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FIELD OF VISION

One center in Brussels has consistently had the lowest HbA1c values in the 4 studies (1994-2009) by the Hvidoere International Study Group on Childhood Diabetes: What are the "recipes"?

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Abstract

The principal aims of therapeutic management of the child, adolescent and adult with type 1 diabetes are to allow good quality of life and to avoid long-term complications (retinopathy, neuropathy, nephropathy, cardiovascular disease, *etc.*) by maintaining blood glucose concentrations close to normal level. Glycated hemoglobin levels (HbA1c) provide a good criterion of overall glycemic control. The Hvidoere Study Group (HSG) on Childhood Diabetes, founded in 1994, is an international group representing about twenty highly experienced pediatric centers from Europe, North America, Japan and Australia. Four international comparisons of metabolic control (1995, 1998, 2005, 2009) have been performed. The one center that has consistently had the lowest HbA1c values (approximate 7.3% or 56.3 mmol/mol) is my center in Brussels. This is more often obtained with a twice-daily free-mixed regimen with additional supplemental fast insulins ad hoc. The so-called "Dorchy's recipes" are summarized. The conclusion is that the number of daily insulin injections, 2 or $\geqslant$ 4, or the use of pumps, by itself does not necessarily give better results. Intensified therapy should not depend upon the number of insulin doses per day, by syringe, pen or pump but rather should be redefined as to intent-to-treat ascertainment (*i.e.*, goals). When there are no mutually agreed upon goals for BG and/or HbA1c, when there is insufficient education and psychosocial support by the medical team or at home, there is likely to be poor outcomes, as shown by the HSG. One of our recipes is not to systematically replace rapid-acting human insulins by fast-acting analogues. Because the multicenter studies of the HSG, performed in developed countries without financial restriction, show that treatment of childhood diabetes is inadequate in general and that levels of HbA1c are very different, diabetes treatment teams should individually explore the reasons for failure, without any prejudice or bias. Any dogmatism must be avoided. Treatment cost *vs* results must also be taken into account.

Key words: Type 1 diabetes mellitus; Insulin regimen; Diabetic children; Glycated hemoglobin; Conventional treatment; Intensive treatment

Core tip: Four international comparisons of the Glycated hemoglobin levels (HbA1c) levels (1995, 1998, 2005, 2009) have been performed by the Hvidoere Study Group on childhood diabetes in about twenty pediatric diabetology centers from about twenty industrialized countries in Europe, North America, Japan and Australia. The one center that has consistently had the lowest HbA1c values (approximate 7.3% or 56.3 mmol/mol) is my center in Brussels. This is more often obtained with a twice-daily free-mixed insulin regimen. The so-called "Dorchy's recipes" are summarized.

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COMMENTARY ON HOT TOPICS

Diabetes Control and Complications Trial research group

The principal aims of therapeutic management of the child, adolescent and adult with type 1 diabetes are to allow good quality of life[1,2] and to avoid long-term complications (retinopathy, neuropathy, nephropathy, cardiovascular disease, *etc.*) by maintaining blood glucose concentrations close to the normal level[3,4] while always minimizing hypoglycemia. Glycated hemoglobin levels (HbA1c) provide a good criterion of overall glycemic control. According to the diabetes control and complications trial research group (DCCT), they must be, in adults, under 7% (53 mmol/mol), if the upper normal limit is about 6% (42 mmol/mol)[3]. The DCCT obtained such results utilizing targeted blood glucose (BG) treatment decisions usually with multidose insulin (MDI) (≥ 4 shots/d) and/or insulin pump treatment compared to a relatively fixed insulin dose schedule in the control group[3]. Such MDI and continuous subcutaneous insulin infusion (CSII) treatment was subsequently known as intensive treatment when it really should have been defined as the targeted BG intent to reach BG goals and HbA1c goals safely that was "intensive treatment". However, in our experience, this is possible even in diabetic children and adolescents with the twice-daily free mixing insulin regimen as well as with the basal-bolus regimen, as we have shown since the 90 s[5-7].

Hvidoere Study Group on childhood diabetes

After the publication of the conclusions of the DCCT and of my own results, causing some debate about "Dorchy's recipes"[6], The Hvidoere Study Group on childhood diabetes (HSG) evolved in 1994, during a workshop, to discuss strategies that could be important in improving quality of pediatric and adolescent diabetes care and, therefore good HbA1c levels. Four international comparisons of metabolic control (1995, 1998, 2005, 2009) have been performed in about twenty pediatric diabetology centers from about twenty industrialized countries in Europe, North America, Japan and Australia[2,8-10]. A capillary blood sample was provided by participants and analyzed centrally at the Steno Diabetes Center in Denmark. HbA1c was DCCT aligned: normal range 4.4%-6.3% or 25-45 mmol/mol, mean 5.4% or 35.5 mmol/mol. The mean is 0.3% higher than the DCCT laboratory level: normal range 4.05%-6.05%, mean 5.05%.

Cameron *et al*[11] have reviewed the major studies of the HSG, both cross-sectional and longitudinal, and summarized the body of work published in 28 peer reviewed medical and scientific journals (Table 1). The authors note that "The one center that has consistently had the lowest HbA1c values from 1995 to 2009" is my center in Brussels. They comment: "The Hvidoere member in question is highly charismatic and has a very prescriptive, 'recipe'-based approach to managing diabetes in his clinic. He prescribes mostly twice-daily free mixing injections of insulin and eschews, a flexible approach to dietary intake. This does not appear to be at the expense of either hypoglycemia or QOL in his patient group. Although many aspects of his practice are shared by other Hvidoere members, it has proved very difficult to translate this total approach into other contexts for a variety of reasons. However, this experience is emblematic that consistently excellent outcomes can be achieved by simple, 'non-intensive' insulin regimens that are underpinned by a strong philosophy of care"[11].

Cameron *et al*[11] conclude in their review: "Therapeutic strategies in and of themselves are not enough to obtained desired clinical outcomes. While all clinical regimens have some clinical utility, it is the underlying therapeutic philosophy based on a qualified common training for all team members delivering diabetes care and education to the families that drives improvement. The clinical aphorism of 'Ask for mediocrity and you will receive' holds true. Thus, it appears that the best results will be obtained by physicians who are target-driven and teams and families that have unanimity of purpose. Perhaps the conclusions relating the best clinical practice drawn from the entire body of work of the Hvidoere studies can be best summarized as -be dogmatic about outcome but flexible in approach".

In the studies of the HSG, the different insulin regimens were: "conventional" twice daily (CT), CTpremix, CTfreemix, CTfreemix + (used only in center number one, *i.e.*, in my center in Brussels), basal-bolus injections (MDI), CSII. In the 4th study[10], there was confusion between CTfreemix and CTfreemix +, fortunately corrected by an erratum[10]. The lowest HbA1c levels were found in the CTfreemix+ group, 7.3% ± 0.5% (56.3 mmol/mol); approximately the same values were obtained in the three preceding studies by the Brussels team. In 2007, after three HSG studies, de Beaufort *et al*[9] noted:

Table 1 HbA1c comparisons in the 4 studies by the Hvidoere international study group on childhood diabetes (1995-2009)

Hvidoere studies	Number of countries of pediatric centers	Number of subjects	Age (yr)	Mean HbA1c (%) ± SD (mean DCCT aligned)	Spread in center mean HbA1C (%) (DCCT aligned)	Conclusions
1995[8]	18 (Europe, North America, Japan) 22	2873	0-18	8.6 ± 1.7 (8.3)	7.6-10.2 (7.3-9.9)	No difference in glycemic control was found among adolescents treated with two, three, and four or more daily injections. Girls on 4 injections had higher BMI
1998[2]	17 (Europe, North America, Japan) 21	2101	11-18	8.7 ± 1.7 (8.4)	7.7-10.1 (7.4-9.8)	The differences between centers were not explicable by differences in insulin regimens. The centers with the lowest mean HbA1c also had lowest rates of severe hypoglycemia and reported better QOL
2005[9]	19 (Europe, North America, Japan, Australia) 21	2093	11-18	8.6 ± 1.7 (8.2)	7.7-9.5 (7.4-9.2)	Intensified insulin regimens (MDI and CSII) showed no lower HbA1c compared with twice daily free-mixing (lowest HbA1c)
2009[10]	17 (Europe, North America, Japan, Australia) 18	1113	< 11	8.3 ± 1.3 (8.0)	7.6-9.2 (7.3-8.9)	despite major changes in management (> 99% on analog insulins and 33% with CSII), the lowest HbA1c levels were found in the twice daily free-mixing insulin regimen in Brussels (7.3% ± 0.5%)

MDI: Multidose insulin; CSII: Continuous subcutaneous insulin infusion.

"The management of children and adolescents with type 1 diabetes has undergone many changes over the past decade, aiming to improve glycemic control and reduce risks of vascular complications, without sacrificing quality of life. These have included increased usage of insulin analogues, basal-bolus regimens, and CSII. Despite these substantial changes, it has been difficult to demonstrate significant improvements in metabolic outcome. This study in 21 international centers was initiated to investigate the impact of treatment changes on glycemic control and to establish whether the previously reported differences between centers were diminishing. The results confirm that there has been no improvement in glycemic control over a decade, with mean A1c levels of 8.6% or 70.5 mmol/mol (1995), 8.7% or 71.6 mmol/mol (1998), and 8.6% or 70.5 mmol/mol (2005), and the substantial differences between centers have remained stable." That means that more expensive and technically complicated treatments have nearly no impact on HbA1c. Only two centers showed a significant reduction (≥ 0.5%) in A1c from 1998 to 2005 and one center had a significant increase in A1c. The conclusion is that, in countries unable to afford a sophisticated and expensive treatment, it is possible to obtain good results without necessarily using expensive insulins and pumps.

As my team has consistently had the lowest HbA1c values during the comparisons of the HSG from 1995 to 2009, I think that I am allowed to summarize the so-called "Dorchy's recipes..."[6,12-15].

Dorchy's recipes

Two daily insulin free-mixed regimen in children or even teenagers: Two daily insulin free-mixed regimen with an human rapid-acting insulin or a fast-acting analogue and NPH (*i.e.,* 4 insulins per day as in the basal-bolus regimen) in children < 15-16 years is easy and effective in countries where the meal schedule allows correct allocation of diet. The first injection (and insulin dose alteration) is done before going to school and the second injection (and insulin dose alteration) after returning from school, before dinner, with the facultative help of the parents. Diabetic children have to eat a snack in the middle of the morning and afternoon periods with their friends, without the need to give an additional insulin injection or to measure blood glucose. This reduces the risk of insulin omission. The doses of the 4 insulins are adjusted according to the results of 4 daily blood glucose measurements of the preceding days (retroactive analysis) and not only to the present glycemia (reactive responsitivity). A third injection with a fast-acting analogue may be done to allow a greater snack or to correct hyperglycemia (= CTfreemix +).

Basal-bolus regimen in adolescents but more complicated: Basal-bolus regimen in adolescents: increased flexibility in daily life and dietary freedom, but more complicated; no simplistic sliding scales according to the present glycemia; insulin dose alteration must be triple: (1) retrospective, according to previous BG analysis, trial and error experiments, in order to enjoy more freedom for meals, sports, *etc*; (2) prospective according to programmed changes in meals and sports (*i.e.,* add more insulin if overeating or temporarily reduce insulin dose to prevent activity-related expected hypoglycemia); and (3) with only a "touch" of compensatory adaptation (reactive dose changes) according to the present glycemia. This needs psychological maturity and ongoing education support and teaching of child, adolescent and family

members, otherwise the multiple injection system lead to anarchy, "cheating" and obesity, especially but not only in adolescent girls. Before or after the meals, there is an injection of rapid human insulin or fast-acting analogue, and before sleeping, an injection of a stable long-acting analogue[16]. The doses of the 4 insulin injections are adjusted according to analysis of the results of at least 4 daily blood glucose measurements (if three meals; otherwise more blood glucose determinations and injections) of the preceding days and not only to the present BG level.

