mentioned, longitudinal studies have demonstrated a two-way relationship between diabetes and periodontitis, with more severe periodontal tissue destruction in diabetic patients and poorer glycemic control in diabetic subjects with periodontal disease[27-30].

Effect of diabetes on periodontal disease and periodontal treatment

Diabetes has been associated to different oral diseases such as salivary and taste dysfunction, oral bacterial and fungal infections (*i.e.*, candidiasis), and oral mucosa lesions (*i.e.*, stomatitis, geographic tongue, traumatic ulcer, lichen planus,...)[31,32]. Diminished salivary flow and burning mouth are other oral characteristics in diabetic patients with poor glycemic control. Also, different oral pathologies such as, lichen planus, leukoplakia and lichenoid reactions are associated to diabetic subjects due to immunosuppression and/or drugs used. In addition, delayed mucosal wound healing, mucosal neuro-sensory disorders, decay lesions and tooth loss have been reported in diabetic patients[33]. Xerostomia is a frequent symptom found in diabetic patients on oral hypoglycemic agents, and it may facilitate the onset of some fungal opportunistic infection. Candidiasis has been reported in patients with poorly controlled diabetes (Figure 6).

Evidence suggests that diabetes leads to worsening of periodontal disease, and a significant association between diabetes and periodontitis has been demonstrated. Periodontal disease has a higher incidence in diabetic patients, and it is more prevalent and severe if compared with a healthy population[27,34]. Lalla *et al*[35] determined the prevalence of periodontitis in different age cohorts. It was 4.8 times higher among diabetic patients compared to non diabetics when the 15 to 24-year age cohort was considered, and 2.3 higher in the 25-34 year group. Also, CAL was higher in diabetic patients when the 15 to 55-year age cohort was considered. Lim *et al*[36] estimated that the glycemic control was the most important risk factor related to severity and extent of periodontitis. Other authors like Lalla *et al*[37] established that the rate of periodontal destruction is related to inappropriate glycemic control in diabetic patients so that accurate metabolic control could be important to prevent periodontal complications. Thus, glycemic control and the diabetes onset are critical factors in periodontal disease progression but it should be considered that substantial heterogeneity exists within diabetics[38].

Glycosylated hemoglobin (HbA1c) allows the control of serum glucose levels in an interval of 120 d and is a useful decision-making tool. Diabetes micro- and macrovascular complications are related to increased levels of HbA1c. The risk of periodontitis is 3-fold times higher among diabetic patients[39], being its prevalence and severity even greater in diabetic patients presenting elevated HbA1c levels[40].

Different hypotheses have been proposed to explain the influence of diabetes mellitus on periodontitis but they are all currently under investigation and remain somewhat controversial. Two similar but distinct pathogenic pathways may justify the biologic plausibility, a possible common origin of the two diseases which results in a host susceptible to either diseases[41], or a direct causal relationship in which, through the effects of advanced glycosylation end products (AGEs), diabetes triggers an increased inflammatory phenotype in cells[5,27]. Studies have shown how chronic hyperglycemia produces AGEs that can bind to specific receptors (RAGE) on different cells such as fibroblast, endothelial cells and macrophages[42]. Thereby, macrophages are transformed into hypereactive cells that produce pro-inflammatory cytokines such as interleukins 1β and 6 (IL-1β, IL-6)

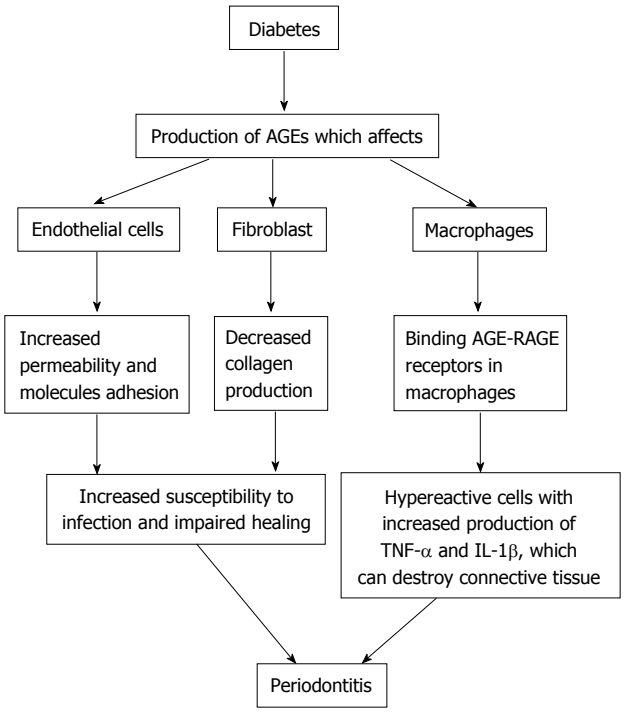

Figure 7 How diabetes mellitus could contribute to the development of periodontal disease (Llambés F, Caffesse R, Arias S). TNF: Tumor necrosis factor; IL-1β: Interleukins 1β; AGE: Advanced glycosylation end product; RAGE: Receptors AGE.

and TNF-α. AGEs can also alter endothelial cells which will become hyperpermeable and hyperexpressive for adhesion molecules, while fibroblasts will show decreased collagen production[43]. Therefore, AGEs produced by chronic hyperglycemia can produce hyper inflammatory responses, vascular modifications, altered healing and increased predisposition to infections (Figure 7). Lalla *et al*[44] supported the hypothesis that the activation of RAGE contributes to pathogenesis of periodontitis in diabetic patients. Increased accumulation of AGEs and their interaction with RAGE in diabetic gingiva leads to hyper production of proinflammatory cytokines, vascular dysfunction, and loss of effective tissue integrity and barrier function.

Despite these facts, periodontal treatment can be successful in diabetic patients. Short term effects of periodontal treatment are similar in diabetic patients and healthy population[45-47] but, more recurrence of periodontal disease can be expected in non well controlled diabetic individuals[26].

Effect of periodontal disease and its treatment on diabetes

The National Health and Nutrition Examination Survey 2009-2010 reported that prevalence of diabetes was 12.5% among periodontal patients, but only 6.3% in subjects without periodontitis[48].

If diabetic individuals are at a higher risk for periodontitis, it is also important to determine what effects periodontitis and its treatment may have on diabetes. It would be reasonable to think that periodontal inflammation, as any other infections, can have an adverse effect on diabetes glycemic control, compromising diabetes management in these individuals. Most evidence on this issue is derived from interventional and observational studies, indicating that periodontitis affects the glycemic control of diabetic patients. HbA1c values < 7% are related with proper glycemic levels whilst > 8% values represents poorly controlled glycemia.

Longitudinal studies have demonstrated that severe periodontitis is associated with poorly controlled glycemia, higher HbA1c levels and development of diabetic systemic complications[1,30,49]. It also has been reported that periodontitis is associated with a slight elevation of HbA1c in non-diabetic subjects (periodontitis may potentially increase the incidence of diabetes), although a clear-cut association could not be established[50].

Studies assumed that periodontal infection may impair glycemic control by increasing insulin tissue resistance[26]. Hence, glycemic level could be improved by non-surgical periodontal treatment removing bacterial plaque accumulation and decreasing gingival inflammation. This assumption is based on studies that observed an improvement in diabetes glycemic control following periodontal therapy[46,51]. It should be considered that other studies did not find such causal relationship, maybe due to inadequate time for periodontal tissues healing, or because periodontitis had not been properly resolved[30,52]. Another reason may be the influence of factors such as diet, physical exercise or use of antidiabetics that can alter significantly HbA1c, and make more difficult to observe the metabolic effect of periodontal treatment[45].

Effect of non-surgical periodontal therapy on diabetes glycemic control

Several studies have investigated the effect of non-surgical periodontal therapy on the glycemic control of diabetic patients. Both non-diabetic and diabetic patients show similar short-term outcomes after non-surgical periodontal therapy in terms of probing depth reductions, gain in CAL and changes in subgingival microbiota[53]. If glycemic control is considered as treatment outcome after non-surgical periodontal therapy, results vary (Table 1).

Different studies on patients with type 1 diabetes mellitus have not found an additional beneficial effect of periodontal treatment in glycemic control. Llambés *et al*[45] obtained changes in mean HbA1c of about 0.07%, without statistical significant difference after non-surgical periodontal treatment in type 1 diabetic patients after 3-mo. Similarly, Seppälä *et al*[54] reported that in poorly-controlled type 1 diabetic patients, non-surgical periodontal therapy had no effect on HbA1c. The same results were observed in the study performed by Aldridge *et al*[55] who stated no changes in HbA1c levels after non-surgical periodontal therapy in 22 type 1 diabetics with severe periodontitis.

On the other hand, Faria-Almeida *et al*[46] reported

Table 1 How periodontal therapy affects diabetes glycemic control

	Ref.	Design	Sample	Follow-up	Outcome	Results
Type 1	Aldridge *et al*[55]	Randomized clinical trial	23 subjects	2 mo	HbA1c	No changes
	Smith *et al*[47]	Controlled clinical trial	18 subjects	2 mo	HbA1c	No changes
	Christgau *et al*[53]	Cohort study	7 subjects	4 mo	HbA1c	No changes
	Llambés *et al*[45]	Randomized clinical trial	30 subjects	3 mo	HbA1c	0.06% reduction (no changes)
Type 2	Stewart *et al*[75]	Controlled clinical trial	72 subjects	10 mo	HbA1c	6% reduction
	Kiran *et al*[51]	Randomized clinical trial	44 subjects	3 mo	HbA1c	0.8% reduction
	Faria-Almeida *et al*[46]	Cohort study	20 subjects	6 mo	HbA1c	5.7% reduction
	Dağ *et al*[56]	Controlled clinical trial	45 subjects	3 mo	HbA1c	No changes
	Auyeung *et al*[57]	Cohort study	75 subjects	12 mo	HbA1c	No changes
	Engebretson *et al*[58]	Randomized clinical trial	257 subjects	6 mo	HbA1c	No changes
	Gay *et al*[59]	Randomized clinical trial	126 subjects	4 mo	HbA1c	No changes

that non-surgical periodontal therapy significantly reduce HbA1c levels about 5.7% in type 2 diabetics, while Dağ *et al*[56] and Auyeung *et al*[57] reported that this therapy alone significantly reduced HbA1c levels only in well-controlled diabetics. Smith *et al*[47] reported that mechanical periodontal therapy alone did not produce a significant change in glycemic control in diabetic patients.

Recently, Engebretson *et al*[58] indicated that non-surgical periodontal therapy in type 2 diabetics with chronic periodontitis did not improve diabetes glycemic control. According to these findings the use of nonsurgical periodontal treatment in order to reduce levels of HbA1c would not be justified. Lately, Gay *et al*[59] in a randomized clinical trial where 152 type 2 diabetic patients with periodontitis were treated, determined that no statistically significant differences were found in the changes of HbA1c levels.

Furthermore, current systematic reviews report glycemic control improvement, with a HbA1c reduction of approximately 0.4%, after non-surgical periodontal treatment[60]. A mean reduction of -0.36% of glycosylated HbA1c in subjects with type 2 diabetes has been determined recently[61]. However, the clinical significance of this effect is still unknown. It has been reported that each 1% reduction of HbA1c may be associated with 35% reduction in the risk of microvascular complications[62]. To the best of our knowledge, no studies have evaluated changes in HbA1c levels in non-diabetic patients after non-surgical periodontal therapy.

Effect of non-surgical periodontal therapy in combination with antimicrobials on diabetes glycemic control

Two studies have examined the added benefit of chlorhexidine as adjunct to non-surgical periodontal therapy in diabetic patients. Christgau *et al*[53] demonstrated that non-surgical periodontal therapy in combination with subgingival irrigation with 0.2% chlorhexidine did not improve HbA1c levels. The same results were achieved when 0.12% chlorhexidine was considered[63].

Iwamoto *et al*[64] demonstrated a 0.8% reduction in HbA1c in type 2 diabetics after non-surgical periodontal therapy and subgingival use of minocycline gel.

Studies in which systemic antibiotics were used along with mechanical therapy showed a significant improvement in glycemic control in diabetic patients. This may be due to the additional benefits of systemic antibiotics, such as their antimicrobial and host modulation effects, as well as their inhibition of non-enzymatic glycosylation[63,65-67].

Non-surgical periodontal therapy combined with 100 mg doxycycline is associated with a mean HbA1c reduction of 0.6% in type 2 diabetics patients[65]. There is not enough evidence about the use of tetracyclines but it seems to play a role in limiting tissue destruction. Lately, a modest improvement in glycemic control was detected after nonsurgical therapy plus azithromycin[68]. However, Llambés *et al*[45] show that non-surgical periodontal treatment combined with systemic doxycycline has no effect on HbA1c of type 1 diabetic patients[43].

Effect of surgical periodontal therapy on diabetes glycemic control

Scarce available evidence makes it impossible to determine the response after periodontal surgical treatment in diabetic patients. Diabetic subjects usually show improved periodontitis after surgical periodontal treatment. However, if poor diabetic control is present, more recurrence of periodontal pockets and unfavorable long term response is expected after surgical treatment[53,69]. Effects of surgical periodontal treatment on HbA1c are currently unknown.

The exact mechanism linking periodontitis/periodontal inflammation and HbA1c levels is still not clearly known. In periodontitis, there is an increased production of pro-inflammatory mediators, such as TNF-α, IL-6, IL-1β and interferon gamma (IF-α), and increased levels of acute-phase proteins, such as C-reactive protein (CRP). All these mediators have important effects on glucose and lipid metabolism. TNF-α, IL-6 and IL-1β are insulin antagonist and lipid metabolism is hampered by TNF-α. Elevated levels of CRP lead to insulin resistance. IF-α induces apoptosis of pancreatic β cells[70]. Non-enzymatic glycosylation of hemoglobin is not induced by inflammation, but rather results from hyperglycemia caused by insulin resistance[44]. Thus, this could explain

why subjects with periodontitis have high HbA1c levels.

According to these reports, it can be presumed that control of periodontal inflammation after therapy may reduce the levels of local and circulatory mediators, such as IL-6 and TNF-α. Both may trigger acute phase proteins such as CRP, and impair intracellular insulin signaling. Consequently, if these mediators were reduced by periodontal treatment, this could theoretically, help in diabetes control. However, this mechanism remains to be confirmed. Some studies have shown that periodontal disease severity is correlated with blood CRP levels in diabetic patients[71,72], however CRP levels are not reduced after periodontal treatment[73,74].

CONCLUSION

Within the limits of this review we can conclude that: Periodontitis is a highly prevalent infectious disease that relates to some systemic disorders, including diabetes mellitus.

Diabetes has been associated to different oral diseases such as: xerostomia, neuro-sensory disorders, several oral mucosa diseases, tooth decay and periodontal disease. It is well documented in the literature that periodontal disease is more prevalent and severe in diabetic individuals than in healthy subjects. However, it has to be kept in mind that the level of metabolic control and duration of diabetes appear to influence the risk for periodontal disease, with a significant heterogeneity among diabetic individuals.

Periodontal treatment is effective in diabetic patients, but more long-term recurrence can be expected when diabetes is not well controlled.

Severe periodontitis is more frequently found in diabetic subjects with high HbA1c levels and systemic diabetic complications; however, the influence of periodontal treatment on HbA1c is not that well established. The beneficial effects of periodontal treatment on HbA1c levels seem to be more apparent in type 2 diabetics and when antibiotics are associated to local periodontal therapy, although other reports did not find any improvement in diabetes control after periodontal treatment. More research on type 1 and type 2 diabetic subjects will be needed to know how periodontal treatment affects diabetes metabolic control. In those, it will be paramount to control other factors that may affect HbA1c levels, such as diabetic medication, diet and physical exercise.

HbA1c reduction after periodontal treatment is usually less than 0.5%. New studies are needed to evaluate the clinical significance of this improvement.

Additionally, it may be necessary to explore the effects of different modalities of periodontal therapy in patients with different types of diabetes and different degrees of metabolic control.

Further analysis of inflammatory mediators, such as CRP, may help to explain the relationship between diabetes and periodontal disease, and the individual variations detected in samples from different severities of diabetes and periodontal disease.

Any improvement in the control of diabetes and/or periodontal disease has the potential to improve significantly the quality of life in diabetic subjects.

REFERENCES

1 **Listgarten MA**, Schifter CC, Laster L. 3-year longitudinal study of the periodontal status of an adult population with gingivitis. *J Clin Periodontol* 1985; **12**: 225-238 [PMID: 3856578 DOI: 10.1111/j.1600-051X.1985.tb00920.x]

2 **Löe H**, Anerud A, Boysen H, Morrison E. Natural history of periodontal disease in man. Rapid, moderate and no loss of attachment in Sri Lankan laborers 14 to 46 years of age. *J Clin Periodontol* 1986; **13**: 431-445 [PMID: 3487557 DOI: 10.1111/j.1600-051X.1986.tb01487.x]

3 **Page RC**. Milestones in periodontal research and the remaining critical issues. *J Periodontal Res* 1999; **34**: 331-339 [PMID: 10685357 DOI: 10.1111/j.1600-0765.1999.tb02262.x]

4 **Armitage GC**. Development of a classification system for periodontal diseases and conditions. *Ann Periodontol* 1999; **4**: 1-6 [PMID: 10863370 DOI: 10.1902/annals.1999.4.1.1]

5 **Soskolne WA**, Klinger A. The relationship between periodontal diseases and diabetes: an overview. *Ann Periodontol* 2001; **6**: 91-98 [PMID: 11887477 DOI: 10.1902/annals.2001.6.1.91]

6 **Eke PI**, Dye BA, Wei L, Thornton-Evans GO, Genco RJ. Prevalence of periodontitis in adults in the United States: 2009 and 2010. *J Dent Res* 2012; **91**: 914-920 [PMID: 22935673 DOI: 10.1177/0022034512457373]

7 **Eke PI**, Page RC, Wei L, Thornton-Evans G, Genco RJ. Update of the case definitions for population-based surveillance of periodontitis. *J Periodontol* 2012; **83**: 1449-1454 [PMID: 22420873 DOI: 10.1902/jop.2012.110664]

8 **Trombelli L**, Tatakis DN, Scapoli C, Bottega S, Orlandini E, Tosi M. Modulation of clinical expression of plaque-induced gingivitis. II. Identification of "high-responder" and "low-responder" subjects. *J Clin Periodontol* 2004; **31**: 239-252 [PMID: 15016251 DOI: 10.1111/j.1600-051x.2004.00478.x]

9 **Offenbacher S**. Periodontal diseases: pathogenesis. *Ann Periodontol* 1996; **1**: 821-878 [PMID: 9118282 DOI: 10.1902/annals.1996.1.1.821]

10 **Socransky SS**, Haffajee AD. The bacterial etiology of destructive periodontal disease: current concepts. *J Periodontol* 1992; **63**: 322-331 [PMID: 1573546 DOI: 10.1902/jop.1992.63.4s.322]

11 **Umeda M**, Kobayashi H, Takeuchi Y, Hayashi J, Morotome-Hayashi Y, Yano K, Aoki A, Ohkusa T, Ishikawa I. High prevalence of Helicobacter pylori detected by PCR in the oral cavities of periodontitis patients. *J Periodontol* 2003; **74**: 129-134 [PMID: 12593608 DOI: 10.1902/jop.2003.74.1.129]

12 **Salehi MR**, Shah Aboei M, Naghsh N, Hajisadeghi S, Ajami E. A Comparison in Prevalence of Helicobacter pylori in the Gingival Crevicular Fluid from Subjects with Periodontitis and Healthy Individuals using Polymerase Chain Reaction. *J Dent Res Dent Clin Dent Prospects* 2013; **7**: 238-243 [PMID: 24578823 DOI: 10.5681/joddd.2013.038]

13 **Bouziane A**, Ahid S, Abouqal R, Ennibi O. Effect of periodontal therapy on prevention of gastric Helicobacter pylori recurrence: a systematic review and meta-analysis. *J Clin Periodontol* 2012; **39**: 1166-1173 [PMID: 23151293 DOI: 10.1111/jcpe.12015]

14 **Mariotti A**. Dental plaque-induced gingival diseases. *Ann Periodontol* 1999; **4**: 7-19 [PMID: 10863371 DOI: 10.1902/annals.1999.4.1.7]

15 **Newman MG**. Classification of diseases and conditions affecting the periodontium. In: Elsevier/Saunders. Carranza's clinical periodontology. St. Louis, Mo, 2012: 64-74

16 **Kinane DF**, Lindhe J, Trombelli L. Chronic periodontitis. In: Blackwell. Clinical periodontology and implant dentistry. Oxford, 2003: 420-428

17 **Mohangi GU**, Singh-Rambirich S, Volchansky A. Periodontal

disease: Mechanisms of infection and inflammation and possible impact on miscellaneous systemic diseases and conditions. *SADJ* 2013; **68**: 462, 464-467 [PMID: 24660421]
18 **Linden GJ**, Lyons A, Scannapieco FA. Periodontal systemic associations: review of the evidence. *J Periodontol* 2013; **84**: S8-S19 [PMID: 23631586 DOI: 10.1902/jop.2013.1340010]
19 **Figuero E**, Sánchez-Beltrán M, Cuesta-Frechoso S, Tejerina JM, del Castro JA, Gutiérrez JM, Herrera D, Sanz M. Detection of periodontal bacteria in atheromatous plaque by nested polymerase chain reaction. *J Periodontol* 2011; **82**: 1469-1477 [PMID: 21453047 DOI: 10.1902/jop.2011.100719]
20 **Aquino AR**, Lima KC, Paiva MS, Rôças IN, Siqueira JF. Molecular survey of atheromatous plaques for the presence of DNA from periodontal bacterial pathogens, archaea and fungi. *J Periodontal Res* 2011; **46**: 303-309 [PMID: 21261623 DOI: 10.1111/j.1600-0765.2010.01343.x]
21 **Si Y**, Fan H, Song Y, Zhou X, Zhang J, Wang Z. Association between periodontitis and chronic obstructive pulmonary disease in a Chinese population. *J Periodontol* 2012; **83**: 1288-1296 [PMID: 22248220 DOI: 10.1902/jop.2012.110472]
22 **Sharma N**, Shamsuddin H. Association between respiratory disease in hospitalized patients and periodontal disease: a cross-sectional study. *J Periodontol* 2011; **82**: 1155-1160 [PMID: 21219100]
23 **Renvert S**, Berglund J, Persson RE, Persson GR. Osteoporosis and periodontitis in older subjects participating in the Swedish National Survey on Aging and Care (SNAC-Blekinge). *Acta Odontol Scand* 2011; **69**: 201-207 [PMID: 21254955 DOI: 10.3109/00016357.2010.549501]
24 **Fratzl P**, Roschger P, Fratzl-Zelman N, Paschalis EP, Phipps R, Klaushofer K. Evidence that treatment with risedronate in women with postmenopausal osteoporosis affects bone mineralization and bone volume. *Calcif Tissue Int* 2007; **81**: 73-80 [PMID: 17612779 DOI: 10.1007/s00223-007-9039-8]
25 **Gonçalves LS**, Gonçalves BM, Fontes TV. Periodontal disease in HIV-infected adults in the HAART era: Clinical, immunological, and microbiological aspects. *Arch Oral Biol* 2013; **58**: 1385-1396 [PMID: 23755999 DOI: 10.1016/j.archoralbio.2013.05.002]
26 **Bascones-Martínez A**, Muñoz-Corcuera M, Bascones-Ilundain J. [Diabetes and periodontitis: A bidirectional relationship]. *Med Clin* (Barc) 2015; **145**: 31-35 [PMID: 25192582 DOI: 10.1016/j.medcli.2014.07.019]
27 **Grossi SG**, Genco RJ. Periodontal disease and diabetes mellitus: a two-way relationship. *Ann Periodontol* 1998; **3**: 51-61 [PMID: 9722690 DOI: 10.1902/annals.1998.3.1.51]
28 **Lalla E**, Papapanou PN. Diabetes mellitus and periodontitis: a tale of two common interrelated diseases. *Nat Rev Endocrinol* 2011; **7**: 738-748 [PMID: 21709707 DOI: 10.1038/nrendo.2011.106]
29 **Preshaw PM**, Alba AL, Herrera D, Jepsen S, Konstantinidis A, Makrilakis K, Taylor R. Periodontitis and diabetes: a two-way relationship. *Diabetologia* 2012; **55**: 21-31 [PMID: 22057194 DOI: 10.1007/s00125-011-2342-y]
30 **Chapple IL**, Genco R; working group 2 of the joint EFP/AAP workshop. Diabetes and periodontal diseases: consensus report of the Joint EFP/AAP Workshop on Periodontitis and Systemic Diseases. *J Periodontol* 2013; **84**: S106-S112 [PMID: 23631572 DOI: 10.1902/jop.2013.1340011]
31 **Saini R**, Al-Maweri SA, Saini D, Ismail NM, Ismail AR. Oral mucosal lesions in non oral habit diabetic patients and association of diabetes mellitus with oral precancerous lesions. *Diabetes Res Clin Pract* 2010; **89**: 320-326 [PMID: 20488573 DOI: 10.1016/j.diabres.2010.04.016]
32 **Sandberg GE**, Sundberg HE, Fjellstrom CA, Wikblad KF. Type 2 diabetes and oral health: a comparison between diabetic and non-diabetic subjects. *Diabetes Res Clin Pract* 2000; **50**: 27-34 [PMID: 10936666 DOI: 10.1016/S0168-8227(00)00159-5]
33 **Lamster IB**, Lalla E, Borgnakke WS, Taylor GW. The relationship between oral health and diabetes mellitus. *J Am Dent Assoc* 2008; **139** Suppl: 19S-24S [PMID: 18809650 DOI: 10.14219/jada.archive.2008.0363]
34 **Papapanou PN**. Periodontal diseases: epidemiology. *Ann Periodontol* 1996; **1**: 1-36 [PMID: 9118256 DOI: 10.1902/annals.1996.1.1.1]
35 **Lalla E**, Lamster IB, Schmidt AM. Enhanced interaction of advanced glycation end products with their cellular receptor RAGE: implications for the pathogenesis of accelerated periodontal disease in diabetes. *Ann Periodontol* 1998; **3**: 13-19 [PMID: 9722686]
36 **Lim LP**, Tay FB, Sum CF, Thai AC. Relationship between markers of metabolic control and inflammation on severity of periodontal disease in patients with diabetes mellitus. *J Clin Periodontol* 2007; **34**: 118-123 [PMID: 17309586 DOI: 10.1111/j.1600-051X.2006.01032.x]
37 **Lalla E**, Cheng B, Lal S, Kaplan S, Softness B, Greenberg E, Goland RS, Lamster IB. Diabetes-related parameters and periodontal conditions in children. *J Periodontal Res* 2007; **42**: 345-349 [PMID: 17559632 DOI: 10.1111/j.1600-0765.2006.00955.x]
38 **Taylor JJ**, Preshaw PM, Lalla E. A review of the evidence for pathogenic mechanisms that may link periodontitis and diabetes. *J Clin Periodontol* 2013; **40** Suppl 14: S113-S134 [PMID: 23627323 DOI: 10.1111/jcpe.12059]
39 **Taylor GW**, Burt BA, Becker MP, Genco RJ, Shlossman M, Knowler WC, Pettitt DJ. Severe periodontitis and risk for poor glycemic control in patients with non-insulin-dependent diabetes mellitus. *J Periodontol* 1996; **67**: 1085-1093 [PMID: 8910827 DOI: 10.1902/jop.1996.67.10s.1085]
40 **Demmer RT**, Holtfreter B, Desvarieux M, Jacobs DR, Kerner W, Nauck M, Völzke H, Kocher T. The influence of type 1 and type 2 diabetes on periodontal disease progression: prospective results from the Study of Health in Pomerania (SHIP). *Diabetes Care* 2012; **35**: 2036-2042 [PMID: 22855731 DOI: 10.2337/dc11-2453]
41 **Emrich LJ**, Shlossman M, Genco RJ. Periodontal disease in non-insulin-dependent diabetes mellitus. *J Periodontol* 1991; **62**: 123-131 [PMID: 2027060 DOI: 10.1902/jop.1991.62.2.123]
42 **Brownlee M**. Glycation products and the pathogenesis of diabetic complications. *Diabetes Care* 1992; **15**: 1835-1843 [PMID: 1464241 DOI: 10.2337/diacare.15.12.1835]
43 **Seppälä B**, Sorsa T, Ainamo J. Morphometric analysis of cellular and vascular changes in gingival connective tissue in long-term insulin-dependent diabetes. *J Periodontol* 1997; **68**: 1237-1245 [PMID: 9444601 DOI: 10.1902/jop.1997.68.12.1237]
44 **Lalla E**, Lamster IB, Drury S, Fu C, Schmidt AM. Hyperglycemia, glycoxidation and receptor for advanced glycation endproducts: potential mechanisms underlying diabetic complications, including diabetes-associated periodontitis. *Periodontol 2000* 2000; **23**: 50-62 [PMID: 11276765 DOI: 10.1034/j.1600-0757.2000.2230104.x]
45 **Llambés F**, Silvestre FJ, Hernández-Mijares A, Guiha R, Caffesse R. The effect of periodontal treatment on metabolic control of type 1 diabetes mellitus. *Clin Oral Investig* 2008; **12**: 337-343 [PMID: 18446389 DOI: 10.1007/s00784.008.0201.0]
46 **Faria-Almeida R**, Navarro A, Bascones A. Clinical and metabolic changes after conventional treatment of type 2 diabetic patients with chronic periodontitis. *J Periodontol* 2006; **77**: 591-598 [PMID: 16584339 DOI: 10.1902/jop.2006.050084]
47 **Smith GT**, Greenbaum CJ, Johnson BD, Persson GR. Short-term responses to periodontal therapy in insulin-dependent diabetic patients. *J Periodontol* 1996; **67**: 794-802 [PMID: 8866319 DOI: 10.1902/jop.1996.67.8.794]
48 **Arora N**, Papapanou PN, Rosenbaum M, Jacobs DR, Desvarieux M, Demmer RT. Periodontal infection, impaired fasting glucose and impaired glucose tolerance: results from the Continuous National Health and Nutrition Examination Survey 2009-2010. *J Clin Periodontol* 2014; **41**: 643-652 [PMID: 24708451 DOI: 10.1111/jcpe.12258]
49 **Karjalainen KM**, Knuuttila ML, von Dickhoff KJ. Association of the severity of periodontal disease with organ complications in type 1 diabetic patients. *J Periodontol* 1994; **65**: 1067-1072 [PMID: 7853131 DOI: 10.1902/jop.1994.65.11.1067]
50 **Wolff RE**, Wolff LF, Michalowicz BS. A pilot study of glycosylated hemoglobin levels in periodontitis cases and healthy

controls. *J Periodontol* 2009; **80**: 1057-1061 [PMID: 19563284 DOI: 10.1902/jop.2009.080664]
51 **Kiran M**, Arpak N, Unsal E, Erdoğan MF. The effect of improved periodontal health on metabolic control in type 2 diabetes mellitus. *J Clin Periodontol* 2005; **32**: 266-272 [PMID: 15766369 DOI: 10.1111/j.1600-051X.2005.00658.x]
52 **Sgolastra F**, Severino M, Pietropaoli D, Gatto R, Monaco A. Effectiveness of periodontal treatment to improve metabolic control in patients with chronic periodontitis and type 2 diabetes: a meta-analysis of randomized clinical trials. *J Periodontol* 2013; **84**: 958-973 [PMID: 23106512 DOI: 10.1902/jop.2012.120377]
53 **Christgau M**, Palitzsch KD, Schmalz G, Kreiner U, Frenzel S. Healing response to non-surgical periodontal therapy in patients with diabetes mellitus: clinical, microbiological, and immunologic results. *J Clin Periodontol* 1998; **25**: 112-124 [PMID: 9495610 DOI: 10.1111/j.1600-051X.1998.tb02417.x]
54 **Seppälä B**, Ainamo J. A site-by-site follow-up study on the effect of controlled versus poorly controlled insulin-dependent diabetes mellitus. *J Clin Periodontol* 1994; **21**: 161-165 [PMID: 8157767 DOI: 10.1111/j.1600-051X.1994.tb00297.x]
55 **Aldridge JP**, Lester V, Watts TL, Collins A, Viberti G, Wilson RF. Single-blind studies of the effects of improved periodontal health on metabolic control in type 1 diabetes mellitus. *J Clin Periodontol* 1995; **22**: 271-275 [PMID: 7622632 DOI: 10.1111/j.1600-051X.1995.tb00147.x]
56 **Dağ A**, Firat ET, Arikan S, Kadiroğlu AK, Kaplan A. The effect of periodontal therapy on serum TNF-alpha and HbA1c levels in type 2 diabetic patients. *Aust Dent J* 2009; **54**: 17-22 [PMID: 19228128 DOI: 10.1111/j.1834-7819.2008.01083.x]
57 **Auyeung L**, Wang PW, Lin RT, Hsieh CJ, Lee PY, Zhuang RY, Chang HW. Evaluation of periodontal status and effectiveness of non-surgical treatment in patients with type 2 diabetes mellitus in Taiwan for a 1-year period. *J Periodontol* 2012; **83**: 621-628 [PMID: 21692625 DOI: 10.1902/jop.2011.110133]
58 **Engebretson SP**, Hyman LG, Michalowicz BS, Schoenfeld ER, Gelato MC, Hou W, Seaquist ER, Reddy MS, Lewis CE, Oates TW, Tripathy D, Katancik JA, Orlander PR, Paquette DW, Hanson NQ, Tsai MY. The effect of nonsurgical periodontal therapy on hemoglobin A1c levels in persons with type 2 diabetes and chronic periodontitis: a randomized clinical trial. *JAMA* 2013; **310**: 2523-2532 [PMID: 24346989 DOI: 10.1001/jama.2013.282431]
59 **Gay IC**, Tran DT, Cavender AC, Weltman R, Chang J, Luckenbach E, Tribble GD. The effect of periodontal therapy on glycaemic control in a Hispanic population with type 2 diabetes: a randomized controlled trial. *J Clin Periodontol* 2014; **41**: 673-680 [PMID: 24797222 DOI: 10.1111/jcpe.12268]
60 **Simpson TC**, Needleman I, Wild SH, Moles DR, Mills EJ. Treatment of periodontal disease for glycaemic control in people with diabetes. *Cochrane Database Syst Rev* 2010; (**5**): CD004714 [PMID: 20464734 DOI: 10.1002/14651858.CD004714.pub2]
61 **Engebretson S**, Kocher T. Evidence that periodontal treatment improves diabetes outcomes: a systematic review and meta-analysis. *J Periodontol* 2013; **84**: S153-S169 [PMID: 23631575 DOI: 10.1902/jop.2013.1340017]
62 **Stratton IM**, Adler AI, Neil HA, Matthews DR, Manley SE, Cull CA, Hadden D, Turner RC, Holman RR. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. *BMJ* 2000; **321**: 405-412 [PMID: 10938048 DOI: 10.1136/bmj.321.7258.405]
63 **Grossi SG**, Skrepcinski FB, DeCaro T, Robertson DC, Ho AW, Dunford RG, Genco RJ. Treatment of periodontal disease in diabetics reduces glycated hemoglobin. *J Periodontol* 1997; **68**: 713-719 [PMID: 9287060 DOI: 10.1902/jop.1997.68.8.713]
64 **Iwamoto Y**, Nishimura F, Nakagawa M, Sugimoto H, Shikata K, Makino H, Fukuda T, Tsuji T, Iwamoto M, Murayama Y. The effect of antimicrobial periodontal treatment on circulating tumor necrosis factor-alpha and glycated hemoglobin level in patients with type 2 diabetes. *J Periodontol* 2001; **72**: 774-778 [PMID: 11453240 DOI: 10.1902/jop.2001.72.6.774]
65 **Miller LS**, Manwell MA, Newbold D, Reding ME, Rasheed A, Blodgett J, Kornman KS. The relationship between reduction in periodontal inflammation and diabetes control: a report of 9 cases. *J Periodontol* 1992; **63**: 843-848 [PMID: 1403592 DOI: 10.1902/jop.1992.63.10.843]
66 **Jones JA**, Miller DR, Wehler CJ, Rich SE, Krall-Kaye EA, McCoy LC, Christiansen CL, Rothendler JA, Garcia RI. Does periodontal care improve glycemic control? The Department of Veterans Affairs Dental Diabetes Study. *J Clin Periodontol* 2007; **34**: 46-52 [PMID: 17137468]
67 **Llambés F**, Silvestre FJ, Hernández-Mijares A, Guiha R, Caffesse R. Effect of non-surgical periodontal treatment with or without doxycycline on the periodontium of type 1 diabetic patients. *J Clin Periodontol* 2005; **32**: 915-920 [PMID: 15998278]
68 **Botero JE**, Yepes FL, Ochoa SP, Hincapie JP, Roldan N, Ospina CA, Castrillon CA, Becerra MA. Effects of periodontal non-surgical therapy plus azithromycin on glycemic control in patients with diabetes: a randomized clinical trial. *J Periodontal Res* 2013; **48**: 706-712 [PMID: 23441920 DOI: 10.1111/jre.12058]
69 **Tervonen T**, Knuuttila M, Pohjamo L, Nurkkala H. Immediate response to nonsurgical periodontal treatment in subjects with diabetes mellitus. *J Clin Periodontol* 1991; **18**: 65-68 [PMID: 2045520 DOI: 10.1111/j.1600-051X.1991.tb01121.x]
70 **Souza KL**, Gurgul-Convey E, Elsner M, Lenzen S. Interaction between pro-inflammatory and anti-inflammatory cytokines in insulin-producing cells. *J Endocrinol* 2008; **197**: 139-150 [PMID: 18372240 DOI: 10.1677/JOE-07-0638]
71 **Llambés F**, Silvestre FJ, Hernández-Mijares A, Guiha R, Bautista D, Caffesse R. Efect of periodontal disease and non surgical periodontal treatment on C-reactive protein. Evaluation of type 1 diabetic patients. *Med Oral Patol Oral Cir Bucal* 2012; **17**: e562-e568 [PMID: 22322513 DOI: 10.4317/medoral.17793]
72 **Priyanka N**, Kumari M, Kalra N, Arjun P, Naik SB, Pradeep AR. Crevicular fluid and serum concentrations of progranulin and high sensitivity CRP in chronic periodontitis and type 2 diabetes. *Dis Markers* 2013; **35**: 389-394 [PMID: 24191130 DOI: 10.1155/2013/803240]
73 **Dasanayake AP**. C-reactive protein levels are elevated in patients with periodontitis and their CRP levels may go down after periodontal therapy. *J Evid Based Dent Pract* 2009; **9**: 21-22 [PMID: 19269611 DOI: 10.1016/j.jebdp.2008.12.010]
74 **Pejcic A**, Kesic L, Milasin J. Association between Periodontopathogens and CRP Levels in Patients with Periodontitis in Serbia. *J Dent Res Dent Clin Dent Prospects* 2011; **5**: 10-16 [PMID: 23019501 DOI: 10.5681/joddd.2011.003]
75 **Stewart JE**, Wager KA, Friedlander AH, Zadeh HH. The effect of periodontal treatment on glycemic control in patients with type 2 diabetes mellitus. *J Clin Periodontol* 2001; **28**: 306-310 [PMID: 11314885 DOI: 10.1034/j.1600-051x.2001.028004306.x]

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REVIEW

Fetal programming of polycystic ovary syndrome

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Abstract

Polycystic ovary syndrome (PCOS) is a common endocrine disorder that affects up to 6.8% of reproductive age women. Experimental research and clinical observations suggest that PCOS may originate in the very early stages of development, possibly even during intrauterine life. This suggests that PCOS is either genetically-transmitted or is due to epigenetic alterations that develop in the intrauterine microenvironment. Although familial cases support the role of genetic factors, no specific genetic pattern has been defined in PCOS. Several candidate genes have been implicated in its pathogenesis, but none can specifically be implicated in PCOS development. Hypotheses based on the impact of the intrauterine environment on PCOS development can be grouped into two categories. The first is the "thrifty" phenotype hypothesis, which states that intrauterine nutritional restriction in fetuses causes decreased insulin secretion and, as a compensatory mechanism, insulin resistance. Additionally, an impaired nutritional environment can affect the methylation of some specific genes, which can also trigger PCOS. The second hypothesis postulates that fetal exposure to excess androgen can induce changes in differentiating tissues, causing the PCOS phenotype to develop in adult life. This review aimed to examine the role of fetal programming in development of PCOS.

Key words: Polycystic ovary syndrome; Androgens; Fetal programming; Intrauterine growth retardation; Genetic

Core tip: Polycystic ovary syndrome (PCOS) is a highly complex and heterogeneous disorder that is significantly influenced by genetic and environmental factors. There is some evidence that the development of PCOS may begin during the intrauterine period. Fetuses exposed to intrauterine nutritional restriction often have lowered insulin secretion and, as a compensatory mechanism, insulin resistance, which is known as the "thrifty" phenotype. Additionally, an impaired intrauterine nutritional environment can affect the methylation of some specific genes, which can trigger PCOS. The other hypothesis postulates that fetal exposure to excess androgen can induce changes in differentiating tissues, causing the PCOS phenotype and related disorders to develop in adult life.

Gur EB, Karadeniz M, Turan GA. Fetal programming of polycystic ovary syndrome. *World J Diabetes* 2015; 6(7): 936-942 Available from: URL: http://www.wjgnet.com/1948-9358/full/v6/i7/936.htm DOI: http://dx.doi.org/10.4239/wjd.v6.i7.936

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a complex disorder characterized by defects in primary cellular control mechanisms that can result in hyperandrogenemia, hyperinsulinemia, insulin resistance, and chronic anovulation. PCOS is the most common endocrinologic disorder among women of reproductive age. Its prevalence typically ranges between 4% and 8% in diverse populations, but it has been reported to be as high as 25%[1]. The variations in the reported prevalence of PCOS have been attributed to the use of different diagnostic criteria. Three main diagnostic criteria systems that are currently accepted for PCOS are those from the National Institutes of Health (NIH, 1990), Rotterdam (ASRM/ESHRE, 2003), and Androgen Excess Society (AES, 2006). According to the Rotterdam criteria, the diagnosis of PCOS is based on the presence of at least two of the following three clinical features: polycystic ovarian morphology, oligo/amenorrhea and hyperandrogenism. However, the NIH criteria require only oligo/amenorrhea and hyperandrogenism for a diagnosis, while the AES criteria require a combination of biochemical or clinical hyperandrogenism along with chronic anovulation or polycystic ovarian morphology[1,2]. Although it is considered to be a disorder of reproductive age women (based on its classical symptoms of amenorrhea, hirsutism, and infertility), it can affect a woman any time during her life. Affected persons have a lifetime risk of disorders, including glucose metabolism, cardiovascular diseases, endometrial hyperplasia and/or cancer[2].

The underlying causes of PCOS are not known. However, its signs and symptoms typically appear during or close to the onset of puberty. Signs of precocious pubarche and adolescent hyperandrogenemia with or without insulin resistance may indicate the early stages of PCOS[3]. Further, epidemiologic studies have shown that adolescents with the aforementioned signs of PCOS had lower birth weights than those of controls[4]. These results suggest the hypothesis that PCOS is a continuum of a process that begins during intrauterine life.

PCOS is also believed to be caused by several genetic and environmental factors. The prevalence of PCOS has risen in populations where the gene pool has been relatively constant, which indicates that environmental factors may be playing a more important role in its development[5]. Further, obesity has been linked to the development of PCOS in susceptible individuals. A recent study revealed that, when compared with matched controls, non-obese women with PCOS had higher levels of glycotoxins, hyperandrogenemia, and advanced glycation end products, which were positively correlated with insulin resistance indices[6]. Some recent animal studies and observational human studies have suggested that impaired nutrition and steroidal environment during intrauterine life may play an important role in the development of PCOS[7-9].

GENETICS OF PCOS

Although case reports indicate that PCOS clusters within families, genetic studies have been inconclusive[10]. Twin studies have shown a heritability of 79% for PCOS with a correlation of 0.71 between monozygotic twins and 0.38 between dizygotic twins[11]. The clinical presentation of PCOS varies widely and there is currently no consensus on its diagnostic criteria[12,13]. Studies aimed at determining a genetic model of PCOS have produced different results when varying diagnostic criteria were used. For instance, some studies accepted hirsutism and ovaries with a polycystic appearance as diagnostic criteria (Rotterdam criteria) for the disease; these studies suggested that PCOS may have an autosomal dominant or X-linked simple Mendelian trait. Other studies using oligomenorrhea and hirsutism as the diagnostic criteria (NIH criteria) have reported lower genetic penetration rates[14-17]. On the other hand, some other recent studies have shown that the genetic aspect of insulin resistance is more prominent than that of hyperandrogenism in PCOS patients[18]. In conclusion, there is not yet a clearly established genetic model of PCOS. This is due to the diversity of both the diagnostic criteria and the clinical presentations of the disease, differences in its prevalence among various ethnic populations, and the limitations of some prior studies with respect to the number of subjects and statistical analyses used[10].