CSII is very rarely recommended: In our experience, CSII is very rarely recommended and used in about 1% of our patients at their request. We do not promote use of expensive insulin pump regimens and believe that patients and their family members can do as well with pens and syringes. Pumps in children and adolescents have not been associated with significant improvements in daily BG results or in A1c according to results of the HSG[11] and also by the PedPump study in 30 centers of 17 countries[17]. In that study, the use of less than 6.7 daily boluses was a significant predictor of an HbA1c level > 7.5% or 58.5 mmol/mol, despite increasing blood glucose measurements and the added expense that this entails. We suspect this reflects insufficient education and motivation and inconsistent team target goal setting as the explanation. In a Belgian retrospective cross-sectional study among 12 pediatric centers, A1c actually was higher among patients with insulin pump therapy[18].

No rejection of non-analogue older human insulins: No systematic (automatic or dogmatic) replacement of rapid-acting human insulins by fast-acting analogues such as more expensive aspart, lispro or glulisine[14]. In the two daily insulin free-mixed regimen as well as in the basal-bolus regimen, the choice of a fast-acting analogue is made if the time period between the injection and the following glycemia, allowing to judge the insulin injected before, is less than 3 or 4 h, *i.e.,* the duration of action of the fast-acting analogue. Otherwise, we use a human rapid-acting insulin whose duration of action reaches 6 to 8 h rather than the newer and more expensive fast-acting analogues.

No carbohydrate counting: The dietician never gives rigid meal plans or exchange lists. "Diet" is never prescribed. No carbohydrate counting is recommended because there is no linear correlation between the metabolization of X grams of glucose by Y units of insulin[12,13,15]. The dietician builds up a picture of the family's and child or teen's usual habits and life style. When possible, the family is encouraged to adopt a similar and normal eating pattern so that the child and adolescent with diabetes does not have to eat specially prepared meals. The main problem with the twice-daily insulin regimen is the allocation of carbohydrates in 6 meals according to the cumulated action of the insulins. The dietician must know perfectly the actions of the insulins and their adjustment. While being criticized for this being too difficult, our glycemic control and A1c results certainly prove that this is feasible to accomplish with large numbers of children, adolescents and young adults. In addition, all members of the professional diabetes team must have the same treatment philosophy, as promulgated by the DCCT, to provide the same message and same target BG and A1c goals[19].

Screening for subclinical complications: Screening for subclinical and asymptomatic complications by sensitive methods from puberty in order to increase the motivation of both the patient and the doctor[20]. After age 13 and/or 3 years of diabetes duration, we perform every year: retinal fluorescein angiography (rather than just direct ophthalmoscopy), measurement of motor and sensitive conduction velocities (which is different from a painful electromyography), sympathetic cutaneous response or heart rate variability and dosage of microalbuminuria. It is important to do a diagnosis at the stage of functional and reversible abnormalities before the installation of irreversible lesions. It is important to be able to say to the patient, for example, "you have no complaint, but as you can see on this photograph, there are two leakages of fluorescein in your left eye; it is reversible if you improve your HbA1c; otherwise, that will become an irreversible lesion leading later to overt complications". The same message for the slowing of conduction velocity or the presence of abnormal microalbuminuria. Every year, we also perform lipid analyses, thyroid and celiac screenings, measurement of blood pressure, *etc.,*[21,22]. Early identification of such abnormalities allows potential early treatment, *i.e.,* medication to control hypertension or early nephropathy, lower lipids, *etc.*

Knowledge of HbA1c target: One hundred percent of our patients and/or their parents as well as the members of the multidisciplinary team know the HbA1c target, *i.e.,* less than 7% or 53 mmol/mol, and one hundred percent of our patients and/or their families know the result of their HbA1c results obtained, on average, every two months before the consultation. This is strongly associated with HbA1c outcome as shown by the HSG[23].

Friendly and personal contacts: Friendly and personal contacts with a large dose of psychological support are indispensable in the long-term relationship of a patient with a chronic disease and diabetes is perhaps the best example because of the multitude of daily behavioral decisions that must be acknowledged and accomplished. The diabetologist must know the whole story of the life of his or her patient, and must adapt his or her psychology to the psychology of the patients and of their families, and not the reverse. The diabetologist is not interchangeable as it can be the case for some other pediatric specialties or practices and this too may help explain our consistent excellent A1c results compared to

others in Hvidoere.

Observers are not allowed during consultations: In the office of the diabetologist, at the outpatient clinic, we do not allow observers: temporary assistants or students. This is most important especially with adolescents, in an effort not to disrupt the mutual trust and to preserve privacy. Patients are not undressed (except to search for lipodystrophies)... They are not ill... It is important on a psychological point of view, mainly with adolescents and with Muslims.

Education is made-to-measure: Nearly 50% of our patients are immigrants and mainly of Moghrabin origin (especially from Morocco). Because of the such cultural, economic and social differences, the education we offer must be adapted to the family and their food choices with individualized teaching modules and concepts but with the same overall glycemic goals in mind. Education must be made-to-measure.

High frequency of long-duration consultations: The duration of the medical consultation varies between 30 to 60 min and is preceded by a consultation with a nurse specialized in pediatrics as well as in diabetology. If necessary further consultation takes place with the dietician, the psychologist or the social worker.

Treatment is nearly costless; the nurses are allowed to go to schools: Care is provided in a specialized pediatric and adolescent/young adult diabetology clinic of a high ranking Belgian university public hospital, recognized by the Belgian Social Security ministry in an official manner. Medical and paramedical (nurse, dietician, psychologist, social worker) "consultations" and material necessary for treatment are nearly costless. Nurses are also allowed and compensated for time to do home and school visits and this is especially helpful for those in poor financial, psychological or immigrant circumstances where more teaching time is required to reach goals and sustain them. We believe that such multidisciplinary ongoing care helps explain our good results even among otherwise potentially more problematic patient populations.

We follow our patients into adulthood: We follow our patients into adulthood and do not automatically suggest their transition to adult physicians after an arbitrary age of 18 years. We believe this allows us to assist with transition through adolescence and into better self-care behaviors as young adults. We also believe that this allows our professional team to be better aware of the actual complications that may only occur after longer duration of diabetes. At the onset of 2014, we followed 527 children and adolescents with diabetes aged < 18 year and 495 aged ≥ 18 year.

Influence of family characteristics and alexithymia on HbA1c: The HSG has shown that family factors, particularly dynamic and communication factors such as parental over-involvement and adolescent-parent concordance on responsibility for diabetes care appear be important determinants of metabolic outcomes in adolescents with diabetes[24]. We tried to determine the family characteristics and the psychological factors influencing A1c. The maternal perception of family cohesiveness and maternal alexithymia predict on glycemic control in children and adolescents with diabetes[25]. We showed, for the first time, that children who have difficulties in expressing their feelings to others are more at risk of poor glycemic control. In future, it will be useful to identify the diabetic young people who have such difficulties and to consider interventions designed specifically for them[26].

Confusion between conventional and intensive therapy

The team that I have created in Brussels believes it is inappropriate to automatically designate the term "intensive treatment" only to imply insulin pumps or multidose insulin regimens when, in fact, it is the goals of glycemic control and A1c achievement that should define intensified treatment not the manner or number of insulin doses each day. It is inadequate to systematically assign the multiple injection regimen, or the pump therapy, to "intensive" treatment, and some forms of the twice-daily injection regimen abusively called "conventional" (there are different strategies as shown by the HSG, premix insulins giving the worst results and the CTfreemix + obtaining the best HbA1c levels) to a non-intensified therapeutic category of insulin therapy. Indeed, a multiple injection regimen, or the use of pumps, not associated with a good intensified and complete education, as well with the application of the consecutive knowledge, may have deleterious effects on HbA1c, as shown by the HSG. The confusion between "conventional therapy" and "intensive therapy" was born from misinterpretation of how the DCCT was structured in 1993[3]. In their "conventional group" with one or two daily injections, there was no insulin adjustment except in order to avoid clinical symptoms such as polyuria and polydipsia with hyperglycemia or symptoms and signs reflecting excessive hypoglycemia and there were very few consultation visits except for every three months follow-up assessments for overall monitoring and lab work. There was no blood glucose target established for the conventional treatment group, no specific amount of monitoring and minimal education sessions that took place; all of this was designed to mimic the type of "non-intensive" usual treatment at that time[3]. Bolli[27] wrote: "One concept should be clear. The difference between intensive and non-intensive therapy is limited to the glycemic targets (mean glycemia < 150 mg/dL which gives an HbA1c < 7%). Non-intensive therapy is defined as a model of insulin treatment (2 or ≥ 4 injections, CSII, diet, HBGM, education, *etc.*) giving a mean blood glucose concentration and % of HbA1c above the values indicated by the DCCT".

General conclusion

Because recent multicenter studies, even those performed in developed countries without financial restriction, show that treatment of childhood, adolescent and young adults diabetes is inadequate in general and that levels of HbA1c are very different, diabetes treatment teams should individually explore the reasons for failure, without any prejudice or bias, in their own centers especially when center average A1c results are over 8%. The number of daily insulin injections, 2 or $\geqslant$ 4 or the use of pumps, by itself does not necessarily give better results. Merely increasing the number of daily insulin injections or encouraging insulin pump treatment does not automatically produce better results although may offer greater flexibility for the patient and family. Key remains unified education by a team of diabetes professionals who know their patient and his/her family, work to educate and re-educate and mutually sets goals known and greed upon by not only the entire team providing such care but also the patient and his or her family. Any dogmatism must be avoided. Treatment cost *vs* results must also be taken into account.