While the etiology of PCOS remains unclear, intrinsic abnormalities in the synthesis and secretion of androgens, insulin and gonadotropins provide a plausible basis for the syndrome. Therefore, it has been suggested that specific primary enzyme abnormalities in these steroidogenic pathways may be an important cause of PCOS. Many different genes encoding these enzymes have been studied to determine the etiology of PCOS; these genes have altered expression, suggesting that the genetic abnormalities in PCOS affects signal transduction pathways controlling steroidogenesis, steroid hormone action, gonadotropin action and regulation, insulin action and secretion, energy homeostasis, chronic inflammation, and more (Table 1)[19-27]. These genes may each contribute separately, or they might act collectively. Moreover, different variation in the same gene (allelic heterogeneity) and possible gene-environment interactions may have different effect on gene function. Data suggests that as of yet, there are no gene defects considered to be responsible for the etiology of PCOS; however, several studies have looked at many candidate genes and have suggested that alterations in these genes may contribute to the development of PCOS. Nevertheless, future studies are needed to determine

Table 1 Genetics of polycystic ovary syndrome

The genes associated with PCOS	Genetic mutation (specific enzyme, protein or receptor)
Genes involved in ovarian and adrenal steroidogenesis	CYP11A (P450 cytochrome)
	CYP21 (21-hydroxylase)
	CYP17 (17α-hydroxylase and 17,20-lyase)
	CYP19 (the enzyme complex aromatase: cytochrome P450 aromatase, the NADPH cytochrome P450 reductase 30, and P450 arom)
Genes involved in steroid hormone actions	AR-VNTR polymorphism (the androgen receptors)
	4-kb gene - A pentanucleotide repeat polymorphism (SHBG)
Genes involved in gonadotropin action and regulation	Trp8Arg and Ilg15Thr (the β-subunit of LH)
Genes involved in insulin action and secretion	INS -VNTR (insulin)
	INSR-SNP (insulin receptor)
	Gly972Arg for IRS1, Gly1057Asp for IRS2 (insulin receptor substrates)
	112/121 haplotype of CAPN10 (calpain-10)
	ApaI; rs680-SNP (IGF-1, IGF-2)
Genes involved in energy homeostasis	T45G in exon 2 and G276T in intron 2 (adipocytokines)
Genes involved in chronic inflammation	Mutation 308 A alleles (TNF-α)
	TNFR2, IL-6 signal transducer gp 130, IL-6 receptor genes (type-2 TNF receptor, IL-6)
	Polymorphism 4G/5G (PAI-1)

PCOS: Polycystic ovary syndrome; PAI-1: Plasminogen activator inhibitor-1; IL-6: Interleukin-6; TNF-α: Tumor necrosis factor α; IRS: Insulin receptor substrates; INSR: Insulin receptor.

which genes are the most appropriate PCOS biomarkers. In addition, more recent genetic approaches, namely genome-wide association studies, may begin a new era in PCOS research[28].

EXPERIMENTAL ANIMAL STUDIES

Experimental evidence supports the hypothesis that the phenotypic expression of PCOS is strongly influenced by the intrauterine environment. Initial studies on rats have shown that elevated testosterone (T) levels early in intrauterine life are related to anovulatory sterility and ovarian polycystic changes in offspring[29-31]. Prenatally T-treated monkeys and sheep serve as good models of PCOS because follicular differentiation in these species is similar to that in humans[32]. The results of studies on prenatally androgenized rhesus monkeys are summarized as follows: (1) Following cessation of maternal testosterone treatment in the early period (beginning on days 40-44 of gestation; term 165 ± 10 d), fetuses of these mothers had irregular ovulatory menstrual cycles, ovarian hyperandrogenism, enlarged polyfollicular ovaries and luteinizing hormone (LH) hypersecretion, insulin resistance, diminished insulin secretion, increased incidence of type 2 diabetes, visceral adiposity, and hyperlipidemia. These conditions may be related to an increase in Gonadotropin-releasing hormone (GnRH) secretion, a reduced negative feedback effect of steroids on LH release and/or increased gonadotropin response to GnRH[33,34]. Normally, healthy fetuses undergo a "critical hypothalamic hormonal period" during sexual differentiation. During this period, a sufficient androgenic stimulus in the brain allows for tonic gonadotropin release and contributes to male-type development; on the other hand, an insufficient androgenic stimulation level promotes the development of a female-type synaptology that is characterized by cyclic GnRH release[35]. An increased frequency and amplitude of GnRH release increases LH levels and impairs folliculogenesis, resulting in the anovulatory clinical picture that is characteristic of PCOS. Female offspring of monkeys that were androgenized during fetal life and have high LH levels have hormonal profiles similar to those of the normal male-type hormonal profile; (2) female monkeys similarly exposed to androgen excess during late gestation (100-110 gestation days) also exhibit an adult PCOS-like phenotype, but they do not have obvious abnormalities in LH and insulin secretion or in insulin action[33]; (3) prenatally androgenized monkeys have high blood levels of androstenedione at birth and their androgens of adrenal origin continue to increase for a period of 4-25 mo after birth, suggesting that prenatal androgen exposure may permanently alter adrenal androgen production[36]; (4) fetuses that are androgenized during the prenatal period have an increased number of primary, growing preantral, and small antral follicles and an accelerated proliferation of granulosa cells. In addition, an excess of prenatal androgen increases the mRNA expression of follicle-stimulating hormone receptor, insulin-like growth factor Ⅰ (IGF-Ⅰ) and the IGF-Ⅰ receptor in granulosa cells. These morphological changes are similar to the increased follicular development from the primordial follicle pool that is seen in PCOS patients. Furthermore, prenatally androgenized fetuses have increased 5α-reductase and decreased aromatase activities, which are similar to mechanisms involved in the impaired follicular maturation of PCOS patients[37,38]; (5) prenatally androgenized female monkeys exhibit enhanced insulin secretion in both the fetal and infant zona reticularis. Therefore, an excess of fetal androgen may induce relative insulin hypersecretion in exposed female fetuses and infants, which in turn programs adrenal hyperandrogenism. In addition, the amelioration of impaired insulin action has beneficial

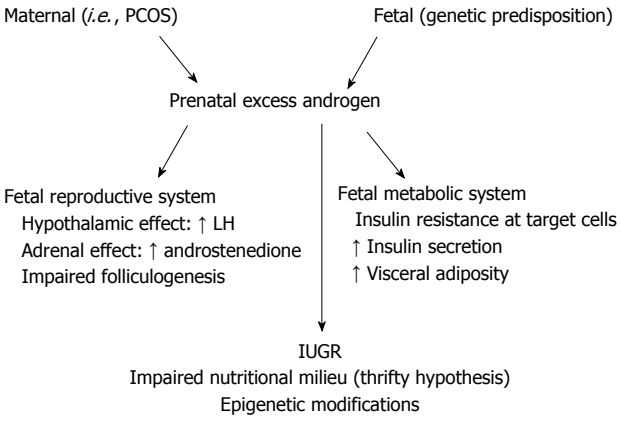

Figure 1 Possible effects of prenatal excess androgen on the fetus. PCOS: Polycystic ovary syndrome; LH: Luteinizing hormone; IUGT: Intrauterine growth restriction.

glucoregulatory effects in both PCOS patients and in prenatally androgenized female monkeys. Treatment with Pioglitazone (a thiazolidinedione-based insulin sensitizer) in prenatally androgenized female monkeys diminishes the aspects of adrenal androgen excess and normalizes menstrual cyclicity[34]; (6) the hypothesis that metabolic disorders are programmed during the fetal stage is supported by the finding that, despite normal T levels after birth, prenatally androgenized male fetuses have insulin resistance and pancreatic beta-cell defects similar to those observed in females[39]; and (7) T excess, when introduced prenatally, decreases birth weight in rodent and sheep offspring. In addition, in humans, impaired placental aromatization is accompanied by diminished uteroplacental perfusion and low infant birth weight[40-44]. It has been suggested that maternal T excess may reduce fetal growth and birth weight *via* impaired placental function (Figure 1).

CLINICAL OBSERVATIONS FOR THE DEVELOPMENTAL ORIGIN OF PCOS

There is some evidence that female fetuses exposed to high androgen levels during the intrauterine period develop the clinical features of PCOS later in life. In humans, it is not possible to perform controlled studies to observe the fetal consequences of maternal androgens; this is left to animal research. However, some observations have been made in humans to support the validity of this hypothesis. Female fetuses having a congenital virilizing tumor or congenital adrenal hyperplasia due to 21-hydroxylase deficiency have been shown to display features of PCOS later in life, even after eliminating the hyperandrogenemia with postnatal therapies[45]. Similarly, it has been reported that female fetuses of women with defects in the p-450 aromatase gene and sex hormone-binding globulin gene, which are rare conditions that cause androgenization, also develop PCOS later in life[46]. Furthermore, it has been shown that exposure to androgen-like chemicals (*e.g.*, Bisphenol A) can lead to PCOS[47,48].

Another study showed that the maternal androgen level is significantly higher in pregnant mothers with PCOS compared to that in healthy pregnant women[49]. While the reason for elevated androgen levels in pregnant women with PCOS has not yet been clarified, it is hypothesized that it may be due to hCG-stimulated androgen production in maternal theca cells or the placenta. Under normal conditions, maternal androgens or fetal adrenal androgens are rapidly converted to estrogens by the activity of the placental enzyme aromatase. However, when the activity of this enzyme is inhibited, the availability of androgens may increase. Insulin has been shown to inhibit aromatase activity in human cytotrophoblasts and can stimulate 3-hydroxysteroid dehydrogenase activity[50]. Therefore, hyperinsulinemia appears to coincide with elevated maternal androgen levels in the development of PCOS in offspring of pregnant women with the same disease. Furthermore, this hypothesis may be supported by the observation that the fetuses of diabetic mothers using insulin have increased levels of macrosomia and fetal pancreatic β-cell hyperplasia, as well as hirsutism, ovarian theca-lutein cysts, ovarian theca cell hyperplasia, and high T and hCG levels in the amniotic fluid[32].

In addition to having increased androgen levels during pregnancy, women with PCOS may also deliver small-for-gestational age newborns at a higher prevalence than do normal control mothers[51]. It is hypothesized that prenatal exposure to androgens in the offspring of women with PCOS may cause the development of the PCOS phenotype later in life, and it may also be the reason for low birth weight during the intrauterine period. With this in mind, recent studies in girls have shown that low birth weight is related to the development of premature pubarche followed by functional hyperandrogenism, insulin resistance with hyperinsulinism, and dyslipidemia during adolescence. It has been suggested that these manifestations may have a common early origin[3,52]. A study in which the authors followed pregnant women during their entire pregnancies reported that pregnant women with PCOS had a progressive increase in both maternal androgens (testosterone and androstenedione) and insulin resistance during their pregnancies, and that these women were exposed to adverse pregnancy-related events significantly more often than those in the control group with a similar body mass index[49]. Fetuses of mothers with PCOS can have developmental delay, which may be related to an elevated T level and insulin resistance. It has been shown that increased insulin resistance during pregnancy is related to adverse pregnancy outcomes including gestational diabetes, preeclampsia, preterm labor, and intrauterine growth restriction (IUGR)[53,54]. This hypothesis is also supported by the fact that male children of mothers with PCOS also have increased prevalence of impaired glucose tolerance, insulin resistance, type-2 diabetes, dyslipidemia and pancreatic beta-cell defects later in life[55].

Another possible mechanism related to the fetal

programming of PCOS involves an impaired intrauterine environment. Independent of elevated androgen levels, intrauterine nutritional insufficiency for any reason may lower insulin secretion and insulin resistance in target tissues as an adaptive mechanism (the thrifty hypothesis). The development of insulin resistance is believed to be directly related to the body "predicting" a life of starvation for the developing fetus. This fetus or infant will have retarded growth and will likely develop PCOS when exposed to nutritional surplus later in life. Epidemiologic studies have demonstrated that babies born with IUGR have an increased prevalence of metabolic syndrome, type-2 diabetes, and hypertension later in life[48]. A recent study showed that urine from neonatal infants with IUGR contained significantly increased levels of metabolic syndrome-associated markers[56]. Although conclusive evidence is lacking, it has been suggested that an impaired intrauterine nutritional environment causes epigenetic changes that trigger metabolic disorders in adult life. The best evidence for this is that there is hypomethylation in the 11p15 imprinting center region that is responsible for the etiology of Silver-Russell syndrome, which is characterized by severe IUGR, lack of catch-up after birth, and specific dysmorphisms[57].

CONCLUSION

In conclusion, PCOS is a highly complex and heterogeneous disorder that is significantly influenced by both genetic and environmental factors. Environmental factors may play a role in the early stages of human development by helping to convert a predisposed genotype to the phenotypic expression of PCOS. In this review, the possible roles of intrauterine environmental factors in PCOS were summarized. Experimental animal studies suggest that maternal hyperandrogenism at a critical stage of fetal development may cause permanent changes in fetal physiology that can trigger PCOS development later in adult life. In humans, it is not possible to perform controlled studies to observe the fetal consequences of maternal androgens; however, some observations have been made in humans to support the validity of this hypothesis. In addition to having increased androgen levels during pregnancy may also deliver small-for-gestational age newborns at a higher prevalence that do normal control mothers. Furthermore, an insufficient intrauterine nutritional environment may also affect PCOS development by affecting cellular metabolism in target tissues or by causing epigenetic alterations to specific genes.

Mechanisms triggering PCOS may be eliminated by making improvements to the maternal hormonal environment and to the intrauterine nutritional environment. Future studies are necessary in order to determine whether insulin-sensitizing treatment of pregnant women with PCOS, or prenatally androgenized animals, will prevent postnatal PCOS in their daughters/female offspring. Results from such studies may help to identify a specific programming mechanism for PCOS.

REFERENCES

1 **Teede H**, Deeks A, Moran L. Polycystic ovary syndrome: a complex condition with psychological, reproductive and metabolic manifestations that impacts on health across the lifespan. *BMC Med* 2010; **8**: 41 [PMID: 20591140 DOI: 10.1186/1741-7015-8-41]

2 **Ehrmann DA**. Polycystic ovary syndrome. *N Engl J Med* 2005; **352**: 1223-1236 [PMID: 15788499 DOI: 10.1056/NEJMra041536]

3 **Ibáñez L**, Potau N, Francois I, de Zegher F. Precocious pubarche, hyperinsulinism, and ovarian hyperandrogenism in girls: relation to reduced fetal growth. *J Clin Endocrinol Metab* 1998; **83**: 3558-3562 [PMID: 9768664 DOI: 10.1210/jcem.83.10.5205]

4 **Sir-Petermann T**, Hitchsfeld C, Maliqueo M, Codner E, Echiburú B, Gazitúa R, Recabarren S, Cassorla F. Birth weight in offspring of mothers with polycystic ovarian syndrome. *Hum Reprod* 2005; **20**: 2122-2126 [PMID: 15802312 DOI: 10.1093/humrep/dei009]

5 **Diamanti-Kandarakis E**, Piperi C, Spina J, Argyrakopoulou G, Papanastasiou L, Bergiele A, Panidis D. Polycystic ovary syndrome: the influence of environmental and genetic factors. *Hormones* (Athens) 2006; **5**: 17-34 [PMID: 16728382 DOI: 10.14310/horm.2002.11165]

6 **Diamanti-Kandarakis E**, Christakou C, Marinakis E. Phenotypes and enviromental factors: their influence in PCOS. *Curr Pharm Des* 2012; **18**: 270-282 [PMID: 22229564 DOI: 10.2174/138161212799040457]

7 **Diamanti-Kandarakis E**, Piperi C, Kalofoutis A, Creatsas G. Increased levels of serum advanced glycation end-products in women with polycystic ovary syndrome. *Clin Endocrinol* (Oxf) 2005; **62**: 37-43 [PMID: 15638868 DOI: 10.1111/j.1365-2265.2004.02170.x]

8 **Xita N**, Tsatsoulis A. Review: fetal programming of polycystic ovary syndrome by androgen excess: evidence from experimental, clinical, and genetic association studies. *J Clin Endocrinol Metab* 2006; **91**: 1660-1666 [PMID: 16522691 DOI: 10.1210/jc.2005-2757]

9 **Dumesic DA**, Schramm RD, Abbott DH. Early origins of polycystic ovary syndrome. *Reprod Fertil Dev* 2005; **17**: 349-360 [PMID: 15745643 DOI: 10.1071/RD04092]

10 **Menke MN**, Strauss JF. Genetic approaches to polycystic ovarian syndrome. *Curr Opin Obstet Gynecol* 2007; **19**: 355-359 [PMID: 17625418 DOI: 10.1097/GCO.0b013e328220e877]

11 **Vink JM**, Sadrzadeh S, Lambalk CB, Boomsma DI. Heritability of polycystic ovary syndrome in a Dutch twin-family study. *J Clin Endocrinol Metab* 2006; **91**: 2100-2104 [PMID: 16219714 DOI: 10.1210/jc.2005-1494]

12 **Urbanek M**, Wu X, Vickery KR, Kao LC, Christenson LK, Schneyer A, Legro RS, Driscoll DA, Strauss JF, Dunaif A, Spielman RS. Allelic variants of the follistatin gene in polycystic ovary syndrome. *J Clin Endocrinol Metab* 2000; **85**: 4455-4461 [PMID: 11134093 DOI: 10.1210/jcem.85.12.7026]

13 **Urbanek M**, Du Y, Silander K, Collins FS, Steppan CM, Strauss JF, Dunaif A, Spielman RS, Legro RS. Variation in resistin gene promoter not associated with polycystic ovary syndrome. *Diabetes* 2003; **52**: 214-217 [PMID: 12502516 DOI: 10.2337/diabetes.52.1.214]

14 **Hague WM**, Adams J, Reeders ST, Peto TE, Jacobs HS. Familial polycystic ovaries: a genetic disease? *Clin Endocrinol* (Oxf) 1988; **29**: 593-605 [PMID: 3076848 DOI: 10.1111/j.1365-2265.1988.tb03707.x]

15 **Givens JR**. Ovarian hyperthecosis. *N Engl J Med* 1971; **285**: 691 [PMID: 5563490 DOI: 10.1056/NEJM197109162851221]

16 **Wilroy RS**, Givens JR, Wiser WL, Coleman SA, Andersen RN, Summitt RL. Hyperthecosis: an inheritable form of polycystic ovarian disease. *Birth Defects Orig Artic Ser* 1975; **11**: 81-85 [PMID: 1156689]

17 **Lunde O**, Magnus P, Sandvik L, Høglo S. Familial clustering in the polycystic ovarian syndrome. *Gynecol Obstet Invest* 1989; **28**: 23-30 [PMID: 2777131]

18 **Diamanti-Kandarakis E**, Alexandraki K, Bergiele A, Kandarakis H, Mastorakos G, Aessopos A. Presence of metabolic risk factors in

non-obese PCOS sisters: evidence of heritability of insulin resistance. *J Endocrinol Invest* 2004; **27**: 931-936 [PMID: 15762040 DOI: 10.1007/BF03347535]

19 **Franks S**, McCarthy MI, Hardy K. Development of polycystic ovary syndrome: involvement of genetic and environmental factors. *Int J Androl* 2006; **29**: 278-285; discussion 286-290 [PMID: 16390494 DOI: 10.1111/j.1365-2605.2005.00623.x]

20 **Shah NA**, Antoine HJ, Pall M, Taylor KD, Azziz R, Goodarzi MO. Association of androgen receptor CAG repeat polymorphism and polycystic ovary syndrome. *J Clin Endocrinol Metab* 2008; **93**: 1939-1945 [PMID: 18303071 DOI: 10.1210/jc.2008-0038]

21 **Guo R**, Zheng Y, Yang J, Zheng N. Association of TNF-alpha, IL-6 and IL-1beta gene polymorphisms with polycystic ovary syndrome: a meta-analysis. *BMC Genet* 2015; **16**: 5 [PMID: 25634659 DOI: 10.1186/s12863-015-0165-4]

22 **Kommoju UJ**, Maruda J, Kadarkarai S, Irgam K, Kotla JP, Velaga L, Mohan Reddy B. No detectable association of IGF2BP2 and SLC30A8 genes with type 2 diabetes in the population of Hyderabad, India. *Meta Gene* 2013; **1**: 15-23 [PMID: 25606370 DOI: 10.1016/j.mgene.2013.09.003]

23 **Zhang TT**, Yuan L, Yang YM, Ren Y. The -675 4G/5G polymorphism in the PAI-1 gene may not contribute to the risk of PCOS. *Eur Rev Med Pharmacol Sci* 2014; **18**: 2326-2331 [PMID: 25219833]

24 **Song do K**, Lee H, Oh JY, Hong YS, Sung YA. FTO Gene Variants Are Associated with PCOS Susceptibility and Hyperandrogenemia in Young Korean Women. *Diabetes Metab J* 2014; **38**: 302-310 [PMID: 25215277 DOI: 10.4093/dmj.2014.38.4.302]

25 **T Mutib M**, B Hamdan F, R Al-Salihi A. INSR gene variation is associated with decreased insulin sensitivity in Iraqi women with PCOs. *Iran J Reprod Med* 2014; **12**: 499-506 [PMID: 25114673]

26 **Ramezani Tehrani F**, Daneshpour M, Hashemi S, Zarkesh M, Azizi F. Relationship between polymorphism of insulin receptor gene, and adiponectin gene with PCOS. *Iran J Reprod Med* 2013; **11**: 185-194 [PMID: 24639745]

27 **Dowling AR**, Nedorezov LB, Qiu X, Marino JS, Hill JW. Genetic factors modulate the impact of pubertal androgen excess on insulin sensitivity and fertility. *PLoS One* 2013; **8**: e79849 [PMID: 24278193 DOI: 10.1371/journal.pone.0079849]

28 **Urbanek M**. The genetics of the polycystic ovary syndrome. *Nat Clin Pract Endocrinol Metab* 2007; **3**: 103-111 [PMID: 17237837 DOI: 10.1038/ncpendmet0400]

29 **Lander ES**, Schork NJ. Genetic dissection of complex traits. *Science* 1994; **265**: 2037-2048 [PMID: 8091226 DOI: 10.1126/science.8091226]

30 **Franks S**, Gharani N, McCarthy M. Candidate genes in polycystic ovary syndrome. *Hum Reprod Update* 2001; **7**: 405-410 [PMID: 11476353 DOI: 10.1093/humupd/7.4.405]

31 **Foecking EM**, Szabo M, Schwartz NB, Levine JE. Neuroendocrine consequences of prenatal androgen exposure in the female rat: absence of luteinizing hormone surges, suppression of progesterone receptor gene expression, and acceleration of the gonadotropin-releasing hormone pulse generator. *Biol Reprod* 2005; **72**: 1475-1483 [PMID: 15744016 DOI: 10.1095/biolreprod.105.039800]

32 **Dumesic DA**, Abbott DH, Padmanabhan V. Polycystic ovary syndrome and its developmental origins. *Rev Endocr Metab Disord* 2007; **8**: 127-141 [PMID: 17659447 DOI: 10.1007/s11154-007-9046-0]

33 **Abbott DH**, Barnett DK, Bruns CM, Dumesic DA. Androgen excess fetal programming of female reproduction: a developmental aetiology for polycystic ovary syndrome? *Hum Reprod Update* 2005; **11**: 357-374 [PMID: 15941725 DOI: 10.1093/humupd/dmi013]

34 **Zhou R**, Bruns CM, Bird IM, Kemnitz JW, Goodfriend TL, Dumesic DA, Abbott DH. Pioglitazone improves insulin action and normalizes menstrual cycles in a majority of prenatally androgenized female rhesus monkeys. *Reprod Toxicol* 2007; **23**: 438-448 [PMID: 17306503 DOI: 10.1016/j.reprotox.2006.12.009]

35 **Matalliotakis I**, Kourtis A, Koukoura O, Panidis D. Polycystic ovary syndrome: etiology and pathogenesis. *Arch Gynecol Obstet* 2006; **274**: 187-197 [PMID: 16685527 DOI: 10.1007/s00404-006-0171-x]

36 **Plant TM**, Zorub DS. A study of the role of the adrenal glands in the initiation of the hiatus in gonadotropin secretion during prepubertal development in the male rhesus monkey (Macaca mulatta). *Endocrinology* 1984; **114**: 560-565 [PMID: 6418533 DOI: 10.1210/endo-114-2-560]

37 **Weil SJ**, Vendola K, Zhou J, Adesanya OO, Wang J, Okafor J, Bondy CA. Androgen receptor gene expression in the primate ovary: cellular localization, regulation, and functional correlations. *J Clin Endocrinol Metab* 1998; **83**: 2479-2485 [PMID: 9661631 DOI: 10.1210/jcem.83.7.4917]

38 **Dumesic DA**, Schramm RD, Bird IM, Peterson E, Paprocki AM, Zhou R, Abbott DH. Reduced intrafollicular androstenedione and estradiol levels in early-treated prenatally androgenized female rhesus monkeys receiving follicle-stimulating hormone therapy for in vitro fertilization. *Biol Reprod* 2003; **69**: 1213-1219 [PMID: 12801996 DOI: 10.1095/biolreprod.102.015164]

39 **Bruns CM**, Baum ST, Colman RJ, Eisner JR, Kemnitz JW, Weindruch R, Abbott DH. Insulin resistance and impaired insulin secretion in prenatally androgenized male rhesus monkeys. *J Clin Endocrinol Metab* 2004; **89**: 6218-6223 [PMID: 15579780 DOI: 10.1210/jc.2004-0918]

40 **Padmanabhan V**, Manikkam M, Recabarren S, Foster D. Prenatal testosterone excess programs reproductive and metabolic dysfunction in the female. *Mol Cell Endocrinol* 2006; **246**: 165-174 [PMID: 16413112 DOI: 10.1016/j.mce.2005.11.016]

41 **McGivern RF**. Low birthweight in rats induced by prenatal exposure to testosterone combined with alcohol, pair-feeding, or stress. *Teratology* 1989; **40**: 335-338 [PMID: 2814895 DOI: 10.1002/tera.1420400405]

42 **Manikkam M**, Crespi EJ, Doop DD, Herkimer C, Lee JS, Yu S, Brown MB, Foster DL, Padmanabhan V. Fetal programming: prenatal testosterone excess leads to fetal growth retardation and postnatal catch-up growth in sheep. *Endocrinology* 2004; **145**: 790-798 [PMID: 14576190 DOI: 10.1210/en.2003-0478]

43 **Tanguy G**, Thoumsin HJ, Zorn JR, Cedard L. DHEA-S-loading test in cases of intrauterine growth retardation: relationship between the pattern of the maternal plasma metabolites and the fetoplacental dysfunction. *Gynecol Obstet Invest* 1981; **12**: 305-316 [PMID: 6457777 DOI: 10.1159/000299660]

44 **Thoumsin HJ**, Alsat E, Cedard L. In vitro aromatization of androgens into estrogens in placental insufficiency. *Gynecol Obstet Invest* 1982; **13**: 37-43 [PMID: 6459977 DOI: 10.1159/000299482]

45 **Hague WM**, Adams J, Rodda C, Brook CG, de Bruyn R, Grant DB, Jacobs HS. The prevalence of polycystic ovaries in patients with congenital adrenal hyperplasia and their close relatives. *Clin Endocrinol* (Oxf) 1990; **33**: 501-510 [PMID: 2225492 DOI: 10.1111/j.1365-2265.1990.tb03887.x]

46 **Morishima A**, Grumbach MM, Simpson ER, Fisher C, Qin K. Aromatase deficiency in male and female siblings caused by a novel mutation and the physiological role of estrogens. *J Clin Endocrinol Metab* 1995; **80**: 3689-3698 [PMID: 8530621 DOI: 10.1210/jcem.80.12.8530621]

47 **Fernández M**, Bourguignon N, Lux-Lantos V, Libertun C. Neonatal exposure to bisphenol a and reproductive and endocrine alterations resembling the polycystic ovarian syndrome in adult rats. *Environ Health Perspect* 2010; **118**: 1217-1222 [PMID: 20413367 DOI: 10.1289/ehp.0901257]

48 **Kandaraki E**, Chatzigeorgiou A, Livadas S, Palioura E, Economou F, Koutsilieris M, Palimeri S, Panidis D, Diamanti-Kandarakis E. Endocrine disruptors and polycystic ovary syndrome (PCOS): elevated serum levels of bisphenol A in women with PCOS. *J Clin Endocrinol Metab* 2011; **96**: E480-E484 [PMID: 21193545 DOI: 10.1210/jc.2010-1658]

49 **Mehrabian F**, Kelishadi R. Comparison of the metabolic parameters and androgen level of umbilical cord blood in newborns of mothers with polycystic ovary syndrome and controls. *J Res Med Sci* 2012; **17**: 207-211 [PMID: 23267369]

50 **Nestler JE**. Modulation of aromatase and P450 cholesterol side-

chain cleavage enzyme activities of human placental cytotrophoblasts by insulin and insulin-like growth factor I. *Endocrinology* 1987; **121**: 1845-1852 [PMID: 3311719 DOI: 10.1210/endo-121-5-1845]

51 **Melo AS**, Vieira CS, Barbieri MA, Rosa-E-Silva AC, Silva AA, Cardoso VC, Reis RM, Ferriani RA, Silva-de-Sá MF, Bettiol H. High prevalence of polycystic ovary syndrome in women born small for gestational age. *Hum Reprod* 2010; **25**: 2124-2131 [PMID: 20573680 DOI: 10.1093/humrep/deq162]

52 **Ibañez L**, Hall JE, Potau N, Carrascosa A, Prat N, Taylor AE. Ovarian 17-hydroxyprogesterone hyperresponsiveness to gonadotropin-releasing hormone (GnRH) agonist challenge in women with polycystic ovary syndrome is not mediated by luteinizing hormone hypersecretion: evidence from GnRH agonist and human chorionic gonadotropin stimulation testing. *J Clin Endocrinol Metab* 1996; **81**: 4103-4107 [PMID: 8923867 DOI: 10.1210/jcem.81.11.8923867]

53 **Hauth JC**, Clifton RG, Roberts JM, Myatt L, Spong CY, Leveno KJ, Varner MW, Wapner RJ, Thorp JM Jr, Mercer BM, Peaceman AM, Ramin SM, Carpenter MW, Samuels P, Sciscione A, Tolosa JE, Saade G, Sorokin Y, Anderson GD; Eunice Kennedy Shriver National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network. Maternal insulin resistance and preeclampsia. *Am J Obstet Gynecol* 2011; **204**: 327.e1-327.e6 [PMID: 21458622 DOI: 10.1016/j.ajog.2011.02.024]

54 **Mastrogiannis DS**, Spiliopoulos M, Mulla W, Homko CJ. Insulin resistance: the possible link between gestational diabetes mellitus and hypertensive disorders of pregnancy. *Curr Diab Rep* 2009; **9**: 296-302 [PMID: 19640343 DOI: 10.1007/s11892-009-0046-1]

55 **Hunter A**, Vimplis S, Sharma A, Eid N, Atiomo W. To determine whether first-degree male relatives of women with polycystic ovary syndrome are at higher risk of developing cardiovascular disease and type II diabetes mellitus. *J Obstet Gynaecol* 2007; **27**: 591-596 [PMID: 17896258 DOI: 10.1080/01443610701497520]

56 **Dessì A**, Atzori L, Noto A, Visser GH, Gazzolo D, Zanardo V, Barberini L, Puddu M, Ottonello G, Atzei A, De Magistris A, Lussu M, Murgia F, Fanos V. Metabolomics in newborns with intrauterine growth retardation (IUGR): urine reveals markers of metabolic syndrome. *J Matern Fetal Neonatal Med* 2011; **24** Suppl 2: 35-39 [PMID: 21767100 DOI: 10.3109/14767058.2011.605868]

57 **Eggermann T**, Gonzalez D, Spengler S, Arslan-Kirchner M, Binder G, Schönherr N. Broad clinical spectrum in Silver-Russell syndrome and consequences for genetic testing in growth retardation. *Pediatrics* 2009; **123**: e929-e931 [PMID: 19364767 DOI: 10.1542/peds.2008-3228]

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REVIEW

Contractile apparatus dysfunction early in the pathophysiology of diabetic cardiomyopathy

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Abstract

Diabetes mellitus significantly increases the risk of cardiovascular disease and heart failure in patients. Independent of hypertension and coronary artery disease, diabetes is associated with a specific cardiomyopathy, known as diabetic cardiomyopathy (DCM). Four decades of research in experimental animal models and advances in clinical imaging techniques suggest that DCM is a progressive disease, beginning early after the onset of type 1 and type 2 diabetes, ahead of left ventricular remodeling and overt diastolic dysfunction. Although the molecular pathogenesis of early DCM still remains largely unclear, activation of protein kinase C appears to be central in driving the oxidative stress dependent and independent pathways in the development of contractile dysfunction. Multiple subcellular alterations to the cardiomyocyte are now being highlighted as critical events in the early changes to the rate of force development, relaxation and stability under pathophysiological stresses. These changes include perturbed calcium handling, suppressed activity of aerobic energy producing enzymes, altered transcriptional and posttranslational modification of membrane and sarcomeric cytoskeletal proteins, reduced actin-myosin cross-bridge cycling and dynamics, and changed myofilament calcium sensitivity. In this review, we will present and discuss novel aspects of the molecular pathogenesis of early DCM, with a special focus on the sarcomeric contractile apparatus.

Key words: Diabetes; Prediabetes; Insulin resistance; Myocardium; Sarcomere; Protein kinase C; Rho kinase

Core tip: Pathological changes in cardiac muscle underlie diabetic cardiomyopathy (DCM) independent of hypertension and atherosclerosis. In advanced diabetes, fibrosis, hypertrophy and apoptosis, reduce myocardial compliance, which leads to increased diastolic filling pressures and overt left ventricular diastolic dysfunction. However, detrimental changes in sarcomeric and other cytoskeletal proteins in the cardiomyocytes of animal models of diabetes precede remodeling of the cardiac extracellular matrix, which has until now been considered the main contributor to diabetic diastolic dysfunction. An important target for preventing early DCM are the protein kinase C/rho-kinase pathways that drive oxidative stress and reduce myosin head cycling and prolong Ca^{2+} transients.

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INTRODUCTION

Diabetes mellitus is a rapidly escalating global epidemic with the International Diabetes Federation predicting that the incidence of diabetes will rise to 552 million people worldwide by 2030[1]. Diabetes is a metabolic disorder characterized by hyperglycemia, insulin deficiency and or resistance. Type 1 diabetes mellitus (T1DM) is triggered by an autoimmune mechanism and accounts for approximately 5%-10% of diabetes cases. Type 2 diabetes mellitus (T2DM) represents 90%-95% of diabetes cases and is initiated by the interaction of genetic, environmental and lifestyle factors[2].

Diabetes is associated with a number of complications such as retinopathy[3], nephropathy[4], peripheral neuropathy[5] and cardiovascular disease, which are similar in their pathophysiological mechanisms in both T1DM and T2DM. Cardiovascular disease is the most common complication of diabetes and a leading cause of morbidity and mortality in patients[6]. Several lines of evidence have established that chronic diabetes is associated with pathological changes to the cardiac muscle[7-9], coronary vasculature[10-12] and cardiac autonomic nerves[13-15], all of which contribute to the increased risk of cardiovascular disease[16,17].

Independent of other cardiovascular complications, a large body of evidence indicates that diabetes is associated with a specific cardiomyopathy known as diabetic cardiomyopathy (DCM)[18-20]. In the long term, people with diabetes more frequently have cardiovascular complications and double the mortality rate[21]. Moreover, we have known for some time from the Framingham Heart study that diabetic men and women are 2 and 5 times more likely to develop heart failure respectively, independent of hypertension and coronary artery disease[22].

Initially, DCM was recognized by impaired myocardial relaxation and left ventricle (LV) stiffening with progressive development of LV interstitial fibrosis and cardiomyocyte hypertrophy[23]. Although the natural history of DCM has never been directly studied, extensive research utilizing experimental animal models of T1DM and T2DM and advances in clinical cardiac imaging over the past four decades support the notion that DCM is a progressive disease, beginning in the early time course of diabetes[24].

Clinical investigations indicate that features of DCM are present early after the onset of T1DM. In T1DM patients, early DCM is identified by subtly reduced peak myocardial systolic velocity and early diastolic velocity preceding overt diastolic dysfunction[25]. Early abnormalities in diastolic function are also apparent in patients with well-controlled T2DM in the absence of LV hypertrophy[26]. Similarly, in one of the most common animal models, the streptozotocin (STZ) induced diabetic rat, depressed rates of pressure development and decay, reduced end systolic developed pressure and prolonged relaxation times are evident as early as 2-3 wk post diabetes induction[27,28], prior to LV remodeling[28].

In the advanced stages of diabetes, a myriad of cellular signaling pathways are chronically activated within the heart, leading to fibrosis, hypertrophy and apoptosis, resulting in inadequate myocardial compliance, increased diastolic filling pressures and the development of overt LV diastolic dysfunction[24]. There is strong evidence from prevention and intervention studies using animal models that specifically targeting the structural manifestations of advanced DCM improves LV function[29-31]. However, in the clinical setting, there is still uncertainty as to whether structural remodeling is a cause or consequence of DCM[32]. Functional abnormalities in isovolumetric contraction and relaxation rates, as well as reduced systolic developed pressure are common to both early and advanced DCM. Moreover, it is not easy to diagnose when structural change has occurred. As these cardiac indices reflect the functional status of mechanisms regulating intracellular events within cardiomyocytes including the initiation and regulation of contraction and relaxation, there is sufficient evidence that demonstrates that subcellular changes to the cardiomyocytes can account for the functional abnormalities observed in early DCM.

In this review, we will focus on and discuss the multiple cellular biochemical derangements, intracellular signaling cascades as well as the structural and functional changes to the cardiomyocyte and sarcomere that establish the beginnings of contractile dysfunction in DCM ahead of myocardial remodeling and overt LV

diastolic dysfunction.

EARLY BIOCHEMICAL DYSFUNCTION AND THE CELLULAR SIGNALING PATHWAYS INVOLVED IN DCM

Diabetes is associated with hyperglycemia, hyperlipidemia, hyperinsulinemia and or insulin resistance. However, unlike T1DM, abnormal glucose homeostasis, hyperinsulinemia and insulin resistance frequently precede development of T2DM[33]. Regardless of the etiology of the disease, diabetes results in multiple cellular metabolic derangements, including increased production and accumulation of advanced glycation end-products in the extracellular space, increased polyol and hexosamine pathway flux and activation of various protein kinase C (PKC) isoforms[34] (Figure 1). In the myocardium, these biochemical derangements drive the generation of oxidative stress, a key contributor to the development of DCM[35] (Figure 1A).

Oxidative stress

The development of oxidative stress has long been recognized as a cardinal molecular event in the initiation and progression of DCM. Importantly, in the long-term, diabetes and insulin resistance induce myocardial oxidative stress from a number of sources leading to enhanced production of reactive oxygen species (ROS) and reactive nitrogen species, whilst also diminishing the heart's antioxidant defense system (reviewed by[36]). The specific mechanisms of how each of these sources generate oxidative stress are beyond the scope of this review and are described in detail by Huynh *et al*[37].

In the setting of chronic experimental diabetes, oxidative stress has been consistently implicated in myocardial remodeling and diastolic dysfunction. Studies conducted by our group and others demonstrate that elevated ROS expression and compromised antioxidant defense are associated with myocardial fibrosis, hypertrophy and diastolic dysfunction in STZ induced T1DM rats[38,39]. Similar findings have been reported in genetic models of advanced T2DM including the db/db mouse[40], the Goto Kakizaki (GK) rat[38] and the Otsuka Long-Evans Tokushima Fatty (OLETF) rat[41].

A role for oxidative stress in the initiation of DCM has also been shown to occur early after the induction of diabetes in animals. One source of myocardial oxidative stress that certainly plays a role in early DCM is nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. Inhibition of NADPH oxidase shortly after the onset of diabetes attenuates subsequent interstitial fibrosis and completely restores systolic and diastolic function in diabetic rats[42]. Three to five weeks post induction of diabetes in rats, there is a significant increase in myocardial oxidative stress (nitrotyrosine and lipid peroxidation), which is associated with contractile dysfunction[43,44]. Antioxidant treatment with N-acetylcystine (NAC) significantly improved the prolonged rates of LV pressure decay, but not the rates of pressure development[43]. While myocardial remodeling was not directly assessed by Cheng *et al*[43], the improvements in LV diastolic indices they observed are most likely caused through actions on cardiomyocyte and sarcomeric function (Figure 1A).

Oxidative stress has been widely reported to induce posttranslational modifications of a large variety of contractile proteins within the sarcomere with redox modifications of such proteins playing a pivotal role in the evolution of heart failure (reviewed extensively by references[45,46]). Therefore, in early DCM, cardiac contractile dysfunction is likely to arise in some part as a consequence of oxidative stress, through posttranslational modifications of contractile proteins as opposed to myocardial structural alterations derived from the increasing burden of chronic oxidative stress associated with advanced DCM. Two distinct cellular signaling cascades that drive generation of oxidative stress through NADPH production are the renin angiotensin aldosterone system (RAAS)[47,48] and the PKCβ_2 pathway[49,50] (Figure 1A). Both have been implicated in the early pathogenesis of DCM.

RAAS

It has been known for many years that the RAAS is a key contributor to the development and progression of renal[51], vascular[52] and cardiac[53] complications in diabetic patients, mainly by its effector molecule, angiotensin Ⅱ (ANGⅡ). Since systemic RAAS activity is unchanged or downregulated in diabetes[54,55], local RAAS in the heart is thought to be responsible for the local production of ANGⅡ. All the components of the classic RAAS have been identified in the heart including renin, angiotensinogen, ANGⅠ, angiotensin converting enzyme (ACE), ANGⅡ and ANGⅡ type 1 receptors (AT_1R)[56]. Traditionally, it is accepted that chronic activation of the RAAS in advanced T1DM and T2DM diabetes increases myocardial levels of ANGⅡ. In addition to its potent vasoconstrictor actions, ANGⅡ promotes myocardial remodeling by driving collagen production, extracellular matrix protein accumulation[57], fibrosis, myocyte hypertrophy, apoptosis, impaired calcium handling, myofibrillar dysfunction and oxidative stress, all of which combine to evoke overt LV diastolic dysfunction[48,58-65] (Figure 1B). Newer evidence suggests that in addition to the activation of classic RAAS, diabetes sequesters the activity of the cardiac ACE2-ANG-(1-7)-Mas receptor axis (Figure 1B), which opposes the pressor, fibrotic and hypertrophic effects of ANGⅡ (reviewed in references[66-68]). Although RAAS research in the context of DCM is in its infancy, the few studies conducted have reinforced the notion that diabetes suppresses cardiac ACE2-ANG-(1-7)-Mas receptor pathway in the hearts of rodents with chronic T1DM[58,69] and T2DM[62], resulting in myocardial remodeling and diastolic dysfunction in the long term (Figure 1B). Therefore, restoring the balance of the pathological and cardioprotective arms of local RAAS in chronic DCM has been proposed as a novel and more effective therapeutic strategy[66,68].

In prediabetic, insulin resistant and early T1DM

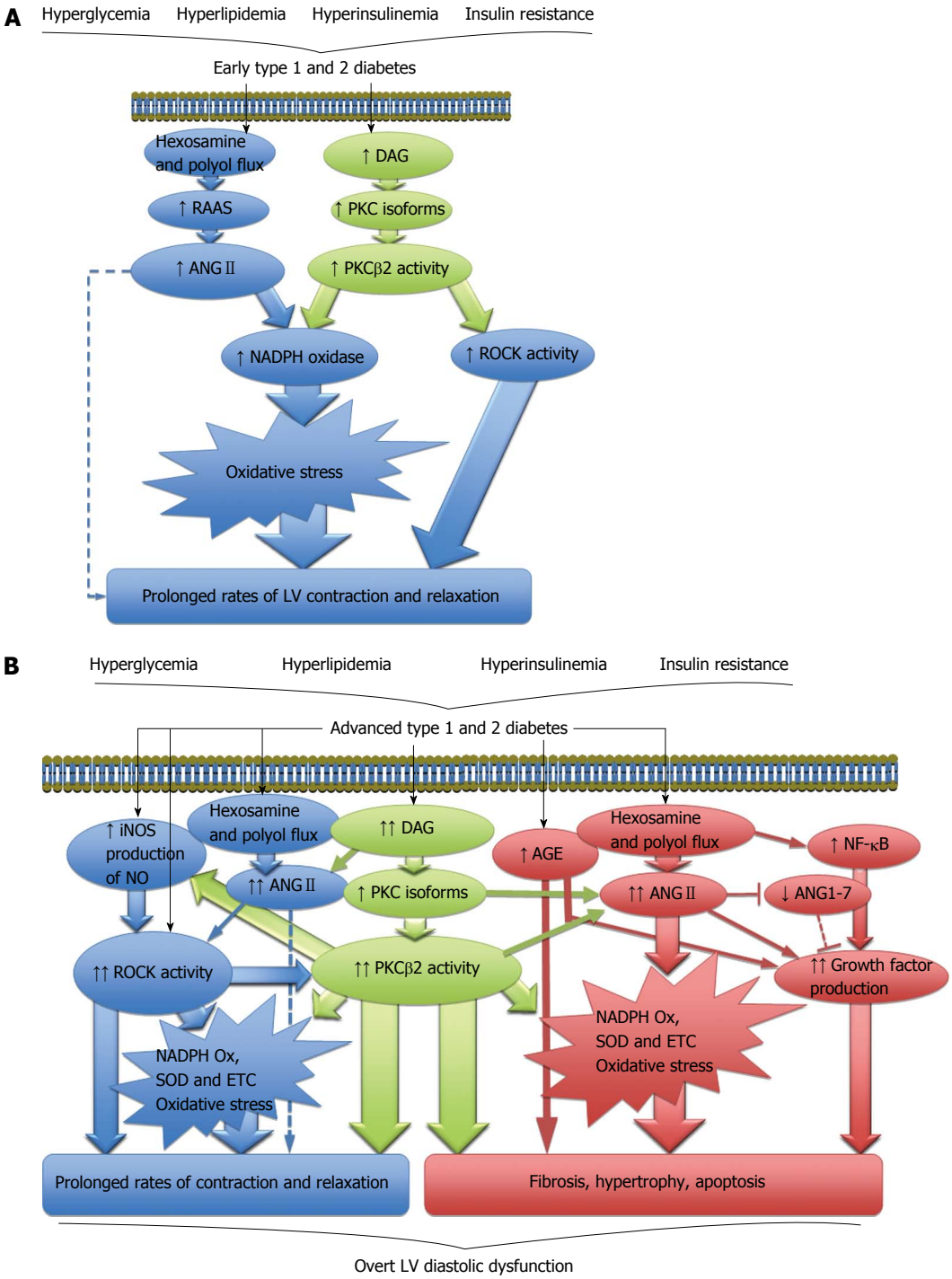

Figure 1 Cellular signaling pathways. A: Cellular signaling pathways driving contractile dysfunction in early diabetic cardiomyopathy; B: Cellular signaling pathways involved in the development of overt LV diastolic dysfunction in diabetes. ANG Ⅱ: Angiotensin Ⅱ; DAG: Diacylglycerol; NADPH: Nicotinamide adenine dinucleotide phosphate; PKC: Protein kinase C; RAAS: Renin angiotensin aldosterone system; ROCK: Rho kinase; AGE: Advanced glycation end products; ANG1-7: Angiotensin 1-7; ETC: Electron transport chain; iNOS: Inducible nitric oxide synthase; NF-κB: Nuclear factor-κB; NO: Nitric oxide; SOD: Superoxide dismutase; LV: Left ventricle.

animal models, there is also a potential role for elevated myocardial ANG Ⅱ and activation of the classic RAAS arm in the initiation of cardiomyopathy. AT_1R-dependent NADPH oxidase-mediated oxidative stress is apparent in the hearts of insulin resistant, prediabetic OLETF rats[70]. Normal myocardial nitric oxide production is restored after AT_1R blockade in a dietary induced rat model of obesity and insulin resistance[71]. Moreover, one week of STZ diabetes in rats significantly increases intracellular ANG Ⅱ content in cardiomyocytes and is associated with myocardial oxidative stress and apoptosis[72]. Unfortunately, as cardiac function was not assessed in these studies, it is not possible to say if the activation of the classic RAAS is responsible for LV contractile dysfunction documented in these early stages of diabetes. However, in support of this possibility, other studies have shown that increased RAAS activity in the hearts of diabetic animals is associated with impaired

calcium handling[60,73] and reduced myofibrillar ATPase activity[64]. Thus, there is a strong possibility for an early involvement of the RAAS in the pathogenesis of DCM as these subcellular alterations are often associated with early LV contractile dysfunction in diabetes (Figure 1A, broken line). Further studies are needed to clarify the involvement of classic RAAS and the ACE2-ANG-(1-7)-Mas receptor axis in contractile dysfunction at this time point, and if other angiotensin peptides[74] and the AT_2R have any role in DCM development.

PKCβ_2 pathway

There are multiple PKC isoforms in existence (α, β_1, β_2, γ, δ, ε, η, θ, ξ, ι/λ), which all regulate a large variety of cellular functions. In diabetes, hyperglycemia causes *de novo* synthesis of diacylglycerols, in turn activating several PKC isoforms in various tissues including the eye, kidney, vasculature and heart (reviewed extensively elsewhere[75,76]) (Figure 1A). Of particular interest is the PKCβ_2 isoform, which is found in the right and left ventricles of the heart[77]. In rodent models of chronic STZ-induced T1DM, increased PKCβ_2 expression and activity results in cardiomyocyte hypertrophy, fibrosis and impaired cardiomyocyte calcium handling capabilities, ultimately prolonging active diastolic relaxation and reducing passive compliance[78-81] (Figure 1B). Elevated myocardial PKCβ_2 expression levels are also reported in a rat model of dietary induced insulin resistance and T2DM[82].

In the context of early DCM, one month after STZ induced diabetes in pigs, both cardiac PKCβ_2 mRNA and protein expression is significantly increased compared to euglycemic controls[83]. Consistent with this finding, two weeks post STZ diabetes induction, PKCβ_2 protein activity is significantly increased in the hearts of rats[84]. Furthermore, selective PKCβ_2 inhibition from shortly after the onset of STZ diabetes prevented cardiomyocyte hypertrophy and NADPH oxidase mediated oxidative stress and thereby restored cardiac function to that of control rats[85]. A significant finding of that study was antioxidant treatment with NAC had comparable effects to PKCβ_2 inhibition in attenuating NADPH induced myocardial oxidative stress and hypertrophy. However, PKCβ_2 inhibition was superior in preventing cardiac dysfunction by completely restoring isovolumetric relaxation times[85]. This suggests that PKCβ_2 also acts through oxidative stress independent pathways in the development of LV contractile dysfunction in early diabetes.

The Rhoa/Rho kinase pathway axis - a possible mechanism for oxidative stress independent contractile dysfunction in early DCM

The Rho Kinases (ROCK), ROCK1 and ROCK2, are Rho-associated kinases activated by the small GTP-binding protein RhoA. ROCK1 is ubiquitously expressed, whereas ROCK2 appears to be brain and cardiac specific[86]. The RhoA/ROCK pathway controls a diverse range of cellular processes in the cardiovascular system (see review[87]), but primary actions of ROCK that are pertinent to this review are the regulation of actin-myosin interactions[88] and maintenance of cytoskeletal structure[89].

A large body of evidence has established that ROCK is a key mediator of diastolic dysfunction in various rodent models of heart disease including hypertensive cardiomyopathy[90-93], pressure-overload hypertrophy[94] and myocardial infarction in mice[95], as well as in patients with chronic heart failure[96]. As a consequence, ROCK has gained attention as a promising therapeutic target for diabetic patients with diastolic dysfunction and preserved ejection fraction[97]. In a small cohort of T2DM patients, daily administration of fasudil, a specific ROCK inhibitor, for two weeks significantly improved isovolumetric relaxation time, deceleration time, E/A wave ratio and E/e' wave ratio in comparison to baseline measures and placebo treated patients[97]. Although not specifically identified in the aforementioned clinical study, elevated ROCK activity leads to diastolic dysfunction in chronic diabetes by inducing fibrosis, hypertrophy and cardiac ultrastructural remodeling[98,99]. However, considering that ROCK is pivotal in regulating contractility at the level of the sarcomere and given the marked improvement in active diastolic indices in patients treated with fasudil, we speculate that elevated ROCK activity primarily drives diastolic dysfunction by modulation of sarcomeric proteins.

Early ROCK activation may also contribute to the initiation of DCM. Acute inhibition of ROCK in diabetic rats rapidly alleviates cardiac contractile dysfunction and increases developed force[100], confirming ROCK has a direct interaction with the cardiomyocyte's contractile apparatus. Work conducted by our group demonstrates depressed actin-myosin dynamics and LV contractile dysfunction[101] is associated with modestly elevated myocardial ROCK1 and ROCK2 expression[102] suggesting that in the early diabetic rat heart, ROCK may drive contractile dysfunction by impairing actin-myosin interaction. Interestingly, we found all of these changes to occur largely independent of oxidative stress[102]. Given the recent studies describing complex interactions between PKCβ_2 and ROCK in chronic experimental DCM[103,104] and the hypothesis that PKCβ_2 is able to modulate cardiac function by oxidative stress independent mechanisms[85], it is conceivable that PKCβ_2 may indeed be acting through ROCK to prolong diastolic relaxation times (Figure 1A). Thus, there is strong evidence that implicates the PKCβ_2/RhoA/ROCK pathway in the pathogenesis and progression of DCM[104]. Future investigations that examine the roles of ROCK at the level of the sarcomere in early diabetes may provide a clearer understanding of the initiating factors involved in DCM.

EARLY STRUCTURAL AND FUNCTIONAL CHANGES TO THE CONTRACTILE APPARATUS IN DCM

It has been widely held for some time that the diastolic

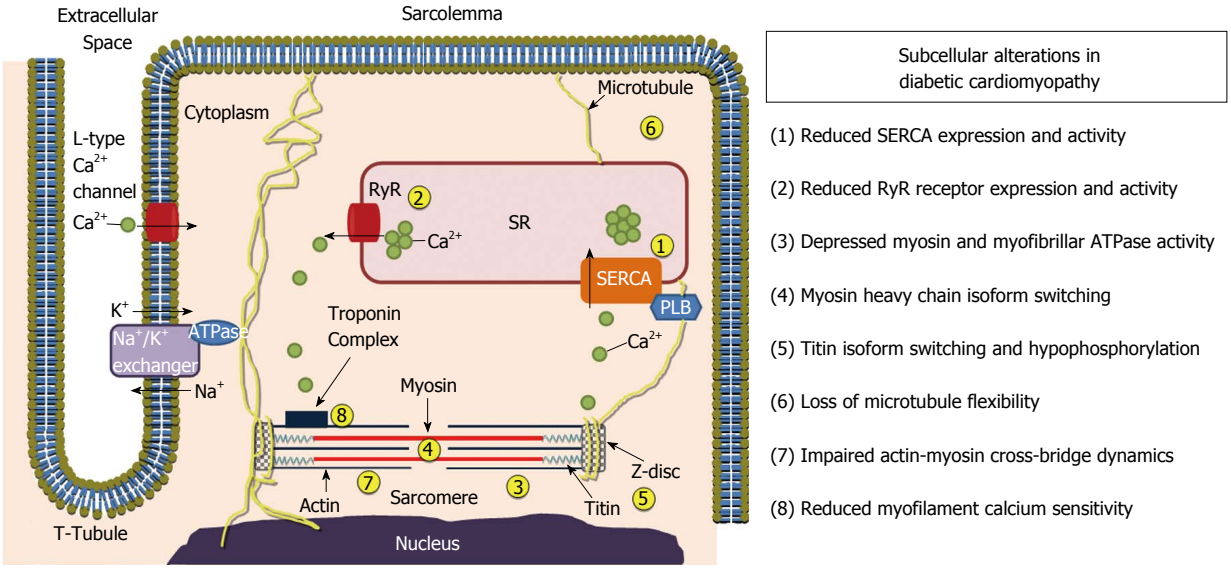

Figure 2 Subcellular alterations in various compartments of the cardiomyocyte in diabetic cardiomyopathy. PLB: Phospholamban; SERCA: Sarcoplasmic reticulum Ca^{2+}-ATPase; SR: Sarcoplasmic reticulum; RyR: Ryanodine receptor.

dysfunction associated with DCM is primarily attributed to increased LV fibrosis and hypertrophy, causing stiffening of the myocardium. However, a growing number of research findings provide evidence that insulin resistance and hyperglycemia also alter cardiomyocyte intracellular processes responsible for initiating and regulating cardiac contraction and force development on a beat-to-beat basis (see reviews[105,106]). These include declines in cardiomyocyte calcium handling abilities, aerobic energy producing enzyme activity, integrity of cytoskeletal structures as well as sarcomeric dysfunction (Figure 2). In the following section we will discuss how all of these subcellular alterations contribute to contractile dysfunction in early DCM in both T1DM and T2DM (Table 1).

Alterations in calcium handling proteins and excitation-contraction coupling

Sufficient and timely transport of calcium ions (Ca^{2+}) in and out of the cardiomyocyte and sarcoplasmic reticulum (SR) is vital for the maintenance of normal cardiac function. During systole, Ca^{2+} enters through sarcolemma L-type Ca^{2+} channels and Ca^{2+} clusters are released spontaneously from the SR Ca^{2+} stores *via* the ryanodine receptor (RyR). The release of Ca^{2+} clusters causes Ca^{2+} sparks and initiates contraction by excitation-contraction coupling. In diastole, Ca^{2+} is pumped back into the SR *via* SR Ca^{2+}-ATPase (SERCA) and out of the cytosolic spaces by the Na^{+}/Ca^{2+} exchanger, driving myocardial relaxation.

Systolic and diastolic Ca^{2+} handling impairment is characteristic of T1DM, even in the early stages. Three weeks post STZ, SR Ca^{2+} reuptake is prolonged and SERCA activity is decreased in comparison to the control group of normoglycemic rats[107]. Slowed rates of contraction and relaxation in diabetic rats were attributed to prolonged Ca^{2+} transients and SR reuptake in isolated papillary muscle preparations[108]. In the whole heart, Takeda *et al*[109] also reported that prolonged myocardial relaxation in early diabetes was associated with depressed sarcolemma Ca^{2+} extrusion and SR Ca^{2+} reuptake, and reduced SERCA activity. More recently, it has been demonstrated that a progressive loss of cardiomyocyte Ca^{2+} handling abilities occurs ahead of LV contractile dysfunction in diabetic rats[110].

Whether impaired Ca^{2+} handling is also an early event in the insulin resistant state and early T2DM is unclear. For example, in sucrose fed rats with insulin resistance[111] and early T2DM[112], impaired ventricular and cardiomyocyte relaxation is associated with decreases in SERCA and RyR expression and prolonged SR Ca^{2+} reuptake. However, in young prediabetic GK rats, appreciable changes in gene expression of Ca^{2+} handling proteins only resulted in moderate prolongation of shortening and a slower decay of Ca^{2+} transients[113]. Similarly, in cardiomyocytes from young Zucker diabetic fatty (ZDF) rats, prolonged relaxation times cannot be fully explained by disruptions in Ca^{2+} handling[114]. In stark contrast, Fredersdorf *et al*[115] have reported increased SERCA expression and Ca^{2+} sequestration in ZDF rats with early DCM, suggesting enhanced Ca^{2+} handling capabilities may be a compensatory mechanism to preserve cardiac function in the presence of hyperglycemia. Thus, subtle or moderately compromised cardiac contractile performance is not always attributable to changes in Ca^{2+} handling, except in early T1DM. Altered Ca^{2+} handling appears to be more important later in the course of diabetes.

Myosin heavy chain, myofibrillar/myosin ATPases and energy depletion

For cardiac contraction to occur, ATP bound to myosin must be hydrolysed to ADP and phosphate. Once the phosphate is released, ADP causes myosin to be strongly

Table 1 Reported subcellular alterations to cardiomyocyte structure and function in early type 1 and type 2 diabetes and gaps in our current knowledge

Cardiomyocyte function change		Type 1 diabetes	Type 2 diabetes
Excitation-contraction coupling, calcium release-reuptake and calcium sensitivity	(-) ↑	↑↑ cTnI phosphorylation[181] (-) cTnI phosphorylation, Ca^{2+} sensitivity[123]	↑ Ca^{2+} transient times[113] ↑ SERCA expression, SR Ca^{2+} reuptake[115]
	↓	↑ PKCβ2 mediated AGE increase, Ca^{2+} release[50] ↓ SERCA activity[107,109,110] ↓ SERCA and RyR expression[110] ↓ Diastolic Ca^{2+} extrusion[109] ↑ SR Ca^{2+} reuptake time[108,109]	↓ SERCA, RyR expression[111,112] ↑ SR Ca^{2+} reuptake time[111,112]
Aerobic energy production	(-)	(-) ATP turnover[1][125]	?
Sarcomere organization		(-) Myofibrillar, sarcomeric order[130]	?
Contractile force development	(-)		↑ Systolic LV pressure, CB formation[139]
	↓	↓ Myosin, myofibrillar ATPase activity[117-120,143] ↓ CB cycling, myosin-actin proximity[101,123,124] ↓ MLC2 phosphorylation, (-) cMyBP-C phosphorylation[123] ↑ ROCK expression, (-) nitrosylation[102]	?
Contractile force transmission, sliding velocity and compliance	↓	↑ V_3 myosin isozyme expression[117,122-124] ↓ Myosin head extension[101] ↓ Relaxation rate[101,123,124] ↓ MLC2 phosphorylation, (-) cMyBP-C phosphorylation[123] ↑ Nitrosylation, lipid peroxidation, prolonged relaxation[43,44]	↑ Diastolic myosin head separation[139] ↑ Ventricular, myocyte relaxation[111,112] ↑ Shortening time[113] ↑ Relaxation time[114]
Cardiomyocyte hypertrophy and apoptosis	↑	↑ ANG Ⅱ mediated oxidative stress[72] ↑ PKCβ2 mediated oxidative stress, apoptosis[50] ↑ PKCβ2 mediated oxidative stress, hypertrophy[85]	↑ ANGII mediated NADPH Ox[70]

References quoted as they appear in text. [1]Study conducted in humans; (-): No change compared to controls; AGE: Advanced glycation endproducts; ANG Ⅱ: Angiotensin Ⅱ; CB: Cross-bridge; cMyBP-C: Cardiac myosin binding protein C; cTnI: Cardiac troponin I; MLC2: Myosin light chain 2; NADPH Ox: Nicotinamide adenine dinucleotide phosphate oxidase; PKC: Protein kinase C; ROCK: Rho kinase; RyR: Ryanodine receptor; SERCA: Sarcoplasmic reticulum Ca^{2+}-ATPase; SR: Sarcoplasmic reticulum; LV: Left ventricle.

bound to actin and induces the force-producing power stroke. Therefore, the rate-limiting step of cardiac contraction is ATP hydrolysis by myofibrillar and myosin ATPase[116]. It has been known for several decades now that reduced myosin and myofibrillar ATPase activity is associated with contractile dysfunction in early diabetes. Three to four weeks post induction of T1DM, myosin and myofibrillar ATPase activities are significantly reduced in diabetic rats compared to normoglycemic, age-matched controls[117,118]. Other studies have demonstrated that decreased myosin and myofibrillar ATPase activity is associated with *in vivo* contractile dysfunction in early diabetes in rats[119] and reduced force development in isolated muscle preparations[120]. Depression of myosin and myofibrillar ATPase activity in the diabetic heart is due to myosin isozyme switching from V_1 to V_3. Myosin isozyme expression is determined by the predominant myosin heavy chain (MHC) dimer[121]. In the adult rodent heart, the V_1 ($MHC_{\alpha\alpha}$ homodimer, α-MHC) is present[105], however isozyme switching to the V_3 ($MHC_{\beta\beta}$ homodimer, β-MHC) is commonly exhibited in diabetic rodents. Such a V_3 isozyme shift has been reported along with depressed myosin ATPase activity in early diabetic rats, with some demonstrating a concomitant prolongation of tension development (without change in net tension development) and contraction times in isolated papillary muscle preparations[117,122]. Notably, it has been shown that marked expression of β-MHC slows actin-myosin kinetics and thereby contributes to contractile dysfunction in early diabetes[123,124].

Despite these convincing studies using animal models, clinical experiments utilizing phosphorus magnetic resonance spectroscopy in T2DM patients with early DCM have found no association between diastolic dysfunction and ATP turnover[125]. Thus, it is unclear if altered energetics of contraction is a cause or consequence of diabetes.

Changes in the structural compliance and order of the cardiomyocyte cytoskeleton network

The cardiomyocyte cytoskeleton is a highly organized and complex subcellular structure. The cytoskeleton can be divided into four main structures, this being the sarcomeric, extra-sarcomeric, membrane-sub-membrane and nuclear cytoskeleton[126]. Although the cytoskeleton is considered a unified structure throughout the cardiomyocyte, there is a clear functional division between the contractile part, the sarcomere, and the non-contractile parts that transmit the developed power and ensures structural integrity of the cell and the functional syncytium[127]. Arguably, the extra-sarcomeric cytoskeleton, comprising of microtubules, actin myofilaments and intermediate desmin filaments are as important for myocyte contraction as the sarcomeres, since these structures transfer the power produced by the sarcomeres through tethering of the sarcomere at the Z-disc to the submembrane skeleton and sarcolemma.

In the diabetic heart, there are inconsistent findings concerning the possible involvement of structural alterations to the sarcomere in the development of contractile dysfunction. One study using electron microscopy reported that hearts with advanced DCM

exhibited disarray of normal sarcomeric order, which was associated with contractile dysfunction[128]. However, others have been unable to corroborate this finding. In contrast, ventricular ultrastructure, and especially the contractile apparatus, remained largely unchanged 8 mo post T1DM induction in rats[129]. Jackson *et al*[130] demonstrated that in the initial stages of DCM sarcomeric and myofibrillar assembly remain unchanged from that of age-matched, normoglycemic controls, despite depressed contractile function. A unique feature of the sarcomeric cytoskeleton is the interaction of a myriad of proteins that provide exceptional stability. One of the major scaffolding proteins within the sarcomere is titin, a giant protein that spans half of the sarcomere from the Z-disc to the M-line, which provides spring-like recoil of the sarcomere to its relaxed length and facilitates power transmission of the sarcomere through the Z-disc. Titin exists in two isoforms; the stiffer N2B and the more elastic N2BA[131]. The expression ratio and phosphorylation state of both isoforms are tightly controlled to regulate myocyte compliance and sarcomeric order[132]. In the adult heart, the N2B isoform is predominantly expressed[133], although a transition toward the N2BA isoform has been observed during the progression to heart failure[134]. Indeed, titin isoform switching from N2B to N2BA has been demonstrated in the hearts of rats with advanced T1DM and diastolic dysfunction[135]. Interestingly, in obese rats with T2DM, titin N2B hypophosphorylation contributes to contractile dysfunction in the absence of a change of isoform composition[136]. Nevertheless, it is plausible that changes in composition and or phosphorylation of sarcomeric cytoskeletal proteins may be more responsible for contractile dysfunction in diabetes as opposed to overt disruption of sarcomeric order and organization.

Diabetes also affects microtubule function in the extra-sarcomeric compartment of the cytoskeleton. Microtubules are hollow protein cylinders created by the polymerization of α and β tubulin heterodimers. Amongst other functions, microtubules regulate the flexibility of the cytoskeleton and thus the contractile capacity[127]. In the hearts of diabetic rats, microtubule flexibility is diminished[137,138]. The lack of microtubule flexibility impedes adequate transfer of the power generated by the sarcomere and thus, contributes to contractile dysfunction[137,138]. Polymerization of cytoskeletal actin filaments is increased in diabetes and essential for activation of the PKCβ2/RhoA/ROCK pathways[104]. The contribution of the extra-sarcomeric cytoskeleton is commonly overlooked in the pathogenesis of DCM and warrants further investigation, not only in areas of mechanical power translation but signal transduction between the sarcolemma, sarcomeres and the nucleus.

Actin-myosin cross-bridge dysregulation

The formation and dissociation of the actin-myosin cross-bridges (CBs) in the cardiac sarcomere is a pivotal determinant of force development and contractility. Various experimental approaches including X-ray diffraction[139], simultaneous recording of heat generation[140] and the measurement of the work of isolated muscle[141] have been used to investigate cardiac performance at the level of the sarcomere and cardiomyocyte. These approaches show that impaired cyclic transfer of myosin heads to actin filaments contributes to contractile dysfunction at the sarcomere level at the onset of diabetes. Moreover, CB dysregulation is also observed in the prediabetic state in the heart of insulin resistant animals.

A significant reduction in CB cycling has been reported in isolated cardiac muscle from early diabetic rats, due to a preferential shift to the less efficient β-MHC isoform[123,124]. Indeed the time required for CB detachment is inversely related to the proportion of α-MHC expression in isolated ferret papillary muscle, attesting to a clear role for β-MHC content in prolonging CB kinetics[142]. Joseph *et al*[143] demonstrated a reduced rate of CB attachment and detachment due to diabetes in *ex vivo* papillary muscle, as well as a significant reduction in the total number of CBs recruited, without a change in force developed per CB. Impaired CB dynamics in this preparation was attributed to a slower myosin ATPase turnover[143]. Accordingly, T1DM is claimed to have no discernible effect on CB mechanical efficiency in isolated trabeculae from rats[144]. Although it is important to recognize that in contrast to Joseph *et al*[143], the estimated number of CBs attached during contraction did not differ between control and diabetic rats in the study of Han *et al*[144]. Recent X-ray diffraction studies conducted by our group affirm these previous findings when CB dynamics are determined across the different layers of the ventricular muscle *in situ*, in the hearts of early diabetic[101] and prediabetic insulin resistant rats[139]. We demonstrated significant myosin head displacement from actin filaments throughout the cardiac cycle, but especially at end diastole, in the rat heart three weeks post STZ induction[101]. Reduction in the proximity of myosin heads to actin filaments at end diastole was directly correlated with a prolonged LV pressure decay rate in diabetic rats. Further, a transmural gradient was found in actin-myosin separation, being most pronounced in the deeper subendocardial layer[101]. However, we also found that the change in CB transfer to actin under β-adrenergic stimulation was comparable in control and diabetic rats suggesting that the impairment of the contractile apparatus precedes that of the β-adrenoceptor signaling[101]. In young prediabetic GK rats, we also found significant myosin head displacement from actin at end diastole in the subendocardial layer of the LV wall[139]. It is still unclear if these altered CB dynamics translate to significant LV dysfunction, but fiber shortening and Ca^{2+} transients are reported to be more prolonged in young GK rats[113].

It is noteworthy that X-ray diffraction recordings from the *in situ* beating heart demonstrate in both STZ induced diabetic rats and young prediabetic GK rats that myosin-myosin separation (interfilament spacing) does not differ from that of controls, and therefore altered myosin head distribution cannot be explained by a

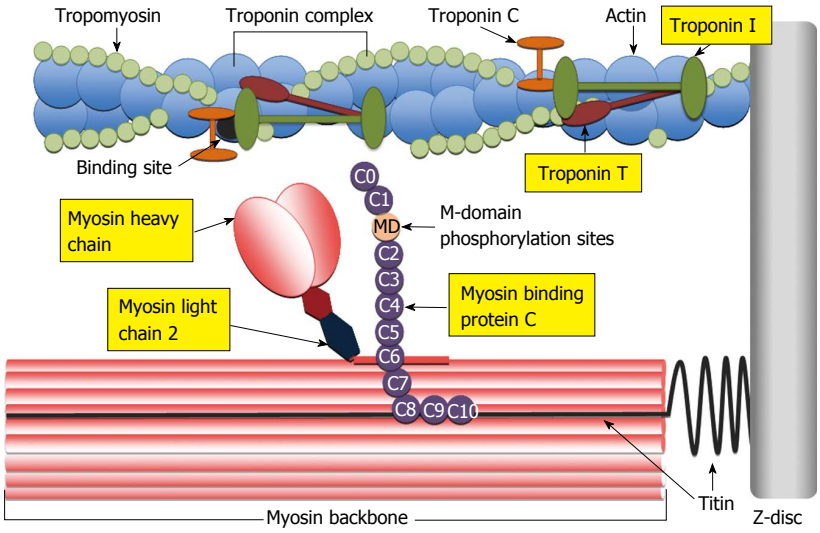

Figure 3 Illustration of the cardiac sarcomere indicating the location of actin thin-filament and myosin thick-filament accessory proteins involved in the regulation of actin-myosin cross-bridge dynamics and kinetics. C0-C10: Immunoglobulin-like and fibronectin-like domains of myosin binding protein C; MD: M-domain of myosin binding protein C containing protein kinase phosphorylation sites.

difference in sarcomere length[101]. Since alterations in thin filament complex cannot explain changes in myosin head extension (despite being a critical determinant of Ca^{2+} sensitivity and CB cycling rates), this implies that modifications to thick filament proteins that regulate myosin head extension are likely to be involved in the impaired CB dynamics and contractile dysfunction in early DCM.

The thick filament accessory proteins - regulators of myosin head extension

Two major proteins reside within the myosin thick filament complex that regulate myosin head extension, namely myosin light chain-2 (MLC2), otherwise known as regulatory light chain and cardiac myosin binding protein-C (cMyBP-C) (Figure 3). Detailed information on the complex molecular structure and broader physiological functions of both these proteins is beyond the scope of this paper but has been reviewed extensively by others (see reviews[145-149]).

It is well accepted that MLC2 and cMyBP-C regulate myosin head extension by means of kinase phosphorylation. In the case of MLC2, phosphorylation is tightly regulated by MLC kinase and Ca^{2+}/calmodulin-dependent protein kinase (CaMK), and is dephosphorylated by MLC phosphatase (see review[150]). X-ray diffraction experiments conducted by Colson *et al*[151] reveal that the negative charge created on the myosin neck when MLC2 is phosphorylated, repels the negative charge of the myosin backbone and thus, subsequently the myosin heads are displaced toward the actin filament.

cMyBP-C is a multifunctional protein within the sarcomere that is primarily involved in the regulation of contractility[152-155] and the organization and structural maintenance of the sarcomere[156-158]. cMyBP-C prevents myosin head projection toward actin by a "tethering" mechanism[159], however, upon phosphorylation, the molecular tether mechanism is released and myosin heads are freed to be displaced towards actin[160,161]. As protein kinase A (PKA) is the primary kinase that phosphorylates cMyBP-C, it is thought that under β-adrenergic stimulation, cMyBP-C works in cooperation with the troponin complex to maintain force development and adequate relaxation and LV filling at higher heart rates[151]. In support of this proposed role, cMyBP-C phosphorylation by PKA evokes radial displacement of myosin heads toward actin in isolated cardiac muscle[162]. To date, seventeen phosphorylation sites have been identified on cMyBP-C[163], however three (Ser273, Ser282, Ser302)[164] have been identified as crucial regulators of contractility, which are all located within the M-domain of cMyBP-C (Figure 3). CaMK is also able to phosphorylate cMyBP-C at these sites in a Ca^{2+} dependent manner and thereby play a potentially important role in regulating contractile function independent of changes in intracellular Ca^{2+} transients[165,166].

Several studies have demonstrated diminished phosphorylation of cMyBP-C in experimental[167,168] and human[163,168,169] non-diabetic cardiac disease. Although there is paucity of information in the literature, some evidence suggests that MLC2 and cMyBP-C phosphorylation are also reduced in DCM. In the hearts of diabetic rats, a significant depression of MLC2 phosphorylation is reported, accompanied by prolonged LV pressure development and decay rates[170]. Diabetic swine also exhibit significantly reduced cMyBP-C phosphorylation[171]. Most recently, in a rat model of early DCM, MLC2 phosphorylation was significantly reduced in comparison to normoglycemic controls while cMyBP-C phosphorylation remained unchanged[123].

It should also be appreciated that other kinases including PKCβ2 and ROCK can directly phosphorylate cMyBP-C, and thus, potentially impact on contractile

function, without appreciably altering pan phosphorylation state. In isolated rat cardiomyocytes, increased PKCβ2 expression did not change pan phosphorylation of cMyBP-C despite prolonging rates of shortening and relaxation, but phosphorylation of Ser302 in particular was significantly increased[172]. Taken together with the finding that phosphorylation Ser302 on cMyBP-C modulates CB kinetics[173], this suggests that the contractile dysfunction observed in the isolated cardiomyocytes may, in part, be a result of altered CB dynamics. Others have also demonstrated active ROCK directly phosphorylates cMyBP-C in cardiomyocytes[174]. Therefore, it is possible that cMyBP-C phosphorylation by PKCβ2 and ROCK may not alter pan phosphorylation, but may indeed impair CB dynamics by displacing myosin heads from the thin filaments and slowing rates of CB attachment and detachment. At the very least, thick filament accessory proteins probably play a role in contractile dysfunction in early DCM, although this requires further investigation.

The actin thin filament troponin complex-could changes in calcium sensitivity contribute to early DCM?

Much of our previous discussion has focused on diabetes impact on the myosin thick filament, however we cannot overlook the importance of the actin thin filament in modulating cardiac contractile properties. The heterotrimeric cardiac troponin (cTn) complex is comprised of cTnC (Ca^{2+} reception site), cTnT and cTnI, the latter two are involved in the transduction of the Ca^{2+}-binding signal (Figure 3). The multiple interactions of cTnI and cTnT with actin and tropomyosin regulate CB kinetics and the number of recruited CBs (reviewed in[175]) (Figure 3).

cTnI is widely recognized to be the principle regulator of CB kinetics and myofilament sensitivity of the thin filament. Under physiological conditions, increased cTnI phosphorylation at Ser23/24 by PKA (which usually occurs under β-adrenergic stimulation) leads to decreased myofilament Ca^{2+} sensitivity and accelerated CB dissociation, and thus hastened relaxation[176]. However, under pathophysiological conditions when expression and activity of PKC isoforms are elevated, PKC rather than PKA predominantly phosphorylates cTnI[177]. Ca^{2+} sensitivity is decreased by PKC phosphorylation at Ser23/24 of cTnI in a manner similar to physiological phosphorylation by PKA, but in contrast, phosphorylation of other sites (Ser44/45 and Thr144) slow CB cycling and sliding velocity and impair relaxation[176]. Thus, PKC-mediated phosphorylation of cTnI may partly explain contractile dysfunction in advanced DCM. Many studies have demonstrated that depressed myofilament Ca^{2+} sensitivity and PKC-induced increases in cTnI phosphorylation are observed in the hearts of rats after eight to twelve weeks of T1DM[178-180].

In early DCM however, there are some conflicting findings regarding the contribution of cTnI. One study found that cellular PKCε translocation paralleled a 5-fold increase in cTnI phosphorylation[181]. Importantly, neither cardiomyocyte function nor myofilament Ca^{2+} sensitivity was examined in this study. Conversely, others reported no changes in cTnI phosphorylation or myofilament calcium sensitivity four weeks post T1DM induction[123]. Therefore, there are some uncertainties as to the role of cTnI changes in early DCM. Given the strong evidence implicating cTnI in chronic diabetes models, this may only be a prominent driver of contractile dysfunction in the advanced stages of DCM.

Interestingly, ROCK is able to phosphorylate multiple sarcomeric proteins. Vahebi *et al*[174] revealed a direct role for ROCK activation in depressing myofilament tension development and ATPase activity *via* phosphorylation of cTnT of the thin filament complex, independent of MLC2 phosphorylation. A possible limitation of these findings is that the authors used constitutively active ROCK2, and whilst many other studies have demonstrated functional changes in diabetes with selective ROCK inhibition, not all have found an increase in ROCK phosphorylation[102,182]. Nonetheless, there is evidence to suggest an increase in ROCK activation in diabetes could contribute to reduced CB cycling through modulation of the thin filament, as well as the thick filament.

OTHER EXACERBATING RISK FACTORS OF DCM

In the clinical setting, DCM is rarely diagnosed independent of any additional risk factors, however, the cumulative effects of multiple risk factors in insulin resistance and diabetes has been rarely considered in preclinical studies. Risk factors that have been shown to exacerbate these disease states include hypertension and obesity, both commonly present with T2DM in patients (reviewed by reference[183]). Hypertension alone is a serious risk factor for the development of diastolic heart failure. Raised systolic blood pressure associated with hypertension evokes an increase in cardiac afterload and accordingly, vascular remodeling and LV hypertrophy ensue as pathophysiological adaptations to maintain cardiac output. Several decades earlier, Factor *et al*[184] showed that diabetes combined with hypertension increased microvessel tortuosity, microaneurysms and focal constrictions more than either disease state alone, which would increase the risk of myocardial ischemia. Activation of the RAAS and sympathetic nervous system (SNS) in the hypertensive state leads to fibrosis and cardiomyocyte hypertrophy, ultimately leading to diastolic dysfunction (see review[185]). In the context of early DCM where LV structural remodeling is normally absent, the additive effect of hypertension may accelerate cardiomyocyte subcellular dysfunction. Jeong *et al*[186] have demonstrated that diastolic dysfunction in deoxycorticosterone acetate treated hypertensive mice is driven by oxidative stress modifications to cMyBP-C, which subsequently suppresses CB kinetics[186]. Further, chronic activation of ROCK plays a central role in most forms of hypertension and diabetic coronary

dysfunction[87,102]. Thus, hypertension may exacerbate the development of cardiac dysfunction in early DCM by redox modifications of various sarcomeric proteins, disturbed CB dynamics and increased oxidative stress.

Obesity (in particular abdominal obesity) is a risk factor for the development cardiac dysfunction and heart failure, independent of diabetes[187]. Several metabolic and neurohormonal mechanisms have been postulated to contribute to obesity-induced cardiomyopathy (see review[188]). Of particular interest, is the interplay of the SNS and the RAAS, which occurs during obesity[189,190]. Classically, obesity leads to activation of the SNS, which in turn rapidly activates the production of renin from the renal juxtaglomerular apparatus as well as angiotensinogen from adipocytes and thus, promotes the excessive production of ANG Ⅱ. Consequently, systemic hypertension ensues (reviewed in[189]). Given our previous discussion on hypertension-induced modulation of sarcomeric proteins leading to diastolic dysfunction[186], it is plausible that obesity may exacerbate DCM in patients *via* the promotion of systemic hypertension. On the other hand, obesity may also exacerbate DCM by directly increasing SNS outflow to the heart. In normotensive obese patients, increased LV mass correlates with elevated cardiac SNS activity[191]. Considering the hypertrophic and fibrotic actions of ANG Ⅱ, elevated local RAAS activity driven by activation of the cardiac SNS could, in part, explain increased LV mass in obese patients. In addition, one study has shown that a high fat diet induced mitochondrial lesions and depressed mitochondrial density in the hearts of GK rats[192]. Therefore, obesity induced activation of the SNS in combination with diabetes may indeed exacerbate DCM.

Lastly, gender must also be considered as an additional risk factor for the development, progression and severity of DCM (reviewed in detail by[193]). Indeed, the Framingham Heart study revealed that diabetic women had twice the frequency of heart failure in comparison to diabetic men[22]. More recent clinical evidence suggests that although non-diabetic women appear to have more pronounced endothelium-mediated dilation (due to higher rates of nitric oxide production), T2DM abrogates these sex differences in vascular function[194]. Female mice also lose this vascular protection with T2DM[182]. Although, similar vascular dysfunction is observed in both sexes, the molecular mechanisms underlying this dysfunction in males appears to be elevated ROCK activity, however this does not appear to be the case in female mice[182]. The impact of sex on myocardial function in diabetes is less clear. Early in the time course of experimental T1DM, it appears that male rats are more vulnerable to cardiac dysfunction. Five to six weeks of STZ induced diabetes in rats caused greater prolongation of the rates of pressure development and decay in diabetic male rats in comparison to their female counterparts[195-197]. However, others have demonstrated that female rats after four weeks from STZ induction exhibit significantly lower MLC2 phosphorylation compared to diabetic males despite equivalent impairment in cardiac muscle tension development in both male and female diabetic rats[123]. Sex differences in the loss of cardiomyocytes may contribute to differential changes in contractile function in diabetes. Females respond to metabolic stresses with greater myocardial glycogen accumulation and elevated glycogen-induced autophagy than males[198,199]. Thus, the underlying mechanisms of sex differences in DCM require a great deal of research to appreciate which of the factors we have identified in this review differ between sexes and how the pathophysiology of diabetes might be compounded by other risk factors in females.

CONCLUSION

In summary, DCM is a progressive disease beginning with contractile dysfunction ahead of LV remodeling. A number of subcellular alterations to the cardiomyocyte contribute to contractile dysfunction in early DCM. While changes in Ca^{2+} handling protein expressions are evident and likely contribute to the prolongation of systole and relaxation, diminished intracellular Ca^{2+} transients appear to be associated only with advanced DCM. Diastolic LV dysfunction has its origins in changes in sarcomeric and other cytoskeletal proteins, both due to transcription and posttranslation changes. Upregulation of PKCβ2 activity is proposed as a central factor in the development of DCM, promoting the activity of both oxidative stress dependent and independent pathways to drive contractile dysfunction in early DCM. Independent of oxidative stress we suggest that hyperglycemia alters modulation of the sarcomeric thick-thin filaments through PKCβ2/ROCK activation. It is not known if any of these pathological changes in sarcomere function are exacerbated by other risk factors such as hypertension and obesity.