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REFERENCES

1 **Dorchy H**, Olinger S. [Well-being of insulin-dependent diabetics. Evaluation of 100 adolescents and young adults in relation to their metabolic control]. *Presse Med* 1997; **26**: 1420-1424 [PMID: 9404353]
2 **Hoey H**, Aanstoot HJ, Chiarelli F, Daneman D, Danne T, Dorchy H, Fitzgerald M, Garandeau P, Greene S, Holl R, Hougaard P, Kaprio E, Kocova M, Lynggaard H, Martul P, Matsuura N, McGee HM, Mortensen HB, Robertson K, Schoenle E, Sovik O, Swift P, Tsou RM, Vanelli M, Aman J. Good metabolic control is associated with better quality of life in 2,101 adolescents with type 1 diabetes. *Diabetes Care* 2001; **24**: 1923-1928 [PMID: 11679458 DOI: 10.2337/diacare.24.11.1923]
3 The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group. *N Engl J Med* 1993; **329**: 977-986 [PMID: 8366922 DOI: 10.1056/NEJM199309303291401]
4 **Verougstraete CM**, Libert JA, Dorchy HR. Discordant diabetic retinopathy in homozygous twins: the importance of good metabolic control. *J Pediatr* 1999; **134**: 658 [PMID: 10228306 DOI: 10.1016/S0022-3476(99)70257-X]
5 **Dorchy H**. [What glycemic control can be achieved in young diabetics without residual secretion of endogenous insulin? What is the frequency of severe hypoglycemia and subclinical complications?]. *Arch Pediatr* 1994; **1**: 970-981 [PMID: 7834046]
6 **Dorchy H**. Dorchy's recipes explaining the "Intriguing efficacity of Belgian conventional therapy". *Diabetes Care* 1994; **17**: 458-460 [PMID: 8062621]
7 **Dorchy H**, Roggemans MP, Willems D. Glycated hemoglobin and related factors in diabetic children and adolescents under 18 years of age: a Belgian experience. *Diabetes Care* 1997; **20**: 2-6 [PMID: 9028684 DOI: 10.2337/diacare.20.1.2]
8 **Mortensen HB**, Hougaard P. Comparison of metabolic control in a cross-sectional study of 2,873 children and adolescents with IDDM from 18 countries. The Hvidøre Study Group on Childhood Diabetes. *Diabetes Care* 1997; **20**: 714-720 [PMID: 9135932 DOI: 10.2337/diacare.20.5.714]
9 **de Beaufort CE**, Swift PG, Skinner CT, Aanstoot HJ, Aman J, Cameron F, Martul P, Chiarelli F, Daneman D, Danne T, Dorchy H, Hoey H, Kaprio EA, Kaufman F, Kocova M, Mortensen HB, Njølstad PR, Phillip M, Robertson KJ, Schoenle EJ, Urakami T, Vanelli M. Continuing stability of center differences in pediatric diabetes care: do advances in diabetes treatment improve outcome? The Hvidoere Study Group on Childhood Diabetes. *Diabetes Care* 2007; **30**: 2245-2250 [PMID: 17540955 DOI: 10.2337/dc07-0475]
10 **de Beaufort CE**, Lange K, Swift PG, Aman J, Cameron F, Castano L, Dorchy H, Fisher LK, Hoey H, Kaprio E, Kocova M, Neu A, Njolstad PR, Phillip M, Schoenle E, Robert JJ, Urukami T, Vanelli M, Danne T, Barrett T, Chiarelli F, Aanstoot HJ, Mortensen HB. Metabolic outcomes in young children with type 1 diabetes differ between treatment centers: the Hvidoere Study in Young Children 2009. *Pediatr Diabetes* 2013; **14**: 422-428 [PMID: 22957743 DOI: 10.1111/j.1399-5448.2012.00922.x]
11 **Cameron FJ**, de Beaufort C, Aanstoot HJ, Hoey H, Lange K, Castano L, Mortensen HB. Lessons from the Hvidoere International Study Group on childhood diabetes: be dogmatic about outcome and flexible in approach. *Pediatr Diabetes* 2013; **14**: 473-480 [PMID: 23627895 DOI: 10.1111/pedi.12036]
12 **Dorchy H**. Insulin regimens and insulin adjustments in diabetic children, adolescents and young adults: personal experience. *Diabetes Metab* 2000; **26**: 500-507 [PMID: 11173723]
13 **Dorchy H**. Dietary management for children and adolescents with diabetes mellitus: personal experience and recommendations. *J Pediatr Endocrinol Metab* 2003; **16**: 131-148 [PMID: 12713249 DOI: 10.1515/JPEM.2003.16.2.131]
14 **Dorchy H**. [Rational use of insulin analogues in the treatment of type 1 diabetic children and adolescents: personal experience]. *Arch Pediatr* 2006; **13**: 1275-1282 [PMID: 16920339 DOI: 10.1016/j.arcped.2006.06.015]
15 **Dorchy H**. [Management of type 1 diabetes (insulin, diet, sport): "Dorchy's recipes"]. *Rev Med Brux* 2010; **31**: S37-S53 [PMID: 21812215]
16 **Heise T**, Nosek L, Rønn BB, Endahl L, Heinemann L, Kapitza C, Draeger E. Lower within-subject variability of insulin detemir in comparison to NPH insulin and insulin glargine in people with type 1 diabetes. *Diabetes* 2004; **53**: 1614-1620 [PMID: 15161770 DOI: 10.2337/diabetes.53.6.1614]
17 **Danne T**, Battelino T, Jarosz-Chobot P, Kordonouri O, Pánkowska E, Ludvigsson J, Schober E, Kaprio E, Saukkonen T, Nicolino M, Tubiana-Rufi N, Klinkert C, Haberland H, Vazeou A, Madacsy L, Zangen D, Cherubini V, Rabbone I, Toni S, de Beaufort C, Bakker-van Waarde W, van den Berg N, Volkov I, Barrio R, Hanas R, Zumsteg U, Kuhlmann B, Aebi C, Schumacher U, Gschwend S, Hindmarsh P, Torres M, Shehadeh N, Phillip M. Establishing glycaemic control with continuous subcutaneous insulin infusion in children and adolescents with type 1 diabetes: experience of the PedPump Study in 17 countries. *Diabetologia* 2008; **51**: 1594-1601 [PMID:

18592209 DOI: 10.1007/s00125-008-1072-2]
18 **Doggen K**, Debacker N, Beckers D, Casteels K, Coeckelberghs M, Dooms L, Dorchy H, Lebrethon M, Logghe K, Maes M, Massa G, Mouraux T, Rooman R, Thiry-Counson G, Van Aken S, Vanbesien J, Van Casteren V. Care delivery and outcomes among Belgian children and adolescents with type 1 diabetes. *Eur J Pediatr* 2012; **171**: 1679-1685 [PMID: 22875314 DOI: 10.1007/s00431-012-1809-2]
19 **Brink SJ**, Miller M, Moltz KC. Education and multidisciplinary team care concepts for pediatric and adolescent diabetes mellitus. *J Pediatr Endocrinol Metab* 2002; **15**: 1113-1130 [PMID: 12387509 DOI: 10.1515/JPEM.2002.15.8.1113]
20 **Dorchy H**. Screening for subclinical complications in young type 1 diabetic patients: experience acquired in Brussels. *Pediatr Endocrinol Rev* 2004; **1**: 380-403 [PMID: 16437030]
21 **Messaaoui A,** Tenoutasse S, Van der Auwera B, Mélot C, Dorchy H. Autoimmune thyroid, celiac and Addison's diseases related to HLA-DQ types in young patients with type 1 diabetes in Belgium. *OJEMD* 2012; **2**: 70-73 [DOI: 10.4236/ojemd.2012.24011]
22 **Messaaoui A**, Willems D, Mélot C, Dorchy H. Risk markers for cardiovascular disease in young type 1 diabetic patients: lipoproteins, high-sensitivity C-reactive protein and adiponectin. *Acta Clin Belg* 2012; **67**: 79-82 [PMID: 22712161]
23 **Swift PG**, Skinner TC, de Beaufort CE, Cameron FJ, Aman J, Aanstoot HJ, Castaño L, Chiarelli F, Daneman D, Danne T, Dorchy H, Hoey H, Kaprio EA, Kaufman F, Kocova M, Mortensen HB, Njølstad PR, Phillip M, Robertson KJ, Schoenle EJ, Urakami T, Vanelli M, Ackermann RW, Skovlund SE. Target setting in intensive insulin management is associated with metabolic control: the Hvidoere childhood diabetes study group centre differences study 2005. *Pediatr Diabetes* 2010; **11**: 271-278 [PMID: 19895567 DOI: 10.1111/j.1399-5448.2009.00596.x]
24 **Cameron FJ**, Skinner TC, de Beaufort CE, Hoey H, Swift PG, Aanstoot H, Aman J, Martul P, Chiarelli F, Daneman D, Danne T, Dorchy H, Kaprio EA, Kaufman F, Kocova M, Mortensen HB, Njølstad PR, Phillip M, Robertson KJ, Schoenle EJ, Urakami T, Vanelli M, Ackermann RW, Skovlund SE. Are family factors universally related to metabolic outcomes in adolescents with Type 1 diabetes? *Diabet Med* 2008; **25**: 463-468 [PMID: 18294223 DOI: 10.1111/j.1464-5491.2008.02399.x]
25 **Meunier J**, Dorchy H, Luminet O. Does family cohesiveness and parental alexithymia predict glycaemic control in children and adolescents with diabetes? *Diabetes Metab* 2008; **34**: 473-481 [PMID: 18783976 DOI: 10.1016/j.diabet.2008.03.005]
26 **Housiaux M**, Luminet O, Van Broeck N, Dorchy H. Alexithymia is associated with glycaemic control of children with type 1 diabetes. *Diabetes Metab* 2010; **36**: 455-462 [PMID: 20863735 DOI: 10.1016/j.diabet.2010.06.004]
27 **Bolli GB**. Rational use of insulin analogues in the treatment of type 1 diabetes mellitus. *Pediatr Endocrinol Rev* 2003; **1**: 9-21 [PMID: 16437009]

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TOPIC HIGHLIGHT

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Insulin sensitivity and complications in type 1 diabetes: New insights

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Abstract

Despite improvements in glucose, lipids and blood pressure control, vascular complications remain the most important cause of morbidity and mortality in patients with type 1 diabetes. For that reason, there is a need to identify additional risk factors to utilize in clinical practice or translate to novel therapies to prevent vascular complications. Reduced insulin sensitivity is an increasingly recognized component of type 1 diabetes that has been linked with the development and progression of both micro- and macrovascular complications. Adolescents and adults with type 1 diabetes have reduced insulin sensitivity, even when compared to their non-diabetic counterparts of similar adiposity, serum triglycerides, high-density lipoprotein cholesterol, level of habitual physical activity, and in adolescents, pubertal stage. Reduced insulin sensitivity is thought to contribute both to the initiation and progression of macro- and microvascular complications in type 1 diabetes. There are currently clinical trials underway examining the benefits of improving insulin sensitivity with regards to vascular complications in type 1 diabetes. Reduced insulin sensitivity is an increasingly recognized component of type 1 diabetes, is implicated in the pathogenesis of vascular complications and is potentially an important therapeutic target to prevent vascular complications. In this review, we will focus on the pathophysiologic contribution of insulin sensitivity to vascular complications and summarize related ongoing clinical trials.

Key words: Type 1 diabetes; Insulin sensitivity; Vascular complications; Hyperfiltration; Cystatin C; Creatinine; Glomerular filtration rate

Core tip: Adolescents and adults with type 1 diabetes have reduced insulin sensitivity compared to their non-diabetic counterparts. Reduced insulin sensitivity is implicated in the development and progression of micro and macrovascular complications in type 1 diabetes. Clinical trials are underway investigating

insulin sensitivity as a therapeutic target to prevent vascular complications in type 1 diabetes. Methods are needed to identify which patients with type 1 diabetes would benefit from treatment of insulin resistance and translation of this to clinical practice.

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INTRODUCTION

The public health burden of type 1 diabetes (T1D), a disease affecting approximately 1.4 million people in the United States and 30 million globally[1], is progressively increasing, largely due to the prevalence of the associated macro- and microvascular complications[1-3]. Macrovascular disease in the form of coronary artery disease (CAD) is the major cause of morbidity and mortality in patients with T1D[3-7]. Annually, up to 1%-2% of young adults (28-38 years of age) with T1D develop CAD[3-5,8,9]. By their mid-forties, over 70% of men and 50% of women with T1D develop measureable coronary artery calcification (CAC) by computed tomography (CT) scan[3-5,10,11] - a marker of significant atherosclerotic plaque burden. In addition to macrovascular disease, microvascular disease continues to cause morbidity and mortality; for example, diabetic nephropathy remains the leading cause of end-stage renal disease in the United States[12-14], and diabetic retinopathy, another form of microvascular disease, is the single most common cause of new-onset blindness[15].

Despite significant improvements in conventional risk factors (*e.g.*, blood pressure, glucose and lipid control) during the past two decades, vascular complications remain a major concern in T1D[14,16-19]. There is a need for improved methods to identify people at risk for, and prevent the development and progression of, these complications.

Adolescents and adults with T1D demonstrate reduced insulin sensitivity, even when compared to non-diabetic counterparts of similar adiposity, body fat distribution, serum triglycerides, high-density lipoprotein cholesterol, level of habitual physical activity, and in adolescents, pubertal stage[17,20-25]. In addition to the insulin resistance documented historically in adolescents with very poor glycemic control[22], more recently significant insulin resistance has been documented in adolescents and adults with T1D, despite modern advances in technology and better glycemic control[20]. Increasing rates of obesity in T1D most likely also contribute to impaired insulin sensitivity[26]. Moreover, a subset of participants in The Diabetes Control and Complications Trial (DCCT) in the intensive treatment arm experienced greater weight gain and worse CVD profiles, which may suggest that lowering HbA1c is not without potential negative effects[27].

The role of insulin sensitivity in the development and progression of macro-[28-30] and microvascular complications[31] in T1D is increasingly recognized. Prospective studies are needed to test the hypothesis that reduced insulin sensitivity is a unifying risk factor for the development of both micro- and macrovascular complications. However, there is increasing evidence implicating reduced insulin sensitivity in the path-ogenesis of vascular complications in T1D[26,32]. It is therefore important to better understand the role of insulin sensitivity in the development of micro- and macrovascular complications in T1D to enable us to intervene early in the pathophysiologic course to halt or slow progression. Accordingly, in this review, we examine the current evidence addressing insulin sensitivity and vascular complications in T1D.

PATHOPHYSIOLOGY OF REDUCED INSULIN SENSITIVITY IN T1D

Historically, when glycemic control was poorer, reduced insulin sensitivity in people with T1D was thought to be solely related to adiposity and high HbA1c[22,33], but recent data have challenged this assumption and suggest that reduced insulin sensitivity cannot simply be explained by excess weight or poor glycemic control[20,21]. In fact, insulin resistance in multiple tissues has recently been documented in T1D subjects with glycemic control much improved from the pre-DCCT era (7.5% ± 0.9% and 8.6% ± 1.6% in adults and adolescents) and with BMI similar to that of non-diabetic individuals[20,21]. These data suggest that resistance to insulin's action on glucose utilization, hepatic glucose release and non-esterified fatty acid suppression is also mediated by factors beyond prevailing adiposity or glycemia[20,21].