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REFERENCES

1 **Whiting DR**, Guariguata L, Weil C, Shaw J. IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. *Diabetes Res Clin Pract* 2011; **94**: 311-321 [PMID: 22079683 DOI: 10.1016/j.diabres.2011.10.029]

2 **Laakso M**, Kuusisto J. Insulin resistance and hyperglycaemia in cardiovascular disease development. *Nat Rev Endocrinol* 2014; **10**: 293-302 [PMID: 24663222 DOI: 10.1038/nrendo.2014.29]

3 **Wilkinson-Berka JL**, Miller AG. Update on the treatment of diabetic retinopathy. *ScientificWorldJournal* 2008; **8**: 98-120 [PMID: 18264628 DOI: 10.1100/tsw.2008.25]

4 **Molitch ME**, DeFronzo RA, Franz MJ, Keane WF, Mogensen CE, Parving HH. Diabetic nephropathy. *Diabetes Care* 2003; **26** Suppl 1: S94-S98 [PMID: 12502629]

5 **Mehra M**, Merchant S, Gupta S, Potluri RC. Diabetic peripheral neuropathy: resource utilization and burden of illness. *J Med Econ* 2014; **17**: 637-645 [PMID: 24888404 DOI: 10.3111/13696998.201

4.928639]
6 **Candido R**, Srivastava P, Cooper ME, Burrell LM. Diabetes mellitus: a cardiovascular disease. *Curr Opin Investig Drugs* 2003; **4**: 1088-1094 [PMID: 14582453]
7 **van Heerebeek L**, Hamdani N, Handoko ML, Falcao-Pires I, Musters RJ, Kupreishvili K, Ijsselmuiden AJ, Schalkwijk CG, Bronzwaer JG, Diamant M, Borbély A, van der Velden J, Stienen GJ, Laarman GJ, Niessen HW, Paulus WJ. Diastolic stiffness of the failing diabetic heart: importance of fibrosis, advanced glycation end products, and myocyte resting tension. *Circulation* 2008; **117**: 43-51 [PMID: 18071071 DOI: 10.1161/CIRCULATIONAHA.107.728550]
8 **Devereux RB**, Roman MJ, Paranicas M, O'Grady MJ, Lee ET, Welty TK, Fabsitz RR, Robbins D, Rhoades ER, Howard BV. Impact of diabetes on cardiac structure and function: the strong heart study. *Circulation* 2000; **101**: 2271-2276 [PMID: 10811594 DOI: 10.1161/01.cir.101.19.2271]
9 **de Simone G**, Mureddu GF, Vaccaro O, Greco R, Sacco M, Rivellese A, Contaldo F, Riccardi G. Cardiac abnormalities in type 1 diabetes. *Ital Heart J* 2000; **1**: 493-499 [PMID: 10933333]
10 **De Vriese AS**, Verbeuren TJ, Van de Voorde J, Lameire NH, Vanhoutte PM. Endothelial dysfunction in diabetes. *Br J Pharmacol* 2000; **130**: 963-974 [PMID: 10882379 DOI: 10.1038/sj.bjp.0703393]
11 **Lumsden NG**, Andrews KL, Bobadilla M, Moore XL, Sampson AK, Shaw JA, Mizrahi J, Kaye DM, Dart AM, Chin-Dusting JP. Endothelial dysfunction in patients with type 2 diabetes post acute coronary syndrome. *Diab Vasc Dis Res* 2013; **10**: 368-374 [PMID: 23673378 DOI: 10.1177/1479164113482593]
12 **Llauradó G**, Ceperuelo-Mallafré V, Vilardell C, Simó R, Albert L, Berlanga E, Vendrell J, González-Clemente JM. Impaired endothelial function is not associated with arterial stiffness in adults with type 1 diabetes. *Diabetes Metab* 2013; **39**: 355-362 [PMID: 23643350 DOI: 10.1016/j.diabet.2013.03.006]
13 **Dimitropoulos G**, Tahrani AA, Stevens MJ. Cardiac autonomic neuropathy in patients with diabetes mellitus. *World J Diabetes* 2014; **5**: 17-39 [PMID: 24567799 DOI: 10.4239/wjd.v5.i1.17]
14 **Rosengård-Bärlund M**, Bernardi L, Fagerudd J, Mäntysaari M, Af Björkesten CG, Lindholm H, Forsblom C, Wadén J, Groop PH. Early autonomic dysfunction in type 1 diabetes: a reversible disorder? *Diabetologia* 2009; **52**: 1164-1172 [PMID: 19340407 DOI: 10.1007/s00125-009-1340-9]
15 **Sacre JW**, Franjic B, Jellis CL, Jenkins C, Coombes JS, Marwick TH. Association of cardiac autonomic neuropathy with subclinical myocardial dysfunction in type 2 diabetes. *JACC Cardiovasc Imaging* 2010; **3**: 1207-1215 [PMID: 21163448 DOI: 10.1016/j.jcmg.2010.09.014]
16 **Kannel WB**, McGee DL. Diabetes and cardiovascular disease. The Framingham study. *JAMA* 1979; **241**: 2035-2038 [PMID: 430798]
17 **Barr EL**, Zimmet PZ, Welborn TA, Jolley D, Magliano DJ, Dunstan DW, Cameron AJ, Dwyer T, Taylor HR, Tonkin AM, Wong TY, McNeil J, Shaw JE. Risk of cardiovascular and all-cause mortality in individuals with diabetes mellitus, impaired fasting glucose, and impaired glucose tolerance: the Australian Diabetes, Obesity, and Lifestyle Study (AusDiab). *Circulation* 2007; **116**: 151-157 [PMID: 17576864 DOI: 10.1161/CIRCULATIONAHA.106.685628]
18 **Battiprolu PK**, Lopez-Crisosto C, Wang ZV, Nemchenko A, Lavandero S, Hill JA. Diabetic cardiomyopathy and metabolic remodeling of the heart. *Life Sci* 2013; **92**: 609-615 [PMID: 23123443 DOI: 10.1016/j.lfs.2012.10.011]
19 **Boudina S**, Abel ED. Diabetic cardiomyopathy revisited. *Circulation* 2007; **115**: 3213-3223 [PMID: 17592090 DOI: 10.1161/circulationaha.106.679597]
20 **Poornima IG**, Parikh P, Shannon RP. Diabetic cardiomyopathy: the search for a unifying hypothesis. *Circ Res* 2006; **98**: 596-605 [PMID: 16543510 DOI: 10.1161/01.RES.0000207406.94146.c2]
21 **Preis SR**, Pencina MJ, Hwang SJ, D'Agostino RB, Savage PJ, Levy D, Fox CS. Trends in cardiovascular disease risk factors in individuals with and without diabetes mellitus in the Framingham Heart Study. *Circulation* 2009; **120**: 212-220 [PMID: 19581493 DOI: 10.1161/circulationaha.108.846519]
22 **Kannel WB**, Hjortland M, Castelli WP. Role of diabetes in congestive heart failure: the Framingham study. *Am J Cardiol* 1974; **34**: 29-34 [PMID: 4835750 DOI: 10.1016/0002-9149(74)90089-7]
23 **Rubler S**, Dlugash J, Yuceoglu YZ, Kumral T, Branwood AW, Grishman A. New type of cardiomyopathy associated with diabetic glomerulosclerosis. *Am J Cardiol* 1972; **30**: 595-602 [PMID: 4263660]
24 **Fang ZY**, Prins JB, Marwick TH. Diabetic cardiomyopathy: evidence, mechanisms, and therapeutic implications. *Endocr Rev* 2004; **25**: 543-567 [PMID: 15294881 DOI: 10.1210/er.2003-0012]
25 **Fang ZY**, Najos-Valencia O, Leano R, Marwick TH. Patients with early diabetic heart disease demonstrate a normal myocardial response to dobutamine. *J Am Coll Cardiol* 2003; **42**: 446-453 [PMID: 12906970 DOI: 10.1016/S0735-1097(03)00654-5]
26 **Diamant M**, Lamb HJ, Groeneveld Y, Endert EL, Smit JW, Bax JJ, Romijn JA, de Roos A, Radder JK. Diastolic dysfunction is associated with altered myocardial metabolism in asymptomatic normotensive patients with well-controlled type 2 diabetes mellitus. *J Am Coll Cardiol* 2003; **42**: 328-335 [PMID: 12875772 DOI: 10.1016/S0735-1097(03)00625-9]
27 **Borges GR**, de Oliveira M, Salgado HC, Fazan R. Myocardial performance in conscious streptozotocin diabetic rats. *Cardiovasc Diabetol* 2006; **5**: 26 [PMID: 17144912 DOI: 10.1186/1475-2840-5-26]
28 **Litwin SE**, Raya TE, Anderson PG, Daugherty S, Goldman S. Abnormal cardiac function in the streptozotocin-diabetic rat. Changes in active and passive properties of the left ventricle. *J Clin Invest* 1990; **86**: 481-488 [PMID: 2200804 DOI: 10.1172/jci114734]
29 **Kelly DJ**, Zhang Y, Connelly K, Cox AJ, Martin J, Krum H, Gilbert RE. Tranilast attenuates diastolic dysfunction and structural injury in experimental diabetic cardiomyopathy. *Am J Physiol Heart Circ Physiol* 2007; **293**: H2860-H2869 [PMID: 17720766 DOI: 10.1152/ajpheart.01167.2006]
30 **Tan SM**, Zhang Y, Wang B, Tan CY, Zammit SC, Williams SJ, Krum H, Kelly DJ. FT23, an orally active antifibrotic compound, attenuates structural and functional abnormalities in an experimental model of diabetic cardiomyopathy. *Clin Exp Pharmacol Physiol* 2012; **39**: 650-656 [PMID: 22612418 DOI: 10.1111/j.1440-1681.2012.05726.x]
31 **Zhang Y**, Edgley AJ, Cox AJ, Powell AK, Wang B, Kompa AR, Stapleton DI, Zammit SC, Williams SJ, Krum H, Gilbert RE, Kelly DJ. FT011, a new anti-fibrotic drug, attenuates fibrosis and chronic heart failure in experimental diabetic cardiomyopathy. *Eur J Heart Fail* 2012; **14**: 549-562 [PMID: 22417655 DOI: 10.1093/eurjhf/hfs011]
32 **Campbell DJ**, Somaratne JB, Jenkins AJ, Prior DL, Yii M, Kenny JF, Newcomb AE, Schalkwijk CG, Black MJ, Kelly DJ. Impact of type 2 diabetes and the metabolic syndrome on myocardial structure and microvasculature of men with coronary artery disease. *Cardiovasc Diabetol* 2011; **10**: 80 [PMID: 21929744 DOI: 10.1186/1475-2840-10-80]
33 **Freeman JS**. A physiologic and pharmacological basis for implementation of incretin hormones in the treatment of type 2 diabetes mellitus. *Mayo Clin Proc* 2010; **85**: S5-S14 [PMID: 21106868 DOI: 10.4065/mcp.2010.0467]
34 **Brownlee M**. Biochemistry and molecular cell biology of diabetic complications. *Nature* 2001; **414**: 813-820 [PMID: 11742414]
35 **Cai L**, Kang YJ. Oxidative stress and diabetic cardiomyopathy: a brief review. *Cardiovasc Toxicol* 2001; **1**: 181-193 [PMID: 12213971]
36 **Varga ZV**, Giricz Z, Liaudet L, Haskó G, Ferdinandy P, Pacher P. Interplay of oxidative, nitrosative/nitrative stress, inflammation, cell death and autophagy in diabetic cardiomyopathy. *Biochim Biophys Acta* 2015; **1852**: 232-242 [PMID: 24997452 DOI: 10.1016/j.bbadis.2014.06.030]
37 **Huynh K**, Bernardo BC, McMullen JR, Ritchie RH. Diabetic cardiomyopathy: mechanisms and new treatment strategies targeting antioxidant signaling pathways. *Pharmacol Ther* 2014; **142**: 375-415 [PMID: 24462787 DOI: 10.1016/j.pharmthera.2014.

01.003]
38 **Connelly KA**, Advani A, Advani SL, Zhang Y, Kim YM, Shen V, Thai K, Kelly DJ, Gilbert RE. Impaired cardiac antioxidant activity in diabetes: human and correlative experimental studies. *Acta Diabetol* 2014; **51**: 771-782 [PMID: 24925443 DOI: 10.1007/s00592-014-0608-9]
39 **Khong FL**, Zhang Y, Edgley AJ, Qi W, Connelly KA, Woodman OL, Krum H, Kelly DJ. 3',4'-Dihydroxyflavonol antioxidant attenuates diastolic dysfunction and cardiac remodeling in streptozotocin-induced diabetic m(Ren2)27 rats. *PLoS One* 2011; **6**: e22777 [PMID: 21829510 DOI: 10.1371/journal.pone.0022777]
40 **Huynh K**, Kiriazis H, Du XJ, Love JE, Jandeleit-Dahm KA, Forbes JM, McMullen JR, Ritchie RH. Coenzyme Q10 attenuates diastolic dysfunction, cardiomyocyte hypertrophy and cardiac fibrosis in the db/db mouse model of type 2 diabetes. *Diabetologia* 2012; **55**: 1544-1553 [PMID: 22374176 DOI: 10.1007/s00125-012-2495-3]
41 **Lee JE**, Yi CO, Jeon BT, Shin HJ, Kim SK, Jung TS, Choi JY, Roh GS. α-Lipoic acid attenuates cardiac fibrosis in Otsuka Long-Evans Tokushima Fatty rats. *Cardiovasc Diabetol* 2012; **11**: 111 [PMID: 22992429 DOI: 10.1186/1475-2840-11-111]
42 **Maalouf RM**, Eid AA, Gorin YC, Block K, Escobar GP, Bailey S, Abboud HE. Nox4-derived reactive oxygen species mediate cardiomyocyte injury in early type 1 diabetes. *Am J Physiol Cell Physiol* 2012; **302**: C597-C604 [PMID: 22031600 DOI: 10.1152/ajpcell.00331.2011]
43 **Cheng X**, Xia Z, Leo JM, Pang CC. The effect of N-acetylcysteine on cardiac contractility to dobutamine in rats with streptozotocin-induced diabetes. *Eur J Pharmacol* 2005; **519**: 118-126 [PMID: 16111676 DOI: 10.1016/j.ejphar.2005.06.037]
44 **Gao X**, Xu Y, Xu B, Liu Y, Cai J, Liu HM, Lei S, Zhong YQ, Irwin MG, Xia Z. Allopurinol attenuates left ventricular dysfunction in rats with early stages of streptozotocin-induced diabetes. *Diabetes Metab Res Rev* 2012; **28**: 409-417 [PMID: 22389139 DOI: 10.1002/dmrr.2295]
45 **Steinberg SF**. Oxidative stress and sarcomeric proteins. *Circ Res* 2013; **112**: 393-405 [PMID: 23329794 DOI: 10.1161/circresaha.111.300496]
46 **Sumandea MP**, Steinberg SF. Redox signaling and cardiac sarcomeres. *J Biol Chem* 2011; **286**: 9921-9927 [PMID: 21257753 DOI: 10.1074/jbc.R110.175489]
47 **Privratsky JR**, Wold LE, Sowers JR, Quinn MT, Ren J. AT1 blockade prevents glucose-induced cardiac dysfunction in ventricular myocytes: role of the AT1 receptor and NADPH oxidase. *Hypertension* 2003; **42**: 206-212 [PMID: 12847113 DOI: 10.1161/01.hyp.0000082814.62655.85]
48 **Maeda Y**, Inoguchi T, Takei R, Hendarto H, Ide M, Inoue T, Kobayashi K, Urata H, Nishiyama A, Takayanagi R. Chymase inhibition prevents myocardial fibrosis through the attenuation of NOX4-associated oxidative stress in diabetic hamsters. *J Diabetes Investig* 2012; **3**: 354-361 [PMID: 24843590 DOI: 10.1111/j.2040-1124.2012.00202.x]
49 **Wang Z**, Zhang Y, Guo J, Jin K, Li J, Guo X, Scott GI, Zheng Q, Ren J. Inhibition of protein kinase C βII isoform rescues glucose toxicity-induced cardiomyocyte contractile dysfunction: role of mitochondria. *Life Sci* 2013; **93**: 116-124 [PMID: 23770211 DOI: 10.1016/j.lfs.2013.06.002]
50 **Zhang L**, Huang D, Shen D, Zhang C, Ma Y, Babcock SA, Chen B, Ren J. Inhibition of protein kinase C βII isoform ameliorates methylglyoxal advanced glycation endproduct-induced cardiomyocyte contractile dysfunction. *Life Sci* 2014; **94**: 83-91 [PMID: 24269213 DOI: 10.1016/j.lfs.2013.11.011]
51 **Kobori H**, Kamiyama M, Harrison-Bernard LM, Navar LG. Cardinal role of the intrarenal renin-angiotensin system in the pathogenesis of diabetic nephropathy. *J Investig Med* 2013; **61**: 256-264 [PMID: 23266706 DOI: 10.231/JIM.0b013e31827c28bb]
52 **Schmieder RE**, Hilgers KF, Schlaich MP, Schmidt BM. Renin-angiotensin system and cardiovascular risk. *Lancet* 2007; **369**: 1208-1219 [PMID: 17416265 DOI: 10.1016/S0140-6736(07)60242-6]
53 **Kumar R**, Yong QC, Thomas CM, Baker KM. Intracardiac intracellular angiotensin system in diabetes. *Am J Physiol Regul Integr Comp Physiol* 2012; **302**: R510-R517 [PMID: 22170614 DOI: 10.1152/ajpregu.00512.2011]
54 **Cassis LA**. Downregulation of the renin-angiotensin system in streptozotocin-diabetic rats. *Am J Physiol* 1992; **262**: E105-E109 [PMID: 1733240]
55 **Giacchetti G**, Sechi LA, Rilli S, Carey RM. The renin-angiotensin-aldosterone system, glucose metabolism and diabetes. *Trends Endocrinol Metab* 2005; **16**: 120-126 [PMID: 15808810 DOI: 10.1016/j.tem.2005.02.003]
56 **Dostal DE**, Baker KM. The cardiac renin-angiotensin system: conceptual, or a regulator of cardiac function? *Circ Res* 1999; **85**: 643-650 [PMID: 10506489 DOI: 10.1161/01.res.85.7.643]
57 **Wong TC**, Piehler KM, Kang IA, Kadakkal A, Kellman P, Schwartzman DS, Mulukutla SR, Simon MA, Shroff SG, Kuller LH, Schelbert EB. Myocardial extracellular volume fraction quantified by cardiovascular magnetic resonance is increased in diabetes and associated with mortality and incident heart failure admission. *Eur Heart J* 2014; **35**: 657-664 [PMID: 23756336 DOI: 10.1093/eurheartj/eht193]
58 **Dong B**, Yu QT, Dai HY, Gao YY, Zhou ZL, Zhang L, Jiang H, Gao F, Li SY, Zhang YH, Bian HJ, Liu CX, Wang N, Xu H, Pan CM, Song HD, Zhang C, Zhang Y. Angiotensin-converting enzyme-2 overexpression improves left ventricular remodeling and function in a rat model of diabetic cardiomyopathy. *J Am Coll Cardiol* 2012; **59**: 739-747 [PMID: 22340266 DOI: 10.1016/j.jacc.2011.09.071]
59 **Kajstura J**, Fiordaliso F, Andreoli AM, Li B, Chimenti S, Medow MS, Limana F, Nadal-Ginard B, Leri A, Anversa P. IGF-1 overexpression inhibits the development of diabetic cardiomyopathy and angiotensin II-mediated oxidative stress. *Diabetes* 2001; **50**: 1414-1424 [PMID: 11375343 DOI: 10.2337/diabetes.50.6.1414]
60 **Liu X**, Suzuki H, Sethi R, Tappia PS, Takeda N, Dhalla NS. Blockade of the renin-angiotensin system attenuates sarcolemma and sarcoplasmic reticulum remodeling in chronic diabetes. *Ann N Y Acad Sci* 2006; **1084**: 141-154 [PMID: 17151298 DOI: 10.1196/annals.1372.003]
61 **Westermann D**, Rutschow S, Jäger S, Linderer A, Anker S, Riad A, Unger T, Schultheiss HP, Pauschinger M, Tschöpe C. Contributions of inflammation and cardiac matrix metalloproteinase activity to cardiac failure in diabetic cardiomyopathy: the role of angiotensin type 1 receptor antagonism. *Diabetes* 2007; **56**: 641-646 [PMID: 17327431 DOI: 10.2337/db06-1163]
62 **Mori J**, Patel VB, Abo Alrob O, Basu R, Altamimi T, Desaulniers J, Wagg CS, Kassiri Z, Lopaschuk GD, Oudit GY. Angiotensin 1-7 ameliorates diabetic cardiomyopathy and diastolic dysfunction in db/db mice by reducing lipotoxicity and inflammation. *Circ Heart Fail* 2014; **7**: 327-339 [PMID: 24389129 DOI: 10.1161/circheartfailure.113.000672]
63 **Guo Z**, Zheng C, Qin Z, Wei P. Effect of telmisartan on the expression of cardiac adiponectin and its receptor 1 in type 2 diabetic rats. *J Pharm Pharmacol* 2011; **63**: 87-94 [PMID: 21155820 DOI: 10.1111/j.2042-7158.2010.01157.x]
64 **Machackova J**, Liu X, Lukas A, Dhalla NS. Renin-angiotensin blockade attenuates cardiac myofibrillar remodelling in chronic diabetes. *Mol Cell Biochem* 2004; **261**: 271-278 [PMID: 15362513]
65 **Connelly KA**, Kelly DJ, Zhang Y, Prior DL, Martin J, Cox AJ, Thai K, Feneley MP, Tsoporis J, White KE, Krum H, Gilbert RE. Functional, structural and molecular aspects of diastolic heart failure in the diabetic (mRen-2)27 rat. *Cardiovasc Res* 2007; **76**: 280-291 [PMID: 17716638 DOI: 10.1016/j.cardiores.2007.06.022]
66 **Keidar S**, Kaplan M, Gamliel-Lazarovich A. ACE2 of the heart: From angiotensin I to angiotensin (1-7). *Cardiovasc Res* 2007; **73**: 463-469 [PMID: 17049503 DOI: 10.1016/j.cardiores.2006.09.006]
67 **Greenberg B**, Cowling RT. An ACE for my sweet heart. *J Am Coll Cardiol* 2012; **59**: 748-750 [PMID: 22340267 DOI: 10.1016/j.jacc.2011.11.021]
68 **Tikellis C**, Bernardi S, Burns WC. Angiotensin-converting enzyme 2 is a key modulator of the renin-angiotensin system in

cardiovascular and renal disease. *Curr Opin Nephrol Hypertens* 2011; **20**: 62-68 [PMID: 21099686 DOI: 10.1097/MNH.0b013e328341164a]

69 **Singh K**, Singh T, Sharma PL. Beneficial effects of angiotensin (1-7) in diabetic rats with cardiomyopathy. *Ther Adv Cardiovasc Dis* 2011; **5**: 159-167 [PMID: 21558085 DOI: 10.1177/1753944711409281]

70 **Vázquez-Medina JP**, Popovich I, Thorwald MA, Viscarra JA, Rodriguez R, Sonanez-Organis JG, Lam L, Peti-Peterdi J, Nakano D, Nishiyama A, Ortiz RM. Angiotensin receptor-mediated oxidative stress is associated with impaired cardiac redox signaling and mitochondrial function in insulin-resistant rats. *Am J Physiol Heart Circ Physiol* 2013; **305**: H599-H607 [PMID: 23771688 DOI: 10.1152/ajpheart.00101.2013]

71 **Huisamen B**, Pêrel SJ, Friedrich SO, Salie R, Strijdom H, Lochner A. ANG II type I receptor antagonism improved nitric oxide production and enhanced eNOS and PKB/Akt expression in hearts from a rat model of insulin resistance. *Mol Cell Biochem* 2011; **349**: 21-31 [PMID: 21153603 DOI: 10.1007/s11010-010-0656-6]

72 **Singh VP**, Le B, Khode R, Baker KM, Kumar R. Intracellular angiotensin II production in diabetic rats is correlated with cardiomyocyte apoptosis, oxidative stress, and cardiac fibrosis. *Diabetes* 2008; **57**: 3297-3306 [PMID: 18829990 DOI: 10.2337/db08-0805]

73 **Yaras N**, Bilginoglu A, Vassort G, Turan B. Restoration of diabetes-induced abnormal local Ca2+ release in cardiomyocytes by angiotensin II receptor blockade. *Am J Physiol Heart Circ Physiol* 2007; **292**: H912-H920 [PMID: 17012347 DOI: 10.1152/ajpheart.00824.2006]

74 **Yugandhar VG**, Clark MA. Angiotensin III: a physiological relevant peptide of the renin angiotensin system. *Peptides* 2013; **46**: 26-32 [PMID: 23692861 DOI: 10.1016/j.peptides.2013.04.014]

75 **Geraldes P**, King GL. Activation of protein kinase C isoforms and its impact on diabetic complications. *Circ Res* 2010; **106**: 1319-1331 [PMID: 20431074 DOI: 10.1161/circresaha.110.217117]

76 **Koya D**, King GL. Protein kinase C activation and the development of diabetic complications. *Diabetes* 1998; **47**: 859-866 [PMID: 9604860 DOI: 10.2337/diabetes.47.6.859]

77 **Shin HG**, Barnett JV, Chang P, Reddy S, Drinkwater DC, Pierson RN, Wiley RG, Murray KT. Molecular heterogeneity of protein kinase C expression in human ventricle. *Cardiovasc Res* 2000; **48**: 285-299 [PMID: 11054475 DOI: 10.1016/s0008-6363(00)00185-1]

78 **Connelly KA**, Kelly DJ, Zhang Y, Prior DL, Advani A, Cox AJ, Thai K, Krum H, Gilbert RE. Inhibition of protein kinase C-beta by ruboxistaurin preserves cardiac function and reduces extracellular matrix production in diabetic cardiomyopathy. *Circ Heart Fail* 2009; **2**: 129-137 [PMID: 19808328 DOI: 10.1161/circheartfailure.108.765750]

79 **Way KJ**, Isshiki K, Suzuma K, Yokota T, Zvagelsky D, Schoen FJ, Sandusky GE, Pechous PA, Vlahos CJ, Wakasaki H, King GL. Expression of connective tissue growth factor is increased in injured myocardium associated with protein kinase C beta2 activation and diabetes. *Diabetes* 2002; **51**: 2709-2718 [PMID: 12196463 DOI: 10.2337/diabetes.51.9.2709]

80 **Xia Z**, Kuo KH, Nagareddy PR, Wang F, Guo Z, Guo T, Jiang J, McNeill JH. N-acetylcysteine attenuates PKCbeta2 overexpression and myocardial hypertrophy in streptozotocin-induced diabetic rats. *Cardiovasc Res* 2007; **73**: 770-782 [PMID: 17250813 DOI: 10.1016/j.cardiores.2006.11.033]

81 **Wang M**, Zhang WB, Zhu JH, Fu GS, Zhou BQ. Breviscapine ameliorates hypertrophy of cardiomyocytes induced by high glucose in diabetic rats via the PKC signaling pathway. *Acta Pharmacol Sin* 2009; **30**: 1081-1091 [PMID: 19597526 DOI: 10.1038/aps.2009.95]

82 **Latha R**, Shanthi P, Sachdanandam P. Kalpaamruthaa modulates oxidative stress in cardiovascular complication associated with type 2 diabetes mellitus through PKC-β/Akt signaling. *Can J Physiol Pharmacol* 2013; **91**: 901-912 [PMID: 24117257 DOI: 10.1139/cjpp-2012-0443]

83 **Guo M**, Wu MH, Korompai F, Yuan SY. Upregulation of PKC genes and isozymes in cardiovascular tissues during early stages of experimental diabetes. *Physiol Genomics* 2003; **12**: 139-146 [PMID: 12441406 DOI: 10.1152/physiolgenomics.00125.2002]

84 **Inoguchi T**, Battan R, Handler E, Sportsman JR, Heath W, King GL. Preferential elevation of protein kinase C isoform beta II and diacylglycerol levels in the aorta and heart of diabetic rats: differential reversibility to glycemic control by islet cell transplantation. *Proc Natl Acad Sci USA* 1992; **89**: 11059-11063 [PMID: 1438315]

85 **Liu Y**, Lei S, Gao X, Mao X, Wang T, Wong GT, Vanhoutte PM, Irwin MG, Xia Z. PKCβ inhibition with ruboxistaurin reduces oxidative stress and attenuates left ventricular hypertrophy and dysfunction in rats with streptozotocin-induced diabetes. *Clin Sci* (Lond) 2012; **122**: 161-173 [PMID: 21892921 DOI: 10.1042/cs20110176]

86 **Shimokawa H**, Rashid M. Development of Rho-kinase inhibitors for cardiovascular medicine. *Trends Pharmacol Sci* 2007; **28**: 296-302 [PMID: 17482681 DOI: 10.1016/j.tips.2007.04.006]

87 **Noma K**, Oyama N, Liao JK. Physiological role of ROCKs in the cardiovascular system. *Am J Physiol Cell Physiol* 2006; **290**: C661-C668 [PMID: 16469861 DOI: 10.1152/ajpcell.00459.2005]

88 **Kureishi Y**, Kobayashi S, Amano M, Kimura K, Kanaide H, Nakano T, Kaibuchi K, Ito M. Rho-associated kinase directly induces smooth muscle contraction through myosin light chain phosphorylation. *J Biol Chem* 1997; **272**: 12257-12260 [PMID: 9139666]

89 **Pulinilkunnil T**, An D, Ghosh S, Qi D, Kewalramani G, Yuen G, Virk N, Abrahani A, Rodrigues B. Lysophosphatidic acid-mediated augmentation of cardiomyocyte lipoprotein lipase involves actin cytoskeleton reorganization. *Am J Physiol Heart Circ Physiol* 2005; **288**: H2802-H2810 [PMID: 15681706 DOI: 10.1152/ajpheart.01162.2004]

90 **Akahori H**, Tsujino T, Naito Y, Matsumoto M, Sasaki N, Iwasaku T, Eguchi A, Sawada H, Hirotani S, Masuyama T. Atorvastatin ameliorates cardiac fibrosis and improves left ventricular diastolic function in hypertensive diastolic heart failure model rats. *J Hypertens* 2014; **32**: 1534-1541; discussion 1541 [PMID: 24759122 DOI: 10.1097/hjh.0000000000000184]

91 **Fukui S**, Fukumoto Y, Suzuki J, Saji K, Nawata J, Tawara S, Shinozaki T, Kagaya Y, Shimokawa H. Long-term inhibition of Rho-kinase ameliorates diastolic heart failure in hypertensive rats. *J Cardiovasc Pharmacol* 2008; **51**: 317-326 [PMID: 18356698 DOI: 10.1097/FJC.0b013e31816533b7]

92 **Kobayashi N**, Horinaka S, Mita S, Nakano S, Honda T, Yoshida K, Kobayashi T, Matsuoka H. Critical role of Rho-kinase pathway for cardiac performance and remodeling in failing rat hearts. *Cardiovasc Res* 2002; **55**: 757-767 [PMID: 12176125 DOI: 10.1016/s0008-6363(02)00457-1]

93 **Satoh S**, Ueda Y, Koyanagi M, Kadokami T, Sugano M, Yoshikawa Y, Makino N. Chronic inhibition of Rho kinase blunts the process of left ventricular hypertrophy leading to cardiac contractile dysfunction in hypertension-induced heart failure. *J Mol Cell Cardiol* 2003; **35**: 59-70 [PMID: 12623300]

94 **Phrommintikul A**, Tran L, Kompa A, Wang B, Adrahtas A, Cantwell D, Kelly DJ, Krum H. Effects of a Rho kinase inhibitor on pressure overload induced cardiac hypertrophy and associated diastolic dysfunction. *Am J Physiol Heart Circ Physiol* 2008; **294**: H1804-H1814 [PMID: 18245565 DOI: 10.1152/ajpheart.01078.2007]

95 **Hattori T**, Shimokawa H, Higashi M, Hiroki J, Mukai Y, Tsutsui H, Kaibuchi K, Takeshita A. Long-term inhibition of Rho-kinase suppresses left ventricular remodeling after myocardial infarction in mice. *Circulation* 2004; **109**: 2234-2239 [PMID: 15096457 DOI: 10.1161/01.cir.0000127939.16111.58]

96 **Do e Z**, Fukumoto Y, Sugimura K, Miura Y, Tatebe S, Yamamoto S, Aoki T, Nochioka K, Nergui S, Yaoita N, Satoh K, Kondo M, Nakano M, Wakayama Y, Fukuda K, Nihei T, Kikuchi Y, Takahashi J, Shimokawa H. Rho-kinase activation in patients with heart failure. *Circ J* 2013; **77**: 2542-2550 [PMID: 23883874 DOI: 10.1253/circj.CJ-13-0397]

97 **Guo R**, Su Y, Yan J, Sun H, Wu J, Liu W, Xu Y. Fasudil improves short-term echocardiographic parameters of diastolic function in patients with type 2 diabetes with preserved left ventricular ejection fraction: a pilot study. *Heart Vessels* 2015; **30**: 89-97 [PMID: 24390764 DOI: 10.1007/s00380-013-0458-3]

98 **Guan SJ**, Ma ZH, Wu YL, Zhang JP, Liang F, Weiss JW, Guo QY, Wang JY, Ji ES, Chu L. Long-term administration of fasudil improves cardiomyopathy in streptozotocin-induced diabetic rats. *Food Chem Toxicol* 2012; **50**: 1874-1882 [PMID: 22429817 DOI: 10.1016/j.fct.2012.03.006]

99 **Zhou H**, Li YJ, Wang M, Zhang LH, Guo BY, Zhao ZS, Meng FL, Deng YG, Wang RY. Involvement of RhoA/ROCK in myocardial fibrosis in a rat model of type 2 diabetes. *Acta Pharmacol Sin* 2011; **32**: 999-1008 [PMID: 21743486 DOI: 10.1038/aps.2011.54]

100 **Lin G**, Craig GP, Zhang L, Yuen VG, Allard M, McNeill JH, MacLeod KM. Acute inhibition of Rho-kinase improves cardiac contractile function in streptozotocin-diabetic rats. *Cardiovasc Res* 2007; **75**: 51-58 [PMID: 17428455 DOI: 10.1016/j.cardiores.2007.03.009]

101 **Jenkins MJ**, Pearson JT, Schwenke DO, Edgley AJ, Sonobe T, Fujii Y, Ishibashi-Ueda H, Kelly DJ, Yagi N, Shirai M. Myosin heads are displaced from actin filaments in the in situ beating rat heart in early diabetes. *Biophys J* 2013; **104**: 1065-1072 [PMID: 23473489 DOI: 10.1016/j.bpj.2013.01.037]

102 **Pearson JT**, Jenkins MJ, Edgley AJ, Sonobe T, Joshi M, Waddingham MT, Fujii Y, Schwenke DO, Tsuchimochi H, Yoshimoto M, Umetani K, Kelly DJ, Shirai M. Acute Rho-kinase inhibition improves coronary dysfunction in vivo, in the early diabetic microcirculation. *Cardiovasc Diabetol* 2013; **12**: 111 [PMID: 24059472 DOI: 10.1186/1475-2840-12-111]

103 **Lin G**, Brownsey RW, Macleod KM. Complex regulation of PKCβ2 and PDK-1/AKT by ROCK2 in diabetic heart. *PLoS One* 2014; **9**: e86520 [PMID: 24466133 DOI: 10.1371/journal.pone.0086520]

104 **Soliman H**, Gador A, Lu YH, Lin G, Bankar G, MacLeod KM. Diabetes-induced increased oxidative stress in cardiomyocytes is sustained by a positive feedback loop involving Rho kinase and PKCβ2. *Am J Physiol Heart Circ Physiol* 2012; **303**: H989-H1000 [PMID: 22865386 DOI: 10.1152/ajpheart.00416.2012]

105 **Dhalla NS**, Liu X, Panagia V, Takeda N. Subcellular remodeling and heart dysfunction in chronic diabetes. *Cardiovasc Res* 1998; **40**: 239-247 [PMID: 9893715 DOI: 10.1016/s0008-6363(98)00186-2]

106 **Dhalla NS**, Takeda N, Rodriguez-Leyva D, Elimban V. Mechanisms of subcellular remodeling in heart failure due to diabetes. *Heart Fail Rev* 2014; **19**: 87-99 [PMID: 23436108 DOI: 10.1007/s10741-013-9385-8]

107 **Zarain-Herzberg A**, Yano K, Elimban V, Dhalla NS. Cardiac sarcoplasmic reticulum Ca(2+)-ATPase expression in streptozotocin-induced diabetic rat heart. *Biochem Biophys Res Commun* 1994; **203**: 113-120 [PMID: 8074644 DOI: 10.1006/bbrc.1994.2156]

108 **Ishikawa T**, Kajiwara H, Kurihara S. Alterations in contractile properties and Ca2+ handling in streptozotocin-induced diabetic rat myocardium. *Am J Physiol* 1999; **277**: H2185-H2194 [PMID: 10600836]

109 **Takeda N**, Dixon IM, Hata T, Elimban V, Shah KR, Dhalla NS. Sequence of alterations in subcellular organelles during the development of heart dysfunction in diabetes. *Diabetes Res Clin Pract* 1996; **30** Suppl: 113-122 [PMID: 8964185 DOI: 10.1016/S0168-8227(96)80047-7]

110 **Zhao SM**, Wang YL, Guo CY, Chen JL, Wu YQ. Progressive decay of Ca2+ homeostasis in the development of diabetic cardiomyopathy. *Cardiovasc Diabetol* 2014; **13**: 75 [PMID: 24712865 DOI: 10.1186/1475-2840-13-75]

111 **Wold LE**, Dutta K, Mason MM, Ren J, Cala SE, Schwanke ML, Davidoff AJ. Impaired SERCA function contributes to cardiomyocyte dysfunction in insulin resistant rats. *J Mol Cell Cardiol* 2005; **39**: 297-307 [PMID: 15878173 DOI: 10.1016/j.yjmcc.2005.03.014]

112 **Bai SZ**, Sun J, Wu H, Zhang N, Li HX, Li GW, Li HZ, He W, Zhang WH, Zhao YJ, Wang LN, Tian Y, Yang BF, Yang GD, Wu LY, Wang R, Xu CQ. Decrease in calcium-sensing receptor in the progress of diabetic cardiomyopathy. *Diabetes Res Clin Pract* 2012; **95**: 378-385 [PMID: 22137362 DOI: 10.1016/j.diabres.2011.11.007]

113 **Salem KA**, Adrian TE, Qureshi MA, Parekh K, Oz M, Howarth FC. Shortening and intracellular Ca2+ in ventricular myocytes and expression of genes encoding cardiac muscle proteins in early onset type 2 diabetic Goto-Kakizaki rats. *Exp Physiol* 2012; **97**: 1281-1291 [PMID: 22581745 DOI: 10.1113/expphysiol.2012.066639]

114 **Howarth FC**, Qureshi MA, Hassan Z, Al Kury LT, Isaev D, Parekh K, Yammahi SR, Oz M, Adrian TE, Adeghate E. Changing pattern of gene expression is associated with ventricular myocyte dysfunction and altered mechanisms of Ca2+ signalling in young type 2 Zucker diabetic fatty rat heart. *Exp Physiol* 2011; **96**: 325-337 [PMID: 21216827 DOI: 10.1113/expphysiol.2010.055574]

115 **Fredersdorf S**, Thumann C, Zimmermann WH, Vetter R, Graf T, Luchner A, Riegger GA, Schunkert H, Eschenhagen T, Weil J. Increased myocardial SERCA expression in early type 2 diabetes mellitus is insulin dependent: In vivo and in vitro data. *Cardiovasc Diabetol* 2012; **11**: 57 [PMID: 22621761 DOI: 10.1186/1475-2840-11-57]

116 **Meijs MF**, Asselbergs FW, Doevendans PA. Omecamtiv mecarbil: a promising new drug in systolic heart failure. *Eur J Heart Fail* 2012; **14**: 232-233 [PMID: 22210833 DOI: 10.1093/eurjhf/hfr178]

117 **Malhotra A**, Penpargkul S, Fein FS, Sonnenblick EH, Scheuer J. The effect of streptozotocin-induced diabetes in rats on cardiac contractile proteins. *Circ Res* 1981; **49**: 1243-1250 [PMID: 6458419 DOI: 10.1161/01.res.49.6.1243]

118 **Pierce GN**, Dhalla NS. Mechanisms of the defect in cardiac myofibrillar function during diabetes. *Am J Physiol* 1985; **248**: E170-E175 [PMID: 3155918]

119 **Garber DW**, Neely JR. Decreased myocardial function and myosin ATPase in hearts from diabetic rats. *Am J Physiol* 1983; **244**: H586-H591 [PMID: 6220613]

120 **Moreira CM**, Meira EF, Vestena L, Stefanon I, Vassallo DV, Padilha AS. Tension cost correlates with mechanical and biochemical parameters in different myocardial contractility conditions. *Clinics* (Sao Paulo) 2012; **67**: 489-496 [PMID: 22666794]

121 **Dillmann WH**. Hormonal influences on cardiac myosin ATPase activity and myosin isoenzyme distribution. *Mol Cell Endocrinol* 1984; **34**: 169-181 [PMID: 6232163 DOI: 10.1016/0303-7207(84)90173-4]

122 **Takeda N**, Nakamura I, Hatanaka T, Ohkubo T, Nagano M. Myocardial mechanical and myosin isoenzyme alterations in streptozotocin-diabetic rats. *Jpn Heart J* 1988; **29**: 455-463 [PMID: 2846906]

123 **Chung CS**, Mitov MI, Callahan LA, Campbell KS. Increased myocardial short-range forces in a rodent model of diabetes reflect elevated content of β myosin heavy chain. *Arch Biochem Biophys* 2014; **552-553**: 92-99 [PMID: 24012810 DOI: 10.1016/j.abb.2013.08.013]

124 **Rundell VL**, Geenen DL, Buttrick PM, de Tombe PP. Depressed cardiac tension cost in experimental diabetes is due to altered myosin heavy chain isoform expression. *Am J Physiol Heart Circ Physiol* 2004; **287**: H408-H413 [PMID: 15001437 DOI: 10.1152/ajpheart.00049.2004]

125 **Rijzewijk LJ**, van der Meer RW, Lamb HJ, de Jong HW, Lubberink M, Romijn JA, Bax JJ, de Roos A, Twisk JW, Heine RJ, Lammertsma AA, Smit JW, Diamant M. Altered myocardial substrate metabolism and decreased diastolic function in nonischemic human diabetic cardiomyopathy: studies with cardiac positron emission tomography and magnetic resonance imaging. *J Am Coll Cardiol* 2009; **54**: 1524-1532 [PMID: 19815124 DOI: 10.1016/j.jacc.2009.04.074]

126 **Kostin S**, Scholz D, Shimada T, Maeno Y, Mollnau H, Hein S, Schaper J. The internal and external protein scaffold of the T-tubular system in cardiomyocytes. *Cell Tissue Res* 1998; **294**: 449-460 [PMID: 9799462 DOI: 10.1007/s004410051196]

127 **Sarantitis I**, Papanastasopoulos P, Manousi M, Baikoussis NG, Apostolakis E. The cytoskeleton of the cardiac muscle cell. *Hellenic J Cardiol* 2012; **53**: 367-379 [PMID: 22995608]

128 **Shimabukuro M**, Higa S, Shinzato T, Nagamine F, Komiya I, Takasu N. Cardioprotective effects of troglitazone in streptozotocin-induced diabetic rats. *Metabolism* 1996; **45**: 1168-1173 [PMID: 8781306 DOI: 10.1016/S0026-0495(96)90018-9]

129 **Howarth FC**, Qureshi MA, Lawrence P, Adeghate E. Chronic effects of streptozotocin-induced diabetes on the ultrastructure of rat ventricular and papillary muscle. *Acta Diabetol* 2000; **37**: 119-124 [PMID: 11277311]

130 **Jackson CV**, McGrath GM, Tahiliani AG, Vadlamudi RV, McNeill JH. A functional and ultrastructural analysis of experimental diabetic rat myocardium. Manifestation of a cardiomyopathy. *Diabetes* 1985; **34**: 876-883 [PMID: 3896897 DOI: 10.2337/diab.34.9.876]

131 **Hoshijima M**. Mechanical stress-strain sensors embedded in cardiac cytoskeleton: Z disk, titin, and associated structures. *Am J Physiol Heart Circ Physiol* 2006; **290**: H1313-H1325 [PMID: 16537787 DOI: 10.1152/ajpheart.00816.2005]

132 **Borbély A**, van Heerebeek L, Paulus WJ. Transcriptional and posttranslational modifications of titin: implications for diastole. *Circ Res* 2009; **104**: 12-14 [PMID: 19118283 DOI: 10.1161/circresaha.108.191130]

133 **Opitz CA**, Leake MC, Makarenko I, Benes V, Linke WA. Developmentally regulated switching of titin size alters myofibrillar stiffness in the perinatal heart. *Circ Res* 2004; **94**: 967-975 [PMID: 14988228 DOI: 10.1161/01.res.0000124301.48193.e1]

134 **Borbély A**, Falcao-Pires I, van Heerebeek L, Hamdani N, Edes I, Gavina C, Leite-Moreira AF, Bronzwaer JG, Papp Z, van der Velden J, Stienen GJ, Paulus WJ. Hypophosphorylation of the Stiff N2B titin isoform raises cardiomyocyte resting tension in failing human myocardium. *Circ Res* 2009; **104**: 780-786 [PMID: 19179657 DOI: 10.1161/circresaha.108.193326]

135 **Krüger M**, Babicz K, von Frieling-Salewsky M, Linke WA. Insulin signaling regulates cardiac titin properties in heart development and diabetic cardiomyopathy. *J Mol Cell Cardiol* 2010; **48**: 910-916 [PMID: 20184888 DOI: 10.1016/j.yjmcc.2010.02.012]

136 **Hamdani N**, Franssen C, Lourenço A, Falcão-Pires I, Fontoura D, Leite S, Plettig L, López B, Ottenheijm CA, Becher PM, González A, Tschöpe C, Díez J, Linke WA, Leite-Moreira AF, Paulus WJ. Myocardial titin hypophosphorylation importantly contributes to heart failure with preserved ejection fraction in a rat metabolic risk model. *Circ Heart Fail* 2013; **6**: 1239-1249 [PMID: 24014826 DOI: 10.1161/circheartfailure.113.000539]

137 **Howarth FC**, Qureshi MA, White E, Calaghan SC. Cardiac microtubules are more resistant to chemical depolymerisation in streptozotocin-induced diabetes in the rat. *Pflugers Arch* 2002; **444**: 432-437 [PMID: 12111253 DOI: 10.1007/s00424-002-0824-y]

138 **Shiels H**, O'Connell A, Qureshi MA, Howarth FC, White E, Calaghan S. Stable microtubules contribute to cardiac dysfunction in the streptozotocin-induced model of type 1 diabetes in the rat. *Mol Cell Biochem* 2007; **294**: 173-180 [PMID: 16838107 DOI: 10.1007/s11010-006-9257-9]

139 **Shirai M**, Schwenke DO, Tsuchimochi H, Umetani K, Yagi N, Pearson JT. Synchrotron radiation imaging for advancing our understanding of cardiovascular function. *Circ Res* 2013; **112**: 209-221 [PMID: 23287456 DOI: 10.1161/circresaha.111.300096]

140 **Gibbs CL**. Cardiac energetics: sense and nonsense. *Clin Exp Pharmacol Physiol* 2003; **30**: 598-603 [PMID: 12890186 DOI: 10.1046/j.1440-1681.2003.03878.x]

141 **Widén C**, Barclay CJ. ATP splitting by half the cross-bridges can explain the twitch energetics of mouse papillary muscle. *J Physiol* 2006; **573**: 5-15 [PMID: 16497711 DOI: 10.1113/jphysiol.2006.104992]

142 **Yagi N**, Saeki Y, Ishikawa T, Kurihara S. Cross-bridge and calcium behavior in ferret papillary muscle in different thyroid states. *Jpn J Physiol* 2001; **51**: 319-326 [PMID: 11492956 DOI: 10.2170/jjphysiol.51.319]

143 **Joseph T**, Coirault C, Dubourg O, Lecarpentier Y. Changes in crossbridge mechanical properties in diabetic rat cardiomyopathy. *Basic Res Cardiol* 2005; **100**: 231-239 [PMID: 15645163 DOI: 10.1007/s00395-005-0512-5]

144 **Han JC**, Tran K, Nielsen PM, Taberner AJ, Loiselle DS. Streptozotocin-induced diabetes prolongs twitch duration without affecting the energetics of isolated ventricular trabeculae. *Cardiovasc Diabetol* 2014; **13**: 79 [PMID: 24731754 DOI: 10.1186/1475-2840-13-79]

145 **Barefield D**, Sadayappan S. Phosphorylation and function of cardiac myosin binding protein-C in health and disease. *J Mol Cell Cardiol* 2010; **48**: 866-875 [PMID: 19962384 DOI: 10.1016/j.yjmcc.2009.11.014]

146 **de Tombe PP**. Myosin binding protein C in the heart. *Circ Res* 2006; **98**: 1234-1236 [PMID: 16728667 DOI: 10.1161/01.RES.0000225873.63162.c4]

147 **Hamdani N**, Kooij V, van Dijk S, Merkus D, Paulus WJ, Remedios CD, Duncker DJ, Stienen GJ, van der Velden J. Sarcomeric dysfunction in heart failure. *Cardiovasc Res* 2008; **77**: 649-658 [PMID: 18055579 DOI: 10.1093/cvr/cvm079]

148 **Sadayappan S**, de Tombe PP. Cardiac myosin binding protein-C: redefining its structure and function. *Biophys Rev* 2012; **4**: 93-106 [PMID: 22707987 DOI: 10.1007/s12551-012-0067-x]

149 **Moss RL**, Fitzsimons DP. Myosin light chain 2 into the mainstream of cardiac development and contractility. *Circ Res* 2006; **99**: 225-227 [PMID: 16888245 DOI: 10.1161/01.RES.0000236793.88131.dc]

150 **Tsukamoto O**, Kitakaze M. Biochemical and physiological regulation of cardiac myocyte contraction by cardiac-specific myosin light chain kinase. *Circ J* 2013; **77**: 2218-2225 [PMID: 23863751 DOI: 10.1253/circj.CJ-13-0627]

151 **Colson BA**, Locher MR, Bekyarova T, Patel JR, Fitzsimons DP, Irving TC, Moss RL. Differential roles of regulatory light chain and myosin binding protein-C phosphorylations in the modulation of cardiac force development. *J Physiol* 2010; **588**: 981-993 [PMID: 20123786 DOI: 10.1113/jphysiol.2009.183897]

152 **Kulikovskaya I**, McClellan G, Levine R, Winegrad S. Effect of extraction of myosin binding protein C on contractility of rat heart. *Am J Physiol Heart Circ Physiol* 2003; **285**: H857-H865 [PMID: 12860568 DOI: 10.1152/ajpheart.00841.2002]

153 **Kunst G**, Kress KR, Gruen M, Uttenweiler D, Gautel M, Fink RH. Myosin binding protein C, a phosphorylation-dependent force regulator in muscle that controls the attachment of myosin heads by its interaction with myosin S2. *Circ Res* 2000; **86**: 51-58 [PMID: 10625305 DOI: 10.1161/01.res.86.1.51]

154 **Pohlmann L**, Kröger I, Vignier N, Schlossarek S, Krämer E, Coirault C, Sultan KR, El-Armouche A, Winegrad S, Eschenhagen T, Carrier L. Cardiac myosin-binding protein C is required for complete relaxation in intact myocytes. *Circ Res* 2007; **101**: 928-938 [PMID: 17823372 DOI: 10.1161/circresaha.107.158774]

155 **Tong CW**, Stelzer JE, Greaser ML, Powers PA, Moss RL. Acceleration of crossbridge kinetics by protein kinase A phosphorylation of cardiac myosin binding protein C modulates cardiac function. *Circ Res* 2008; **103**: 974-982 [PMID: 18802026 DOI: 10.1161/circresaha.108.177683]

156 **Harris SP**, Bartley CR, Hacker TA, McDonald KS, Douglas PS, Greaser ML, Powers PA, Moss RL. Hypertrophic cardiomyopathy in cardiac myosin binding protein-C knockout mice. *Circ Res* 2002; **90**: 594-601 [PMID: 11909824 DOI: 10.1161/01.res.0000012222.70819.64]

157 **McClellan G**, Kulikovskaya I, Flavigny J, Carrier L, Winegrad S. Effect of cardiac myosin-binding protein C on stability of the thick filament. *J Mol Cell Cardiol* 2004; **37**: 823-835 [PMID: 15380673 DOI: 10.1016/j.yjmcc.2004.05.023]

158 **Nyland LR**, Palmer BM, Chen Z, Maughan DW, Seidman CE, Seidman JG, Kreplak L, Vigoreaux JO. Cardiac myosin binding protein-C is essential for thick-filament stability and flexural rigidity. *Biophys J* 2009; **96**: 3273-3280 [PMID: 19383471 DOI: 10.1016/j.bpj.2008.12.3946]

159 **Colson BA**, Bekyarova T, Fitzsimons DP, Irving TC, Moss RL. Radial displacement of myosin cross-bridges in mouse myocardium due to ablation of myosin binding protein-C. *J Mol Biol* 2007; **367**: 36-41 [PMID: 17254601]

160 **Stelzer JE**, Fitzsimons DP, Moss RL. Ablation of myosin-binding protein-C accelerates force development in mouse myocardium. *Biophys J* 2006; **90**: 4119-4127 [PMID: 16513777 DOI: 10.1529/biophysj.105.078147]

161 **Weisberg A**, Winegrad S. Alteration of myosin cross bridges by phosphorylation of myosin-binding protein C in cardiac muscle. *Proc Natl Acad Sci USA* 1996; **93**: 8999-9003 [PMID: 8799143]

162 **Colson BA**, Bekyarova T, Locher MR, Fitzsimons DP, Irving TC, Moss RL. Protein kinase A-mediated phosphorylation of cMyBP-C increases proximity of myosin heads to actin in resting myocardium. *Circ Res* 2008; **103**: 244-251 [PMID: 18599866 DOI: 10.1161/circresaha.108.178996]

163 **Kooij V**, Holewinski RJ, Murphy AM, Van Eyk JE. Characterization of the cardiac myosin binding protein-C phosphoproteome in healthy and failing human hearts. *J Mol Cell Cardiol* 2013; **60**: 116-120 [PMID: 23619294 DOI: 10.1016/j.yjmcc.2013.04.012]

164 **Gresham KS**, Mamidi R, Stelzer JE. The contribution of cardiac myosin binding protein-c Ser282 phosphorylation to the rate of force generation and in vivo cardiac contractility. *J Physiol* 2014; **592**: 3747-3765 [PMID: 24951619 DOI: 10.1113/jphysiol.2014.276022]

165 **McClellan G**, Kulikovskaya I, Winegrad S. Changes in cardiac contractility related to calcium-mediated changes in phosphorylation of myosin-binding protein C. *Biophys J* 2001; **81**: 1083-1092 [PMID: 11463649 DOI: 10.1016/S0006-3495(01)75765-7]

166 **Tong CW**, Gaffin RD, Zawieja DC, Muthuchamy M. Roles of phosphorylation of myosin binding protein-C and troponin I in mouse cardiac muscle twitch dynamics. *J Physiol* 2004; **558**: 927-941 [PMID: 15194741 DOI: 10.1113/jphysiol.2004.062539]

167 **Decker RS**, Decker ML, Kulikovskaya I, Nakamura S, Lee DC, Harris K, Klocke FJ, Winegrad S. Myosin-binding protein C phosphorylation, myofibril structure, and contractile function during low-flow ischemia. *Circulation* 2005; **111**: 906-912 [PMID: 15699252 DOI: 10.1161/01.cir.0000155609.95618.75]

168 **El-Armouche A**, Pohlmann L, Schlossarek S, Starbatty J, Yeh YH, Nattel S, Dobrev D, Eschenhagen T, Carrier L. Decreased phosphorylation levels of cardiac myosin-binding protein-C in human and experimental heart failure. *J Mol Cell Cardiol* 2007; **43**: 223-229 [PMID: 17560599 DOI: 10.1016/j.yjmcc.2007.05.003]

169 **Jacques AM**, Copeland O, Messer AE, Gallon CE, King K, McKenna WJ, Tsang VT, Marston SB. Myosin binding protein C phosphorylation in normal, hypertrophic and failing human heart muscle. *J Mol Cell Cardiol* 2008; **45**: 209-216 [PMID: 18573260 DOI: 10.1016/j.yjmcc.2008.05.020]

170 **Liu X**, Takeda N, Dhalla NS. Myosin light-chain phosphorylation in diabetic cardiomyopathy in rats. *Metabolism* 1997; **46**: 71-75 [PMID: 9005973]

171 **Korte FS**, Mokelke EA, Sturek M, McDonald KS. Exercise improves impaired ventricular function and alterations of cardiac myofibrillar proteins in diabetic dyslipidemic pigs. *J Appl Physiol* (1985) 2005; **98**: 461-467 [PMID: 15465890 DOI: 10.1152/japplphysiol.00551.2004]

172 **Hwang H**, Robinson DA, Stevenson TK, Wu HC, Kampert SE, Pagani FD, Dyke DB, Martin JL, Sadayappan S, Day SM, Westfall MV. PKCβII modulation of myocyte contractile performance. *J Mol Cell Cardiol* 2012; **53**: 176-186 [PMID: 22587992 DOI: 10.1016/j.yjmcc.2012.05.001]

173 **Bardswell SC**, Cuello F, Rowland AJ, Sadayappan S, Robbins J, Gautel M, Walker JW, Kentish JC, Avkiran M. Distinct sarcomeric substrates are responsible for protein kinase D-mediated regulation of cardiac myofilament Ca2+ sensitivity and cross-bridge cycling. *J Biol Chem* 2010; **285**: 5674-5682 [PMID: 20018870 DOI: 10.1074/jbc.M109.066456]

174 **Vahebi S**, Kobayashi T, Warren CM, de Tombe PP, Solaro RJ. Functional effects of rho-kinase-dependent phosphorylation of specific sites on cardiac troponin. *Circ Res* 2005; **96**: 740-747 [PMID: 15774859 DOI: 10.1161/01.RES.0000162457.56568.7d]

175 **Solaro RJ**, Rosevear P, Kobayashi T. The unique functions of cardiac troponin I in the control of cardiac muscle contraction and relaxation. *Biochem Biophys Res Commun* 2008; **369**: 82-87 [PMID: 18162178 DOI: 10.1016/j.bbrc.2007.12.114]

176 **Layland J**, Solaro RJ, Shah AM. Regulation of cardiac contractile function by troponin I phosphorylation. *Cardiovasc Res* 2005; **66**: 12-21 [PMID: 15769444 DOI: 10.1016/j.cardiores.2004.12.022]

177 **Hinken AC**, Hanft LM, Scruggs SB, Sadayappan S, Robbins J, Solaro RJ, McDonald KS. Protein kinase C depresses cardiac myocyte power output and attenuates myofilament responses induced by protein kinase A. *J Muscle Res Cell Motil* 2012; **33**: 439-448 [PMID: 22527640 DOI: 10.1007/s10974-012-9294-9]

178 **Liu X**, Takeda N, Dhalla NS. Troponin I phosphorylation in heart homogenate from diabetic rat. *Biochim Biophys Acta* 1996; **1316**: 78-84 [PMID: 8672554 DOI: 10.1016/0925-4439(96)00007-5]

179 **Wickley PJ**, Shiga T, Murray PA, Damron DS. Propofol decreases myofilament Ca2+ sensitivity via a protein kinase C-, nitric oxide synthase-dependent pathway in diabetic cardiomyocytes. *Anesthesiology* 2006; **104**: 978-987 [PMID: 16645450]

180 **Zhang L**, Ward ML, Phillips AR, Zhang S, Kennedy J, Barry B, Cannell MB, Cooper GJ. Protection of the heart by treatment with a divalent-copper-selective chelator reveals a novel mechanism underlying cardiomyopathy in diabetic rats. *Cardiovasc Diabetol* 2013; **12**: 123 [PMID: 23981320 DOI: 10.1186/1475-2840-12-123]

181 **Malhotra A**, Reich D, Reich D, Nakouzi A, Sanghi V, Geenen DL, Buttrick PM. Experimental diabetes is associated with functional activation of protein kinase C epsilon and phosphorylation of troponin I in the heart, which are prevented by angiotensin II receptor blockade. *Circ Res* 1997; **81**: 1027-1033 [PMID: 9400384 DOI: 10.1161/01.res.81.6.1027]

182 **Nuno DW**, Harrod JS, Lamping KG. Sex-dependent differences in Rho activation contribute to contractile dysfunction in type 2 diabetic mice. *Am J Physiol Heart Circ Physiol* 2009; **297**: H1469-H1477 [PMID: 19666843 DOI: 10.1152/ajpheart.00407.2009]

183 **Vigili de Kreutzenberg S**, Avogaro A. The limited clinical value of a specific diabetic cardiomyopathy. *Nutr Metab Cardiovasc Dis* 2013; **23**: 599-605 [PMID: 23725770 DOI: 10.1016/j.numecd.2013.03.008]

184 **Factor SM**, Minase T, Cho S, Fein F, Capasso JM, Sonnenblick EH. Coronary microvascular abnormalities in the hypertensive-diabetic rat. A primary cause of cardiomyopathy? *Am J Pathol* 1984; **116**: 9-20 [PMID: 6742110]

185 **Gradman AH**, Wilson JT. Hypertension and diastolic heart failure. *Curr Cardiol Rep* 2009; **11**: 422-429 [PMID: 19863866 DOI: 10.1007/s11886-009-0061-5]

186 **Jeong EM**, Monasky MM, Gu L, Taglieri DM, Patel BG, Liu H, Wang Q, Greener I, Dudley SC, Solaro RJ. Tetrahydrobiopterin improves diastolic dysfunction by reversing changes in myofilament properties. *J Mol Cell Cardiol* 2013; **56**: 44-54 [PMID: 23247392 DOI: 10.1016/j.yjmcc.2012.12.003]

187 **Hubert HB**, Feinleib M, McNamara PM, Castelli WP. Obesity as an independent risk factor for cardiovascular disease: a 26-year follow-up of participants in the Framingham Heart Study. *Circulation* 1983; **67**: 968-977 [PMID: 6219830 DOI: 10.1161/01.cir.67.5.968]

188 **Wong C**, Marwick TH. Obesity cardiomyopathy: pathogenesis and pathophysiology. *Nat Clin Pract Cardiovasc Med* 2007; **4**: 436-443 [PMID: 17653116 DOI: 10.1038/ncpcardio.0943]

189 **Kalil GZ**, Haynes WG. Sympathetic nervous system in obesity-related hypertension: mechanisms and clinical implications. *Hypertens Res* 2012; **35**: 4-16 [PMID: 22048570 DOI: 10.1038/hr.2011.173]

190 **Lohmeier TE**, Iliescu R. The sympathetic nervous system in obesity hypertension. *Curr Hypertens Rep* 2013; **15**: 409-416 [PMID: 23677623 DOI: 10.1007/s11906-013-0356-1]

191 **Amador N**, de Jesús Encarnación J, Rodríguez L, Tello A, López M, Guízar JM. Relationship between left ventricular mass and heart sympathetic activity in male obese subjects. *Arch Med Res* 2004; **35**: 411-415 [PMID: 15610911 DOI: 10.1016/j.arcmed.2004.05.003]

192 **Howarth FC**, Qureshi MA, Sobhy ZH, Parekh K, Yammahi SR, Adrian TE, Adeghate E. Structural lesions and changing pattern of

expression of genes encoding cardiac muscle proteins are associated with ventricular myocyte dysfunction in type 2 diabetic Goto-Kakizaki rats fed a high-fat diet. *Exp Physiol* 2011; **96**: 765-777 [PMID: 21666035 DOI: 10.1113/expphysiol.2011.058446]

193 **Reichelt ME**, Mellor KM, Bell JR, Chandramouli C, Headrick JP, Delbridge LM. Sex, sex steroids, and diabetic cardiomyopathy: making the case for experimental focus. *Am J Physiol Heart Circ Physiol* 2013; **305**: H779-H792 [PMID: 23792676 DOI: 10.1152/ajpheart.00141.2013]

194 **Steinberg HO**, Paradisi G, Cronin J, Crowde K, Hempfling A, Hook G, Baron AD. Type II diabetes abrogates sex differences in endothelial function in premenopausal women. *Circulation* 2000; **101**: 2040-2046 [PMID: 10790344 DOI: 10.1161/01.cir.101.17.2040]

195 **Chandler MP**, Morgan EE, McElfresh TA, Kung TA, Rennison JH, Hoit BD, Young ME. Heart failure progression is accelerated following myocardial infarction in type 2 diabetic rats. *Am J Physiol Heart Circ Physiol* 2007; **293**: H1609-H1616 [PMID: 17545473 DOI: 10.1152/ajpheart.01338.2006]

196 **Tuncay E**, Seymen AA, Tanriverdi E, Yaras N, Tandogan B, Ulusu NN, Turan B. Gender related differential effects of Omega-3E treatment on diabetes-induced left ventricular dysfunction. *Mol Cell Biochem* 2007; **304**: 255-263 [PMID: 17530185 DOI: 10.1007/s11010-007-9508-4]

197 **Yaras N**, Tuncay E, Purali N, Sahinoglu B, Vassort G, Turan B. Sex-related effects on diabetes-induced alterations in calcium release in the rat heart. *Am J Physiol Heart Circ Physiol* 2007; **293**: H3584-H3592 [PMID: 17890429 DOI: 10.1152/ajpheart.00619.2007]

198 **Reichelt ME**, Mellor KM, Curl CL, Stapleton D, Delbridge LM. Myocardial glycophagy - a specific glycogen handling response to metabolic stress is accentuated in the female heart. *J Mol Cell Cardiol* 2013; **65**: 67-75 [PMID: 24080183]

199 **Mellor KM**, Varma U, Stapleton DI, Delbridge LM. Cardiomyocyte glycophagy is regulated by insulin and exposure to high extracellular glucose. *Am J Physiol Heart Circ Physiol* 2014; **306**: H1240-H1245 [PMID: 24561860 DOI: 10.1152/ajpheart.00059.2014]

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MINIREVIEWS

Peripheral artery disease in patients with diabetes: Epidemiology, mechanisms, and outcomes

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Abstract

Peripheral artery disease (PAD) is the atherosclerosis of lower extremity arteries and is also associated with atherothrombosis of other vascular beds, including the cardiovascular and cerebrovascular systems. The presence of diabetes mellitus greatly increases the risk of PAD, as well as accelerates its course, making these patients more susceptible to ischemic events and impaired functional status compared to patients without diabetes. To minimize these cardiovascular risks it is critical to understand the pathophysiology of atherosclerosis in diabetic patients. This, in turn, can offer insights into the therapeutic avenues available for these patients. This article provides an overview of the epidemiology of PAD in diabetic patients, followed by an analysis of the mechanisms by which altered metabolism in diabetes promotes atherosclerosis and plaque instability. Outcomes of PAD in diabetic patients are also discussed, with a focus on diabetic ulcers and critical limb ischemia.

Key words: Peripheral artery disease; Epidemiology; Pathophysiology; Outcomes; Diabetes

Core tip: Diabetes mellitus (DM) is a major risk factor of peripheral artery disease (PAD), leading to increased morbidity and mortality as well as an accelerated disease course. As such, a more thorough understanding of the multi-factorial mechanisms underlying disease etiology for both DM and PAD is justified. This review provides clinical insight into the current state of research in the pathophysiology of PAD in diabetic patients, as well as highlights the progress of endovascular interventions for PAD, with a focus on techniques that have shown promise for treatment of critical lower limb ischemia.

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INTRODUCTION

Over 170 million people worldwide have diabetes mellitus (DM) and the worldwide burden is projected to increase to 366 million people by 2030[1,2]. The major causes of DM include impaired insulin secretion or inadequate response to secreted insulin[3]. DM is a major risk factor for atherosclerotic disease as well as cardiovascular mortality and morbidity[3,4]. Atherosclerotic disease is not only increased in incidence in diabetic patients, but its course is also accelerated[4], thereby accounting for as much as 44% of all-cause mortality[5]. DM-associated atherosclerosis can lead to complications in all major of vascular beds, including the coronary arteries, carotid vessels, and lower extremity arteries[5,6]. For example, a study by Haffner *et al*[7], estimated the 7-year incidence of a first-time myocardial infarction (MI) in diabetic patients at 20.2%, compared to 3.5% in nondiabetic patients.

Peripheral artery disease (PAD) is defined as atherosclerotic occlusive disease of lower extremities. PAD is associated with increased risk of lower extremity amputation and is also a marker for atherothrombosis in cardiovascular, cerebrovascular and renovascular beds. Patients with PAD therefore have an increased risk of MI, stroke and death[8]. Additionally, PAD causes significant long-term disability in diabetic patients[5,9]. The treatment of patients with PAD can therefore be expensive, owing to need for a variety of diagnostic tests, therapeutic procedures, and hospitalizations[10].

The purpose of this article is to review the epidemiology and mechanisms that contribute to development of PAD in diabetic patients. The outcomes of PAD in diabetic patients are also compared to nondiabetics, with an emphasis on the prevention of major amputations among patients with DM who have severe PAD.

EPIDEMIOLOGY OF PAD IN PATIENTS WITH DIABETES

PAD affects 12 million people in United States. The most common symptom in PAD is claudication, characterized as a cramping, pain or aching in the calves, thighs or buttocks with exertion and relief with rest[8]. However, many patients have atypical symptoms that may require formal testing with an ankle brachial index test to diagnose PAD[11].

The strongest risk factors for PAD are DM and smoking, with an odds-ratio of 2.72 and 1.88, respectively[12]. With decreased rates of smoking in Western countries, DM is projected to become an increasingly important contributor to the development and progression of PAD. Previous studies have shown that glucose intolerance is associated with a greater than 20% prevalence of an abnormal ankle-brachial index (ABI) relative to 7% in those with normal glucose tolerance[4]. Moreover, 20%-30% of patients with PAD have DM, although this is likely underestimated by the asymptomatic nature of less severe PAD and the altered pain perception in diabetic patients due to peripheral neuropathy[5].

Age, duration of diabetes, and peripheral neuropathy are associated with an increased risk of PAD in patients with pre-existing DM[8,12]. Using ABI to identify PAD, the prevalence of PAD in people with DM over 40 years of age has been estimated to be 20%[13]. This prevalence increases to 29% in patients with DM over 50 years of age[5,14]. The severity and duration of DM are important predictors of both the incidence and the extent of PAD, as observed in United Kingdom Prospective Diabetes Study, where each 1% increase in glycosylated hemoglobin was correlated with a 28% increase in incidence of PAD, and higher rates of death, microvascular complications and major amputation[15,16]. This correlation is particularly strong in men with hypertension or active tobacco use[5]. Patients with PAD who have DM also tend to stay longer in hospital, incur greater costs, and account for greater use of hospital resources compared to patients with PAD alone[10,17].

DM is also associated with more severe below-the-knee PAD (*e.g.,* popliteal, anterior tibial, peroneal and posterior tibial arteries), whereas risk factors such as smoking are associated with more proximal PAD in the aorto-ilio-femoral vessels[8,16]. The prevalence of concomitant PAD and DM is especially high in those patients who have critical lower limb ischemia, with more than 50% of patients with critical limb ischemia (CLI) also having DM[18].

In patients with PAD, the cardiovascular event rate over a 5-year period, including MI and stroke, is 20%, and the overall mortality rate is 30%[19]. Among those with CLI, 30% undergo major amputation, and the 6-mo mortality rate is 20%[20]. Diabetic patients comprise 25%-30% of patients undergoing coronary artery revascularization and up to 60% of patients presenting with acute MI[21-23]. Cardiovascular and cerebrovascular event rates, both fatal and non-fatal, are increased in patients with PAD and DM relative to nondiabetic patients with PAD[8].

Similar to the greater likelihood of diffuse and complex coronary artery disease in diabetic patients, patients with DM also tend to have more diffuse PAD, compared to the more focal disease observed in those without DM[1,5,24]. Although patients with DM tend to present later in the course of disease progression, the incidence of intermittent claudication is also higher than in nondiabetics, as seen in Framingham study[5,25]. In that cohort, the risk of claudication associated with DM was increased by 3.5 fold in men and 8.6 fold in women[25]. Concomitant peripheral neuropathy, which diminishes sensory feedback and leads to a lack of symptoms from minimized pain perception, may predispose patients with DM and PAD to present with more advanced disease, such as an ischemic ulcer or gangrene, compared to patients without DM[8]. The prevalence of major amputation in patients with DM is also higher than in nondiabetics, with rates ranging from 5 to 15 times greater in some studies[8,16]. In a Medicare population, relative

to nondiabetic patients, the relative risk (RR) for lower extremity amputation was 12.7 in diabetic patients. The RR rose to 23.5 in a cohort aged 65-74 years[4].

The risk relationship between PAD and DM is noted to be reciprocal: while DM is a risk factor for PAD, higher rates of PAD, up to 30%, have been found in diabetic patients[26]. The Hoorn study further clarified the discrepancy in prevalence of PAD between diabetic and nondiabetic patients: glucose intolerance was associated with 20.9% prevalence of an ABI less than 0.9, relative to 7% in those with normal glucose tolerance[27]. Moreover, the prevalence of PAD in diabetic patients is likely underestimated by the asymptomatic nature of the condition, lack of reporting by the patients, and the altered pain perception in diabetic patients due to peripheral neuropathy[11,26].

MECHANISMS OF PAD IN PATIENTS WITH DIABETES

DM is characterized by hyperglycemia, dyslipidemia, and insulin resistance[4,28-30]. These pathologic states foster development and progression of PAD through mechanisms similar to that in coronary or carotid artery disease[31,32]. These mechanisms include derangements in the vessel wall through promotion of vascular inflammation and endothelial cell dysfunction; abnormalities in blood cells, including smooth muscle cells and platelets; and factors affecting hemostasis (Table 1). Such vascular abnormalities that cause atherosclerosis in DM patients are often prevalent prior to the diagnosis of DM, and their severity increases with worsening blood glucose control and duration of DM[8,33]. Taken together, these mechanisms likely contribute to increased plaque burden, plaque instability, and greater complexity of vascular disease[3,34-36].

Inflammation

Inflammation is a risk marker for atherothrombosis. Among biomarkers of inflammation, C-reactive protein (CRP) is associated with both the development of PAD and impaired glucose regulation[37]. CRP may also play a direct pathophysiologic role by promoting production of procoagulant tissue factor, leukocyte adhesion molecules, and chemotactic substances. CRP causes derangement in vascular tone by inhibiting endothelial nitric oxide synthase (eNOS), which produces nitric oxide (NO) *via* phosphoinositol-3-kinase dependent pathway[3,8,38]. Moreover, CRP impairs fibrinolysis *via* the production of substances such as plasminogen activator inhibitor (PAI)-1, which blocks the breakdown of plasminogen into plasmin, a fibrinolytic[39]. All of these factors in diabetic patients increase the susceptibility of vascular walls to the development of atherosclerosis[40].

DM is also associated with increased circulating levels of pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α and interleukin-6[41,42]. These cytokines bind to endothelial cell surface receptors and activate nuclear factor (NF)-$\kappa\beta$. This process promotes transcription of endothelial cell adhesion molecules, leading to increased binding of leukocytes and platelets to the endothelial surface, thereby fostering thrombogenesis. Plaque inflammation and instability may also be enhanced due to the increased leukocyte migration, which is associated with an increased risk of rupture and subsequent thrombus formation[3,40,43].

Endothelial dysfunction

Endothelial cells mediate the interaction between blood cell elements and the vascular wall, thereby affecting blood flow, nutrient delivery, coagulation, and the balance between thrombosis and fibrinolysis[8,44]. Endothelial cells also release substances that are critical for blood vessel function and structure, including NO, reactive oxygen species, and endothelin[4,44]. Insulin is critical for the induction of phosphoinositol-3 kinase signaling, leading to production of NO and subsequent smooth muscle cell relaxation[38,45]. NO also inhibits platelet activation and limits vascular smooth muscle cell (VSMC) migration and proliferation[46-48]. By mediating the interaction between leukocytes and the vascular wall, NO also plays an important role in vasodilation and inflammation[8,44].

Hyperglycemia, insulin resistance, and free fatty acid (FFA) production all reduce NO bioavailability in diabetic patients. Hyperglycemia impairs eNOS function, promoting oxidative stress by producing reactive oxygen species in endothelial and VSMCs[38,49]. In turn, these factors inhibit endothelial vasodilation[4,38,44]. Insulin resistance induces excess production of FFAs, which activate protein kinase C (PKC), inhibit phosphatidylinositol (PI)-3 kinase (an important agonist of eNOS), and produce reactive oxygen species[24,28,50,51]. These mediators inhibit NO production and decrease its bioavailability, thereby causing endothelial dysfunction and leading to greater susceptibility of the vascular bed to atherosclerosis[8,24,38,44,49-51].

DM is also associated with the enhanced production of advanced glycation end products (AGEs), which are formed by binding of reducing sugars to free amino groups *via* the Maillard reaction[3,52-54]. The interaction of AGEs with their receptors can upregulate the synthesis of pro-inflammatory transcription factors such as NF-$\kappa\beta$ and activator protein 1[54]. In addition to decreased endothelial function and impaired NO formation, these factors also lead to increased leukocyte chemotaxis, adhesion, transmigration, and transformation into foam cells. The latter process is the first step in the formation of atheromatous plaque[8].

VSMC

VSMC migration from the medial layer into the intimal layer is associated with deposition of complex extracellular matrix, thereby stabilizing the atheroma. This decreases the risk of plaque rupture associated with thrombosis[4,48,55,56]. In diabetic patients, plaques have fewer VSMC, increasing the chance of rupture and

Table 1 Mechanisms of peripheral arterial disease in diabetes mellitus patients

Disease characteristics			Mechanisms of pathology		Disease characteristics	
DM	Hyperglycemia Dyslipidemia	→	Vascular inflammation CRP: promotes leukocyte adhesion, coagulation, and chemotaxis; inhibits eNOS; impairs fibrinolysis	→	Atherosclerosis (Increased plaque burden)	PAD
	Insulin resistance		TNF-α and IL-6: activate NF-κβ, leading to thrombogenesis; promote leukocyte migration and adhesion, increasing plaque instability/rupture		Atherothrombosis (Increased plaque instability/rupture)	
	↑ FFA production		Endothelial cell dysfunction Decreased NO production: inhibits vasodilation Increased reactive oxygen species: inhibits vasodilation Increased AGE production: is proinflammatory; induces leukocyte chemotaxis, adhesion, and transformation into foam cells Vascular smooth muscle cell derangement Tissue factor production: proatherogenic; procoagulation FGF and TGF-α: Extracellular matrix production Impaired synthesis of collagen: destabilizes plaque Apoptosis of VSMC: increases risk of plaque rupture and thrombosis Increased production of endothelin-1, angiotensin Ⅱ, and prostanoids: leads to vasoconstriction Platelet dysfunction Enhanced uptake of glucose: increases oxidative stress; decreased NO production Upregulation of P-selectin, GP Ⅰb, and GP Ⅱb/Ⅲa receptors: promotes platelet adhesion and aggregation Calcium dysregulation: increases platelet aggregation Hypercoagulability Increased tissue factor and FⅦ production: enhances coagulability Decreased antithrombin and protein C synthesis: enhances coagulability Rheology Elevated blood viscosity Increased fibrinogen production Impaired arteriogenesis Inhibited sensing of shear stress Decreased monocyte and growth factor signaling		Restenosis (Increased complexity)	

Note that there is significant interplay between the different mechanisms: for example, impaired NO production can affect inflammation, endothelial cell function and arteriogenesis, while increased reactive oxygen species causes platelet and endothelial cell dysfunction. FFA: Free fatty acids; CRP: C-reactive protein; eNOS: Endothelial nitric oxide synthetase; TNF-α: Tumor necrosis factor-α; IL-6: Interleukin-6; NF-κβ: Nuclear factor-κβ; NO: Nitric oxide; AGE: Advanced glycation end products; FGF: Fibroblast growth factor; TGF-α: Transforming growth factor-α; VSMC: Vascular smooth muscle cell; GP Ⅰb: Glycoprotein Ⅰb; GP Ⅱb/Ⅲa: Glycoprotein Ⅱb/Ⅲa; FⅦ: Factor 7; DM: Diabetes mellitus; PAD: Peripheral artery disease.

thrombosis[57]. Moreover, the lipid modifications noted in diabetic patients, such as glycated oxidized low-density lipoprotein, can promote apoptosis of VSMC[4,46]. The metabolic syndrome that defines DM results in enhanced production of reactive oxygen species, inhibition of PI-3 kinase and upregulation of PKC, AGE receptors and NF-κβ, which in turn further promotes an atherogenic phenotype in VSMCs[4,58]. These factors further contribute to the increased apoptosis of VSMC and upregulation of proatherogenic tissue factor in diabetic patients, while impairing synthesis of collagen, an important plaque-stabilizing compound[8,59]. DM is also associated with increased matrix metalloproteinases, which further break down collagen, leading to plaque instability[60]. Therefore, DM not only promotes atherosclerosis but also destabilizes plaques, triggering thrombus formation and impacting clinical outcomes[8].

DM has also been found to promote upregulation and enhanced activity of endothelin-1, a protein that activates the endothelin-A receptor on VSMCs, leading to enhanced vascular tone[61]. Such dysregulated hyperactivation of endothelin-A receptor can cause pathological vasoconstriction[62]. Endothelin-1 is also responsible for increasing salt and water retention, inducing the renin-angiotensin system, and causing vascular smooth muscle hypertrophy. Other vasoactive substances, such as vasoconstrictor prostanoids and angiotensin Ⅱ, are also increased in production, further inducing vasoconstriction[63].

Platelet function

Platelets mediate the interaction between vascular function and thrombosis. Hence, platelet dysfunction can accelerate atherosclerosis, as well as impact the destabilization of plaque and promote atherothrombosis[8,64]. Platelets take up glucose independent of insulin, which in turn activates protein kinase-C and decreases NO production[39]. Oxidative stress is also increased when platelets take up glucose, thus promoting platelet aggregation. Platelet adhesion is enhanced in diabetic patients due to upregulated expression of P-selectin on platelet surfaces[3].

Diabetic patients also have upregulated expression of platelet receptors, such as glycoprotein Ⅰb (which binds to von Willebrand Factor) and Ⅱb/Ⅲa receptors (integral to platelet-fibrin interaction); these receptors mediate platelet adhesion and aggregation, thus inducing thrombosis[39]. Intra-platelet calcium regulation, important for regulation of platelet shape change and aggregation, as well as for thromboxane production, is also deranged in diabetic patients, further contributing to atherosclerosis in this patient population[4,39,65,66].

Coagulation

DM and hyperglycemic states promote hypercoagulability *via* upregulation of tissue factor by endothelial cells and VSMCs[67,68]. These conditions also increase coagulation factor ⅦA production and decrease anticoagulants, such as antithrombin and protein C production[67,68]. DM also impairs fibrinolytic function and induces PAI-1 production[69]. Taken together, these factors increase the risk of atherosclerotic plaque rupture and subsequent thrombus formation[8,68,70].

Rheology

Elevated blood viscosity and fibrinogen production also occur in patients with DM. This is manifested *via* abnormal ABI in patients with PAD as well as development and complications of PAD[8,71].

Restenosis after angioplasty

Acutely elevated glucose levels may induce inflammation, smooth muscle proliferation, abnormal matrix production, and inactivation of endothelium-derived relaxing factor[72]. Additionally, hyperglycemia may impact expression of fibroblast growth factor and transforming growth factor-α, which in turn promotes proliferation of smooth muscle cells and extracellular matrix production. Increased TNF-α and CRP, as well as oxidative stress and endothelial dysfunction, may also play roles in explaining the restenosis rates in patients with higher blood glucose values at time of angioplasty[73]. Acute hyperglycemia also induces production of monocyte chemoattractant-protein-1, which has been linked with a higher risk of restenosis[73]. Restenosis among patients with DM can therefore be explained by the abnormal inflammatory state, oxidative stress, endothelial and platelet function in patients with acute hyperglycemia[1].

Arteriogenesis

Outward remodeling of pre-existing arteries in response to obstruction of blood flow to restore blood flow distal to the occlusion is termed arteriogenesis[74]. Endothelial shear stress, detected by the vessel wall through integrins, adhesion molecules, tyrosine kinases, and ion channels, is hypothesized to be the main trigger for arteriogenesis[45,75,76]. DM limits the adaptive arteriogenesis response and collateral blood flow development by attenuating the remodeling process[77,78]. Specifically, diabetes attenuates the sensing of shear stress and increases the response to vasodilatory stimuli, which reduces the recruitment and dilation of collateral arteries. Additionally, DM impairs various other factors critical to remodeling, such as the downstream signaling of monocytes, growth factor signaling, and endothelial NO synthetase, thus inhibiting arteriogenesis and contributing to the severity of occlusive disease in these patients[74].

OUTCOMES OF PATIENTS WITH PAD AND DM

The outcomes of patients with coexistent diabetes and PAD depend on the interplay between factors such as patient comorbidities, presence of infection, neuropathy, and immunologic factors[79]. Poor glycemic control has been associated with a higher prevalence of PAD and risk of adverse outcomes, including need for lower extremity bypass surgery, amputation or death[80]. Poor glycemic control is also associated with worse outcomes following vascular surgery or endovascular intervention[80].

It is therefore important to identify therapies that can affect the multifactorial pathophysiologic mechanisms of DM in order to provide effective long-term treatments[3]. Lifestyle interventions, such as weight loss, physical activity, and reduced cholesterol and fat intake, all help reduce the risk of progression from glucose intolerance to diabetes, as well as improve cardiovascular risk factors[81]. Tobacco cessation is also critical and has been associated with improved outcomes after surgical and endovascular interventions. Such secondary risk factor reduction can help reduce the prevalence and severity of PAD in diabetic patients and also minimize adverse events post revascularization[3].

Revascularization in patients with PAD and diabetes

Revascularization, either *via* a surgical or endovascular approach, is an important therapeutic option for treatment of symptomatic PAD in diabetic patients[5]. Due to the greater prevalence of below-the knee disease in patients with DM, some studies have shown that endovascular interventions are associated with worse outcomes in diabetics, especially as distal runoff diminishes[4]. Endovascular interventions were initially therefore considered more appropriate in patients with focal disease above the knee. Diabetic patients were also noted to have greater durability with surgical approach to revascularization, especially in the setting of tibial disease managed *via* bypass with autologous saphenous vein[5]. However, recent studies have suggested that diabetic patients with adequate distal runoff appear to have patency rates comparable to that of nondiabetics[4].

This association of glucose control and vessel patency has been investigated in a single-center retrospective study of outcomes after infrapopliteal balloon angioplasty among diabetic patients. Patients were divided based on median pre-procedure fasting blood glucose (FBG) values into two groups. At one-year follow-up, primary patency,

defined as freedom from restenosis or reintervention based on duplex ultrasound, was 16% for those with FBG values above the median and 46% for patients with below the median FBG values. Amputation rates also trended higher among patients with high pre-procedure FBG compared to low FBG. One-year major adverse limb event rates were significantly higher for patients with FBG values above the median, even after adjusting for insulin use and lesion-specific characteristics. No association between FBG values and overall mortality, amputation-free survival or rates of major adverse cardiovascular events was noted[80]. When the FBG levels were divided into quartiles, a fivefold increase was noted in primary patency in the lowest quartile of FBG relative to those in the highest quartile of FBG. These results remained significant even after adjusting for baseline insulin use. These outcomes failed to show an association between HbA1C and restenosis, therefore implying that glycemic control at the time of intervention may be a better predictor of primary patency than overall glycemic control[80]. Furthermore, these results suggest that the acute metabolic milieu at the time of intervention plays an important role in restenosis.

Treatment of critical limb ischemia

In patients with CLI, revascularization is usually required for successful limb preservation[5]. The prevalence of DM in patients with CLI is extremely high, with some studies suggesting a prevalence of up to 76%[18]. Disease severity at the time of presentation and progression of CLI in diabetics has also been noted to be worse[82]. Current recommendations suggest arterial reconstruction in patients with CLI who have a predicted 1-year amputation-free survival of at least 75%[18].

While patients with CLI may require multiple procedures and close follow-up, the choice of initial revascularization does not appear to influence success in diabetic patients with CLI[18]. Whether chosen initially or subsequently, surgical and endovascular approaches both are associated with similar outcomes in terms of survival without major amputation or repeated target extremity revascularization (TER)[18]. However, that study did confirm that repeat TER is more frequently required in diabetic patients. Despite the increased need for repeat revascularization, repeated procedures were associated with overall success rates comparable to that in nondiabetic patients[18]. Immediate revascularization was also associated with improved outcomes relative to delayed revascularization in patients with CLI, regardless of diabetic status[13]. Additional studies have also shown that an aggressive multidisciplinary approach in diabetic patients who present with CLI had similar limb salvage, 30-d mortality, cumulative survival, amputation-free survival, and major amputation rates, relative to non-diabetic patients[83]. Revascularization rates do appear to be better in this patient population when both endovascular and bypass grafting procedures are available relative to one of the two approaches only[84].

While most patients with CLI can be revascularized, the presence of irreversible gangrene, the absence of a target vessel, and the lack of availability of an autologous vein can limit successful limb preservation. In these patients, amputation may be the best option[5]. In general, however, medical management and use of multidisciplinary approach that includes revascularization can lead to reduced amputation rates in patients with DM[79].

Diabetic foot ulcer is another complication in these patients that is associated with an increased risk of call-cause mortality[79]. In those patients with PAD whose course is complicated by diabetic foot ulcer, similar outcomes in terms of limb salvage rates were seen with endovascular and open surgical approaches[85]. It is important to note, however, that concomitant PAD in patients with diabetic foot ulcers is linked to greater failure rates of wound healing and need for amputation. This association is complex, and different studies have shown that successful revascularization and ulcer healing are not always correlated[79].

CONCLUSION

DM is associated with greater severity and more diffuse PAD relative to nondiabetics. It also correlates to greater risk of mortality and impaired quality of life. The mechanisms by which diabetes induces atherosclerosis are multifactorial and include inflammatory processes, derangements of various cell types within the vascular wall, promotion of coagulation, and inhibition of fibrinolysis. These factors both increase the susceptibility of the vasculature to atherosclerosis, as well as the instability that makes plaque prone to rupture and thrombosis. Thus, it is important for different specialists, from cardiology and internal medicine to vascular surgery, to collaborate and use a multidisciplinary approach to improve the clinical outcomes in this patient population.

Although diabetics have a higher risk of adverse outcomes when compared to nondiabetics, the rates are improving thanks to recent advances in pharmacology and procedural techniques. Nonetheless, further work remains necessary. For instance, while trials such as TRITON-TIMI 38 and PLATO show better clinical outcomes with prasugrel or ticagrelor compared to clopidogrel after percutaneous coronary intervention, it is unclear if similar benefit is seen in DM patients with PAD[3]. Further studies should also include the impact of biochemical factors found in central obesity, which are known to promote atherothrombosis[86]. Better understanding of the mechanisms responsible for restenosis among diabetic patients will also ultimately improve the outcomes of surgical and endovascular procedures in these patients.