The exact mechanism of reduced insulin sensitivity in T1D is poorly understood. Various hypotheses exist to explain reduced insulin sensitivity in T1D[34,35], including prolonged peripheral exposure to supraphysiologic levels of exogenous insulin, weight gain in part caused by intensive insulin therapy, and genetic and environmental factors that contribute to type 2 diabetes (T2D)[26,34,35] (Figure 1). Another proposed mechanism includes lack of delivery of insulin to the portal circulation, causing reduced insulin delivery to the liver, and subsequent lower hepatic IGF-1 production, and lower feedback inhibition to the pituitary leading to higher growth hormone levels, which are known to induce insulin resistance[36] (Figure 1). Abnormal glucagon regulation leading to increased hepatic glucose output has also been implicated in insulin resistance[36]. Another possible mechanistic pathway linking reduced insulin sensitivity to vascular complications in T1D is *via* insulin's effects on overall non-essential fatty acid (NEFA) exposure and lipotoxicity in development of macro- and microangiopathy[20,34,35,37]. Finally, ectopically accumulated fat and its catabolites have been proposed to induce insulin resistance *via* the effects of various signaling

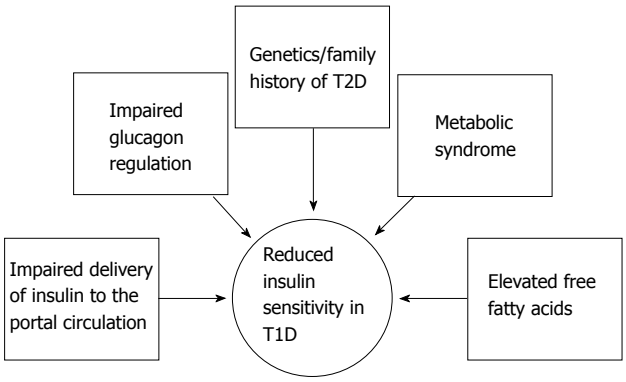

Figure 1 Possible mechanisms of reduced insulin sensitivity in type 1 diabetes. T1D: Type 1 diabetes; T2D: Type 2 diabetes.

pathways including MAPK, protein kinase C, IkB kinases, S6 kinases and ER stress on GLUT4, although the role of ectopic fat remains controversial[38-40] (Figure 1).

There are also robust data demonstrating that subjects with T1D with greater degrees of insulin resistance and/or family history of T2D are at greater risk of micro- and macrovascular complications[41-44] (Figure 1). Furthermore, a high prevalence of metabolic syndrome (38% in men and 40% in women) has been reported in adults with T1D[45]. A close relation between serum uric acid (SUA), insulin sensitivity and metabolic syndrome in non-diabetic subjects has been shown[46]. Conversely, in adolescents and adults with T1D we demonstrated very weak associations between SUA and estimated insulin sensitivity[47].

CURRENT METHODS OF MEASURING AND ESTIMATING INSULIN SENSITIVITY IN T1D

Although insulin resistance is recognized as an important component of vascular complications in T1D, none of the guidelines make specific recommendations about when or how to test for insulin resistance. The insulinopenic nature of T1D prohibits the use of the IV glucose tolerance test (IVGTT) and oral glucose tolerance test (OGTT)-based methods to estimate insulin sensitivity. The gold standard measurement of insulin sensitivity in T1D, glucose disposal rate (GDR) from a hyperinsulinemic-euglycemic clamp, is too cumbersome for use in routine clinical care, but newer insulin sensitivity estimation equations (estimated GDR, eGDR), demonstrate strong agreement with measured insulin sensitivity and offer promise in the clinical setting[11]. Insulin sensitivity prediction equations from the SEARCH Study (eIS-SEARCH)[11], the Pittsburgh Epidemiology of Diabetes Complications Study (eIS-EDC)[48], and the Coronary Artery Calcification in Type 1 diabetes study (eIS-CACTI)[20,31,47] are available, with others currently under development. The eIS-CACTI model includes waist circumference, daily insulin dose per kg body weight, triglycerides and DBP: exp(4.1075 - 0.01299*waist(cm) - 1.05819 *insulin dose [daily units per kg) - 0.00354*triglycerides (mg/dL) - 0.00802*DBP (mm Hg)]. The eIS-CACTI explains 63% of the variance in the GDR in hyperinsulinemic-euglycemic clamp studies, and has been validated in adolescent and adult cohorts with and without T1D[20,31,47]. There remains a need for a commonly accepted measure to estimate insulin sensitivity to be used in the clinical setting to identify patients who would benefit from therapies to improve insulin sensitivity.

INSULIN SENSITIVITY AND MICROVASCULAR COMPLICATIONS

The association between insulin sensitivity and microvascular complications is increasingly recognized, but it is not a recent discovery. In 1968, Martin *et al*[49] showed that microvascular complications were associated with insulin resistance in long-standing T1D subjects. In 1993, Yip *et al*[50] explored insulin resistance as an underlying factor in T1D and found reduced insulin sensitivity, measured by GDR, in a small group with microalbuminuria, while Orchard *et al*[48] later demonstrated that eGDR predicted overt nephropathy in T1D subjects in the EDC cohort. Finally, we and others have reported that higher estimated insulin sensitivity at baseline predicts lower odds of developing diabetic retinopathy, proliferative diabetic retinopathy and diabetic neuropathy independent of other established risk factors[32,48,51]. We have also previously demonstrated that reduced estimated insulin sensitivity predicted incident microalbuminuria and rapid glomerular filtration decline by cystatin C over 6 years[31] and that increased insulin sensitivity predicted regression of albuminuria in adults with T1D[32], similar to data in the EDC study[48,52].

Mechanisms underlying insulin sensitivity and renal pathology remain unclear, but the association between reduced insulin sensitivity and hemodynamic changes in the kidney is increasingly recognized[13]. A growing body of data demonstrates that insulin resistance is associated with an elevation of glomerular hydrostatic pressure causing increased renal vascular permeability and ultimately glomerular hyperfiltration[13,53-60]. Another possible mechanistic pathway linking insulin resistance to diabetic nephropathy is *via* effects on overall non-esterified fatty acid exposure and lipotoxicity, leading to the development of angiopathy[36].

Markers of reduced insulin sensitivity in adults with T1D have also been linked with increased risk of diabetic retinopathy and proliferative diabetic retinopathy in the EURODIAB Prospective Complications Study[61]. However, the investigators in EURODIAB included surrogates for insulin sensitivity including triglycerides and waist-to-hip ratio rather than measured or validated estimates of insulin sensitivity[61]. In the DCCT/EDIC study, higher estimated insulin sensitivity using the Pittsburgh eGDR equation was associated with a lower risk for retinopathy in both the conventionally treated and intensively treated groups, though not independent of HbA1c[30]. Some

studies in adults with T2D demonstrate an association between reduced measured insulin sensitivity and diabetic retinopathy[62,63], but there is a need for more data in T1D. There are also limited data on insulin sensitivity and diabetic neuropathy in T1D, with only a single small study showing an association between estimated insulin sensitivity and diabetic neuropathy in adults with T1D[64]. In contrast, the association between reduced insulin sensitivity and diabetic neuropathy is well recognized in adults with T2D[65-67].

INSULIN SENSITIVITY AND MACROVASCULAR COMPLICATIONS

In the general population, insulin resistance has been implicated as an important contributor to accelerated atherosclerosis[68,69]. In the CACTI study, a longitudinal cohort study of adults with T1D designed to investigate the determinants of early and accelerated atherosclerosis in T1D, insulin sensitivity independently predicted CAC[20,70]. Data from EDC also demonstrated strong associations between insulin sensitivity and coronary artery disease in adults with T1D[30]. In the DCCT, excess weight gain was associated with insulin resistance, as well as more extensive atherosclerosis during EDIC[43]. Moreover, we have also previously shown associations between insulin resistance and cardiac and exercise dysfunction in adolescents with T1D[21]. Renal health, which is known to be associated with better insulin sensitivity, is also strongly associated with higher peak exercise capacity in adolescents with T1D[52]. Finally, we have shown that higher estimated insulin sensitivity in adolescents with T1D is inversely associated with CVD risk factors[44].

CLINICAL TRIALS

Modification of insulin sensitivity therefore holds promise as a therapeutic target to reduce vascular complications in T1D, since both life style changes (diet and exercise) and drugs such as metformin can improve insulin sensitivity in other populations. Metformin is an inexpensive and well tolerated medication. The most common adverse effects associated with metformin are gastrointestinal, with anemia due to vitamin B12 malabsorption and lactic acidosis being rare events when used in T2D[71]. In T2D, metformin is widely considered to protect against cardiovascular complications[71,72]. In contrast, metformin is not advocated in any major national or international guidelines for the management of T1D[72]. In a recent meta-analysis of randomized trials, Vella *et al*[72] found that metformin therapy in T1D was associated with reduced insulin-dose requirements but no clear evidence for an improvement in glycemic control was demonstrated[72]. Moreover, Nadeau *et al*[73] recently showed that low-dose metformin decreased total daily insulin doses in adolescents with T1D, likely representing improvement in insulin sensitivity[73]. Metformin is also associated with reduced LDL cholesterol[74], precursors of advanced glycation end production[75,76] and a decrease in blood pressure[77,78] in adults with T1D and T2D. However, there are currently insufficient data on vascular outcomes and their surrogates to routinely recommend metformin therapy in adolescents and adults with T1D for improving cardiovascular outcomes. For that reason, there is a need for well-designed randomized clinical trials of sufficient size and duration to show clinical benefit of metformin. Another important consideration is the significant variation observed in adults with T2D in response to metformin[71]. The inter-individual variation in metformin may also apply to patients with T1D. Genetic variation may be one of the important determinants of this inter-individual variation, and improved understanding of underlying genes and pathways has the potential to improve the effect of metformin on insulin sensitivity[71].

The BARI-2D study showed no benefit of an insulin sensitizing strategy compared to insulin provision on diabetic nephropathy in older adults with coronary artery disease T2D[79]. In contrast to most contemporary cohorts with T1D, the BARI-2D trial included older adults with T2D, with most participants already having both hypertension and hyperlipidemia. It is plausible that the vascular injuries in older adults with T2D and longstanding pathology may not be responsive to changes in insulin sensitivity. A hypothesis is that early intervention prior to establishment of vascular lesions may result in significant delay of clinical pathology as suggested by the concept of "metabolic memory" in the DCCT-EDIC study[80-84]. Also, clinical cardiovascular disease typically does not manifest until older ages; for example, it took 17 years of follow-up for the benefits of intensive management to manifest in DCCT[27]. Improvements in outcomes due to adjunctive therapy in the era of intensive management may be more subtle. Furthermore, long-term studies in children are lacking[85].