REFERENCES

1 **Armstrong EJ**, Waltenberger J, Rogers JH. Percutaneous coronary intervention in patients with diabetes: current concepts and future directions. *J Diabetes Sci Technol* 2014; **8**: 581-589 [PMID: 24876623 DOI: 10.1177/1932296813517058]

2 **Wild S**, Roglic G, Green A, Sicree R, King H. Global prevalence

of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* 2004; **27**: 1047-1053 [PMID: 15111519 DOI: 10.2337/diacare.27.5.1047]

3 **Armstrong EJ**, Rutledge JC, Rogers JH. Coronary artery revascularization in patients with diabetes mellitus. *Circulation* 2013; **128**: 1675-1685 [PMID: 24100481]

4 **Beckman JA**, Creager MA, Libby P. Diabetes and atherosclerosis: epidemiology, pathophysiology, and management. *JAMA* 2002; **287**: 2570-2581 [PMID: 12020339 DOI: 10.1001/jama.287.19.2570]

5 **Marso SP**, Hiatt WR. Peripheral arterial disease in patients with diabetes. *J Am Coll Cardiol* 2006; **47**: 921-929 [PMID: 16516072 DOI: 10.1016/j.jacc.2005.09.065]

6 **Forbang NI**, McDermott MM, Liao Y, Ix JH, Allison MA, Liu K, Tian L, Evans N, Criqui MH. Associations of diabetes mellitus and other cardiovascular disease risk factors with decline in the ankle-brachial index. *Vasc Med* 2014; **19**: 465-472 [PMID: 25358555]

7 **Haffner SM**, Lehto S, Rönnemaa T, Pyörälä K, Laakso M. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *N Engl J Med* 1998; **339**: 229-234 [PMID: 9673301 DOI: 10.1056/NEJM199807233390404]

8 **American Diabetes Association**. Peripheral arterial disease in people with diabetes. *Diabetes Care* 2003; **26**: 3333-3341 [PMID: 14633825 DOI: 10.2337/diacare.26.12.3333]

9 **Vogt MT**, Cauley JA, Kuller LH, Nevitt MC. Functional status and mobility among elderly women with lower extremity arterial disease: the Study of Osteoporotic Fractures. *J Am Geriatr Soc* 1994; **42**: 923-929 [PMID: 8064098]

10 **Hirsch AT**, Hartman L, Town RJ, Virnig BA. National health care costs of peripheral arterial disease in the Medicare population. *Vasc Med* 2008; **13**: 209-215 [PMID: 18687757 DOI: 10.1177/1358863X08089277]

11 **Criqui MH**, Fronek A, Klauber MR, Barrett-Connor E, Gabriel S. The sensitivity, specificity, and predictive value of traditional clinical evaluation of peripheral arterial disease: results from noninvasive testing in a defined population. *Circulation* 1985; **71**: 516-522 [PMID: 3156007 DOI: 10.1161/01.CIR.71.3.516]

12 **Fowkes FG**, Rudan D, Rudan I, Aboyans V, Denenberg JO, McDermott MM, Norman PE, Sampson UK, Williams LJ, Mensah GA, Criqui MH. Comparison of global estimates of prevalence and risk factors for peripheral artery disease in 2000 and 2010: a systematic review and analysis. *Lancet* 2013; **382**: 1329-1340 [PMID: 23915883 DOI: 10.1016/S0140-6736(13)61249-0]

13 **Elhadd TA**, Robb R, Jung RT, Stonebridge PA, Belch JJF. Pilot study of prevalence of asymptomatic peripheral arterial occlusive disease in patients with diabetes attending a hospital clinic. *Practical Diabetes Int* 1999; **16**: 163-166 [DOI: 10.1002/pdi.1960160605]

14 **Hirsch AT**, Criqui MH, Treat-Jacobson D, Regensteiner JG, Creager MA, Olin JW, Krook SH, Hunninghake DB, Comerota AJ, Walsh ME, McDermott MM, Hiatt WR. Peripheral arterial disease detection, awareness, and treatment in primary care. *JAMA* 2001; **286**: 1317-1324 [PMID: 11560536 DOI: 10.1001/jama.286.11.1317]

15 **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]

16 **Jude EB**, Oyibo SO, Chalmers N, Boulton AJ. Peripheral arterial disease in diabetic and nondiabetic patients: a comparison of severity and outcome. *Diabetes Care* 2001; **24**: 1433-1437 [PMID: 11473082 DOI: 10.2337/diacare.24.8.1433]

17 **Malone M**, Lau NS, White J, Novak A, Xuan W, Iliopoulos J, Crozier J, Dickson HG. The effect of diabetes mellitus on costs and length of stay in patients with peripheral arterial disease undergoing vascular surgery. *Eur J Vasc Endovasc Surg* 2014; **48**: 447-451 [PMID: 25116276 DOI: 10.1016/j.ejvs.2014.07.001]

18 **Dick F**, Diehm N, Galimanis A, Husmann M, Schmidli J, Baumgartner I. Surgical or endovascular revascularization in patients with critical limb ischemia: influence of diabetes mellitus on clinical outcome. *J Vasc Surg* 2007; **45**: 751-761 [PMID: 17306950 DOI: 10.1016/j.jvs.2006.12.022]

19 **Weitz JI**, Byrne J, Clagett GP, Farkouh ME, Porter JM, Sackett DL, Strandness DE Jr, Taylor LM. Diagnosis and treatment of chronic arterial insufficiency of the lower extremities: a critical review. *Circulation* 1996; **94**: 3026-3049 [PMID: 8941154 DOI: 10.1161/01.CIR.94.11.3026]

20 **Dormandy JA**, Rutherford RB. Management of peripheral arterial disease (PAD). TASC Working Group. TransAtlantic Inter-Society Consensus (TASC). *J Vasc Surg* 2000; **31**: S1-S296 [PMID: 10666287]

21 **Aronson D**, Edelman ER. Revascularization for coronary artery disease in diabetes mellitus: angioplasty, stents and coronary artery bypass grafting. *Rev Endocr Metab Disord* 2010; **11**: 75-86 [PMID: 20221852 DOI: 10.1007/s11154-010-9135-3]

22 **Lankisch M**, Füth R, Gülker H, Lapp H, Bufe A, Haastert B, Martin S, Rathmann W. Screening for undiagnosed diabetes in patients with acute myocardial infarction. *Clin Res Cardiol* 2008; **97**: 753-759 [PMID: 18491170 DOI: 10.1007/s00392-008-0674-5]

23 **Shore S**, Borgerding JA, Gylys-Colwell I, McDermott K, Ho PM, Tillquist MN, Lowy E, McGuire DK, Stolker JM, Arnold SV, Kosiborod M, Maddox TM. Association between hyperglycemia at admission during hospitalization for acute myocardial infarction and subsequent diabetes: insights from the veterans administration cardiac care follow-up clinical study. *Diabetes Care* 2014; **37**: 409-418 [PMID: 24089537 DOI: 10.2337/dc13-1125]

24 **Creager MA**, Lüscher TF, Cosentino F, Beckman JA. Diabetes and vascular disease: pathophysiology, clinical consequences, and medical therapy: Part I. *Circulation* 2003; **108**: 1527-1532 [PMID: 14504252 DOI: 10.1161/01.CIR.0000091257.27563.32]

25 **Kannel WB**, McGee DL. Update on some epidemiologic features of intermittent claudication: the Framingham Study. *J Am Geriatr Soc* 1985; **33**: 13-18 [PMID: 3965550]

26 **Setacci C**, de Donato G, Setacci F, Chisci E. Diabetic patients: epidemiology and global impact. *J Cardiovasc Surg* (Torino) 2009; **50**: 263-273 [PMID: 19543188]

27 **Beks PJ**, Mackaay AJ, de Neeling JN, de Vries H, Bouter LM, Heine RJ. Peripheral arterial disease in relation to glycaemic level in an elderly Caucasian population: the Hoorn study. *Diabetologia* 1995; **38**: 86-96 [PMID: 7744233 DOI: 10.1007/BF02369357]

28 **Chahil TJ**, Ginsberg HN. Diabetic dyslipidemia. *Endocrinol Metab Clin North Am* 2006; **35**: 491-510, vii-viii [PMID: 16959582 DOI: 10.1016/j.ecl.2006.06.002]

29 **Grundy SM**. Hypertriglyceridemia, insulin resistance, and the metabolic syndrome. *Am J Cardiol* 1999; **83**: 25F-29F [PMID: 10357572 DOI: 10.1016/S0002-9149(99)00211-8]

30 **Rizzo M**, Pernice V, Frasheri A, Berneis K. Atherogenic lipoprotein phenotype and LDL size and subclasses in patients with peripheral arterial disease. *Atherosclerosis* 2008; **197**: 237-241 [PMID: 17485096 DOI: 10.1016/j.atherosclerosis.2007.03.034]

31 **MacGregor AS**, Price JF, Hau CM, Lee AJ, Carson MN, Fowkes FG. Role of systolic blood pressure and plasma triglycerides in diabetic peripheral arterial disease. The Edinburgh Artery Study. *Diabetes Care* 1999; **22**: 453-458 [PMID: 10097928 DOI: 10.2337/diacare.22.3.453]

32 **Paneni F**, Beckman JA, Creager MA, Cosentino F. Diabetes and vascular disease: pathophysiology, clinical consequences, and medical therapy: part I. *Eur Heart J* 2013; **34**: 2436-2443 [PMID: 23641007 DOI: 10.1093/eurheartj/eht149]

33 **Price JF**, Lee AJ, Fowkes FG. Hyperinsulinaemia: a risk factor for peripheral arterial disease in the non-diabetic general population. *J Cardiovasc Risk* 1996; **3**: 501-505 [PMID: 9100085]

34 **Johnstone MT**, Creager SJ, Scales KM, Cusco JA, Lee BK, Creager MA. Impaired endothelium-dependent vasodilation in patients with insulin-dependent diabetes mellitus. *Circulation* 1993; **88**: 2510-2516 [PMID: 8080489 DOI: 10.1161/01.CIR.88.6.2510]

35 **Steinberg HO**, Tarshoby M, Monestel R, Hook G, Cronin J, Johnson A, Bayazeed B, Baron AD. Elevated circulating free fatty acid levels impair endothelium-dependent vasodilation. *J Clin*

Invest 1997; **100**: 1230-1239 [PMID: 9276741 DOI: 10.1172/JCI119636]
36 **Li Y**, Woo V, Bose R. Platelet hyperactivity and abnormal Ca(2+) homeostasis in diabetes mellitus. *Am J Physiol Heart Circ Physiol* 2001; **280**: H1480-H1489 [PMID: 11247757]
37 **Ridker PM**, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Plasma concentration of C-reactive protein and risk of developing peripheral vascular disease. *Circulation* 1998; **97**: 425-428 [PMID: 9490235 DOI: 10.1161/01.CIR.97.5.425]
38 **Kawashima S**, Yokoyama M. Dysfunction of endothelial nitric oxide synthase and atherosclerosis. *Arterioscler Thromb Vasc Biol* 2004; **24**: 998-1005 [PMID: 15001455 DOI: 10.1161/01.ATV.0000125114.88079.96]
39 **Vinik AI**, Erbas T, Park TS, Nolan R, Pittenger GL. Platelet dysfunction in type 2 diabetes. *Diabetes Care* 2001; **24**: 1476-1485 [PMID: 11473089 DOI: 10.2337/diacare.24.8.1476]
40 **Schaper NC**, Nabuurs-Franssen MH, Huijberts MS. Peripheral vascular disease and type 2 diabetes mellitus. *Diabetes Metab Res Rev* 2000; **16** Suppl 1: S11-S15 [PMID: 11054881]
41 **Pradhan AD**, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *JAMA* 2001; **286**: 327-334 [PMID: 11466099 DOI: 10.1001/jama.286.3.327]
42 **Pickup JC**, Chusney GD, Thomas SM, Burt D. Plasma interleukin-6, tumour necrosis factor alpha and blood cytokine production in type 2 diabetes. *Life Sci* 2000; **67**: 291-300 [PMID: 10983873 DOI: 10.1016/S0024-3205(00)00622-6]
43 **Ross R**. Atherosclerosis is an inflammatory disease. *Am Heart J* 1999; **138**: S419-S420 [PMID: 10539839 DOI: 10.1016/S0002-8703(99)70266-8]
44 **De Vriese AS**, Verbeuren TJ, Van de Voorde J, Lameire NH, Vanhoutte PM. Endothelial dysfunction in diabetes. *Br J Pharmacol* 2000; **130**: 963-974 [PMID: 10882379 DOI: 10.1038/sj.bjp.0703393]
45 **Troidl K**, Schaper W. Arteriogenesis versus angiogenesis in peripheral artery disease. *Diabetes Metab Res Rev* 2012; **28** Suppl 1: 27-29 [PMID: 22271719 DOI: 10.1002/dmrr.2232]
46 **Suzuki LA**, Poot M, Gerrity RG, Bornfeldt KE. Diabetes accelerates smooth muscle accumulation in lesions of atherosclerosis: lack of direct growth-promoting effects of high glucose levels. *Diabetes* 2001; **50**: 851-860 [PMID: 11289052 DOI: 10.2337/diabetes.50.4.851]
47 **Radomski MW**, Palmer RM, Moncada S. The role of nitric oxide and cGMP in platelet adhesion to vascular endothelium. *Biochem Biophys Res Commun* 1987; **148**: 1482-1489 [PMID: 2825688 DOI: 10.1016/S0006-291X(87)80299-1]
48 **Sarkar R**, Meinberg EG, Stanley JC, Gordon D, Webb RC. Nitric oxide reversibly inhibits the migration of cultured vascular smooth muscle cells. *Circ Res* 1996; **78**: 225-230 [PMID: 8575065 DOI: 10.1161/01.RES.78.2.225]
49 **Giacco F**, Brownlee M. Oxidative stress and diabetic complications. *Circ Res* 2010; **107**: 1058-1070 [PMID: 21030723 DOI: 10.1161/CIRCRESAHA.110.223545]
50 **Laakso M**, Sarlund H, Salonen R, Suhonen M, Pyörälä K, Salonen JT, Karhapää P. Asymptomatic atherosclerosis and insulin resistance. *Arterioscler Thromb* 1991; **11**: 1068-1076 [PMID: 2065028 DOI: 10.1161/01.ATV.11.4.1068]
51 **Steinberg HO**, Baron AD. Vascular function, insulin resistance and fatty acids. *Diabetologia* 2002; **45**: 623-634 [PMID: 12107742 DOI: 10.1007/s00125-002-0800-2]
52 **Bierhaus A**, Hofmann MA, Ziegler R, Nawroth PP. AGEs and their interaction with AGE-receptors in vascular disease and diabetes mellitus. I. The AGE concept. *Cardiovasc Res* 1998; **37**: 586-600 [PMID: 9659442 DOI: 10.1016/S0008-6363(97)00233-2]
53 **Miyazawa T**, Nakagawa K, Shimasaki S, Nagai R. Lipid glycation and protein glycation in diabetes and atherosclerosis. *Amino Acids* 2012; **42**: 1163-1170 [PMID: 20957396 DOI: 10.1007/s00726-010-0772-3]
54 **Bierhaus A**, Nawroth PP. Multiple levels of regulation determine the role of the receptor for AGE (RAGE) as common soil in inflammation, immune responses and diabetes mellitus and its complications. *Diabetologia* 2009; **52**: 2251-2263 [PMID: 19636529 DOI: 10.1007/s00125-009-1458-9]
55 **Libby P**. Current concepts of the pathogenesis of the acute coronary syndromes. *Circulation* 2001; **104**: 365-372 [PMID: 11457759 DOI: 10.1161/01.CIR.104.3.365]
56 **Davies MJ**, Richardson PD, Woolf N, Katz DR, Mann J. Risk of thrombosis in human atherosclerotic plaques: role of extracellular lipid, macrophage, and smooth muscle cell content. *Br Heart J* 1993; **69**: 377-381 [PMID: 8518056 DOI: 10.1136/hrt.69.5.377]
57 **Fukumoto H**, Naito Z, Asano G, Aramaki T. Immunohistochemical and morphometric evaluations of coronary atherosclerotic plaques associated with myocardial infarction and diabetes mellitus. *J Atheroscler Thromb* 1998; **5**: 29-35 [PMID: 10077455 DOI: 10.5551/jat1994.5.29]
58 **Quehenberger P**, Bierhaus A, Fasching P, Muellner C, Klevesath M, Hong M, Stier G, Sattler M, Schleicher E, Speiser W, Nawroth PP. Endothelin 1 transcription is controlled by nuclear factor-kappaB in AGE-stimulated cultured endothelial cells. *Diabetes* 2000; **49**: 1561-1570 [PMID: 10969841 DOI: 10.2337/diabetes.49.9.1561]
59 **Geng YJ**, Libby P. Progression of atheroma: a struggle between death and procreation. *Arterioscler Thromb Vasc Biol* 2002; **22**: 1370-1380 [PMID: 12231554 DOI: 10.1161/01.ATV.0000031341.84618.A4]
60 **Uemura S**, Matsushita H, Li W, Glassford AJ, Asagami T, Lee KH, Harrison DG, Tsao PS. Diabetes mellitus enhances vascular matrix metalloproteinase activity: role of oxidative stress. *Circ Res* 2001; **88**: 1291-1298 [PMID: 11420306 DOI: 10.1161/hh1201.092042]
61 **Cardillo C**, Campia U, Bryant MB, Panza JA. Increased activity of endogenous endothelin in patients with type II diabetes mellitus. *Circulation* 2002; **106**: 1783-1787 [PMID: 12356630 DOI: 10.1161/01.CIR.0000032260.01569.64]
62 **Nugent AG**, McGurk C, Hayes JR, Johnston GD. Impaired vasoconstriction to endothelin 1 in patients with NIDDM. *Diabetes* 1996; **45**: 105-107 [PMID: 8522053 DOI: 10.2337/diab.45.1.105]
63 **Hopfner RL**, Gopalakrishnan V. Endothelin: emerging role in diabetic vascular complications. *Diabetologia* 1999; **42**: 1383-1394 [PMID: 10651255 DOI: 10.1007/s001250051308]
64 **Tschoepe D**, Roesen P, Kaufmann L, Schauseil S, Kehrel B, Ostermann H, Gries FA. Evidence for abnormal platelet glycoprotein expression in diabetes mellitus. *Eur J Clin Invest* 1990; **20**: 166-170 [PMID: 2112481 DOI: 10.1111/j.1365-2362.1990.tb02264.x]
65 **Trovati M**, Anfossi G, Cavalot F, Massucco P, Mularoni E, Emanuelli G. Insulin directly reduces platelet sensitivity to aggregating agents. Studies in vitro and in vivo. *Diabetes* 1988; **37**: 780-786 [PMID: 2838353 DOI: 10.2337/diab.37.6.780]
66 **Davì G**, Catalano I, Averna M, Notarbartolo A, Strano A, Ciabattoni G, Patrono C. Thromboxane biosynthesis and platelet function in type II diabetes mellitus. *N Engl J Med* 1990; **322**: 1769-1774 [PMID: 2345567 DOI: 10.1056/NEJM199006213222503]
67 **Kario K**, Matsuo T, Kobayashi H, Matsuo M, Sakata T, Miyata T. Activation of tissue factor-induced coagulation and endothelial cell dysfunction in non-insulin-dependent diabetic patients with microalbuminuria. *Arterioscler Thromb Vasc Biol* 1995; **15**: 1114-1120 [PMID: 7627704 DOI: 10.1161/01.ATV.15.8.1114]
68 **Carr ME**. Diabetes mellitus: a hypercoagulable state. *J Diabetes Complications* 2001; **15**: 44-54 [PMID: 11259926 DOI: 10.1016/S1056-8727(00)00132-X]
69 **Carmassi F**, Morale M, Puccetti R, De Negri F, Monzani F, Navalesi R, Mariani G. Coagulation and fibrinolytic system impairment in insulin dependent diabetes mellitus. *Thromb Res* 1992; **67**: 643-654 [PMID: 1440530 DOI: 10.1016/0049-3848(92)90068-L]
70 **Wilcox JN**, Smith KM, Schwartz SM, Gordon D. Localization of tissue factor in the normal vessel wall and in the atherosclerotic plaque. *Proc Natl Acad Sci USA* 1989; **86**: 2839-2843 [PMID: 2704749 DOI: 10.1073/pnas.86.8.2839]
71 **Cooke JP**. The pathophysiology of peripheral arterial disease: rational targets for drug intervention. *Vasc Med* 1997; **2**: 227-230 [PMID: 9546972]
72 **Nakamura N**, Ueno Y, Tsuchiyama Y, Koike Y, Gohda M, Satani O. Isolated post-challenge hyperglycemia in patients with normal

fasting glucose concentration exaggerates neointimal hyperplasia after coronary stent implantation. *Circ J* 2003; **67**: 61-67 [PMID: 12520154 DOI: 10.1253/circj.67.61]

73 **Marfella R**, Sasso FC, Siniscalchi M, Paolisso P, Rizzo MR, Ferraro F, Stabile E, Sorropago G, Calabrò P, Carbonara O, Cinquegrana G, Piscione F, Ruocco A, D'Andrea D, Rapacciuolo A, Petronella P, Bresciani A, Rubino P, Mauro C, Paolisso G. Peri-procedural tight glycemic control during early percutaneous coronary intervention is associated with a lower rate of in-stent restenosis in patients with acute ST-elevation myocardial infarction. *J Clin Endocrinol Metab* 2012; **97**: 2862-2871 [PMID: 22639289 DOI: 10.1210/jc.2012-1364]

74 **Ruiter MS**, van Golde JM, Schaper NC, Stehouwer CD, Huijberts MS. Diabetes impairs arteriogenesis in the peripheral circulation: review of molecular mechanisms. *Clin Sci* (Lond) 2010; **119**: 225-238 [PMID: 20545627 DOI: 10.1042/CS20100082]

75 **Pipp F**, Boehm S, Cai WJ, Adili F, Ziegler B, Karanovic G, Ritter R, Balzer J, Scheler C, Schaper W, Schmitz-Rixen T. Elevated fluid shear stress enhances postocclusive collateral artery growth and gene expression in the pig hind limb. *Arterioscler Thromb Vasc Biol* 2004; **24**: 1664-1668 [PMID: 15242864 DOI: 10.1161/01.ATV.0000138028.14390.e4]

76 **Li YS**, Haga JH, Chien S. Molecular basis of the effects of shear stress on vascular endothelial cells. *J Biomech* 2005; **38**: 1949-1971 [PMID: 16084198 DOI: 10.1016/j.jbiomech.2004.09.030]

77 **van Golde JM**, Ruiter MS, Schaper NC, Vöö S, Waltenberger J, Backes WH, Post MJ, Huijberts MS. Impaired collateral recruitment and outward remodeling in experimental diabetes. *Diabetes* 2008; **57**: 2818-2823 [PMID: 18633114 DOI: 10.2337/db08-0229]

78 **De Vivo S**, Palmer-Kazen U, Kalin B, Wahlberg E. Risk factors for poor collateral development in claudication. *Vasc Endovascular Surg* 2005; **39**: 519-524 [PMID: 16382273 DOI: 10.1177/153857440503900609]

79 **Forsythe RO**, Jones KG, Hinchliffe RJ. Distal bypasses in patients with diabetes and infrapopliteal disease: technical considerations to achieve success. *Int J Low Extrem Wounds* 2014; **13**: 347-362 [PMID: 25123371]

80 **Singh S**, Armstrong EJ, Sherif W, Alvandi B, Westin GG, Singh GD, Amsterdam EA, Laird JR. Association of elevated fasting glucose with lower patency and increased major adverse limb events among patients with diabetes undergoing infrapopliteal balloon angioplasty. *Vasc Med* 2014; **19**: 307-314 [PMID: 24939930 DOI: 10.1177/1358863X14538330]

81 **Reynolds K**, He J. Epidemiology of the metabolic syndrome. *Am J Med Sci* 2005; **330**: 273-279 [PMID: 16355011 DOI: 10.1097/00000441-200512000-00004]

82 **Kullo IJ**, Bailey KR, Kardia SL, Mosley TH, Boerwinkle E, Turner ST. Ethnic differences in peripheral arterial disease in the NHLBI Genetic Epidemiology Network of Arteriopathy (GENOA) study. *Vasc Med* 2003; **8**: 237-242 [PMID: 15125483 DOI: 10.1191/1358863x03vm511oa]

83 **Awad S**, Karkos CD, Serrachino-Inglott F, Cooper NJ, Butterfield JS, Ashleigh R, Nasim A. The impact of diabetes on current revascularisation practice and clinical outcome in patients with critical lower limb ischaemia. *Eur J Vasc Endovasc Surg* 2006; **32**: 51-59 [PMID: 16488631 DOI: 10.1016/j.ejvs.2005.12.019]

84 **Faglia E**, Clerici G, Losa S, Tavano D, Caminiti M, Miramonti M, Somalvico F, Airoldi F. Limb revascularization feasibility in diabetic patients with critical limb ischemia: results from a cohort of 344 consecutive unselected diabetic patients evaluated in 2009. *Diabetes Res Clin Pract* 2012; **95**: 364-371 [PMID: 22104261 DOI: 10.1016/j.diabres.2011.10.033]

85 **Hinchliffe RJ**, Andros G, Apelqvist J, Bakker K, Friederichs S, Lammer J, Lepantalo M, Mills JL, Reekers J, Shearman CP, Valk G, Zierler RE, Schaper NC. A systematic review of the effectiveness of revascularization of the ulcerated foot in patients with diabetes and peripheral arterial disease. *Diabetes Metab Res Rev* 2012; **28** Suppl 1: 179-217 [PMID: 22271740 DOI: 10.1002/dmrr.2249]

86 **Reisin E**, Alpert MA. The metabolic syndrome: an overview. *Am J Med Sci* 2005; **330**: 263 [PMID: 16355008 DOI: 10.1097/00000441-200512000-00001]

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MINIREVIEWS

Dyslipidaemia of diabetes and the intestine

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Abstract

Atherosclerosis is the major complication of diabetes and has become a major issue in the provision of medical care. In particular the economic burden is growing at an alarming rate in parallel with the increasing world-wide prevalence of diabetes. The major disturbance of lipid metabolism in diabetes relates to the effect of insulin on fat metabolism. Raised triglycerides being the hallmark of uncontrolled diabetes, *i.e.*, in the presence of hyperglycaemia. The explosion of type 2 diabetes has generated increasing interest on the aetiology of atherosclerosis in diabetic patients. The importance of the atherogenic properties of triglyceride rich lipoproteins has only recently been recognised by the majority of diabetologists and cardiologists even though experimental evidence has been strong for many years. In the post-prandial phase 50% of triglyceride rich lipoproteins come from chylomicrons produced in the intestine. Recent evidence has secured the chylomicron as a major player in the atherogenic process. In diabetes chylomicron production is increased through disturbance in cholesterol absorption, in particular Neimann Pick C1-like1 activity is increased as is intestinal synthesis of cholesterol through 3-hydroxy-3-methyl glutaryl co enzyme A reductase. ATP binding cassette proteins G5 and G8 which regulate cholesterol in the intestine is reduced leading to chylomicronaemia. The chylomicron particle itself is atherogenic but the increase in the triglyceride-rich lipoproteins lead to an atherogenic low density lipoprotein and low high density lipoprotein. The various steps in the absorption process and the disturbance in chylomicron synthesis are discussed.

Key words: Triglyceride; Cholesterol chylomicrons; Microsomal triglyceride transfer protein; Niemann Pick C1-like1; Lipoproteins; Diabetes; ATP binding cassette proteins G5/G8

Core tip: The explosion of type 2 diabetes has generated increasing interest on the aetiology of atherosclerosis in diabetic patients. Evidence is mounting on the importance of the atherogenic properties of triglyceride rich lipoproteins. In the post-prandial phase 50% of triglyceride rich lipoproteins come from chylomicrons produced in the intestine. Recent evidence has secured the chylomicron as a major player in the atherogenic process. In diabetes chylomicron production is increased through disturbance in cholesterol absorption. This paper reviews recent literature in relation to diabetes, the intestine and dyslipidaemia with a view to understanding new targets for treatment.

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INTRODUCTION

The increasing incidence and prevalence of diabetes is of concern to patients their relatives and the medical profession. Politicians who do not fit into the above groups are also concerned because of the health budget considerations. Management of chronic conditions is expensive! A major cost of diabetes care goes on the management of cardiovascular atherosclerotic complications which are so much more common in both diabetes and pre-diabetes. Although statins remain at the centre of dyslipidaemia treatment for diabetes, there is generally an unawareness of the importance of the chylomicron and triglyceride-rich proteins being atherogenic in their own right but also in forming atherogenic low density lipoprotein (LDL). This lack of appreciation of the importance of hypertriglyceridaemia has for example encouraged Tenenbaum *et al*[1] to write an article entitled "Hypertriglyceridaemia: too long an unfairly neglected major cardiovascular risk factor". Another important milestone in bringing the intestine to the notice of diabetologists and cardiologists has been the validation of ezetimibe, a drug that inhibits the absorption of intestinal cholesterol, which has been shown beyond doubt in the IMPROVE-IT study, demonstrating significantly lower primary combined endpoints in moderate to high risk patients who stabilise following acute coronary syndrome[2]. This article reviews dyslipidaemia in diabetes with a focus on the intestine as a dysfunctional regulator of cholesterol metabolism.

Insulin deficiency is associated with disturbance in both carbohydrate and fat metabolism. In fact even before diabetes becomes manifest, in the pre diabetes phase of the condition free fatty acids fail to be suppressed after a glucose load leading to the suggestion that diabetes should be defined as lipidus rather than mellitus[3]. Indeed, had the serum rather than the urine been easily available many centuries ago, post prandial chylomicronaemia, as demonstrated by the milky serum, would have been preferred to the sweet taste of the urine as the diagnostic tool of choice!

The chylomicron is a particle containing protein fat and cholesterol. The protein, which is mostly apolipoprotein (apo) B48, is the solubilising protein which facilitates the transport of fatty acids, triglycerides and cholesterol. The chylomicron is assembled in the intestinal mucosa under the influence of microsomal triglyceride transfer protein (MTP) which is the rate limiting enzyme. Very LDL (VLDL) is the major triglyceride-containing particle assembled in the liver and, in the postprandial state, about 50% of triglyceride is carried on the VLDL particle and 50% on the chylomicron. Although the chylomicron by definition is the triglyceride rich particle containing apo B48 and VLDL by definition is the triglyceride-rich particle containing apo B100, the term chylomicron is also used based on density following separation in the ultracentrifuge and thus is a mixture of both chylomicrons and large VLDL particles. Hence there is often confusion about what is meant by the term chylomicron.

CHYLOMICRON AS AN ATHEROGENIC PARTICLE

For many years triglycerides have taken a back seat in the perception and understanding of the aetiology of atherosclerosis. Part of the problem might have been that triglycerides have usually been taken fasting whereas the chylomicron is a post-prandial particle and the hepatic VLDL, the other major triglyceride containing particle, is also mostly produced in the postprandial state. Evidence that Apo B48 is found in the atherosclerotic plaque[4-7] has been around for years confirming the atherogenicity of the chylomicron particle. Trials such as the FIELD Study[8], failed to show cardiovascular benefit for reduction of triglycerides and had an adverse effect on the understanding of the atherosclerotic effect of the chylomicron. This has been rectified particularly by the Danish group who have shown that postprandial triglycerides are indeed associated with an increased in cardiovascular events[9]. Further analysis of the FIELD Study, and in particular understanding that too many people with normal triglycerides were included in the study, has resulted in a number of post hoc analysis of that study showing that reduction in triglycerides did have cardiovascular benefit. An evaluation of the effect of fenofibrate by sex in the FIELD Study was recently reported[10]. In that study the authors found that fenofibrate reduced LDL, non-high density lipoprotein (HDL) cholesterol and apo B more in women than in men irrespective of menopausal status. The prevention of total cardiovascular events was more in women (30% *viz* 13%). In Patients with high triglycerides and low HDL the cardiovascular reduction was less different between the sexes (30% *viz* 24%). In a recent review Varbo *et al*[11] conclude that post hoc subgroup analysis of randomised trials using fibrates in individuals with raised triglycerides show a benefit in lowering triglycerides. Conversely low non fasting triglycerides have been shown to be associated with reduced all cause mortality[12]. The authors examined individuals from the Copenhagen Heart study. Genetically derived low triglycerides were associated with a reduction in all cause mortality and the authors suggest probably due to a reduction in cholesterol in remnant particles. Apo C111 interferes with the uptake of triglyceride-rich apo E containing lipoproteins (both chylomicron and VLDL). Loss of function mutations are associated with lower triglycerides. The effect of these loss of function mutations on the risk of coronary heart disease (CHD) was examined[13]. An aggregate of rare mutations in the gene encoding apo C111 was associated with lower plasma triglycerides.

Levels were 39% lower in carriers when compared to non-carriers and circulating levels of apo C111 were 46% lower than in non-carriers. The study found that the risk of CHD among carriers of any apo C111 mutation was 40% lower than the risk among the non carriers. An other important boost to the importance of triglycerides in CHD. Fasting blood sugar is of course a recognised cause of increased cardiovascular risk[14] and it is very interesting to see that patients with polymorphisms of the various genes known to be associated with raised glucose such as the glucokinase gene (*GCK*) rs4607517 have been shown to be associated with an increased risk of ischaemic heart disease and myocardial infarction as compared to genotypes associated with lower levels[15]. It is likely that the raised glucose is associated with the failure to suppress triglyceride.

Inflammation is a key factor in atherosclerosis progression and obesity is associated with an increase in inflammatory proteins such as tumour necrosis factor alpha and interleucin 6 (IL6). A study genotyping for variants affecting levels of non fasting remnant cholesterol, LDL cholesterol and C-reactive protein (CRP) by both CRP alleles and IL6 receptor alleles found that increasing non-fasting remnant cholesterol was associated with significantly higher CRP. This was not the case for LDL, suggesting that remnant cholesterol may in part cause acceleration of atherosclerosis through an inflammatory process[16-18]. Endothelial dysfunction is a precursor of atherosclerosis. Post prandially, when triglycerides rise neutrophils increase with concomitant production of pro-inflammatory cytokines and oxidative stress suggesting a contributory cause of endothelial dysfunction[19,20]. Further triglycerides have also been shown to increase leucocyte activation markers[21,22].

The mechanisms whereby the postprandial lipoproteins might be pro-inflammatory and stimulate the progression of atherosclerosis has been investigated[23]. One mechanism is through the activation of neutrophils and Klop *et al*[24] have shown in healthy volunteers that post prandially changes occur in the white cell population which are similar to that shown in infection. A good review of post-prandial inflammation and the role of glucose and lipids has recently been published[25].

MTP

Since chylomicronaemia is such an important finding in diabetes it is of interest to examine MTP function in diabetes.

Biosynthesis of lipoproteins requires apo B and MTP. MTP binds and chaperones lipoproteins to the nascent apo B. MTP is an endoplasmic reticulum resident hetero dimeric complex. The liver and intestine are the major organs that express apo B and secrete apoB containing lipoproteins. There is good agreement between apoB levels and activity and in various animal models of diabetes in rats, rabbits and fructose fed hamsters diabetes MTP is up regulated[26-29].

In human studies in type 2 diabetes we demonstrated an increase in MTP mRNA in intestinal biopsies[30,31]. Diabetic patients who were on statin therapy had lower MTP mRNA compared to those not on statins[31]. We found positive correlations between MTP mRNA and chylomicron fraction cholesterol and apo B48[31]. A novel intestinal specific inhibitor of MTP has been shown to ameliorate impaired glucose and lipid metabolism in Zucker diabetic fatty rats but whether this effect was due to impairment of food intake or to inhibition of fat absorption is not clear[32]. The signals that upregulate chylomicron formation to cope with excess fat in the diet are slowly being elucidated. Another non-specific inhibitor of MTP, which reduced serum levels of triglycerides by more than 70%, was also associated with significant improvements in glucose tolerance and insulin sensitivity in Zucker fatty rats[33]. Hepatic MTP mRNA expression is negatively regulated by insulin and it is suggested that insulin might also directly inhibit apo B48 secretion independently of MTP even though it is probable that up-regulation of MTP stimulates apo B secretion[34]. The membrane glycoprotein CD36 binds long chain fatty acids. CD 36 deficiency reduces chylomicron production[35]. It has recently been shown that binding of lipid by CD36 upregulates apo B48 and MTP through CD 36 signalling *via* the ERK 1/2 pathway[36]. Interestingly polymorphisms of MTP which have been associated with differences in serum lipids appear to alter cholesterol absorption but not synthesis in women[37].

ATP BINDING CASSETTE PROTEINS G5/G8

Once cholesterol has been transported across the brush border membrane it faces another regulatory process and may be excreted back into the intestinal lumen rather than being further processed for absorption into the lymphatic circulation. ATP binding cassette proteins G5/G8 (ABC G5/G8) are heterodimers which are mostly confined to the human small intestine and liver[38]. These two proteins act in tandem to re-excrete both cholesterol, and in particular non-cholesterol sterols from the body. Much of the understanding of ABC G5/G8 comes from the rare mutations that cause a defect in ABC G5 and G8 and result in high levels of sitosterol in the blood. Sitosterolemia, is a condition which manifests itself in children as tendon xanthomas or in young adults as severe CHD with massive accumulation of sterols and stanols in monocyte derived macrophages[39]. Ma *et al*[40] found in an animal model, that dietary calcium had a beneficial effect on lipoprotein profile by up-regulating the mRNA levels of intestinal ABC G5/8 and cholesterol-7α-hydroxylase (CYP7A1), whereas it down-regulated the intestinal NPC1L1 and MTP due to enhanced biliary cholesterol excretion. Méndez-González *et al*[41] investigated the effect of ABC G5 and G8 deficiency on lipoproteins in mice. They found that postprandial triglycerides were 5 fold higher in the ABCG5/G8$^{-/-}$ mice due to a lower fractional catabolic rate with lower post heparin lipoprotein lipase activities. They also showed

that liver triglyceride secretion and intestinal triglyceride secretion were higher and there was a relationship between this and the HOMA index as a measure of insulin resistance. Rats with induced diabetes (streptozotosin) had impaired expression of ABC G5/8. Treatment with insulin partially reversed this effect[42]. This trend in impairment was found in Zucker diabetic rats[43,44] and the Psamonas Obesus (sand rat) was found to have the same intestinal impairment[45,46]. Intestinal G5/G8 mRNA in type 2 diabetic subjects produced similar findings[30].

ABC 5/8 genetic variants have been associated with susceptibility to CHD. One polymorphism in particular was shown to be associated with increased triglycerides with a significant gene - tobacco smoking interaction[47]. Another study has shown that ABC G5/8 regulate cholesterol available for chylomicron production. It is interesting to read that ABC G5/8 genotypes that are associated with low LDL cholesterol are protective against myocardial infarction but increase risk of symptomatic gall stone disease[48].

NIEMANN PICK C1-LIKE1

The first step in cholesterol absorption in the intestine appears to be through the multi transmembrane protein Niemann Pick C1-like1 (NPC1L1) which is highly expressed in the jejunum[49]. In humans it is localised to the brush borders of the enterocytes and acts as a unidirectional transporter of cholesterol and non-cholesterol sterols[50]. Zhang *et al*[51] discovered that it is the N-terminal domain of NPC1L1 that binds cholesterol. Twenty rare NCP1L1 alleles have been found in the low cholesterol absorbers and appear to impair NPC1L1 cholesterol uptake through various mechanism[52,53], for review see Calandera[54]. It has been shown that the effectiveness of ezetimibe, which blocks NPC1L1 and inhibits cholesterol absorption, depends on the NPC1L1 genotype.

Cholesterol absorption has been shown to be increased in both animal and human diabetes[55] due to an increase in NPC1-L1[43,44,55]. In an animal model of type 2 diabetes, Sammomas Obesus, the opposite was found even though these animals have an increase in apo B48[45,46]. Ezetimibe inhibits cholesterol absorbtion through inhibition of NPCILI (for review see[56]). NPC1L1 activity appears to be governed by dietary cholesterol[57]. The mechanism of this control is through the nuclear receptor, peroxisome proliferator-activated receptor (PPAR)δ/β[58]. Fenofibrate, a PPARα agonist has been shown to inhibit cholesterol absorption, the mechanism has been shown to be through NCP1L1 transcription by binding to a PPARα response element upstream of the human *NPC1L1* gene[59]. In a human construct Iwayanagi *et al*[60] showed that PPARα positively regulated human NPC1L1 transcription and Valasek *et al*[59] showed that Fenofibrate reduced intestinal cholesterol absorption by PPARα modulation of NPC1L1. HMGCoA reductase inhibition (Atorvastatin) has been shown to increase cholesterol absorption in the intestine and downregulation of NPC1L1[61] in the intestine. Ezetimibe has been shown to improve biomarkers of inflammation and platelet activity[62] as stated above the IMPROVE-IT trial has been presented at the American Heart 2014 but not yet published. Ciriacks *et al*[63] have examined the addition of Ezetimibe to simvastatin in type 1 and type 2 diabetes. The study demonstrated that ezetimibe was at least as effective in lowering cholesterol as simvastatin among type 1 diabetics. Some studies have suggested that there may be a difference in cholesterol absorption rates between type 1 and type 2 diabetic patients[64].

OTHER TRANSPORTERS OF CHOLESTEROL

There are other transporters of cholesterol for example scavenger receptor class B type 1 (SR-B1) which is located both in the apical and basolateral membranes of the enterocyte[65]. SR are cell surface proteins that can bind and internalise modified lipoproteins. SR-B1, which is involved in cholesterol uptake in the intestine, may play an important part in intestinal chylomicron production[66]. The fatty acid transporter CD36 which is also involved in the uptake of oxidised LDL, is another member of the class B scavenger receptor family[66]. Hayashi *et al*[67] investigated gene expression of key proteins involved in the active absorption of dietary fat and cholesterol in response to the development of insulin resistance. They used 2 models of diet induced insulin resistance, the fructose fed hamster and the high fat fed mouse. Expression of SR-B1 was increased in both the fructose fed hamster and the high fat fed mouse models of insulin resistance. In CaCo2 cells SR-B1 over expression increased apo B100 and apo B48 secretion. The authors conclude that apical or basolateral SR-B1 may have an important role in cholesterol absorption and may play a part in cholesterol over absorption in insulin resistant states. SR-B1 in the intestine may play an important role in chylomicron production. CdC42, a member of the Rho family of small Guanidine triphosphotases with numerous functions, has been shown by Xie *et al*[68] to interact with NPC1L1 and to control its movement from endocytic recycling compartment to plasma membrane in a cholesterol dependent manner. Glucose stimulated CDc42 signalling appears to be essential for second stage insulin secretion[69]. It is probable that in insulin resistance the signalling of NPC1L1 is disturbed through this pathway but we have been unable to find any studies in the intestine that have explored the pathway in diabetes/insulin resistance.

THE EFFECT OF HIGH GLUCOSE ON CHOLESTEROL ABSORPTION

Ravid *et al*[70] have shown that high glucose increases intestinal absorption of cholesterol through an increase in the protein expression of NPC1L1. The same group later showed that the effect was through the basolateral

domain suggesting glucose in the circulation rather than in the lumen to be the stimulus[71]. The reason for the up-regulation of cholesterol through NPC1L1 in diabetes has been explored by Malhotra *et al*[72]. Using CaCo2 cells they showed that removal of glucose from the culture medium significantly decreased NPC1L1 mRNA protein expression as well as pro-motor activity. Glucose replenishment significantly increased the promoter activity of NPC1L1 in a dose dependant manner. The authors concluded their experiments by examining mouse jejunum after 24 h fasting which confirmed the CaCo2 cell results.

The role of cholecystokinin on intestinal absorption has been reported by Zhou *et al*[73]. They found that in mice cholecystokinin (CCK) increased cholesterol absorption and increased cell surface associated NPC1L1. Previously Irwin *et al*[74] have shown that an CCK-8 analogue improves insulin sensitivity and triglyceride deposition in liver and muscle but with reduction in weight gain and food intake. The effect therefore on lipid metabolism might be very dependant on dietary intake and the inhibition of apatite and improvement in insulin sensitivity with improvement in glucose tolerance with CCK makes analogues of CCK of interest as a possible treatment in diabetes and the metabolic syndrome.

Interest in bile salt binding drugs for treatment of dyslipidaemia, such as cholestyramine, went out of fashion because of their poor cholesterol lowering effects and their unacceptable side effects. A new formulation colsevelam, is interesting in that not only that it lowers cholesterol and apo B but also lowers blood sugar. A recent study in type 2 diabetic patients demonstrated a 0.32 drop in HbA1c *vs* placebo at 24 wk and a reduction of cholesterol of 6.5%[75,76]. The majority of adverse effects were mild or moderate, the authors concluding that the drug was well tolerated. Thus another option for patients who are near to but not on target with current medication.

CHOLESTEROL SYNTHESIS AND 3-HYDROXY-3-METHYLGLUTARYL CO-ENZYME A REDUCTASE

Cholesterol synthesis in the intestine makes up 25% of *de novo* cholesterol synthesis. Cholesterol synthesis is regulated by 3-hydroxy-3-methylglutaryl co-enzyme A (HMGCo A) reductase the rate limiting enzyme in the synthetic pathway. HMGCo A reductase activity has been shown to be reduced by insulin in the rat hepatocyte[77]. It has been suggested that in type 1 diabetes improved glycaemic control will increase cholesterol synthesis. HMGCoA reductase inhibition has been shown to increase cholesterol absorption through a lowering of ABC G5/G8 and an increase in NPC1L1[78].

HEPATIC STEATOSIS

Hepatic steatosis is common in diabetes, insulin resistance and obesity. Inflammatory stress is present in these conditions and is also associated with obesity insulin resistance and diabetes. It is therefore of interest to read that Zhao *et al*[79] demonstrated that IL1b and IL6 stimulation in Hep G2 cells increased SREBP2 and HMGCoA mRNA. Further high fat loading in mice or LDL loading in HepG2 cells suppressed the above genes but this suppression could be over ridden by the above inflammatory proteins. Severe calorie restriction in patients with steatosis results in rapid reduction of liver fat, insulin resistance and improvement in diabetes control[80]. On the other side of the coin insulin resistance and the accompanying hyperinsulinaemia are associated with an upregulation of SREPB-2 through extracellular signal regulated pathways involving the kinases ERK-1 and 2 another example of the interaction between fat and carbohydrate metabolism, for review see Van Rooyen *et al*[81].

In conclusion, vascular disease in diabetes is complex as would be expected with a condition that impacts on so many metabolic pathways. Examination of the intestine in the search for abnormalities in cholesterol absorption and chylomicron formation has been rewarding. Statins have been very effective in reducing the burden of atherosclerosis in patients with diabetes but even so a large proportion of patients still succumb to events. Reduction in triglycerides is now accepted as being important in those patients who have raised triglycerides and in particular the importance of postprandial disturbance in triglyceride metabolism and its impact on atherosclerosis is now accepted as being an important issue in management of diabetes and in the prevention of macrovascular complications.