The REducing With MetfOrmin Vascular Adverse Lesions in Type 1 Diabetes (REMOVAL, NCT01483560) study is an ongoing double-blind randomized clinical trial with metformin to improve insulin sensitivity in adults with T1D in an attempt to prevent vascular complications, and the Effects of Metformin on Cardiovascular Function in Adolescents With Type 1 Diabetes (EMERALD, NCT01808690) is an ongoing double-blind randomized clinical trial with metformin to evaluate if metformin will improve tissue-specific insulin resistance in T1D adolescents using the hyperinsulinemic-euglycemic clamp technique, as well as improve vascular, cardiac, exercise and muscle mitochondrial function (Table 1). The effect of Metformin on Vascular and Mitochondrial Function in Type 1 Diabetes (MeT1, NCT01813929) study is also underway which seeks to measure the effect of improving insulin sensitivity on vascular function and compliance, and mitochondrial function in adults with T1D. Metformin Therapy for Overweight Adolescents with Type 1 Diabetes (NCT01881828) being performed in the T1D Exchange clinic network seeks to evaluate the efficacy and safety of the use of metformin in

Table 1 Clinical trials investigating insulin sensitivity as a therapeutic target in type 1 diabetes

Clinical trial name(s)	Description
Metformin Vascular Adverse Lesions in Type 1 Diabetes (REMOVAL) trial (NCT01483560)	Double-blind RCT with metformin to improve insulin sensitivity in subjects with T1D in an attempt to prevent vascular complications
Effects of Metformin On Cardiovascular Function In Adolescents with Type 1 Diabetes (EMERALD) study (NCT01808690)	Double-blind RCT with metformin to evaluate if metformin will improve tissue-specific insulin resistance in T1D adolescents using the hyperinsulinemic-euglycemic clamp technique, as well as improve vascular, cardiac, exercise and muscle mitochondrial function
Insulin Clamp Ancillary Study for Assessment of Insulin Resistance (NCT02045290)	Assess if metformin will improve tissue-specific insulin resistance in type 1 diabetes using hyperinsulinemic euglycemic clamps
Metformin Therapy for Overweight Adolescents With Type 1 Diabetes (NCT01881828)	Evaluate the efficacy and safety of the use of metformin in addition to standard insulin therapy in overweight and obese children and adolescents, age 12 - < 20 yr, with type 1 diabetes for at least 1 yr
Effect of Metformin on Vascular and Mitochondrial Function in Type 1 Diabetes (MeT1, NCT01813929)	Measure the effect of insulin sensitivity vascular function and compliance, and mitochondrial function in T1D

RCT: Randomized clinical trial; T1D: Type 1 diabetes.

addition to standard insulin therapy in overweight and obese children and adolescents, age 12 - < 20 years, with type 1 diabetes for at least 1 year. Furthermore, the insulin Clamp Ancillary Study for Assessment of Insulin Resistance (NCT02045290) is an associated study underway to evaluate if metformin will improve tissue-specific insulin resistance in obese T1D adolescents using hyperinsulinemic-euglycemic clamp technique (Table 1). Smaller metformin trials are also underway. Additional studies are also needed to assess the impact of other drugs that influence insulin sensitivity in T2D, including glucagon-like peptide-1 analogues, dipeptidyl peptidase-4 inhibitor, sodium glucose co-transporter 2 inhibitors, thiazolidinediones and bromocriptine, which may have similarly beneficial effects in T1D.

DIET AND EXERCISE

Diet and exercise are important modifiable risk factors in the development of insulin resistance and vascular complications in T1D[85]. We have previously reported that adults with T1D consume higher levels of fat and saturated fat than their non-diabetic peers, and that the high intake of fat is associated with risk factors for coronary heart disease[86,87]. Although studies suggest that nutrition influences vascular complications in adults with T1D[85,88], it remains inconclusive whether insulin resistance or specific nutrients are responsible for this association. The ADA and ISPAD both emphasize incorporation of fruits, vegetables, whole grains, and low-fat food choices[89,90], but further studies related to insulin sensitivity are required. Adolescents with T1D also appear to be more sedentary and less fit than their non-diabetic counterparts[91]. Higher physical fitness among youth with T1D is associated with lower HbA1c[85,91]. In a study of overweight and sedentary nondiabetic children, 3 mo of aerobic training provided dose-response benefits for insulin resistance compared with usual physical activity[92]. Moreover, small T1D studies have demonstrated increased fitness and decreased total daily insulin dosage with aerobic and strength training, compared with normal daily activities[93]. Finally, higher levels of fitness reduced the mortality risk associated with diabetes and CVD in older adult men with diabetes, but those with T1D *vs* T2D were not analyzed separately[94]. Moreover, higher levels of energy expenditure (due to a more active lifestyle) were found to be associated with increased cardiorespiratory fitness in a small study of T1D adults, but not necessarily better glycemic control[95]. Therefore, while physical activity appears very promising to improve insulin resistance and reduce CVD, definitive trials in T1D are still required.

CONCLUSION

One of the major challenges in preventing vascular complications in T1D relates to the accurate identification of high risk patients at an early stage when pathology may be amenable to intervention. A promising potential therapeutic target is insulin sensitivity. Reduced insulin sensitivity is well documented in both adolescents and adults with T1D, and is thought to contribute both to the initiation and progression of macro- and microvascular complications. Novel insulin sensitivity equations derived from easily identified risk factors (*e.g.*, waist circumference and insulin dose) may allow the clinician to estimate insulin sensitivity in the clinical setting. Interventions to improve insulin sensitivity, including diet, exercise and insulin sensitizing medications, are potential therapies to supplement conventional therapies in reducing vascular complications in T1D, but further study is required. Finally, translation of insulin sensitivity into clinical practice as a therapeutic target to reduce vascular complications requires investment in adequately powered clinical trials designed to capture important long-term vascular outcomes.

REFERENCES

1 **Forlenza GP**, Rewers M. The epidemic of type 1 diabetes: what is it telling us? *Curr Opin Endocrinol Diabetes Obes* 2011; **18**: 248-251 [PMID: 21844707 DOI: 10.1097/MED.0b013e32834872ce]
2 **Stark Casagrande S**, Fradkin JE, Saydah SH, Rust KF, Cowie

CC. The prevalence of meeting A1C, blood pressure, and LDL goals among people with diabetes, 1988-2010. *Diabetes Care* 2013; **36**: 2271-2279 [PMID: 23418368 DOI: 10.2337/dc12-2258]

3 **Libby P**, Nathan DM, Abraham K, Brunzell JD, Fradkin JE, Haffner SM, Hsueh W, Rewers M, Roberts BT, Savage PJ, Skarlatos S, Wassef M, Rabadan-Diehl C. Report of the National Heart, Lung, and Blood Institute-National Institute of Diabetes and Digestive and Kidney Diseases Working Group on Cardiovascular Complications of Type 1 Diabetes Mellitus. *Circulation* 2005; **111**: 3489-3493 [PMID: 15983263 DOI: 10.1161/CIRCULATIONAHA.104.529651]

4 **Krolewski AS**, Kosinski EJ, Warram JH, Leland OS, Busick EJ, Asmal AC, Rand LI, Christlieb AR, Bradley RF, Kahn CR. Magnitude and determinants of coronary artery disease in juvenile-onset, insulin-dependent diabetes mellitus. *Am J Cardiol* 1987; **59**: 750-755 [PMID: 3825934]

5 **Olson JC**, Edmundowicz D, Becker DJ, Kuller LH, Orchard TJ. Coronary calcium in adults with type 1 diabetes: a stronger correlate of clinical coronary artery disease in men than in women. *Diabetes* 2000; **49**: 1571-1578 [PMID: 10969842]

6 **de Ferranti SD**, de Boer IH, Fonseca V, Fox CS, Golden SH, Lavie CJ, Magge SN, Marx N, McGuire DK, Orchard TJ, Zinman B, Eckel RH. Type 1 diabetes mellitus and cardiovascular disease: a scientific statement from the American Heart Association and American Diabetes Association. *Circulation* 2014; **130**: 1110-1130 [PMID: 25114208 DOI: 10.1161/CIR.0000000000000034]

7 **Go AS**, Mozaffarian D, Roger VL, Benjamin EJ, Berry JD, Blaha MJ, Dai S, Ford ES, Fox CS, Franco S, Fullerton HJ, Gillespie C, Hailpern SM, Heit JA, Howard VJ, Huffman MD, Judd SE, Kissela BM, Kittner SJ, Lackland DT, Lichtman JH, Lisabeth LD, Mackey RH, Magid DJ, Marcus GM, Marelli A, Matchar DB, McGuire DK, Mohler ER, Moy CS, Mussolino ME, Neumar RW, Nichol G, Pandey DK, Paynter NP, Reeves MJ, Sorlie PD, Stein J, Towfighi A, Turan TN, Virani SS, Wong ND, Woo D, Turner MB. Heart disease and stroke statistics--2014 update: a report from the American Heart Association. *Circulation* 2014; **129**: e28-e292 [PMID: 24352519 DOI: 10.1161/01.cir.0000441139.02102.80]

8 **Pambianco G**, Costacou T, Ellis D, Becker DJ, Klein R, Orchard TJ. The 30-year natural history of type 1 diabetes complications: the Pittsburgh Epidemiology of Diabetes Complications Study experience. *Diabetes* 2006; **55**: 1463-1469 [PMID: 16644706]

9 **Secrest AM**, Becker DJ, Kelsey SF, Laporte RE, Orchard TJ. Cause-specific mortality trends in a large population-based cohort with long-standing childhood-onset type 1 diabetes. *Diabetes* 2010; **59**: 3216-3222 [PMID: 20739685 DOI: 10.2337/db10-0862]

10 **Alman AC**, Maahs DM, Rewers MJ, Snell-Bergeon JK. Ideal cardiovascular health and the prevalence and progression of coronary artery calcification in adults with and without type 1 diabetes. *Diabetes Care* 2014; **37**: 521-528 [PMID: 24130360 DOI: 10.2337/dc13-0997]

11 **Duca LM**, Bergman BC, Kinney G, Maahs D, Nadeau K, Rewers M, Schauer IE, Sippl RM, Snell-Bergeon J. Validating Insulin Sensitivity Prediction Equations in Type 1 Diabetes. USA: American Diabetes Association, 2014: Abstract

12 **Collins AJ**, Foley RN, Herzog C, Chavers B, Gilbertson D, Ishani A, Kasiske B, Liu J, Mau LW, McBean M, Murray A, St Peter W, Guo H, Gustafson S, Li Q, Li S, Li S, Peng Y, Qiu Y, Roberts T, Skeans M, Snyder J, Solid C, Wang C, Weinhandl E, Zaun D, Arko C, Chen SC, Dalleska F, Daniels F, Dunning S, Ebben J, Frazier E, Hanzlik C, Johnson R, Sheets D, Wang X, Forrest B, Constantini E, Everson S, Eggers P, Agodoa L. US Renal Data System 2010 Annual Data Report. *Am J Kidney Dis* 2011; **57**: A8, e1-526 [PMID: 21184928 DOI: 10.1053/j.ajkd.2010.10.007]

13 **Bjornstad P**, Cherney D, Maahs DM. Early Diabetic Nephropathy in Type 1 Diabetes - New Insights. *Current Opinion in Endocrinology, Diabetes & Obesity* 2014; **21**: 279-286 [DOI: 10.1097/MED.0000000000000074]

14 **Marshall SM**. Diabetic nephropathy in type 1 diabetes: has the outlook improved since the 1980s? *Diabetologia* 2012; **55**: 2301-2306 [PMID: 22696035 DOI: 10.1007/s00125-012-2606-1]

15 **Fong DS**, Aiello L, Gardner TW, King GL, Blankenship G, Cavallerano JD, Ferris FL, Klein R. Diabetic retinopathy. *Diabetes Care* 2003; **26**: 226-229 [PMID: 12502685]

16 **Orchard TJ**, Secrest AM, Miller RG, Costacou T. In the absence of renal disease, 20 year mortality risk in type 1 diabetes is comparable to that of the general population: a report from the Pittsburgh Epidemiology of Diabetes Complications Study. *Diabetologia* 2010; **53**: 2312-2319 [PMID: 20665208 DOI: 10.1007/s00125-010-1860-3]

17 **Snell-Bergeon JK**, Hokanson JE, Jensen L, MacKenzie T, Kinney G, Dabelea D, Eckel RH, Ehrlich J, Garg S, Rewers M. Progression of coronary artery calcification in type 1 diabetes: the importance of glycemic control. *Diabetes Care* 2003; **26**: 2923-2928 [PMID: 14514603]

18 **Bjornstad P**, Wadwa RP. Risks and benefits of statin use in young people with type 1 diabetes. *Curr Diab Rep* 2014; **14**: 499 [PMID: 24796934 DOI: 10.1007/s11892-014-0499-8]

19 **Nathan DM**, Zinman B, Cleary PA, Backlund JY, Genuth S, Miller R, Orchard TJ. Modern-day clinical course of type 1 diabetes mellitus after 30 years' duration: the diabetes control and complications trial/epidemiology of diabetes interventions and complications and Pittsburgh epidemiology of diabetes complications experience (1983-2005). *Arch Intern Med* 2009; **169**: 1307-1316 [PMID: 19636033 DOI: 10.1001/archinternmed.2009.193]

20 **Schauer IE**, Snell-Bergeon JK, Bergman BC, Maahs DM, Kretowski A, Eckel RH, Rewers M. Insulin resistance, defective insulin-mediated fatty acid suppression, and coronary artery calcification in subjects with and without type 1 diabetes: The CACTI study. *Diabetes* 2011; **60**: 306-314 [PMID: 20978091 DOI: 10.2337/db10-0328]

21 **Nadeau KJ**, Regensteiner JG, Bauer TA, Brown MS, Dorosz JL, Hull A, Zeitler P, Draznin B, Reusch JEB. Insulin Resistance in Adolescents with Type 1 Diabetes and Its Relationship to Cardiovascular Function. *J ClinEndocr Metab* 2010; **95**: 513-521 [DOI: 10.1210/jc.2009-1756]

22 **Amiel SA**, Sherwin RS, Simonson DC, Lauritano AA, Tamborlane WV. Impaired insulin action in puberty. A contributing factor to poor glycemic control in adolescents with diabetes. *N Engl J Med* 1986; **315**: 215-219 [PMID: 3523245 DOI: 10.1056/NEJM198607243150402]

23 **Heptulla RA**, Stewart A, Enocksson S, Rife F, Ma TY, Sherwin RS, Tamborlane WV, Caprio S. In situ evidence that peripheral insulin resistance in adolescents with poorly controlled type 1 diabetes is associated with impaired suppression of lipolysis: a microdialysis study. *Pediatr Res* 2003; **53**: 830-835 [PMID: 12702748 DOI: 10.1203/01.PDR.0000059552.08913.B7]

24 **Williams KV**, Erbey JR, Becker D, Arslanian S, Orchard TJ. Can clinical factors estimate insulin resistance in type 1 diabetes? *Diabetes* 2000; **49**: 626-632 [PMID: 10871201]

25 **Levy-Marchal C**, Arslanian S, Cutfield W, Sinaiko A, Druet C, Marcovecchio ML, Chiarelli F. Insulin resistance in children: consensus, perspective, and future directions. *J Clin Endocrinol Metab* 2010; **95**: 5189-5198 [PMID: 20829185 DOI: 10.1210/jc.2010-1047]

26 **Cleland SJ**, Fisher BM, Colhoun HM, Sattar N, Petrie JR. Insulin resistance in type 1 diabetes: what is 'double diabetes' and what are the risks? *Diabetologia* 2013; **56**: 1462-1470 [PMID: 23613085 DOI: 10.1007/s00125-013-2904-2]

27 **Nathan DM**, Cleary PA, Backlund JY, Genuth SM, Lachin JM, Orchard TJ, Raskin P, Zinman B. Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. *N Engl J Med* 2005; **353**: 2643-2653 [PMID: 16371630

DOI: 10.1056/NEJMoa052187]
28 **Orchard TJ**, Olson JC, Erbey JR, Williams K, Forrest KY, Smithline Kinder L, Ellis D, Becker DJ. Insulin resistance-related factors, but not glycemia, predict coronary artery disease in type 1 diabetes: 10-year follow-up data from the Pittsburgh Epidemiology of Diabetes Complications Study. *Diabetes Care* 2003; **26**: 1374-1379 [PMID: 12716791]
29 **Soedamah-Muthu SS**, Chaturvedi N, Toeller M, Ferriss B, Reboldi P, Michel G, Manes C, Fuller JH. Risk factors for coronary heart disease in type 1 diabetic patients in Europe: the EURODIAB Prospective Complications Study. *Diabetes Care* 2004; **27**: 530-537 [PMID: 14747240]
30 **Kilpatrick ES**, Rigby AS, Atkin SL. Insulin resistance, the metabolic syndrome, and complication risk in type 1 diabetes: "double diabetes" in the Diabetes Control and Complications Trial. *Diabetes Care* 2007; **30**: 707-712 [PMID: 17327345 DOI: 10.2337/dc06-1982]
31 **Bjornstad P**, Snell-Bergeon JK, Rewers M, Jalal D, Chonchol MB, Johnson RJ, Maahs DM. Early diabetic nephropathy: a complication of reduced insulin sensitivity in type 1 diabetes. *Diabetes Care* 2013; **36**: 3678-3683 [PMID: 24026551 DOI: 10.2337/dc13-0631]
32 **Bjornstad P,** Maahs DM, Johnson RJ, Rewers M, Snell-Bergeon JK. Estimated insulin sensitivity predicts regression of albuminuria in Type 1 diabetes. *Diabet Med* 2014 [PMID: 25303233 DOI: 10.1111/dme.12572]
33 **Yki-Järvinen H**, Koivisto VA. Natural course of insulin resistance in type I diabetes. *N Engl J Med* 1986; **315**: 224-230 [PMID: 3523247 DOI: 10.1056/NEJM198607243150404]
34 **Perseghin G**, Lattuada G, Danna M, Sereni LP, Maffi P, De Cobelli F, Battezzati A, Secchi A, Del Maschio A, Luzi L. Insulin resistance, intramyocellular lipid content, and plasma adiponectin in patients with type 1 diabetes. *Am J Physiol Endocrinol Metab* 2003; **285**: E1174-E1181 [PMID: 12933352 DOI: 10.1152/ajpendo.00279.2003]
35 **Houstis N**, Rosen ED, Lander ES. Reactive oxygen species have a causal role in multiple forms of insulin resistance. *Nature* 2006; **440**: 944-948 [PMID: 16612386 DOI: 10.1038/nature04634]
36 **Aronoff SL**, Berkowitz K, Shreiner B, Want L. Glucose Metabolism and Regulation: Beyond Insulin and Glucagon. *Diabetes Spectrum* 2004; **17**: 183-190
37 **Bergman BC**, Howard D, Schauer IE, Maahs DM, Snell-Bergeon JK, Eckel RH, Perreault L, Rewers M. Features of hepatic and skeletal muscle insulin resistance unique to type 1 diabetes. *J Clin Endocrinol Metab* 2012; **97**: 1663-1672 [PMID: 22362823 DOI: 10.1210/jc.2011-3172]
38 **Ye J**. Role of insulin in the pathogenesis of free fatty acid-induced insulin resistance in skeletal muscle. *Endocr Metab Immune Disord Drug Targets* 2007; **7**: 65-74 [PMID: 17346204]
39 **Wullaert A**, van Loo G, Heyninck K, Beyaert R. Hepatic tumor necrosis factor signaling and nuclear factor-kappaB: effects on liver homeostasis and beyond. *Endocr Rev* 2007; **28**: 365-386 [PMID: 17431229 DOI: 10.1210/er.2006-0031]
40 **Kaneto H**, Matsuoka TA, Katakami N, Kawamori D, Miyatsuka T, Yoshiuchi K, Yasuda T, Sakamoto K, Yamasaki Y, Matsuhisa M. Oxidative stress and the JNK pathway are involved in the development of type 1 and type 2 diabetes. *Curr Mol Med* 2007; **7**: 674-686 [PMID: 18045145]
41 **Erbey JR**, Kuller LH, Becker DJ, Orchard TJ. The association between a family history of type 2 diabetes and coronary artery disease in a type 1 diabetes population. *Diabetes Care* 1998; **21**: 610-614 [PMID: 9571351]
42 **Roglic G**, Colhoun HM, Stevens LK, Lemkes HH, Manes C, Fuller JH. Parental history of hypertension and parental history of diabetes and microvascular complications in insulin-dependent diabetes mellitus: the EURODIAB IDDM Complications Study. *Diabet Med* 1998; **15**: 418-426 [PMID: 9609365 DOI: 10.1002/(SICI)1096-9136(199805)15: 5<418: : AID-DIA604>3.0.CO; 2-P]
43 **Purnell JQ**, Zinman B, Brunzell JD. The effect of excess weight gain with intensive diabetes mellitus treatment on cardiovascular disease risk factors and atherosclerosis in type 1 diabetes mellitus: results from the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Study (DCCT/EDIC) study. *Circulation* 2013; **127**: 180-187 [PMID: 23212717 DOI: 10.1161/CIRCULATIONAHA.111.077487]
44 **Specht BJ**, Wadwa RP, Snell-Bergeon JK, Nadeau KJ, Bishop FK, Maahs DM. Estimated insulin sensitivity and cardiovascular disease risk factors in adolescents with and without type 1 diabetes. *J Pediatr* 2013; **162**: 297-301 [PMID: 22921593 DOI: 10.1016/j.jpeds.2012.07.036]
45 **Thorn LM**, Forsblom C, Fagerudd J, Thomas MC, Pettersson-Fernholm K, Saraheimo M, Wadén J, Rönnback M, Rosengård-Bärlund M, Björkesten CG, Taskinen MR, Groop PH. Metabolic syndrome in type 1 diabetes: association with diabetic nephropathy and glycemic control (the FinnDiane study). *Diabetes Care* 2005; **28**: 2019-2024 [PMID: 16043748]
46 **Rathmann W**, Funkhouser E, Dyer AR, Roseman JM. Relations of hyperuricemia with the various components of the insulin resistance syndrome in young black and white adults: the CARDIA study. Coronary Artery Risk Development in Young Adults. *Ann Epidemiol* 1998; **8**: 250-261 [PMID: 9590604]
47 **Bjornstad P**, Snell-Bergeon JK, McFann K, Wadwa RP, Rewers M, Rivard CJ, Jalal D, Chonchol MB, Johnson RJ, Maahs DM. Serum uric acid and insulin sensitivity in adolescents and adults with and without type 1 diabetes. *J Diabetes Complications* 2014; **28**: 298-304 [PMID: 24461546 DOI: 10.1016/j.jdiacomp.2013.12.007]
48 **Orchard TJ**, Chang YF, Ferrell RE, Petro N, Ellis DE. Nephropathy in type 1 diabetes: a manifestation of insulin resistance and multiple genetic susceptibilities? Further evidence from the Pittsburgh Epidemiology of Diabetes Complication Study. *Kidney Int* 2002; **62**: 963-970 [PMID: 12164879 DOI: 10.1046/j.1523-1755.2002.00507.x]
49 **Martin FI**, Stocks AE. Insulin sensitivity and vascular disease in insulin-dependent diabetics. *Br Med J* 1968; **2**: 81-82 [PMID: 5646096]
50 **Yip J**, Mattock MB, Morocutti A, Sethi M, Trevisan R, Viberti G. Insulin resistance in insulin-dependent diabetic patients with microalbuminuria. *Lancet* 1993; **342**: 883-887 [PMID: 8105164]
51 **Bjornstad P**, Maahs DM, Cherney DZ, Cree-Green M, West A, Pyle L, Nadeau KJ. Insulin sensitivity is an important determinant of renal health in adolescents with type 2 diabetes. *Diabetes Care* 2014; **37**: 3033-3039 [PMID: 25071077 DOI: 10.2337/dc14-1331]
52 **Bjornstad P,** Cree Green M, Baumgartner A, Maahs D, Cherney D, Pyle L, Regensteiner JG, Reusch JE, Nadeau K. Renal function is associated with peak exercise capacity in adolescents with type 1 diabetes. *Diabetes Care* 2014; In Press
53 **Catalano C**, Muscelli E, Quiñones Galvan A, Baldi S, Masoni A, Gibb I, Torffvit O, Seghieri G, Ferrannini E. Effect of insulin on systemic and renal handling of albumin in nondiabetic and NIDDM subjects. *Diabetes* 1997; **46**: 868-875 [PMID: 9133557]
54 **Cohen AJ**, McCarthy DM, Stoff JS. Direct hemodynamic effect of insulin in the isolated perfused kidney. *Am J Physiol* 1989; **257**: F580-F585 [PMID: 2679144]
55 **Tucker BJ**, Anderson CM, Thies RS, Collins RC, Blantz RC. Glomerular hemodynamic alterations during acute hyperinsulinemia in normal and diabetic rats. *Kidney Int* 1992; **42**: 1160-1168 [PMID: 1453601]
56 **Cherney DZ**, Miller JA, Scholey JW, Nasrallah R, Hébert RL, Dekker MG, Slorach C, Sochett EB, Bradley TJ. Renal hyperfiltration is a determinant of endothelial function responses to cyclooxygenase 2 inhibition in type 1 diabetes. *Diabetes Care* 2010; **33**: 1344-1346 [PMID: 20332349]