REFERENCES

1 **Tenenbaum A**, Klempfner R, Fisman EZ. Hypertriglyceridemia: a too long unfairly neglected major cardiovascular risk factor. *Cardiovasc Diabetol* 2014; **13**: 159 [PMID: 25471221]
2 **Kohno T**. Report of the American Heart Association (AHA) Scientific Sessions 2014, Chicago. *Circ J* 2015; **79**: 34-40 [PMID: 25502168]
3 **Shafrir E**, Raz I. Diabetes: mellitus or lipidus? *Diabetologia* 2003; **46**: 433-440 [PMID: 12687345]
4 **Elsegood CL**, Mamo JC. An investigation by electron microscopy of chylomicron remnant uptake by human monocyte-derived macrophages. *Atherosclerosis* 2006; **188**: 251-259 [PMID: 16310792]
5 **Proctor SD**, Mamo JC. Intimal retention of cholesterol derived from apolipoprotein B100- and apolipoprotein B48-containing lipoproteins in carotid arteries of Watanabe heritable hyperlipidemic rabbits. *Arterioscler Thromb Vasc Biol* 2003; **23**: 1595-1600 [PMID: 12842838]
6 **Pal S**, Semorine K, Watts GF, Mamo J. Identification of lipoproteins of intestinal origin in human atherosclerotic plaque. *Clin Chem Lab Med* 2003; **41**: 792-795 [PMID: 12880143]
7 **Nakano T**, Nakajima K, Niimi M, Fujita MQ, Nakajima Y, Takeichi S, Kinoshita M, Matsushima T, Teramoto T, Tanaka A. Detection of apolipoproteins B-48 and B-100 carrying particles in lipoprotein fractions extracted from human aortic atherosclerotic plaques in sudden cardiac death cases. *Clin Chim Acta* 2008; **390**: 38-43 [PMID: 18206113 DOI: 10.1016/j.cca.2007.12.012]
8 **Scott R**, Best J, Forder P, Taskinen MR, Simes J, Barter P, Keech A. Fenofibrate Intervention and Event Lowering in Diabetes (FIELD)

study: baseline characteristics and short-term effects of fenofibrate [ISRCTN64783481]. *Cardiovasc Diabetol* 2005; **4**: 13 [PMID: 16111499]

9 **Varbo A**, Benn M, Nordestgaard BG. Remnant cholesterol as a cause of ischemic heart disease: evidence, definition, measurement, atherogenicity, high risk patients, and present and future treatment. *Pharmacol Ther* 2014; **141**: 358-367 [PMID: 24287311]

10 **d'Emden MC**, Jenkins AJ, Li L, Zannino D, Mann KP, Best JD, Stuckey BG, Park K, Saltevo J, Keech AC. Favourable effects of fenofibrate on lipids and cardiovascular disease in women with type 2 diabetes: results from the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study. *Diabetologia* 2014; **57**: 2296-2303 [PMID: 25149070]

11 **Varbo A**, Nordestgaard BG. Remnant cholesterol and ischemic heart disease. *Curr Opin Lipidol* 2014; **25**: 266-273 [PMID: 24977981]

12 **Thomsen M**, Varbo A, Tybjærg-Hansen A, Nordestgaard BG. Low nonfasting triglycerides and reduced all-cause mortality: a mendelian randomization study. *Clin Chem* 2014; **60**: 737-746 [PMID: 24436475]

13 **Crosby J**, Peloso GM, Auer PL, Crosslin DR, Stitziel NO, Lange LA, Lu Y, Tang ZZ, Zhang H, Hindy G, Masca N, Stirrups K, Kanoni S, Do R, Jun G, Hu Y, Kang HM, Xue C, Goel A, Farrall M, Duga S, Merlini PA, Asselta R, Girelli D, Olivieri O, Martinelli N, Yin W, Reilly D, Speliotes E, Fox CS, Hveem K, Holmen OL, Nikpay M, Farlow DN, Assimes TL, Franceschini N, Robinson J, North KE, Martin LW, DePristo M, Gupta N, Escher SA, Jansson JH, Van Zuydam N, Palmer CN, Wareham N, Koch W, Meitinger T, Peters A, Lieb W, Erbel R, Konig IR, Kruppa J, Degenhardt F, Gottesman O, Bottinger EP, O'Donnell CJ, Psaty BM, Ballantyne CM, Abecasis G, Ordovas JM, Melander O, Watkins H, Orho-Melander M, Ardissino D, Loos RJ, McPherson R, Willer CJ, Erdmann J, Hall AS, Samani NJ, Deloukas P, Schunkert H, Wilson JG, Kooperberg C, Rich SS, Tracy RP, Lin DY, Altshuler D, Gabriel S, Nickerson DA, Jarvik GP, Cupples LA, Reiner AP, Boerwinkle E, Kathiresan S. Loss-of-function mutations in APOC3, triglycerides, and coronary disease. *N Engl J Med* 2014; **371**: 22-31 [PMID: 24941081 DOI: 10.1056/NEJMoa1307095]

14 **Sarwar N**, Gao P, Seshasai SR, Gobin R, Kaptoge S, Di Angelantonio E, Ingelsson E, Lawlor DA, Selvin E, Stampfer M, Stehouwer CD, Lewington S, Pennells L, Thompson A, Sattar N, White IR, Ray KK, Danesh J. Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies. *Lancet* 2010; **375**: 2215-2222 [PMID: 20609967 DOI: 10.1016/S0140-6736(10)]

15 **Benn M**, Tybjaerg-Hansen A, McCarthy MI, Jensen GB, Grande P, Nordestgaard BG. Nonfasting glucose, ischemic heart disease, and myocardial infarction: a Mendelian randomization study. *J Am Coll Cardiol* 2012; **59**: 2356-2365 [PMID: 22698489 DOI: 10.1016/j.jacc.2012.02.043]

16 **Bansal S**, Buring JE, Rifai N, Mora S, Sacks FM, Ridker PM. Fasting compared with nonfasting triglycerides and risk of cardiovascular events in women. *JAMA* 2007; **298**: 309-316 [PMID: 17635891]

17 **Mora S**, Rifai N, Buring JE, Ridker PM. Fasting compared with nonfasting lipids and apolipoproteins for predicting incident cardiovascular events. *Circulation* 2008; **118**: 993-1001 [PMID: 18711012 DOI: 10.1161/CIRCULATIONAHA.108.777334]

18 **Nordestgaard BG**, Benn M, Schnohr P, Tybjaerg-Hansen A. Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. *JAMA* 2007; **298**: 299-308 [PMID: 17635890]

19 **van Oostrom AJ**, Sijmonsma TP, Verseyden C, Jansen EH, de Koning EJ, Rabelink TJ, Castro Cabezas M. Postprandial recruitment of neutrophils may contribute to endothelial dysfunction. *J Lipid Res* 2003; **44**: 576-583 [PMID: 12562833]

20 **Van Oostrom AJ**, Sijmonsma TP, Rabelink TJ, Van Asbeck BS, Cabezas MC. Postprandial leukocyte increase in healthy subjects. *Metabolism* 2003; **52**: 199-202 [PMID: 12601632]

21 **van Oostrom AJ**, Plokker HW, van Asbeck BS, Rabelink TJ, van Kessel KP, Jansen EH, Stehouwer CD, Cabezas MC. Effects of rosuvastatin on postprandial leukocytes in mildly hyperlipidemic patients with premature coronary sclerosis. *Atherosclerosis* 2006; **185**: 331-339 [PMID: 16098531]

22 **van Oostrom AJ**, Rabelink TJ, Verseyden C, Sijmonsma TP, Plokker HW, De Jaegere PP, Cabezas MC. Activation of leukocytes by postprandial lipemia in healthy volunteers. *Atherosclerosis* 2004; **177**: 175-182 [PMID: 15488881]

23 **Klop B**, Proctor SD, Mamo JC, Botham KM, Castro Cabezas M. Understanding postprandial inflammation and its relationship to lifestyle behaviour and metabolic diseases. *Int J Vasc Med* 2012; **2012**: 947417 [PMID: 21961070 DOI: 10.1155/2012/947417]

24 **Klop B**, van de Geijn GJ, Njo TL, Janssen HW, Rietveld AP, van Miltenburg A, Fernández-Sender L, Elte JW, Castro Cabezas M. Leukocyte cell population data (volume conductivity scatter) in postprandial leukocyte activation. *Int J Lab Hematol* 2013; **35**: 644-651 [PMID: 23663689 DOI: 10.1111/ijlh.12103]

25 **de Vries MA**, Alipour A, Klop B, van de Geijn GJ, Janssen HW, Njo TL, van der Meulen N, Rietveld AP, Liem AH, Westerman EM, de Herder WW, Cabezas MC. Glucose-dependent leukocyte activation in patients with type 2 diabetes mellitus, familial combined hyperlipidemia and healthy controls. *Metabolism* 2015; **64**: 213-217 [PMID: 25456098 DOI: 10.1016/j.metabol]

26 **Phillips C**, Bennett A, Anderton K, Owens D, Collins P, White D, Tomkin GH. Intestinal rather than hepatic microsomal triglyceride transfer protein as a cause of postprandial dyslipidemia in diabetes. *Metabolism* 2002; **51**: 847-852 [PMID: 12077729]

27 **Gleeson A**, Anderton K, Owens D, Bennett A, Collins P, Johnson A, White D, Tomkin GH. The role of microsomal triglyceride transfer protein and dietary cholesterol in chylomicron production in diabetes. *Diabetologia* 1999; **42**: 944-948 [PMID: 10491754]

28 **Qin B**, Qiu W, Avramoglu RK, Adeli K. Tumor necrosis factor-alpha induces intestinal insulin resistance and stimulates the overproduction of intestinal apolipoprotein B48-containing lipoproteins. *Diabetes* 2007; **56**: 450-461 [PMID: 17259391]

29 **Zoltowska M**, Ziv E, Delvin E, Sinnett D, Kalman R, Garofalo C, Seidman E, Levy E. Cellular aspects of intestinal lipoprotein assembly in Psammomys obesus: a model of insulin resistance and type 2 diabetes. *Diabetes* 2003; **52**: 2539-2545 [PMID: 14514638]

30 **Lally S**, Tan CY, Owens D, Tomkin GH. Messenger RNA levels of genes involved in dysregulation of postprandial lipoproteins in type 2 diabetes: the role of Niemann-Pick C1-like 1, ATP-binding cassette, transporters G5 and G8, and of microsomal triglyceride transfer protein. *Diabetologia* 2006; **49**: 1008-1016 [PMID: 16518588]

31 **Phillips C**, Mullan K, Owens D, Tomkin GH. Intestinal microsomal triglyceride transfer protein in type 2 diabetic and non-diabetic subjects: the relationship to triglyceride-rich postprandial lipoprotein composition. *Atherosclerosis* 2006; **187**: 57-64 [PMID: 16183064]

32 **Hata T**, Mera Y, Kawai T, Ishii Y, Kuroki Y, Kakimoto K, Ohta T, Kakutani M. JTT-130, a novel intestine-specific inhibitor of microsomal triglyceride transfer protein, ameliorates impaired glucose and lipid metabolism in Zucker diabetic fatty rats. *Diabetes Obes Metab* 2011; **13**: 629-638 [PMID: 21362121 DOI: 10.1111/j.1463-1326.2011.01387.x]

33 **Dhote V**, Joharapurkar A, Kshirsagar S, Dhanesha N, Patel V, Patel A, Raval S, Jain M. Inhibition of microsomal triglyceride transfer protein improves insulin sensitivity and reduces atherogenic risk in Zucker fatty rats. *Clin Exp Pharmacol Physiol* 2011; **38**: 338-344 [PMID: 21401695 DOI: 10.1111/j.1440-1681.2011.05513.x]

34 **Sparks JD**, Chamberlain JM, O'Dell C, Khatun I, Hussain MM, Sparks CE. Acute suppression of apo B secretion by insulin occurs independently of MTP. *Biochem Biophys Res Commun* 2011; **406**: 252-256 [PMID: 21316344 DOI: 10.1016/j.bbrc.2011.02.028]

35 **Tran TT**, Poirier H, Clément L, Nassir F, Pelsers MM, Petit V, Degrace P, Monnot MC, Glatz JF, Abumrad NA, Besnard P, Niot I. Luminal lipid regulates CD36 levels and downstream signaling to stimulate chylomicron synthesis. *J Biol Chem* 2011; **286**: 25201-25210 [PMID: 21610069 DOI: 10.1074/jbc.M111.233551]

36 **Buttet M**, Traynard V, Tran TT, Besnard P, Poirier H, Niot I. From fatty-acid sensing to chylomicron synthesis: role of intestinal lipid-binding proteins. *Biochimie* 2014; **96**: 37-47 [PMID: 23958439 DOI: 10.1016/j.biochi.2013.08.011]

37 **Wolff E**, Vergnes MF, Defoort C, Planells R, Portugal H, Nicolay A, Lairon D. Cholesterol absorption status and fasting plasma cholesterol are modulated by the microsomal triacylglycerol transfer protein -493 G/T polymorphism and the usual diet in women. *Genes Nutr* 2011; **6**: 71-79 [PMID: 21437032 DOI: 10.1007/s12263-010-0174-x]

38 **Berge KE**, Tian H, Graf GA, Yu L, Grishin NV, Schultz J, Kwiterovich P, Shan B, Barnes R, Hobbs HH. Accumulation of dietary cholesterol in sitosterolemia caused by mutations in adjacent ABC transporters. *Science* 2000; **290**: 1771-1775 [PMID: 11099417]

39 **Silbernagel G**, Fauler G, Renner W, Landl EM, Hoffmann MM, Winkelmann BR, Boehm BO, März W. The relationships of cholesterol metabolism and plasma plant sterols with the severity of coronary artery disease. *J Lipid Res* 2009; **50**: 334-341 [PMID: 18769018 DOI: 10.1194/jlr.P800013-JLR200]

40 **Ma KY**, Yang N, Jiao R, Peng C, Guan L, Huang Y, Chen ZY. Dietary calcium decreases plasma cholesterol by down-regulation of intestinal Niemann-Pick C1 like 1 and microsomal triacylglycerol transport protein and up-regulation of CYP7A1 and ABCG 5/8 in hamsters. *Mol Nutr Food Res* 2011; **55**: 247-258 [PMID: 20715096]

41 **Méndez-González J**, Julve J, Rotllan N, Llaverias G, Blanco-Vaca F, Escolà-Gil JC. ATP-binding cassette G5/G8 deficiency causes hypertriglyceridemia by affecting multiple metabolic pathways. *Biochim Biophys Acta* 2011; **1811**: 1186-1193 [PMID: 21855652 DOI: 10.1016/j.bbalip]

42 **Bloks VW**, Bakker-Van Waarde WM, Verkade HJ, Kema IP, Wolters H, Vink E, Groen AK, Kuipers F. Down-regulation of hepatic and intestinal Abcg5 and Abcg8 expression associated with altered sterol fluxes in rats with streptozotocin-induced diabetes. *Diabetologia* 2004; **47**: 104-112 [PMID: 14618236]

43 **Lally S**, Owens D, Tomkin GH. The different effect of pioglitazone as compared to insulin on expression of hepatic and intestinal genes regulating post-prandial lipoproteins in diabetes. *Atherosclerosis* 2007; **193**: 343-351 [PMID: 17109865]

44 **Lally S**, Owens D, Tomkin GH. Genes that affect cholesterol synthesis, cholesterol absorption, and chylomicron assembly: the relationship between the liver and intestine in control and streptozotosin diabetic rats. *Metabolism* 2007; **56**: 430-438 [PMID: 17292734]

45 **Levy E**, Lalonde G, Delvin E, Elchebly M, Précourt LP, Seidah NG, Spahis S, Rabasa-Lhoret R, Ziv E. Intestinal and hepatic cholesterol carriers in diabetic Psammomys obesus. *Endocrinology* 2010; **151**: 958-970 [PMID: 20130116 DOI: 10.1210/en.2009-0866]

46 **Levy E**, Spahis S, Ziv E, Marette A, Elchebly M, Lambert M, Delvin E. Overproduction of intestinal lipoprotein containing apolipoprotein B-48 in Psammomys obesus: impact of dietary n-3 fatty acids. *Diabetologia* 2006; **49**: 1937-1945 [PMID: 16788801]

47 **Wu G**, Li GB, Yao M, Zhang DQ, Dai B, Ju CJ, Han M. ABCG5/8 variants are associated with susceptibility to coronary heart disease. *Mol Med Rep* 2014; **9**: 2512-2520 [PMID: 24691589 DOI: 10.3892/mmr]

48 **Stender S**, Frikke-Schmidt R, Nordestgaard BG, Tybjaerg-Hansen A. The ABCG5/8 cholesterol transporter and myocardial infarction versus gallstone disease. *J Am Coll Cardiol* 2014; **63**: 2121-2128 [PMID: 24657701 DOI: 10.1016/j.jacc.2013.12.055]

49 **Altmann SW**, Davis HR, Zhu LJ, Yao X, Hoos LM, Tetzloff G, Iyer SP, Maguire M, Golovko A, Zeng M, Wang L, Murgolo N, Graziano MP. Niemann-Pick C1 Like 1 protein is critical for intestinal cholesterol absorption. *Science* 2004; **303**: 1201-1204 [PMID: 14976318]

50 **Sané AT**, Sinnett D, Delvin E, Bendayan M, Marcil V, Ménard D, Beaulieu JF, Levy E. Localization and role of NPC1L1 in cholesterol absorption in human intestine. *J Lipid Res* 2006; **47**: 2112-2120 [PMID: 16829661]

51 **Zhang JH**, Ge L, Qi W, Zhang L, Miao HH, Li BL, Yang M, Song BL. The N-terminal domain of NPC1L1 protein binds cholesterol and plays essential roles in cholesterol uptake. *J Biol Chem* 2011; **286**: 25088-25097 [PMID: 21602275 DOI: 10.1074/jbc.M111.244475]

52 **Fahmi S**, Yang C, Esmail S, Hobbs HH, Cohen JC. Functional characterization of genetic variants in NPC1L1 supports the sequencing extremes strategy to identify complex trait genes. *Hum Mol Genet* 2008; **17**: 2101-2107 [PMID: 18413323 DOI: 10.1093/hmg/ddn108]

53 **Wang LJ**, Wang J, Li N, Ge L, Li BL, Song BL. Molecular characterization of the NPC1L1 variants identified from cholesterol low absorbers. *J Biol Chem* 2011; **286**: 7397-7408 [PMID: 21189420 DOI: 10.1074/jbc.M110.178368]

54 **Calandra S**, Tarugi P, Speedy HE, Dean AF, Bertolini S, Shoulders CC. Mechanisms and genetic determinants regulating sterol absorption, circulating LDL levels, and sterol elimination: implications for classification and disease risk. *J Lipid Res* 2011; **52**: 1885-1926 [PMID: 21862702 DOI: 10.1194/jlr.R017855]

55 **Gleeson A**, Owens D, Collins P, Johnson A, Tomkin GH. The relationship between cholesterol absorption and intestinal cholesterol synthesis in the diabetic rat model. *Int J Exp Diabetes Res* 2000; **1**: 203-210 [PMID: 11467411]

56 **Betters JL**, Yu L. NPC1L1 and cholesterol transport. *FEBS Lett* 2010; **584**: 2740-2747 [PMID: 20307540 DOI: 10.1016/j.febslet.2010.03.030]

57 **Huff MW**, Pollex RL, Hegele RA. NPC1L1: evolution from pharmacological target to physiological sterol transporter. *Arterioscler Thromb Vasc Biol* 2006; **26**: 2433-2438 [PMID: 16973966]

58 **van der Veen JN**, Kruit JK, Havinga R, Baller JF, Chimini G, Lestavel S, Staels B, Groot PH, Groen AK, Kuipers F. Reduced cholesterol absorption upon PPARdelta activation coincides with decreased intestinal expression of NPC1L1. *J Lipid Res* 2005; **46**: 526-534 [PMID: 15604518]

59 **Valasek MA**, Clarke SL, Repa JJ. Fenofibrate reduces intestinal cholesterol absorption via PPARalpha-dependent modulation of NPC1L1 expression in mouse. *J Lipid Res* 2007; **48**: 2725-2735 [PMID: 17726195]

60 **Iwayanagi Y**, Takada T, Tomura F, Yamanashi Y, Terada T, Inui K, Suzuki H. Human NPC1L1 expression is positively regulated by PPARα. *Pharm Res* 2011; **28**: 405-412 [PMID: 20953676 DOI: 10.1007/s11095-010-0294]

61 **Tremblay AJ**, Lamarche B, Lemelin V, Hoos L, Benjannet S, Seidah NG, Davis HR, Couture P. Atorvastatin increases intestinal expression of NPC1L1 in hyperlipidemic men. *J Lipid Res* 2011; **52**: 558-565 [PMID: 21123766 DOI: 10.1194/jlr.M011080]

62 **Miller M**, DiNicolantonio JJ, Can M, Grice R, Damoulakis A, Serebruany VL. The effects of ezetimibe/simvastatin versus simvastatin monotherapy on platelet and inflammatory biomarkers in patients with metabolic syndrome. *Cardiology* 2013; **125**: 74-77 [PMID: 23652826 DOI: 10.1159/000347134]

63 **Ciriacks K**, Coly G, Krishnaswami S, Patel SB, Kidambi S. Effects of simvastatin and ezetimibe in lowering low-density lipoprotein cholesterol in subjects with type 1 and type 2 diabetes mellitus. *Metab Syndr Relat Disord* 2015; **13**: 84-90 [PMID: 25490061]

64 **Gylling H**, Laaksonen DE, Atalay M, Hallikainen M, Niskanen L, Miettinen TA. Markers of absorption and synthesis of cholesterol in men with type 1 diabetes. *Diabetes Metab Res Rev* 2007; **23**: 372-377 [PMID: 17080398]

65 **Wiersma H**, Nijstad N, Gautier T, Iqbal J, Kuipers F, Hussain MM, Tietge UJ. Scavenger receptor BI facilitates hepatic very low density lipoprotein production in mice. *J Lipid Res* 2010; **51**: 544-553 [PMID: 19723664 DOI: 10.1194/jlr.M000844]

66 **Iqbal J**, Hussain MM. Intestinal lipid absorption. *Am J Physiol Endocrinol Metab* 2009; **296**: E1183-E1194 [PMID: 19158321]

67 **Hayashi AA**, Webb J, Choi J, Baker C, Lino M, Trigatti B, Trajcevski KE, Hawke TJ, Adeli K. Intestinal SR-BI is upregulated in insulin-resistant states and is associated with overproduction of intestinal apoB48-containing lipoproteins. *Am J Physiol Gastrointest Liver*

Physiol 2011; **301**: G326-G337 [PMID: 21546579 DOI: 10.1152/ajpgi]

68 **Xie C**, Li N, Chen ZJ, Li BL, Song BL. The small GTPase Cdc42 interacts with Niemann-Pick C1-like 1 (NPC1L1) and controls its movement from endocytic recycling compartment to plasma membrane in a cholesterol-dependent manner. *J Biol Chem* 2011; **286**: 35933-35942 [PMID: 21844200 DOI: 10.1074/jbc.M111.270199]

69 **Wang Z**, Thurmond DC. Differential phosphorylation of RhoGDI mediates the distinct cycling of Cdc42 and Rac1 to regulate second-phase insulin secretion. *J Biol Chem* 2010; **285**: 6186-6197 [PMID: 20028975 DOI: 10.1074/jbc.M109.072421]

70 **Ravid Z**, Bendayan M, Delvin E, Sane AT, Elchebly M, Lafond J, Lambert M, Mailhot G, Levy E. Modulation of intestinal cholesterol absorption by high glucose levels: impact on cholesterol transporters, regulatory enzymes, and transcription factors. *Am J Physiol Gastrointest Liver Physiol* 2008; **295**: G873-G885 [PMID: 18772361 DOI: 10.1152/ajpgi.90376.2008]

71 **Grenier E**, Mailhot G, Dion D, Ravid Z, Spahis S, Bendayan M, Levy E. Role of the apical and basolateral domains of the enterocyte in the regulation of cholesterol transport by a high glucose concentration. *Biochem Cell Biol* 2013; **91**: 476-486 [PMID: 24219290 DOI: 10.1139/bcb-2013-0053]

72 **Malhotra P**, Boddy CS, Soni V, Saksena S, Dudeja PK, Gill RK, Alrefai WA. D-Glucose modulates intestinal Niemann-Pick C1-like 1 (NPC1L1) gene expression via transcriptional regulation. *Am J Physiol Gastrointest Liver Physiol* 2013; **304**: G203-G210 [PMID: 23139223 DOI: 10.1152/ajpgi.00288.2012]

73 **Zhou L**, Yang H, Okoro EU, Guo Z. Up-regulation of cholesterol absorption is a mechanism for cholecystokinin-induced hypercholesterolemia. *J Biol Chem* 2014; **289**: 12989-12999 [PMID: 24692543 DOI: 10.1074/jbc.M113.534388]

74 **Irwin N**, Frizelle P, O'Harte FP, Flatt PR. Metabolic effects of activation of CCK receptor signaling pathways by twice-daily administration of the enzyme-resistant CCK-8 analog, (pGlu-Gln)-CCK-8, in normal mice. *J Endocrinol* 2013; **216**: 53-59 [PMID: 23055535]

75 **Rosenstock J**, Truitt KE, Baz-Hecht M, Ford DM, Tao B, Chou HS. Efficacy and safety of colesevelam in combination with pioglitazone in patients with type 2 diabetes mellitus. *Horm Metab Res* 2014; **46**: 943-949 [PMID: 25054436 DOI: 10.1055/s-0034-1383648]

76 **Sonne DP**, Hansen M, Knop FK. Bile acid sequestrants in type 2 diabetes: potential effects on GLP1 secretion. *Eur J Endocrinol* 2014; **171**: R47-R65 [PMID: 24760535 DOI: 10.1530/EJE-14-0154]

77 **Devery RA**, O'Meara N, Collins PB, Johnson AH, Scott L, Tomkin GH. A comparative study of the rate-limiting enzymes of cholesterol synthesis, esterification and catabolism in the alloxan-induced diabetic rat and rabbit. *Comp Biochem Physiol B* 1987; **88**: 547-550 [PMID: 3427902]

78 **Sittiwet C**, Gylling H, Hallikainen M, Pihlajamäki J, Moilanen L, Laaksonen DE, Niskanen L, Agren JJ, Laakso M, Miettinen TA. Cholesterol metabolism and non-cholesterol sterol distribution in lipoproteins of type 1 diabetes: the effect of improved glycemic control. *Atherosclerosis* 2007; **194**: 465-472 [PMID: 16996519]

79 **Zhao L**, Chen Y, Tang R, Chen Y, Li Q, Gong J, Huang A, Varghese Z, Moorhead JF, Ruan XZ. Inflammatory stress exacerbates hepatic cholesterol accumulation via increasing cholesterol uptake and de novo synthesis. *J Gastroenterol Hepatol* 2011; **26**: 875-883 [PMID: 21488946 DOI: 10.1111/j.1440-1746.2010.06560.x]

80 **Xie X**, Liao H, Dang H, Pang W, Guan Y, Wang X, Shyy JY, Zhu Y, Sladek FM. Down-regulation of hepatic HNF4alpha gene expression during hyperinsulinemia via SREBPs. *Mol Endocrinol* 2009; **23**: 434-443 [PMID: 19179483 DOI: 10.1210/me.2007-0531]

81 **Van Rooyen DM**, Farrell GC. SREBP-2: a link between insulin resistance, hepatic cholesterol, and inflammation in NASH. *J Gastroenterol Hepatol* 2011; **26**: 789-792 [PMID: 21488942 DOI: 10.1111/j.1440]

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MINIREVIEWS

Serum hepcidin concentrations and type 2 diabetes

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Abstract

Hepcidin is a peptide hormone with both paracrine and endocrine functions that help in maintaining body iron stores. Type 2 diabetes (T2D) is one of the sequelae of excess body iron stores; thus, iron regulatory hormone hepcidin may have a direct or at least an indirect role in the aetiopathogenesis of T2D. Both human and animal studies at molecular and genetic levels have attempted to establish a role for hepcidin in the development of T2D, and a few epidemiologic studies have also showed a link between hepcidin and T2D at population level, but the findings are still inconclusive. Recent data have suggested different pathways in which hepcidin could be associated with T2D with much emphasis on its primary or secondary role in insulin resistance. Some of the suggested pathways are *via* transcription modulator of hepcidin (STAT3); ferroportin 1 expression on the cells involved in iron transport; transmembrane protease 6 enzyme; and pro-inflammatory cytokines, interleukin (IL)-1, IL-6, tumor necrosis factor-α and IL-10. This review briefly reports the existing evidence on the possible links between hepcidin and T2D and concludes that more data are needed to confirm or refute hepcidin's role in the development of T2D. Examining this role could provide a further evidence base for iron in the aetiopathogenesis of T2D.

Key words: Serum hepcidin; Body iron; Diabetes; Type 2 diabetes; Insulin resistance

Core tip: Excess body iron has been demonstrated as an independent risk factor of type 2 diabetes (T2D). Lately, manipulation of serum hepcidin concentrations through the use of hepcidin agonist is being suggested in the management of iron overload diseases, of which T2D is one. However, little is known about the role of hepcidin in the development of T2D; hence, the need for a review of the existing evidence linking hepcidin and T2D. We discuss some of the main mechanisms through which hepcidin could be associated with T2D.

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INTRODUCTION

Recently, attention has been shifting towards the iron-regulatory hormone hepcidin and its possible role in the aetiopathogenesis of type 2 diabetes (T2D). Hepcidin is primarily a hepatic peptide synthesized as a preprohepcidin, which is a 84-amino acid peptide. It undergoes enzymatic cleavage into a 60- to 64-residue prohepcidin peptide and finally into a biologically active 25-amino acid peptide hormone, hepcidin[1-3]. Tissues of the kidney[4], pancreatic beta cell[5], macrophages[6] and adipocytes[7] have also been reported to produce hepcidin, but the role of this extra hepatic contribution to serum hepcidin is still unclear.

Hepcidin's role is to maintain iron homeostasis[8,9]. It performs this action by regulating the expression and function of cell membrane-embedded ferroportin (FPN)[10], the cellular iron exporter in iron-transporting cells[8,9]. In response to the level of body iron stores, hepcidin regulates dietary iron absorption from the intestine and iron release from macrophages, by decreasing the cell surface expression of FPN.

Among the known diseases associated with the iron overload syndrome is T2D[11,12]. Even mildly elevated body iron has been demonstrated as a risk factor of T2D[13]. Some recent epidemiologic studies have tried to explore the association between serum hepcidin and T2D, with inconsistent findings[14-16]. Also, serum glucose concentration has been shown to regulate serum hepcidin[17]. As the underlying mechanisms between body iron stores and T2D still need further clarifications, hepcidin could provide some answers. Therefore, we reviewed the emerging links between hepcidin and T2D.

ROLE OF HEPCIDIN IN IRON METABOLISM

Iron is a transition metal that is predominantly absorbed in the duodenum and upper jejunum[18]. The intestinal absorption of iron is in two waves of uptake of iron from intestinal lumen into the intestinal mucosa and from mucosa into the blood[19]. The two known forms of dietary iron are heme and non-heme iron. The heme iron is well absorbed by the body while the non heme iron predominantly in ferric form Fe^{3+} is reduced to ferrous Fe^{2+} form by the duodenal cytochrome b reductase[20]. This is transported by the divalent-cation transporter 1, a member of the natural- resistance-associated macrophage protein family, across the intestinal apical membrane[21], and later stored in the cytosol of ferritin or exported from the basolateral membrane of the enterocytes *via* FPN1. Because of its numerous health effects due to excess or deficiency, there is a need to strictly regulate iron within the physiological range.

Following a feedback mechanism, hepcidin is either up- or down-regulated by hepatocytes, depending on the level of body iron. Genetically, the mRNA of the gene (*HEPC*) coding for hepcidin increases with increase in body iron stores. At molecular level, hepcidin regulates iron transport from iron-exporting tissue into plasma by inhibiting intestinal iron absorption[9], release of iron from macrophages[22] and the placental passage of iron[23]. It performs these functions by binding to FPN1, which is expressed on the duodenal enterocytes, hepatocytes, placental syncytiotrophoblasts and the reticuloendothelia macrophages. It later internalizes and degrades FPN1 leading to a reduction in the ability of these cells to export iron into the plasma[8]. Conversely, there is re-expression of FPN1 on these iron-exporting cells in iron deficiency. However, it must be noted that body iron is not the only stimulus for hepcidin release. Hepcidin also responds to inflammation, infection, or both; as such, hepcidin appears to be a link between iron and inflammation. The antimicrobial properties of hepcidin would require conditions inconsistent with those observed in the serum, further emphasizing its iron-regulatory role rather than its broad-spectrum antibiotic activity[24].

MECHANISMS LINKING HEPCIDIN AND T2D

Insulin resistance is a feature of T2D, and the relationship between iron metabolism and insulin resistance has been suggested to be bidirectional[25]. However, the association between hepcidin and insulin resistance remains vague. Molecular studies have showed that insulin stimulates hepcidin *via* STAT3, which is a novel transcription modulator of hepcidin[26]. A study by Wang *et al*[26], in which they induced diabetes in rats using streptozotocin with or without high-fat diet, showed a significant reduction in hepcidin expression in the liver, mediated by STAT3, causing abnormal elevation of FPN in the intestine, leading to serum iron elevation. Le Guenno *et al*[27] showed a 3.5-fold reduction in hepcidin mRNA in the group with higher insulin resistance when compared to the control group. Some previous studies[15,28,29] also have shown reduced hepcidin and prohepcidin concentrations in T2D subjects, suggesting insulin signal loss among T2D subjects with elevated iron stores.

Another mechanistic link between hepcidin and insulin is through glucose stimulation. One of the extrahepatic sources of serum hepcidin is the beta cell of the pancreas[5]. Insulin and hepcidin release have also been localized to beta cell granules where hepcidin may evoke its paracrine function. Thus, as glucose stimulates insulin release, there is a concomitant production of hepcidin. In the trial arm of a study by Aigner *et al*[17] in which they assessed the effect of glucose on serum iron and hepcidin, they found an increase in serum hepcidin in glucose-treated subjects compared to the control group treated with only water.

To further strengthen the association between hepcidin and T2D, a genome-wide association study (GWAS) by Gan *et al*[30] evaluated the association of transmembrane protease serine 6 (TMPRSS6) variants

Table 1 Studies linking hepcidin/prohepcidin and type 2 diabetes (n = 6)

Ref.	Subject/study design	Measurement of hepcidin/prohepcidin	Assessment of T2D	Results
Sam *et al*[15]	British men and women/case-control	Active serum hepcidin-25 and serum hepcidin-ferritin ratio measured using RIA	HbA1c = 73.17 ± 4.12, mean ± SEM	Student's *t*-test/Mann-Whitney *U*-test to compare hepcidin (ng/mL) and hepcidin-ferritin ratio in T2D *vs* control showed: 20.00 (10.00-41.00) *vs* 33.00 (18.05-54.00), $P < 0.05$ and 0.22 (0.15-0.32) *vs* 0.45 (0.26-0.58), $P < 0.01$
Guo *et al*[16]	Chinese men and women/case-control	Serum hepcidin measured using ELISA	Fasting plasma glucose > 7 mmol/L	Wilcoxon rank test to compare hepcidin (ng/mL) in T2D *vs* control showed: 34.44 ± 26.98 *vs* 32.34 ± 22.75, $P = 0.72$. Logistic regression analysis showed no significant association between serum hepcidin concentrations and onset of T2D: OR = 1.03, 95%CI: 0.87-1.22, $P = 0.75$
Gan *et al*[30]	Chinese men and women/prospective cohort	Two TMPRSS6 SNPs [rs855791 (V736A) and rs4820268 (D521D)] of hepcidin by DNA genotyping	WHO 1999 criteria or previous history of T2D. Fasting plasma glucose < 5.6 mmol/L = normoglycemia	Logistic regression analysis showed both 2 TMPRSS6 SNPs to be significantly associated with decreased risk of T2D:$OR_{rs855791\ (v736A)}$ = 0.801, 95%CI: 0.654-0.98, $P = 0.0314$ and $OR_{rs4820268(D521D)}$ = 0.802, 95%CI: 0.656-0.98, $P = 0.0311$
Jiang *et al*[14]	Chinese men and women/case-control	Serum hepcidin measured using ELISA	Fasting glucose ⩾ 7.0 mmol/L, non-fasting glucose ⩾ 11.1 mmol/L, use of diabetes medication, or a self-reported physician diagnosis	Kruskal-Wallis test/student's *t*-test to compare hepcidin (μg/L) in T2D *vs* control showed: 778.91 ± 175.22 *vs* 513.44 ± 281.73, $P < 0.001$
Aso *et al*[29]	Japanese men and women/case-control	Serum prohepcidin measured using ELIA	Not reported	Mann-Whitney *U* test to compare prohepcidin (ng/mL) in T2D *vs* control showed: 141 ± 42.6 *vs* 198.1 ± 36.7, $P < 0.0001$
Fernández-Real *et al*[28]	Spanish men/cross-sectional and intervention	Serum prohepcidin measured using ELISA	Oral glucose tolerance test	Pearson's test showed significant correlation between circulating prohepcidin and fasting glucose ($r = 0.27$; $P = 0.002$) and HbA1c ($r = 0.31$; $P < 0.0001$). After phlebotomy, prohepcidin decreased significantly in T2D ($P = 0.04$) and in *HFE* gene mutation carrier (0.03) with a negative correlation between serum prohepcidin and insulin sensitivity ($r = -0.50$, $P = 0.04$)

HbA1c: Glycated hemoglobin; T2D: Type 2 diabetes; RIA: Radioimmunoassay; ELISA: Enzyme linked immunosorbent assay; TMPRSS6: Transmembrane protease serine 6; SNPs: Single nucleotide polymorphisms; WHO: World Health Organization.

with risk of T2D. TMPRSS6 is an enzyme that inhibits the expression of hepcidin and its iron-lowering variants were used in their study. They found a reduced risk between the two TMPRSS6 variants and T2D ($P = 0.0277$). It is at least plausible to report that lower hepcidin concentration exacerbates insulin resistance seen in T2D, if causal relationship is yet to be fully established.

T2D is a dysmetabolic state characterized by low-grade chronic inflammation, and some authors have suggested a role for pro-inflammatory cytokines such as interleukins (IL)-1, IL-6 and tumor necrosis factor-α, in the development of insulin resistance[31], and in addition, IL-10 in T2D[32]. Increased circulating concentrations of these pro-inflammatory cytokines have also been observed in T2D subjects[33]. IL-6 is the most important inflammatory cytokine regulating hepcidin, and it performs this function during STAT3 phosphorylation, thus activating STAT3 for hepcidin gene expression[24]. STAT3 has been reported as necessary and sufficient for IL-6 responsiveness of the hepcidin promoter[34]. Thus, a low hepcidin concentration could stimulate IL-6, thereby enhancing its role in the development of T2D. A population-based study by Spranger *et al*[35] showed that IL-1β and IL-6 concentrations predict the risk of T2D. It is therefore enticing to speculate from the available evidence that hepcidin has a role in insulin resistance, the hallmark of T2D, through iron regulation from interrelated signals of STAT3, pro-inflammatory cytokines and the TMPRSS6 enzyme.

STUDIES LINKING HEPCIDIN AND T2D

Existing data across different populations suggest a link between hepcidin and T2D (Table 1). There are four case-control studies[14-16,29]. In Sam *et al*[15] study, the authors measured serum hepcidin and serum hepcidin:ferritin ratio, which has been suggested as a marker of adequate hepcidin production for a particular iron dosage[36]. Aso *et al*[29] also measured serum ferritin, prohepcidin and adiponectin, and both studies showed decrease in serum hepcidin/prohepcidin in T2D subjects when compared with the healthy control[15,29]. Because of the confounding effect of obesity and renal status in serum hepcidin measurement, Sam *et al*[15] matched their control subjects for body mass index (BMI) and serum creatinine. Aso *et al*[29] assessed the correlation between adiponectin and prohepcidin in T2D subjects on the basis that adiponectin has a beneficial role in

glucose homeostasis[37]; hence, in glucose dysregulation observed in T2D, adiponectin and prohepcidin were expected to be low. In keeping with their hypothesis, Aso *et al*[29] found low concentrations of adiponectin and prohepcidin and a positive correlation between them in T2D subjects. The other two case-control studies[14,16] showed increased hepcidin/prohepcidin in T2D subjects when compared with the control group. In Jiang *et al*[14] study, T2D subjects had higher BMI and creatinine than the controls, thus suggesting obesity and renal impairment as possible reasons for the elevated serum hepcidin. Further, inflammatory markers, *i.e.*, IL-6, C-reactive protein and white cell counts, were elevated in T2D subjects compared to the controls, speculating inflammatory signals as the cause of the elevated hepcidin. One factor that could be responsible for the contradictory findings in the hepcidin-T2D association study is the wide range of assays with varying degrees of limitation that were used in evaluating serum hepcidin and serum prohepcidin. The lack of an accurate assay to evaluate serum hepcidin in the past may have influenced some investigators to choose prohepcidin, which is thought to be easier to measure due to its higher immunogenicity. Although some studies have shown that prohepcidin does not accurately reflect iron status and iron absorption[38], others have claimed that it is an indicator of endogenous hepcidin levels[39] in healthy subjects. This could be the reason why some studies used hepcidin while others used prohepcidin in examining the association between hepcidin and T2D. This is of particular interest in chronic renal disease patients in end-stage renal failure, where there is cross-reactivity in serum hepcidin-25 measurement with that of other hepcidins, *i.e.*, hepcidin-20 and hepcidin-22[40]. The question then arises whether the concentration of hepcidin in T2D subjects is primary or secondary to elevated body iron stores. The GWAS with a prospective design by Gan *et al*[30] at least provided further insight into the role of hepcidin as they showed a decreased risk in developing T2D with the iron-lowering variants of TMPRSS6. Also, in the trial arm of the three-in-one study by Fernández-Real *et al*[28], a negative correlation was observed between prohepcidin and insulin sensitivity after phlebotomy to reduce body iron stores, suggesting an association between prohepcidin and body iron in insulin sensitivity.

CONCLUSION

In conclusion, we have briefly reviewed the emerging evidence pertaining to the role of hepcidin in T2D. Although the causative role of body iron in insulin resistance and T2D has been documented in both observational[41] and interventional studies[42], the role of hepcidin in this process is still uncertain. However, in addition to the regulatory role in body iron stores, serum hepcidin concentrations have been linked to pro-inflammatory cytokines, STAT3, and TMPRSS6, all of which have been associated with T2D. Data gathered in this review showed that hepcidin concentrations vary in different populations with T2D. Further, hepcidin has either a primary or secondary role in insulin resistance which characterizes T2D. However, it is still inconclusive from these accumulated data that serum hepcidin is an independent risk factor in the aetiopathogenesis of T2D. Thus, more experimental and clinical studies are needed to confirm or refute the claim that hepcidin has a role in T2D.

REFERENCES

1 **Krause A**, Neitz S, Mägert HJ, Schulz A, Forssmann WG, Schulz-Knappe P, Adermann K. LEAP-1, a novel highly disulfide-bonded human peptide, exhibits antimicrobial activity. *FEBS Lett* 2000; **480**: 147-150 [PMID: 11034317 DOI: 10.1016/S0014-5793(00)01920-7]

2 **Park CH**, Valore EV, Waring AJ, Ganz T. Hepcidin, a urinary antimicrobial peptide synthesized in the liver. *J Biol Chem* 2001; **276**: 7806-7810 [PMID: 11113131 DOI: 10.1074/jbc.M008922200]

3 **Singh B**, Arora S, Agrawal P, Gupta SK. Hepcidin: a novel peptide hormone regulating iron metabolism. *Clin Chim Acta* 2011; **412**: 823-830 [PMID: 21333642]

4 **Kulaksiz H**, Theilig F, Bachmann S, Gehrke SG, Rost D, Janetzko A, Cetin Y, Stremmel W. The iron-regulatory peptide hormone hepcidin: expression and cellular localization in the mammalian kidney. *J Endocrinol* 2005; **184**: 361-370 [PMID: 15684344 DOI: 10.1677/joe.1.05729]

5 **Kulaksiz H**, Fein E, Redecker P, Stremmel W, Adler G, Cetin Y. Pancreatic beta-cells express hepcidin, an iron-uptake regulatory peptide. *J Endocrinol* 2008; **197**: 241-249 [PMID: 18434354 DOI: 10.1677/JOE-07-0528]

6 **Sow FB**, Florence WC, Satoskar AR, Schlesinger LS, Zwilling BS, Lafuse WP. Expression and localization of hepcidin in macrophages: a role in host defense against tuberculosis. *J Leukoc Biol* 2007; **82**: 934-945 [PMID: 17609338 DOI: 10.1189/jlb.0407216]

7 **Bekri S**, Gual P, Anty R, Luciani N, Dahman M, Ramesh B, Iannelli A, Staccini-Myx A, Casanova D, Ben Amor I, Saint-Paul MC, Huet PM, Sadoul JL, Gugenheim J, Srai SK, Tran A, Le Marchand-Brustel Y. Increased adipose tissue expression of hepcidin in severe obesity is independent from diabetes and NASH. *Gastroenterology* 2006; **131**: 788-796 [PMID: 16952548 DOI: 10.1053/j.gastro.2006.07.007]

8 **Nemeth E**, Tuttle MS, Powelson J, Vaughn MB, Donovan A, Ward DM, Ganz T, Kaplan J. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science* 2004; **306**: 2090-2093 [PMID: 15514116 DOI: 10.1126/science.1104742]

9 **Ganz T**. Hepcidin and its role in regulating systemic iron metabolism. *Hematology Am Soc Hematol Educ Program* 2006: 29-35, 507 [PMID: 17124036 DOI: 10.1182/asheducation-2006.1.29]

10 **Franchini M**, Montagnana M, Lippi G. Hepcidin and iron metabolism: from laboratory to clinical implications. *Clin Chim Acta* 2010; **411**: 1565-1569 [PMID: 20620132 DOI: 10.1016/j.cca.2010.07.003]

11 **Kell DB**. Iron behaving badly: inappropriate iron chelation as a major contributor to the aetiology of vascular and other progressive inflammatory and degenerative diseases. *BMC Med Genomics* 2009; **2**: 2 [PMID: 19133145 DOI: 10.1186/1755-8794-2-2]

12 **Adams PC**, Kertesz AE, Valberg LS. Clinical presentation of hemochromatosis: a changing scene. *Am J Med* 1991; **90**: 445-449 [PMID: 2012084 DOI: 10.1016/0002-9343(91)80084-Y]

13 **Aregbesola A**, Voutilainen S, Virtanen JK, Mursu J, Tuomainen TP. Body iron stores and the risk of type 2 diabetes in middle-aged men. *Eur J Endocrinol* 2013; **169**: 247-253 [PMID: 23715774 DOI: 10.1530/EJE-13-0145]

14 **Jiang F**, Sun ZZ, Tang YT, Xu C, Jiao XY. Hepcidin expression and iron parameters change in Type 2 diabetic patients. *Diabetes*

Res Clin Pract 2011; **93**: 43-48 [PMID: 21513996 DOI: 10.1016/j.diabres.2011.03.028]
15 **Sam AH**, Busbridge M, Amin A, Webber L, White D, Franks S, Martin NM, Sleeth M, Ismail NA, Daud NM, Papamargaritis D, Le Roux CW, Chapman RS, Frost G, Bloom SR, Murphy KG. Hepcidin levels in diabetes mellitus and polycystic ovary syndrome. *Diabet Med* 2013; **30**: 1495-1499 [PMID: 23796160 DOI: 10.1111/dme.12262]
16 **Guo X**, Zhou D, An P, Wu Q, Wang H, Wu A, Mu M, Zhang D, Zhang Z, Wang H, He L, Liu Y, Wang F. Associations between serum hepcidin, ferritin and Hb concentrations and type 2 diabetes risks in a Han Chinese population. *Br J Nutr* 2013; **110**: 2180-2185 [PMID: 23742704 DOI: 10.1017/S0007114513001827]
17 **Aigner E**, Felder TK, Oberkofler H, Hahne P, Auer S, Soyal S, Stadlmayr A, Schwenoha K, Pirich C, Hengster P, Datz C, Patsch W. Glucose acts as a regulator of serum iron by increasing serum hepcidin concentrations. *J Nutr Biochem* 2013; **24**: 112-117 [PMID: 22819549 DOI: 10.1016/j.jnutbio.2012.02.017]
18 **Muir A**, Hopfer U. Regional specificity of iron uptake by small intestinal brush-border membranes from normal and iron-deficient mice. *Am J Physiol* 1985; **248**: G376-G379 [PMID: 3976894]
19 **Wheby MS**, Jones LG, Crosby WH. Studies on iron absorption. intestinal regulatory mechanisms. *J Clin Invest* 1964; **43**: 1433-1442 [PMID: 14192524 DOI: 10.1172/JCI105019]
20 **Su D**, Asard H. Three mammalian cytochromes b561 are ascorbate-dependent ferrireductases. *FEBS J* 2006; **273**: 3722-3734 [PMID: 16911521 DOI: 10.1111/j.1742-4658.2006.05381.x]
21 **Gunshin H**, Mackenzie B, Berger UV, Gunshin Y, Romero MF, Boron WF, Nussberger S, Gollan JL, Hediger MA. Cloning and characterization of a mammalian proton-coupled metal-ion transporter. *Nature* 1997; **388**: 482-488 [PMID: 9242408]
22 **Fleming RE**, Sly WS. Hepcidin: a putative iron-regulatory hormone relevant to hereditary hemochromatosis and the anemia of chronic disease. *Proc Natl Acad Sci USA* 2001; **98**: 8160-8162 [PMID: 11459944 DOI: 10.1073/pnas.161296298]
23 **Nicolas G**, Bennoun M, Porteu A, Mativet S, Beaumont C, Grandchamp B, Sirito M, Sawadogo M, Kahn A, Vaulont S. Severe iron deficiency anemia in transgenic mice expressing liver hepcidin. *Proc Natl Acad Sci USA* 2002; **99**: 4596-4601 [PMID: 11930010 DOI: 10.1073/pnas.072632499]
24 **Fleming RE**. Iron and inflammation: cross-talk between pathways regulating hepcidin. *J Mol Med* (Berl) 2008; **86**: 491-494 [PMID: 18425494 DOI: 10.1007/s00109-008-0349-8]
25 **Fernández-Real JM**, López-Bermejo A, Ricart W. Cross-talk between iron metabolism and diabetes. *Diabetes* 2002; **51**: 2348-2354 [PMID: 12145144 DOI: 10.2337/diabetes.51.8.2348]
26 **Wang H**, Li H, Jiang X, Shi W, Shen Z, Li M. Hepcidin is directly regulated by insulin and plays an important role in iron overload in streptozotocin-induced diabetic rats. *Diabetes* 2014; **63**: 1506-1518 [PMID: 24379355 DOI: 10.2337/db13-1195]
27 **Le Guenno G**, Chanséaume E, Ruivard M, Morio B, Mazur A. Study of iron metabolism disturbances in an animal model of insulin resistance. *Diabetes Res Clin Pract* 2007; **77**: 363-370 [PMID: 17350134 DOI: 10.1016/j.diabres.2007.02.004]
28 **Fernández-Real JM**, Equitani F, Moreno JM, Manco M, Ortega F, Ricart W. Study of circulating prohepcidin in association with insulin sensitivity and changing iron stores. *J Clin Endocrinol Metab* 2009; **94**: 982-988 [PMID: 19116239 DOI: 10.1210/jc.2008-1211]
29 **Aso Y**, Takebayashi K, Wakabayashi S, Momobayashi A, Sugawara N, Terasawa T, Naruse R, Hara K, Suetsugu M, Morita K, Inukai T. Relation between serum high molecular weight adiponectin and serum ferritin or prohepcidin in patients with type 2 diabetes. *Diabetes Res Clin Pract* 2010; **90**: 250-255 [PMID: 20888657 DOI: 10.1016/j.diabres.2010.09.008]
30 **Gan W**, Guan Y, Wu Q, An P, Zhu J, Lu L, Jing L, Yu Y, Ruan S, Xie D, Makrides M, Gibson RA, Anderson GJ, Li H, Lin X, Wang F. Association of TMPRSS6 polymorphisms with ferritin, hemoglobin, and type 2 diabetes risk in a Chinese Han population. *Am J Clin Nutr* 2012; **95**: 626-632 [PMID: 22301935 DOI: 10.3945/ajcn.111.025684]
31 **Tilg H**, Moschen AR. Inflammatory mechanisms in the regulation of insulin resistance. *Mol Med* 2008; **14**: 222-231 [PMID: 18235842 DOI: 10.2119/2007-00119.Tilg]
32 **Saxena M**, Srivastava N, Banerjee M. Association of IL-6, TNF-α and IL-10 gene polymorphisms with type 2 diabetes mellitus. *Mol Biol Rep* 2013; **40**: 6271-6279 [PMID: 24057184 DOI: 10.1007/s11033-013-2739-4]
33 **Pickup JC**, Chusney GD, Thomas SM, Burt D. Plasma interleukin-6, tumour necrosis factor alpha and blood cytokine production in type 2 diabetes. *Life Sci* 2000; **67**: 291-300 [PMID: 10983873 DOI: 10.1016/S0024-3205(00)00622-6]
34 **Wrighting DM**, Andrews NC. Interleukin-6 induces hepcidin expression through STAT3. *Blood* 2006; **108**: 3204-3209 [PMID: 16835372 DOI: 10.1182/blood-2006-06-027631]
35 **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]
36 **Piperno A**, Girelli D, Nemeth E, Trombini P, Bozzini C, Poggiali E, Phung Y, Ganz T, Camaschella C. Blunted hepcidin response to oral iron challenge in HFE-related hemochromatosis. *Blood* 2007; **110**: 4096-4100 [PMID: 17724144 DOI: 10.1182/blood-2007-06-096503]
37 **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]
38 **Roe MA**, Spinks C, Heath AL, Harvey LJ, Foxall R, Wimperis J, Wolf C, Fairweather-Tait SJ. Serum prohepcidin concentration: no association with iron absorption in healthy men; and no relationship with iron status in men carrying HFE mutations, hereditary haemochromatosis patients undergoing phlebotomy treatment, or pregnant women. *Br J Nutr* 2007; **97**: 544-549 [PMID: 17313717 DOI: 10.1017/S0007114507336829]
39 **Hadley KB**, Johnson LK, Hunt JR. Iron absorption by healthy women is not associated with either serum or urinary prohepcidin. *Am J Clin Nutr* 2006; **84**: 150-155 [PMID: 16825689]
40 **Peters HP**, Laarakkers CM, Swinkels DW, Wetzels JF. Serum hepcidin-25 levels in patients with chronic kidney disease are independent of glomerular filtration rate. *Nephrol Dial Transplant* 2010; **25**: 848-853 [PMID: 19854845 DOI: 10.1093/ndt/gfp546]
41 **Fernández-Real JM**, López-Bermejo A, Ricart W. Iron stores, blood donation, and insulin sensitivity and secretion. *Clin Chem* 2005; **51**: 1201-1205 [PMID: 15976100 DOI: 10.1373/clinchem.2004.046847]
42 **Facchini FS**. Effect of phlebotomy on plasma glucose and insulin concentrations. *Diabetes Care* 1998; **21**: 2190 [PMID: 9839115 DOI: 10.2337/diacare.21.12.2190a]

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ORIGINAL ARTICLE

Observational Study

CD36 expression and lipid metabolism following an oral glucose challenge in South Asians

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Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at Dryad repository, who will provide a permanent, citable and open-access home for the dataset.

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Abstract

AIM: To investigate lipid metabolism and the relationship with monocyte expression of the fatty acid translocase CD36 in South Asians.

METHODS: An observational study of South Asians whom as an ethnic group have - a higher risk of developing diabetes. The susceptibility to diabetes is coupled with an earlier and more rapid progression of micro-, and macro-vascular complications. Twenty-nine healthy South Asian participants [mean age 34.6 (8.9) years, 76.2% male, mean body-mass index 25.0 (5.2) kg/m^2] were recruited from an urban residential area of central Birmingham (United Kingdom). The main outcomes measured were post prandial (30 min) and post absorptive (120 min) changes from fasting (0 min) in circulating lipoproteins, lipds and hormones, and

monocyte expression of CD36 post injection of a 75 g oral glucose challenge. The inducements of variations of monocyte CD36 expression were analysed.

RESULTS: Our results showed evident changes in monocyte CD36 expression following the glucose challenge ($P < 0.001$). Non-esterified fatty acids (NEFA) levels decreased progressively during the challenge ($P < 0.001$), in contrast to increased cholesterol (but not triglyceride) concentrations within very low density lipoprotein (VLDL) and low density lipoprotein subfractions ($P < 0.01$). Levels of, glucose, serum triglycerides and high density lipoprotein cholesterol remained largely unchanged. Variations of monocyte CD36 were negatively ($r = -0.47$, $P = 0.04$) associated to fat from the diet and positively to carbohydrate from the diet ($r = 0.65$, $P < 0.001$).

CONCLUSION: These data suggest that the initiation of VLDL genesis follows the consumption of glucose within this population, inferring that the sequestration of NEFA from these particles happens due to the increased availability of CD36 receptors. While these are preliminary results, it would appear that lifestyle exposures have a role in moderating the expression of CD36.

Key words: CD36; Lipoprotein; Glucose; South Asians; Diabetes; Micro-vascular; Macro-vascular

Core tip: This study investigated the relationship between the expression of fatty acid translocase CD36 on monocytes and lipid and lipoprotein metabolism in South Asians. Post prandial and post absorptive changes from fasting in circulating lipids, lipoproteins, hormones and monocyte expression of CD36 were recorded subsequent to an oral glucose challenge. Our results showed discernible changes in monocyte CD36 expression post glucose administration. These data suggest that the production of very low density lipoprotein occurs subsequent to the ingestion of glucose within this population. It is presumed that the sequestration of non-esterified fatty acids from these particles happens due to an increase in availability of CD36 receptors.

Patel JV, Banerjee A, Montoro-Garcia S, Shantsila E, Alam M, Flinders P, Houlton KAL, Hughes EA, Lip GYH, Gill PS. CD36 expression and lipid metabolism following an oral glucose challenge in South Asians. *World J Diabetes* 2015; 6(7): 983-989 Available from: URL: http://www.wjgnet.com/1948-9358/full/v6/i7/983.htm DOI: http://dx.doi.org/10.4239/wjd.v6.i7.983

INTRODUCTION

The prevalence of diabetes is increased on the Indian subcontinent[1]. Globally dispersed migrant populations of "South Asians" also have high rates of diabetes, which is evident when compared to the various indigenous populations of where they migrated[2-6]. Nephropathy and retinopathy develop earlier and are more progressive in South Asian diabetics compared to White diabetics[7-11]. The incidence of vascular disease is also higher in South Asians compared to the general White population[12].

Established risk factors such as obesity and urbanised lifestyle "operate" amongst South Asians but it remains unclear as to how far they explain an increased susceptibility to diabetes for this group. Rates of glucose intolerance amongst Indians living in rural village settings appear to be no different to their migrant contemporaries living overseas in Western countries[5,6]. We and others have observed that high glucose excursions in South Asians are underpinned by unregulated non-esterified fatty acids (NEFA) metabolism[13,14]. This phenomenon is likely to be complex and multifactorial, and we were interested to investigate the role of the fatty acid translocase CD36[15], which is the major facilitator of NEFA sequestration from the blood[16]. Specifically, CD36 allows the transport of NEFA that are generated from the lipolysis of triglyceride-rich lipoproteins such as very low density lipoprotein (VLDL) across plasma membranes, and into cells (*e.g.*, monocytes, cardiomyocytes and adipocytes) for fatty acid oxidation and lipid deposition[17]. Cardiomyocytes use glucose in addition to fatty acids for cellular respiration, and the expression of glucose transporter 4 (GLUT4) and the fatty acid transporter CD36 is stimulated by raised insulin concentrations and an increase in cardiac work[18].

Given that abnormalities of increased CD36 expression and NEFA uptake may represent a common cause for diabetes and the progression of its complications[19-21], we measured changes in the expression of CD36 on circulating monocytes and it association with direct indices of NEFA metabolism amongst South Asians during an oral glucose challenge.

MATERIALS AND METHODS

Participants

Healthy South Asian volunteers were recruited between Jan and July 2011 for diabetes research Diabetes Health, Residence and Metabolism in Asians (DHRMA): the DHRMA study - a blinded, randomised, placebo controlled trial at Sandwell and West Birmingham Hospitals NHS Trust (Birmingham United Kingdom) (described in detail elsewhere[22]). All the participants were aged 18 years and over and were of self reported South Asian ethnicity with no known cardiovascular disease or associated medications, body-mass index (BMI) less than 30 kg/m^2, and normal glucose tolerance. The study design was approved by the Local Research Ethics Committee. Written informed consent was collected from all participants.

Table 1 Characteristics of South Asian volunteers

Characteristics of 29 South Asian volunteers	Mean (SD)
Age (yr)	34.6 (8.9)
Weight (kg)	69.6 (18.4)
Body-mass index (kg/m^2)	25 (5.2)
Waist circumference (cm)	86.8 (16.3)
Hip circumference (cm)	101 (10)
Hip to waist ratio	0.892 (0.144)
Systolic BP (mmHg)	119 (13)
Diastolic BP (mmHg)	75.8 (8.8)
Energy from carbohydrates (%)	45 (6.3)
Energy from fats (%)	40.8 (5.8)
Energy from protein (%)	9.4 (6.8)
Energy from sugars (%)	12.6 (7.5)
Energy from starch (%)	32 (8.5)
Energy from saturated fat (%)	9.7 (5.8)
Energy from monounsaturated fat (%)	10.7 (5.2)
Energy from polyunsaturated fat (%)	6.8 (2.9)
Energy from alcohol (%)	0.5 (2.2)

BP: Blood pressure.

Variables

All patients and controls attended a baseline assessment for the DHRMA study at Sandwell and West Birmingham Hospitals. The assessment incorporated an interview-administered medical history questionnaire, and a dietary assessment (24-h food recall) scrutinised using the WISP (Weighed Intake Software Program) nutritional package (version 3, Tinuviel Software, Llanfechell, United Kingdom)[6], and anthropometric measurements, which were measured using Seca scales and stadiometer (Seca Ltd, Birmingham). Waist measurements were recorded from the narrowest circumference above the umbilicus and below the rib, and the hip was recorded as the widest circumference at the buttocks. Both girths were measured in duplicate, and repeated where there were differences more than 2%. Blood pressure (BP) measurement was repeated three times, 1 min apart (analysing the mean) using a semi automated BP monitor, the OMRON 705CP (Omron Healthcare Europe, Mannheim, Germany) in combination with suitable cuff sizes for each participant, after at least five minutes in the sitting position. During this assessment venepucture was performed to collect blood (as serum, fluoride oxalate plasma and EDTA plasma) at fasting, 30 min post administration of a 75 g oral glucose load (Maxijul; SHS Supplies, Liverpool, United Kingdom). Blood collected with fluoride oxalate was analysed for glucose (glucose oxidase method) within 2 h of venepuncture using the Cobas Integra 400 auto analyser (Roche Diagnostics, United Kingdom). EDTA plasma was stored at 4℃ was analysed by: (1) Flow cytometry was recorded using the becton dickinson (BD) FACSCalibur flow cytometer (BD, Oxford, United Kingdom) (described elsewhere[23]). Absolute counts of monocytes analysed for human CD36 antibodies (Miltenyi Biotec GmbH, Germany). Monocyte CD36 was also assessed in subsets of monocytes by order of their co-expression of CD14, CD16 and CCR2, defined as Mon1: CD14(+)CD16(-)CCR2(+), Mon2: CD14(+)CD16(+)CCR2(+), and Mon3: CD14(low)CD16(+)CCR2(-) (as described previously[23]); and (2) Density gradient ultracentrifugation (described elsewhere[24]) was used to separate VLDL, low density lipoprotein (LDL), high density lipoprotein (HDL)$_2$ and HDL$_3$ subfractions using the Optima TLX Ultracentrifuge (Beckman Coulter, High Wycombe UK). Briefly, VLDL subfractions were extracted at a density 1.006 kg/L, LDL at 1.063 kg/L, HDL$_2$ at 1.123 kg/L and HDL$_3$ at 1.21 kg/L. Concentrations of cholesterol and triglyceride were measured on these separated lipoprotein subfractions on the Cobas Integra 400.

Blood collected as serum was separated and stored at -70℃ for batch analysis using commercially availible (1) colourimetric assays for NEFA (Acyl CoA synthase/oxidase method, Randox Laboratories, Co Antrim, United Kingdom); (2) ELISA for Insulin (Abcam, Cambridge, United Kingdom), adiponectin (R and D systems, Abington, United Kingdom), soluble CD36 (Adipo Bioscience Inc., Santa Clara, United States); and (3) automated biochemistry assays for total cholesterol, HDL cholesterol, LDL cholesterol, apolipoprotein AI, apolipoprotein B on the Cobas Integra 400.

Statistical analysis

Statistical review of the study was performed by a biomedical statistician. Data were analysed and validated using SPSS version 16.0 (SPSS Inc., Chicago, IL, United States). The parametric distribution of variables was scrutinised against Kolmogorov-Smirnov plots. Normally distributed data was analysed using ANOVA. The central tendencies of the data were presented as mean and variation by SD. Non-parametrically distributed data were analysed using the Friedman test for related measures, and data were presented as both median and interquartile ranges. A two-tailed bivariate correlation analysis was performed using Spearman's correlation coefficient. Categorical data were analysed using χ^2 tests. A P value < 0.05 was accepted as statistically significant.

Statistical review was performed by Dr. Andrew Blann, a biomedical statistician.

RESULTS

A total of 29 volunteers were consecutively recruited for this study. The characteristics of the cohort are shown in Table 1. South Asians were typically of Indian origin (72.4%) and subscribed to diets where the fat intake was 40% of the total energy intake. Their mean age was 34.6 (8.9) years, 16 were male (76.2%) and mean levels of BMI, fasting blood glucose, fasting serum lipids, and BP were reflective of a healthy cohort (Table 2). Soluble levels of CD36 were unrelated to monocyte expression of CD36. Analysing variables reported in Tables 1 and 2, variations in the percentage expression of monocyte CD36 was associated with anthropometry and dietary intake (Table 3). These correlations were specific to monocyte subsets, where Mon1 was positively

Table 2 Fasting metabolic indices and monocyte CD36 expression amongst South Asian volunteers

Characteristics of 29 South Asian volunteers	Median (interquartile range)
Plasma glucose (mmol/L)	5.09 (4.50, 5.47)
Serum NEFA (mmol/L)	0.416 (0.264, 0.514)
Serum cholesterol (mmol/L)	3.41 (2.95, 3.96)
Serum triglycerides (mmol/L)	0.9 (0.67, 1.44)
HDL cholesterol (mmol/L)	1.27 (0.98, 1.48)
LDL cholesterol (mmol/L)	2.03 (1.56, 2.44)
Apolipoprotein AI (g/L)	1.21 (1.10, 1.43)
Apolipoprotein B (g/L)	0.58 (0.46, 0.77)
VLDL subfraction cholesterol (mmol/L)	1.26 (0.97, 1.44)
LDL subfraction cholesterol (mmol/L)	1.3 (0.80, 1.65)
HDL_2 subfraction cholesterol (mmol/L)	0.58 (0.41, 0.76)
HDL_3 subfraction cholesterol (mmol/L)	0.37 (0.25, 0.51)
VLDL subfraction triglyceride (mmol/L)	0.34 (0.24, 0.43)
LDL subfraction triglyceride (mmol/L)	0.27 (0.17, 0.55)
HDL_2 subfraction triglyceride (mmol/L)	0.08 (0.06, 0.15)
HDL_3 subfraction triglyceride (mmol/L)	0.06 (0.04, 0.08)
Insulin (mU/L)	4.64 (2.60, 8.23)
Adiponectin (ng/mL)	1.79 (0.65, 2.65)
Plasma soluble CD36 (mmol/L)	108 (0, 249)
Total monocyte CD36 (Mon $CD36^+$ per μL)	361 (301, 432)
Monocyte CD36 on Mon1 (%)	81.8 (75.1, 85.8)
Monocyte CD36 on Mon2 (%)	7.2 (5.0, 10.3)
Monocyte CD36 on Mon3 (%)	10.5 (8.7, 16.6)

Monocyte subset. Mon1: CD14(+)CD16(-)CCR2(+); Mon2: CD14(+)-CD16(+)CCR2(+); Mon3: CD14(low)CD16(+)CCR2(-); NEFA: Non-esterified fatty acids; VLDL: Very low density lipoprotein; LDL: Low density lipoprotein; HDL: High density liporprotein.

Table 3 Correlation between characteristics and fasting metabolic indices against percentage monocyte CD36 expression

Characteristics and fasting metabolic indices	Spearman correlation coefficient (*P*)		
	Mon1	Mon2	Mon3
Body-mass index	-0.438 (0.02)	0.156 (0.43)	0.418 (0.027)
Hip circumference	-0.633 (0.002)	0.419 (0.06)	0.483 (0.027)
Serum NEFA	-0.349 (0.06)	0.151 (0.43)	0.478 (0.009)
Apolipoprotein AI	-0.282 (0.14)	0.475 (0.009)	0.149 (0.44)
Energy from total fat (%)	-0.472 (0.036)	0.399 (0.08)	0.458 (0.04)
Energy from monounsaturated fat (%)	-0.691 (< 0.001)	0.724 (< 0.001)	0.230 (0.33)
Energy from carbohydrates (%)	0.645 (0.002)	-0.496 (0.026)	-0.533 (0.016)

Monocyte subset. Mon1: CD14(+)CD16(-)CCR2(+); Mon2: CD14(+)-CD16(+)CCR2(+); Mon3: CD14(low)CD16(+)CCR2(-); NEFA: Non-esterified fatty acids.

associated with carbohydrate intake, and negatively with fat intake and BMI, while such trends appeared reversed in Mon2 and Mon3 subsets, which were additionally associated with apolipoprotein AI and NEFA (Table 3).

On analysis of serial measures of monocyte CD36 expression, there were evident changes in receptor concentrations for all monocytes ($P < 0.001$), as well as across subsets Mon1 ($P < 0.001$), Mon2 ($P = 0.011$) and Mon3 ($P = 0.03$) (Table 4). The profile of monocyte subset changes reflected a decrease post-prandially (30 min after the glucose challenge) and higher levels post-absorbatively (after 120 min) in Mon1 and Mon2

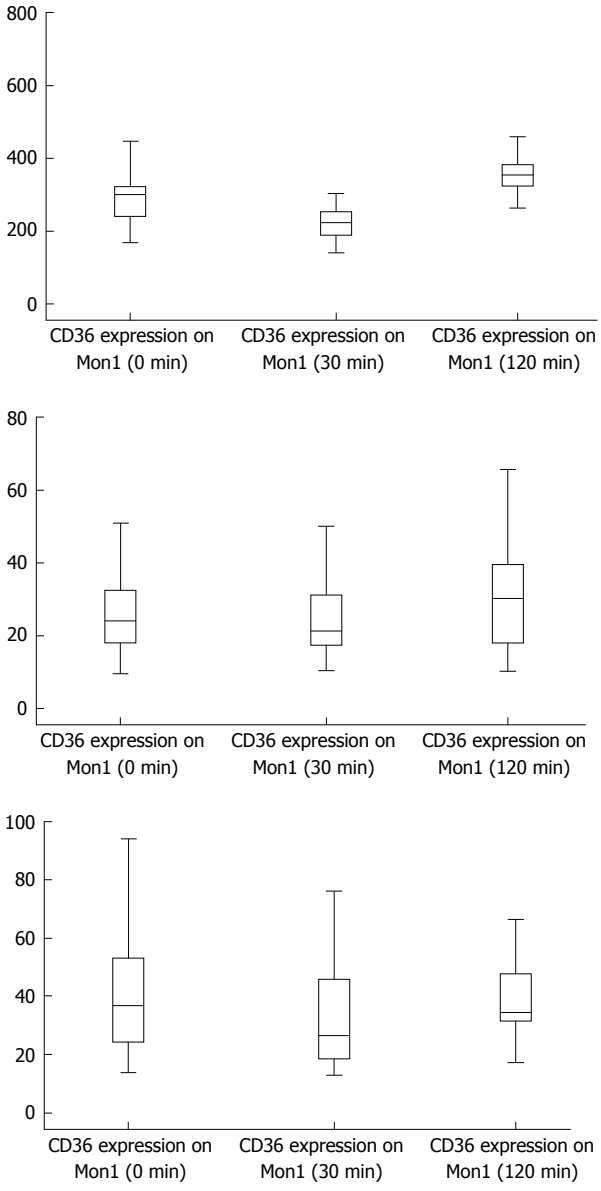

Figure 1 Changes in monocyte subset CD36 expression (Mon $CD36^+$ per μL) pre and post administration of oral glucose. Monocyte subsets. Mon1: CD14(+)CD16(-)CCR2(+); Mon2: CD14(+)CD16(+)CCR2(+); Mon3: CD14(low)CD16(+)CCR2(-).

(Figure 1). NEFA levels progressively decreased during the glucose challenge ($P < 0.001$), whereas cholesterol concentrations within VLDL and LDL subfractions increased ($P < 0.001$). The levels of triglyceride within VLDL particles and HDL particles appeared to decrease during the glucose challenge, but these changes were not significant. Levels of serum triglycerides, glucose, HDL subfraction lipids were largely unchanged following the glucose load. Similarly, there were no significant changes in percentage expression of monocyte CD36 on Mon1, Mon2 or Mon3.

DISCUSSION

Greater CD36 expression following a glucose challenge in healthy South Asians, could reflect a physiological

Table 4 Changes in metabolic factors absolute monocyte CD36 expression pre and post administration of oral glucose

	Circulating concentrations following the administration of glucose			*P*
	0 min	30 min	120 min	
Monocyte CD36 on Mon1 (Mon CD36$^+$ per μL)	300 (240, 324)	223 (187, 254)	353 (323, 382)	< 0.001
Monocyte CD36 on Mon2 (Mon CD36$^+$ per μL)	23.9 (17.9, 32.3)	21.3 (17.2, 30.8)	29.9 (18.1, 39.6)	0.011
Monocyte CD36 on Mon3 (Mon CD36$^+$ per μL)	36.7 (24.3, 53.2)	26.1 (18.4, 45.8)	34.4 (31.3, 48.7)	0.03
Total monocyte CD36 (Mon CD36$^+$ per μL)	361 (308, 432)	278 (240, 324)	422 (394, 458)	< 0.001
Plasma glucose (mmol/L)	5.09 (4.5, 5.47)	6.68 (4.93, 8.12)	5.2 (4.64, 6.07)	0.08
Serum NEFA (mmol/L)	0.416 (0.264, 0.514)	0.215 (0.098, 0.326)	0.094 (0.071, 0.207)	< 0.001
Serum cholesterol (mmol/L)	3.41 (2.95, 3.96)	3.29 (2.95, 3.95)	3.44 (3.06, 3.80)	0.52
Serum triglycerides (mmol/L)	0.9 (0.67, 1.44)	0.83 (0.55, 1.19)	1.01 (0.54, 1.35)	0.26
VLDL cholesterol (mmol/L)[1]	1.26 (0.97, 1.44)	1.31 (0.94, 1.50)	1.42 (1.27, 1.62)	0.001
LDL cholesterol (mmol/L)[1]	1.39 (0.89, 1.71)	1.61 (0.8, 1.82)	1.92 (1.58, 2.24)	0.003
HDL_2 cholesterol (mmol/L)[1]	0.600 (0.425, 0.735)	0.470 (0.360, 0.725)	0.540 (0.415, 0.820)	0.19
HDL_3 cholesterol (mmol/L)[1]	0.395 (0.285, 0.505)	0.330 (0.205, 0.450)	0.325 (0.250, 0.570)	0.43
VLDL triglyceride (mmol/L)[1]	0.338 (0.223, 0.440)	0.263 (0.213, 0.365)	0.265 (0.105, 0.353)	0.37
LDL triglyceride (mmol/L)[1]	0.270 (0.170, 0.505)	0.250 (0.170, 0.360)	0.275 (0.125, 0.385)	0.16
HDL_2 triglyceride (mmol/L)[1]	0.080 (0.055, 0.138)	0.070 (0.043, 0.093)	0.060 (0.028, 0.100)	0.09
HDL_3 triglyceride (mmol/L)[1]	0.058 (0.035, 0.083)	0.035 (0.013, 0.045)	0.038 (0.025, 0.065)	0.12

[1]Subfraction lipids. Data are median (interquartile range). NEFA: Non-esterified fatty acids; VLDL: Very low density lipoprotein; LDL: Low density lipoprotein; HDL: High density liporprotein.

response to counteract the toxicity of excessive plasma glucose. Monocytes have been shown to present an intracellular CD36 pool[25], and the transient expression of CD36 in monocytes during glucose challenge, may serve as a critical process in dictating the functional activity of CD36 during diabetic conditions and perhaps, atherogenesis. These increases in absolute monocyte CD36 concentrations occurred in parallel to an exponential decrease in NEFA, a rise in levels of VLDL and LDL cholesterol, and no changes in plasma glucose, serum triglycerides or HDL cholesterol. It is possible that the oral glucose challenge in South Asians results in the generation of VLDL particles, and the increased availability and action of CD36 results in the liberation of triglyceride from these particles. The expression of CD36 on monocytes is associated with factors such as dietary fat and carbohydrate, and we are tempted to speculate that lifestyle exposures have a role in moderating the expression of CD36.

An increase in the expression of CD36 is seen as a dysfunctional event, and in diabetics it is associated with a down regulation of the GLUT4 receptor and a reduction in glycogen synthesis[18]. In South Asians, this response to increase CD36 expression following a glucose load may reflect a homeostatic process to up-regulate those mechanisms that preferentially store energy as fat. In animal models and cellular models, CD36 is shown to lower circulating NEFA concentrations and to promote the efflux of triglyceride from VLDL[16].

The reasons this "ethnic" preference to process energy in this way are complex. One interesting aspect of CD36 measurement in South Asians is in its dual role in the clearance of red blood cells infected with plasmodium falciparum. The interaction between malaria and CD36 receptors is complex and the subject of debate[26]. South Asians have evolved from a part of the world where malaria was endemic[27], and this may have conferred a survival benefit[28,29].

Hepcidin is upregulated in malarial infected individuals by interleukin-6[29], a protein that supports hepatic gluconeogenesis, by a process that is dysregulated in diabetics. Further research is required to fully understand the link between exposure to malaria and a subsequent susceptibility to diabetes.

Monocytes and macrophages play a fundamental role in the pathogenesis of atherosclerosis, and various subtypes of monocytes are associated with cardiovascular diseases[30]. Functional differences of three Mon1, Mon2 and Mon3 monocyte subsets have been described[23]. Each monocyte subset responds differently to distinctive immunological stimuli. For example, Mon2 and 3 monocytes predominate during inflammatory states and Mon1 in response to Candida albicans[29]. The production of cytokines such as tumor necrosis factor-alpha, associated with diabetes, also varies with stimulus[31]. However, responses in CD36 expression following the glucose challenge were largely similar across these subsets, and as such the metabolic significance of these subclassifications are unclear. The increased surface expression of CD36 on Mon1 was associated with lower BMI and a higher intake of carbohydrate, where as the converse appeared true for Mon1 and Mon2, which were positively associated with dietary fat. Such findings suggest that the expression of CD36 on monocytes can be moderated by diet.

Due to the low numbers of subjects in this study, we only had sufficient statistical power to detect significant associations (alpha at 0.02) where the correlation coefficient was $\geqslant$ 0.55. We found levels of soluble CD36 to be unrelated to monocytes and metabolic measures. However, elsewhere, soluble CD36 has been shown to reflect several aspects of insulin resistance in humans[32]. The isolation of lipoprotein subfractions by ultracentrifugation is prone to losses during the

extraction process. Further work would need to be undertaken to analyse the generalisability of the data generated here. There is considerable diversity within the South Asian ethnic category, it encompasses over 22 different languages and more than 6 different religions. The dietary intake of fat in this cohort was similar to that we have measured in a larger cohort of South Asians Living in the United Kingdom[33]. Nonetheless, the dietary intake of fat in these groups is much greater than that seen amongst South Asians living in rural India[28].

In summary, these data describe changes in lipid metabolism following the oral ingestion of glucose in South Asians which includes the generation of VLDL, and an increase in monocytes expressing CD36. We presume that triglycerides from these particles are cleared by an increase in the availability of CD36 receptors, and it would appear that lifestyle exposures may influence this process.

COMMENTS

Background

The incidence of diabetes in South Asian populations, including those living in the Indian Subcontinent, and those who have migrated away, is significant. Indeed it is higher than in comparable Caucasian populations and the resulting complications of diabetes such as nephropathy and retinopathy occur both earlier and more severely. Despite extensive investigation, the underlying pathophysiological processes explaining this phenomenon remain elusive. For its part, this study investigated lipid and lipoprotein metabolism and its relationship with the expression of the fatty acid translocase CD36 on monocytes in South Asians.

Research frontiers

Gene variants in CD36, a macrophage scavenger receptor, have been implicated in the pathogenesis of type 2 diabetes and its complications.

Innovations and breakthroughs

This study found that following the administration of a glucose load to South Asian individuals, an upregulation of CD36 positive monocytes was demonstrated which has not been previously seen. In keeping with animal models, it is presumed this increased CD36 expression facilitates the sequestration of triglycerides from very low density lipoprotein particles.

Terminology

This finding suggests that CD36 may represent an as yet unexploited target for therapeutic interventions addressing both diabetes and dyslipidaemia.

Peer-review

This is a very interesting study and all the sections of the manuscript are complete; results have been well described in the text and in the Tables and Figure.

REFERENCES

1 **King H**, Aubert RE, Herman WH. Global burden of diabetes, 1995-2025: prevalence, numerical estimates, and projections. *Diabetes Care* 1998; **21**: 1414-1431 [PMID: 9727886 DOI: 10.2337/diacare.21.9.1414]

2 **Cruickshank JK**, Beevers DG, Osbourne VL, Haynes RA, Corlett JC, Selby S. Heart attack, stroke, diabetes, and hypertension in West Indians, Asians, and whites in Birmingham, England. *Br Med J* 1980; **281**: 1108 [PMID: 7427603 DOI: 10.2337/diacare.21.9.1414]

3 **Verma NP**, Mehta SP, Madhu S, Mather HM, Keen H. Prevalence of known diabetes in an urban Indian environment: the Darya Ganj diabetes survey. *Br Med J* (Clin Res Ed) 1986; **293**: 423-424 [PMID: 3091141 DOI: 10.1136/bmj.281.6248.1108]

4 **Dowse GK**, Gareeboo H, Zimmet PZ, Alberti KG, Tuomilehto J, Fareed D, Brissonnette LG, Finch CF. High prevalence of NIDDM and impaired glucose tolerance in Indian, Creole, and Chinese Mauritians. Mauritius Noncommunicable Disease Study Group. *Diabetes* 1990; **39**: 390-396 [PMID: 2307296 DOI: 10.2337/diab.39.3.390]

5 **Ramaiya KL**, Denver E, Yudkin JS. Diabetes, impaired glucose tolerance and cardiovascular disease risk factors in the Asian Indian Bhatia community living in Tanzania and in the United Kingdom. *Diabet Med* 1995; **12**: 904-910 [PMID: 8846682 DOI: 10.1111/j.1464-5491.1995.tb00394.x]

6 **Patel JV**, Vyas A, Prabhakaran D, Bhatnagar D, Durrington PN, Heald A, Hughes EA, Mackness MI, Reddy KS, Cruickshank JK. Nonesterified fatty acids as mediators of glucose intolerance in Indian Asian populations. *Diabetes Care* 2005; **28**: 1505-1507 [PMID: 15920081 DOI: 10.2337/diacare.28.6.1505]

7 **Chandie Shaw PK**, Baboe F, van Es LA, van der Vijver JC, van de Ree MA, de Jonge N, Rabelink TJ. South-Asian type 2 diabetic patients have higher incidence and faster progression of renal disease compared with Dutch-European diabetic patients. *Diabetes Care* 2006; **29**: 1383-1385 [PMID: 16732026 DOI: 10.2337/dc06-0003]

8 **Burden AC**, McNally PG, Feehally J, Walls J. Increased incidence of end-stage renal failure secondary to diabetes mellitus in Asian ethnic groups in the United Kingdom. *Diabet Med* 1992; **9**: 641-645 [PMID: 1511571 DOI: 10.1111/j.1464-5491.1992.tb01860.x]

9 **Middelkoop BJ**, Kesarlal-Sadhoeram SM, Ramsaransing GN, Struben HW. Diabetes mellitus among South Asian inhabitants of The Hague: high prevalence and an age-specific socioeconomic gradient. *Int J Epidemiol* 1999; **28**: 1119-1123 [PMID: 10661656 DOI: 10.1093/ije/28.6.1119]

10 **Stolk RP**, van Schooneveld MJ, Cruickshank JK, Hughes AD, Stanton A, Lu J, Patel A, Thom SA, Grobbee DE, Vingerling JR. Retinal vascular lesions in patients of Caucasian and Asian origin with type 2 diabetes: baseline results from the ADVANCE Retinal Measurements (AdRem) study. *Diabetes Care* 2008; **31**: 708-713 [PMID: 18184903 DOI: 10.2337/dc07-1657]

11 **Raymond NT**, Varadhan L, Reynold DR, Bush K, Sankaranarayanan S, Bellary S, Barnett AH, Kumar S, O'Hare JP. Higher prevalence of retinopathy in diabetic patients of South Asian ethnicity compared with white Europeans in the community: a cross-sectional study. *Diabetes Care* 2009; **32**: 410-415 [PMID: 19074992 DOI: 10.2337/dc08-1422]

12 **Patel JV**, Lim HS, Gunarathne A, Tracey I, Durrington PN, Hughes EA, Lip GY. Ethnic differences in myocardial infarction in patients with hypertension: effects of diabetes mellitus. *QJM* 2008; **101**: 231-236 [PMID: 18204065 DOI: 10.1093/qjmed/hcm151]

13 **Kooner JS**, Baliga RR, Wilding J, Crook D, Packard CJ, Banks LM, Peart S, Aitman TJ, Scott J. Abdominal obesity, impaired nonesterified fatty acid suppression, and insulin-mediated glucose disposal are early metabolic abnormalities in families with premature myocardial infarction. *Arterioscler Thromb Vasc Biol* 1998; **18**: 1021-1026 [PMID: 9672061 DOI: 10.1161/01.ATV.18.7.1021]

14 **Hanley AJ**, Williams K, Stern MP, Haffner SM. Homeostasis model assessment of insulin resistance in relation to the incidence of cardiovascular disease: the San Antonio Heart Study. *Diabetes Care* 2002; **25**: 1177-1184 [PMID: 12087016 DOI: 10.2337/diacare.25.7.1177]

15 **Endemann G**, Stanton LW, Madden KS, Bryant CM, White RT, Protter AA. CD36 is a receptor for oxidized low density lipoprotein. *J Biol Chem* 1993; **268**: 11811-11816 [PMID: 7685021]

16 **Habets DD**, Coumans WA, Voshol PJ, den Boer MA, Febbraio M, Bonen A, Glatz JF, Luiken JJ. AMPK-mediated increase in myocardial long-chain fatty acid uptake critically depends on sarcolemmal CD36. *Biochem Biophys Res Commun* 2007; **355**: 204-210 [PMID: 17292863 DOI: 10.1016/j.bbrc.2007.01.141]

17 **Bharadwaj KG**, Hiyama Y, Hu Y, Huggins LA, Ramakrishnan R, Abumrad NA, Shulman GI, Blaner WS, Goldberg IJ. Chylomicron- and VLDL-derived lipids enter the heart through different pathways: in vivo evidence for receptor- and non-receptor-mediated fatty acid

uptake. *J Biol Chem* 2010; **285**: 37976-37986 [PMID: 20852327 DOI: 10.1074/jbc.M110.174458]
18 **Steinbusch LK**, Schwenk RW, Ouwens DM, Diamant M, Glatz JF, Luiken JJ. Subcellular trafficking of the substrate transporters GLUT4 and CD36 in cardiomyocytes. *Cell Mol Life Sci* 2011; **68**: 2525-2538 [PMID: 21547502 DOI: 10.1007/s00018-011-0690-x]
19 **Schwenk RW**, Luiken JJ, Bonen A, Glatz JF. Regulation of sarcolemmal glucose and fatty acid transporters in cardiac disease. *Cardiovasc Res* 2008; **79**: 249-258 [PMID: 18469026 DOI: 10.1093/cvr/cvn116]
20 **Aguer C**, Mercier J, Man CY, Metz L, Bordenave S, Lambert K, Jean E, Lantier L, Bounoua L, Brun JF, Raynaud de Mauverger E, Andreelli F, Foretz M, Kitzmann M. Intramyocellular lipid accumulation is associated with permanent relocation ex vivo and in vitro of fatty acid translocase (FAT)/CD36 in obese patients. *Diabetologia* 2010; **53**: 1151-1163 [PMID: 20333349 DOI: 10.1007/s00125-010-1708-x]
21 **Sun Y**, Scavini M, Orlando RA, Murata GH, Servilla KS, Tzamaloukas AH, Schrader R, Bedrick EJ, Burge MR, Abumrad NA, Zager PG. Increased CD36 expression signals monocyte activation among patients with type 2 diabetes. *Diabetes Care* 2010; **33**: 2065-2067 [PMID: 20551015 DOI: 10.2337/dc10-0460]
22 **Patel JV**, Hughes EA, Lip GY, Gill PS. Diabetes Health, Residence & Metabolism in Asians: the DHRMA study, research into foods from the Indian subcontinent - a blinded, randomised, placebo controlled trial. *BMC Cardiovasc Disord* 2011; **11**: 70 [PMID: 22136261 DOI: 10.1186/1471-2261-11-70]
23 **Shantsila E**, Wrigley B, Tapp L, Apostolakis S, Montoro-Garcia S, Drayson MT, Lip GY. Immunophenotypic characterization of human monocyte subsets: possible implications for cardiovascular disease pathophysiology. *J Thromb Haemost* 2011; **9**: 1056-1066 [PMID: 21342432 DOI: 10.1111/j.1538-7836.2011.04244.x]
24 **Winocour PH**, Durrington PN, Bhatnagar D, Ishola M, Mackness M, Arrol S. Influence of early diabetic nephropathy on very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), and low density lipoprotein (LDL) composition. *Atherosclerosis* 1991; **89**: 49-57 [PMID: 1772471 DOI: 10.1016/0021-9150(91)90006-O]
25 **Huh HY**, Pearce SF, Yesner LM, Schindler JL, Silverstein RL. Regulated expression of CD36 during monocyte-to-macrophage differentiation: potential role of CD36 in foam cell formation. *Blood* 1996; **87**: 2020-2028 [PMID: 8634453]
26 **Cabrera A**, Neculai D, Kain KC. CD36 and malaria: friends or foes? A decade of data provides some answers. *Trends Parasitol* 2014; **30**: 436-444 [PMID: 25113859 DOI: 10.1016/j.pt.2014.07.006]
27 **Clegg JB**, Weatherall DJ. Thalassemia and malaria: new insights into an old problem. *Proc Assoc Am Physicians* 1999; **111**: 278-282 [PMID: 10417734 DOI: 10.1046/j.1525-1381.1999.99235.x]
28 **Das SK**, Talukder G. A review on the origin and spread of deleterious mutants of the beta-globin gene in Indian populations. *Homo* 2001; **52**: 93-109 [PMID: 11802568 DOI: 10.1078/0018-442X-00022]
29 **Hassall OW**, Tillyer ML, Old JM. Prevalence and molecular basis of alpha thalassaemia in British South Asians. *J Med Screen* 1998; **5**: 31-33 [PMID: 9575457 DOI: 10.1136/jms.5.1.31]
30 **Gratchev A**, Sobenin I, Orekhov A, Kzhyshkowska J. Monocytes as a diagnostic marker of cardiovascular diseases. *Immunobiology* 2012; **217**: 476-482 [PMID: 22325375 DOI: 10.1016/j.imbio.2012.01.008]
31 **Chua CL**, Brown G, Hamilton JA, Rogerson S, Boeuf P. Monocytes and macrophages in malaria: protection or pathology? *Trends Parasitol* 2013; **29**: 26-34 [PMID: 23142189 DOI: 10.1016/j.pt.2012.10.002]
32 **Handberg A**, Højlund K, Gastaldelli A, Flyvbjerg A, Dekker JM, Petrie J, Piatti P, Beck-Nielsen H. Plasma sCD36 is associated with markers of atherosclerosis, insulin resistance and fatty liver in a nondiabetic healthy population. *J Intern Med* 2012; **271**: 294-304 [PMID: 21883535 DOI: 10.1111/j.1365-2796.2011.02442.x]
33 **Patel JV**, Vyas A, Cruickshank JK, Prabhakaran D, Hughes E, Reddy KS, Mackness MI, Bhatnagar D, Durrington PN. Impact of migration on coronary heart disease risk factors: comparison of Gujaratis in Britain and their contemporaries in villages of origin in India. *Atherosclerosis* 2006; **185**: 297-306 [PMID: 16005463 DOI: 10.1016/j.atherosclerosis.2005.06.005]

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