57 **Cherney DZ**, Perkins BA, Soleymanlou N, Maione M, Lai V, Lee A, Fagan NM, Woerle HJ, Johansen OE, Broedl UC, von Eynatten M. Renal hemodynamic effect of sodium-glucose cotransporter 2 inhibition in patients with type 1 diabetes mellitus. *Circulation* 2014; **129**: 587-597 [PMID: 24334175 DOI: 10.1161/CIRCULATIONAHA.113.005081]

58 **Cherney DZ**, Reich HN, Jiang S, Har R, Nasrallah R, Hébert RL, Lai V, Scholey JW, Sochett EB. Hyperfiltration and effect of nitric oxide inhibition on renal and endothelial function in humans with uncomplicated type 1 diabetes mellitus. *Am J Physiol Regul Integr Comp Physiol* 2012; **303**: R710-R718 [PMID: 22855276]

59 **Cherney DZ**, Scholey JW, Sochett E, Bradley TJ, Reich HN. The acute effect of clamped hyperglycemia on the urinary excretion of inflammatory cytokines/chemokines in uncomplicated type 1 diabetes: a pilot study. *Diabetes Care* 2011; **34**: 177-180 [PMID: 20841614 DOI: 10.2337/dc10-1219]

60 **Cherney DZ**, Sochett EB. Evolution of renal hyperfiltration and arterial stiffness from adolescence into early adulthood in type 1 diabetes. *Diabetes Care* 2011; **34**: 1821-1826 [PMID: 21636797 DOI: 10.2337/dc11-0167]

61 **Chaturvedi N**, Sjoelie AK, Porta M, Aldington SJ, Fuller JH, Songini M, Kohner EM. Markers of insulin resistance are strong risk factors for retinopathy incidence in type 1 diabetes. *Diabetes Care* 2001; **24**: 284-289 [PMID: 11213880]

62 **Parvanova A**, Iliev I, Filipponi M, Dimitrov BD, Vedovato M, Tiengo A, Trevisan R, Remuzzi G, Ruggenenti P. Insulin resistance and proliferative retinopathy: a cross-sectional, case-control study in 115 patients with type 2 diabetes. *J Clin Endocrinol Metab* 2004; **89**: 4371-4376 [PMID: 15356034 DOI: 10.1210/jc.2003-032076]

63 **Anan F**, Takayuki M, Takahashi N, Nakagawa M, Eshima N, Saikawa T, Yoshimatsu H. Diabetic retinopathy is associated with insulin resistance and cardiovascular autonomic dysfunction in type 2 diabetic patients. *Hypertens Res* 2009; **32**: 299-305 [PMID: 19262488 DOI: 10.1038/hr.2009.8]

64 **Chillarón JJ**, Goday A, Flores-Le-Roux JA, Benaiges D, Carrera MJ, Puig J, Cano-Pérez JF, Pedro-Botet J. Estimated glucose disposal rate in assessment of the metabolic syndrome and microvascular complications in patients with type 1 diabetes. *J Clin Endocrinol Metab* 2009; **94**: 3530-3534 [PMID: 19584183 DOI: 10.1210/jc.2009-0960]

65 **Cho YN**, Lee KO, Jeong J, Park HJ, Kim SM, Shin HY, Hong JM, Ahn CW, Choi YC. The role of insulin resistance in diabetic neuropathy in Koreans with type 2 diabetes mellitus: a 6-year follow-up study. *Yonsei Med J* 2014; **55**: 700-708 [PMID: 24719137 DOI: 10.3349/ymj.2014.55.3.700]

66 **Lee KO**, Nam JS, Ahn CW, Hong JM, Kim SM, Sunwoo IN, Moon JS, Na SJ, Choi YC. Insulin resistance is independently associated with peripheral and autonomic neuropathy in Korean type 2 diabetic patients. *Acta Diabetol* 2012; **49**: 97-103 [PMID: 20130937 DOI: 10.1007/s00592-010-0176-6]

67 **Lehtinen JM**, Niskanen L, Hyvönen K, Siitonen O, Uusitupa M. Nerve function and its determinants in patients with newly-diagnosed type 2 (non-insulin-dependent) diabetes mellitus and in control subjects--a 5-year follow-up. *Diabetologia* 1993; **36**: 68-72 [PMID: 8436256]

68 **Howard G**, O'Leary DH, Zaccaro D, Haffner S, Rewers M, Hamman R, Selby JV, Saad MF, Savage P, Bergman R. Insulin sensitivity and atherosclerosis. The Insulin Resistance Atherosclerosis Study (IRAS) Investigators. *Circulation* 1996; **93**: 1809-1817 [PMID: 8635260]

69 **Ginsberg HN**. Insulin resistance and cardiovascular disease. *J Clin Invest* 2000; **106**: 453-458 [PMID: 10953019 DOI: 10.1172/JCI10762]

70 **Rodrigues TC,** Haarhues MD, Kinney GL, Rewers M, Snell-Bergeon JK, Veyna AM. Obesity and coronary artery calcium in diabetes: the coronary artery calcification in type 1 diabetes (CACTI) study. *Diabetes Technol Ther* 2011; **13**: 991

71 **Pawlyk AC**, Giacomini KM, McKeon C, Shuldiner AR, Florez JC. Metformin pharmacogenomics: current status and future directions. *Diabetes* 2014; **63**: 2590-2599 [PMID: 25060887 DOI: 10.2337/db13-1367]

72 **Vella S**, Buetow L, Royle P, Livingstone S, Colhoun HM, Petrie JR. The use of metformin in type 1 diabetes: a systematic review of efficacy. *Diabetologia* 2010; **53**: 809-820 [PMID: 20057994 DOI: 10.1007/s00125-009-1636-9]

73 **Nadeau KJ**, Chow K, Alam S, Lindquist K, Campbell S, McFann K, Klingensmith G, Walravens P. Effects of low dose metformin in adolescents with type I diabetes mellitus: a randomized, double-blinded placebo-controlled study. *Pediatr Diabetes* 2014 Apr 3; Epub ahead of print [PMID: 24698216 DOI: 10.1111/pedi.12140]

74 **DeFronzo RA**, Goodman AM. Efficacy of metformin in patients with non-insulin-dependent diabetes mellitus. The Multicenter Metformin Study Group. *N Engl J Med* 1995; **333**: 541-549 [PMID: 7623902 DOI: 10.1056/NEJM199508313330902]

75 **Beisswenger PJ**, Howell SK, Touchette AD, Lal S, Szwergold BS. Metformin reduces systemic methylglyoxal levels in type 2 diabetes. *Diabetes* 1999; **48**: 198-202 [PMID: 9892243]

76 **Nagi DK**, Yudkin JS. Effects of metformin on insulin resistance, risk factors for cardiovascular disease, and plasminogen activator inhibitor in NIDDM subjects. A study of two ethnic groups. *Diabetes Care* 1993; **16**: 621-629 [PMID: 8462390]

77 **Giugliano D**, Quatraro A, Consoli G, Minei A, Ceriello A, De Rosa N, D'Onofrio F. Metformin for obese, insulin-treated diabetic patients: improvement in glycaemic control and reduction of metabolic risk factors. *Eur J Clin Pharmacol* 1993; **44**: 107-112 [PMID: 8453955]

78 **Landin K**, Tengborn L, Smith U. Treating insulin resistance in hypertension with metformin reduces both blood pressure and metabolic risk factors. *J Intern Med* 1991; **229**: 181-187 [PMID: 1900072]

79 **August P**, Hardison RM, Hage FG, Marroquin OC, McGill JB, Rosenberg Y, Steffes M, Wall BM, Molitch M. Change in albuminuria and eGFR following insulin sensitization therapy versus insulin provision therapy in the BARI 2D study. *Clin J Am Soc Nephrol* 2014; **9**: 64-71 [PMID: 24178969 DOI: 10.2215/CJN.12281211]

80 Effect of Intensive Diabetes Therapy on the Progression of Diabetic Retinopathy in Patients with Type 1 Diabetes: 18 Years of Follow-up in the DCCT/EDIC. *Diabetes* 2014 Sep 9; Epub ahead of print [PMID: 25204977 DOI: 10.2337/db14-0930]

81 **de Boer IH**, Rue TC, Cleary PA, Lachin JM, Molitch ME, Steffes MW, Sun W, Zinman B, Brunzell JD, White NH, Danis RP, Davis MD, Hainsworth D, Hubbard LD, Nathan DM. Long-term renal outcomes of patients with type 1 diabetes mellitus and microalbuminuria: an analysis of the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications cohort. *Arch Intern Med* 2011; **171**: 412-420 [PMID: 21403038 DOI: 10.1001/archinternmed.2011.16]

82 **de Boer IH**, Sun W, Cleary PA, Lachin JM, Molitch ME, Steffes MW, Zinman B. Intensive diabetes therapy and glomerular filtration rate in type 1 diabetes. *N Engl J Med* 2011; **365**: 2366-2376 [PMID: 22077236 DOI: 10.1056/NEJMoa1111732]

83 **Group DR.** The Effect of Intensive Treatment of Diabetes on the Development and Progression of Long-Term Complications in Insulin-Dependent Diabetes Mellitus. *N Engl J Med* 1993; **329**: 977-986 [PMID: 8366922 DOI: 10.1056/NEJM199309303291401]

84 **Purnell JQ**, Hokanson JE, Marcovina SM, Steffes MW, Cleary PA, Brunzell JD. Effect of excessive weight gain with intensive therapy of type 1 diabetes on lipid levels and blood pressure: results from the DCCT. Diabetes Control and Complications Trial. *JAMA* 1998; **280**: 140-146 [PMID: 9669786]

85 **Maahs DM**, Daniels SR, de Ferranti SD, Dichek HL, Flynn J, Goldstein BI, Kelly AS, Nadeau KJ, Martyn-Nemeth P, Osganian SK, Quinn L, Shah AS, Urbina E. Cardiovascular

disease risk factors in youth with diabetes mellitus: a scientific statement from the american heart association. *Circulation* 2014; **130**: 1532-1558 [PMID: 25170098 DOI: 10.1161/CIR.0000000000000094]

86 **Bishop FK**, Maahs DM, Snell-Bergeon JK, Ogden LG, Kinney GL, Rewers M. Lifestyle risk factors for atherosclerosis in adults with type 1 diabetes. *Diab Vasc Dis Res* 2009; **6**: 269-275 [PMID: 20368221 DOI: 10.1177/1479164109346359]

87 **Snell-Bergeon JK**, Chartier-Logan C, Maahs DM, Ogden LG, Hokanson JE, Kinney GL, Eckel RH, Ehrlich J, Rewers M. Adults with type 1 diabetes eat a high-fat atherogenic diet that is associated with coronary artery calcium. *Diabetologia* 2009; **52**: 801-809 [PMID: 19219420 DOI: 10.1007/s00125-009-1280-4]

88 **Albu JB**, Heilbronn LK, Kelley DE, Smith SR, Azuma K, Berk ES, Pi-Sunyer FX, Ravussin E. Metabolic changes following a 1-year diet and exercise intervention in patients with type 2 diabetes. *Diabetes* 2010; **59**: 627-633 [PMID: 20028945 DOI: 10.2337/db09-1239]

89 Standards of medical care in diabetes--2014. *Diabetes Care* 2014; **37** Suppl 1: S14-S80 [PMID: 24357209 DOI: 10.2337/dc14-S014]

90 **Acerini C**, Craig ME, de Beaufort C, Maahs DM, Hanas R. Introduction to ISPAD Clinical Practice Consensus Guidelines 2014 Compendium. *Pediatr Diabetes* 2014; **15** Suppl 20: 1-3 [PMID: 25182304 DOI: 10.1111/pedi.12182]

91 **Robertson K**, Riddell MC, Guinhouya BC, Adolfsson P, Hanas R. Exercise in children and adolescents with diabetes. *Pediatr Diabetes* 2014; **15** Suppl 20: 203-223 [PMID: 25182315 DOI: 10.1111/pedi.12176]

92 **Davis CL**, Pollock NK, Waller JL, Allison JD, Dennis BA, Bassali R, Meléndez A, Boyle CA, Gower BA. Exercise dose and diabetes risk in overweight and obese children: a randomized controlled trial. *JAMA* 2012; **308**: 1103-1112 [PMID: 22990269 DOI: 10.1001/2012.jama.10762]

93 **D'hooge R**, Hellinckx T, Van Laethem C, Stegen S, De Schepper J, Van Aken S, Dewolf D, Calders P. Influence of combined aerobic and resistance training on metabolic control, cardiovascular fitness and quality of life in adolescents with type 1 diabetes: a randomized controlled trial. *Clin Rehabil* 2011; **25**: 349-359 [PMID: 21112904 DOI: 10.1177/0269215510386254]

94 **McAuley P**, Myers J, Emerson B, Oliveira RB, Blue CL, Pittsley J, Froelicher VF. Cardiorespiratory fitness and mortality in diabetic men with and without cardiovascular disease. *Diabetes Res Clin Pract* 2009; **85**: e30-e33 [PMID: 19524317 DOI: 10.1016/j.diabres.2009.05.012]

95 **Valletta JJ**, Chipperfield AJ, Clough GF, Byrne CD. Daily energy expenditure, cardiorespiratory fitness and glycaemic control in people with type 1 diabetes. *PLoS One* 2014; **9**: e97534 [PMID: 24826899 DOI: 10.1371/journal.pone.0097534]

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TOPIC HIGHLIGHT

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Utility of different glycemic control metrics for optimizing management of diabetes

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Abstract

The benchmark for assessing quality of long-term glycemic control and adjustment of therapy is currently glycated hemoglobin (HbA1c). Despite its importance as an indicator for the development of diabetic complications, recent studies have revealed that this metric has some limitations; it conveys a rather complex message, which has to be taken into consideration for diabetes screening and treatment. On the basis of recent clinical trials, the relationship between HbA1c and cardiovascular outcomes in long-standing diabetes has been called into question. It becomes obvious that other surrogate and biomarkers are needed to better predict cardiovascular diabetes complications and assess efficiency of therapy. Glycated albumin, fructosamin, and 1,5-anhydroglucitol have received growing interest as alternative markers of glycemic control. In addition to measures of hyperglycemia, advanced glucose monitoring methods became available. An indispensible adjunct to HbA1c in routine diabetes care is self-monitoring of blood glucose. This monitoring method is now widely used, as it provides immediate feedback to patients on short-term changes, involving fasting, preprandial, and postprandial glucose levels. Beyond the traditional metrics, glycemic variability has been identified as a predictor of hypoglycemia, and it might also be implicated in the pathogenesis of vascular diabetes complications. Assessment of glycemic variability is thus important, but exact quantification requires frequently sampled glucose measurements. In order to optimize diabetes treatment, there is a need for both key metrics of glycemic control on a day-to-day basis and for more advanced, user-friendly monitoring methods. In addition to traditional discontinuous glucose testing, continuous glucose sensing has become a useful tool to reveal insufficient glycemic management. This new technology is particularly effective in patients with complicated diabetes and provides the opportunity to characterize glucose dynamics. Several continuous glucose monitoring (CGM) systems, which have shown usefulness in clinical practice, are presently on the market. They can broadly be divided into systems providing retrospective or real-time information on glucose patterns. The widespread clinical application of CGM is still hampered by the lack of generally

accepted measures for assessment of glucose profiles and standardized reporting of glucose data. In this article, we will discuss advantages and limitations of various metrics for glycemic control as well as possibilities for evaluation of glucose data with the special focus on glycemic variability and application of CGM to improve individual diabetes management.

Key words: Markers of glycemic control; Hemoglobin A1c; Postprandial glucose; Risk of hyperglycemia and hypoglycemia; Continuous glucose monitoring; Glycemic variability; Glucose dynamics; Standardization; Diabetes mellitus

Core tip: Hemoglobin A1c is the gold standard to assess glycemic control and a surrogate for diabetes-associated complications. Self-monitoring of blood glucose complements daily diabetes management but is insufficient in providing complete information on short-term changes in glucose levels induced by effects of food or antidiabetic medication. Key metrics beyond HbA1c are needed for glycemic control on a day-to-day basis as well as more advanced monitoring methods. Herein, we will review advantages and limitations of different metrics for glycemic control as well as possibilities for characterization of glucose dynamics with the special focus on glycemic variability and continuous glucose monitoring.

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INTRODUCTION

Since landmark studies have provided evidence that glycated hemoglobin (HbA1c) is linked to vascular complications of diabetes[1,2], this biomarker of glycemia emerged as the benchmark for current diabetes management. Thus, optimal diabetes control aims to restore levels of HbA1c to as normal as possible to reduce or prevent diabetic complications. However, HbA1c has some important limitations and is a rather complex measure of hyperglycemia. It represents an indicator for overall glucose exposure, integrating fasting, preprandial as well as postprandial hyperglycemia, but their relative contribution varies with the quality of glycemic control[3]. Apart from several medical conditions that can cause inaccurate test results, HbA1c neither captures glucose fluctuations nor does it provide any information on glucose dynamics.

Chronic sustained hyperglycemia is well known to increase the risk for micro- and macrovascular complications in type 1 as well as in type 2 diabetes. Especially postprandial/postchallenge hyperglycemia, independent of HbA1c or fasting glucose, has been associated with cardiovascular disease[4], and this could be confirmed very recently in a post-hoc analysis of the "Effects of prandial *vs* fasting glycemia on cardiovascular outcomes in type 2 diabetes (HEART2D)" study[5].

As generally accepted and laid down in the American Diabetes Association (ADA) and International Diabetes Federation (IDF) guidelines, strict glycemic control, implicating comprehensive diabetes evaluation, is needed to prevent or delay diabetes complications. Nevertheless, the outcomes of the ACCORD[6] and ADVANCE[7] trials have taught us that HbA1c levels should be tailored to the patients' health status-older age and extensive comorbid conditions require less stringent targets. In the overwhelming majority of large clinical trials, HbA1c has been used to predict long-term outcomes related to morbidity and mortality in people with type 1 and type 2 diabetes, but the strength of association with macrovascular end points was weaker than with microvascular end points. Furthermore, it remains still unclear how various measures of glycemia predict diabetes complications and whether a combination of several markers might even be more strongly related to adverse outcomes than a single biomarker. A recent analysis of data from the Diabetes Control and Complication Trial/Epidemiology of Diabetes Interventions and Complication Study by Nathan *et al*[8] supports the suggestion of using two glycemic markers to strengthen risk prediction. Thus, it would not be surprising if in the near future a combination of shorter and longer term glycemic markers could be used to predict cardiovascular outcomes more precisely. Now, we believe that time has come to move from measurement of HbA1c to other markers, allowing for assessment of short-time and intermediate-time changes in glycemia.

Although self-monitoring of blood glucose (SMBG) is still the predominant mode of glucose monitoring, the use of advanced technology, such as continuous glucose monitoring (CGM) has shown remarkable benefits and expanded significantly during recent years. One of the major problems in utilization such systems are appropriate evaluation of the great amount of data provided by CGM and the lack of standardization.

The purpose of the present review is to give an insight into the problems of choosing the most relevant markers of glycemic control and how to evaluate CGM data properly to optimize management of diabetes in order to avoid long-term complications.

MARKERS OF GLYCEMIC CONTROL

Glycemic markers are indispensable in routine practice as well as in clinical trials to guide therapy and to investigate the efficacy of medications on patients' glycemic control. A summary of useful glucose measures is shown in Table 1. As discussed in the following, not only do these markers cover different timeframes of glycemic control,

Table 1 Traditional and alternative markers of glycemic control

Marker	Time span of glycemic control	Ref.
Hemoglobin A1c	1-3 mo	Cohen[15], 2007
Glycated serum proteins	2-3 wk	Takahashi *et al*[33], 2007
1,5-Anhydroglucitol	1-2 wk	Dungan *et al*[43], 2008
Glycemic variability indices	24-72 h	Rodbard[54], 2009
Mean plasma glucose	24-72 h	Bergenstal *et al*[30], 2013
Fasting plasma glucose	8-10 h	Monami *et al*[22], 2013
Postprandial plasma glucose	2-4 h	Standl *et al*[23], 2011

they also provide different information on glucose metabolism and may reflect different pathways.

HbA1c

HbA1c is formed by nonenzymatic glycation as adduct of glucose and the hemoglobin molecule. The HbA1c value reflects average glucose over 1-3 mo. The National Glycohemoglobin Standardization Program is the organization that evaluates, sets standards for accuracy, and certifies methods for measurement of HbA1c. Besides laboratory tests, even home monitors for patients have been approved, *e.g.*, Bayer A1cNow Selfcheck At-HomeA1c System or BioRad's Micromat™ Ⅱ Hemoglobin Instrument.

HbA1c has been used as a biomarker for more than three decades as universally accepted means for monitoring glycemic control and as clinical surrogate endpoint in diabetes. In both patients with type 1 and type 2 diabetes, it is well documented that HbA1c predicts the occurrence of diabetes complications. A review by Khaw *et al*[9] examined HbA1c as a risk predictor for cardiovascular disease and found that a 1% increment in absolute concentration of HbA1c was associated with about 10%-20% increase in cardiovascular risk[9]. Elley *et al*[10] confirmed in a large prospective cohort study of 48444 people with type 2 diabetes that increased HbA1c is an independent risk factor for cardiovascular disease, after adjusting for traditional risk factors. This is consistent with work by Ma *et al*[11] who suggested from data of a retrospective study in older patients with diabetes that elevated HbA1c values are an independent predictor of complex coronary lesions. However, a very recent analysis in subjects without diabetes and cardiovascular disease obtained little additional benefit for prediction of first-onset cardiovascular disease[12]. Prior to the Emerging Risk Factors Collaboration study[12] large trials, such as ACCORD[6] and ADVANCE[7], also failed to demonstrate the ability to alter cardiovascular outcomes upon lowering HbA1c values in patients with long-standing diabetes. This is in contrast to the effects of tight glycemic control in reducing microvascular complications. As a corollary, the uncertainty around HbA1c results in relation to clinical outcomes was augmented. Moreover, deeper

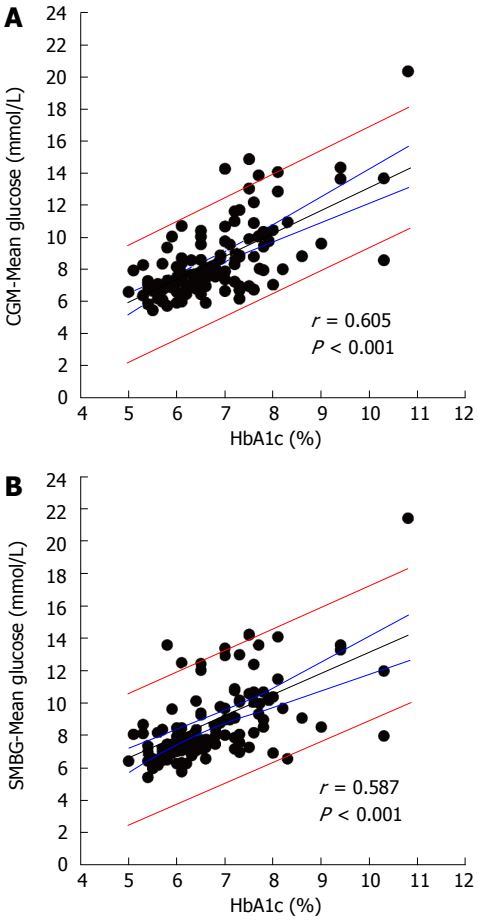

Figure 1 Relationship between hemoglobin A1c and mean glucose obtained from (A) continuous glucose monitoring and (B) self-monitoring of blood glucose in a cohort of 114 non-insulin treated type 2 diabetic patients. Medians (25th-75th percentile) for age, diabetes duration, and HbA1c were 59.0-68.0 yr, 2.0-10.0 yr, and 6.0%-7.3% (42-56 mmol/mol), respectively. The lines denote the regression lines (black), 95%CI (blue), and prediction intervals (red) (Kohnert *et al*, Unpublished data). CGM: Continuous glucose monitoring; HbA1c: Hemoglobin A1c.

insight into the pathogenesis of diabetes has disclosed important limitations of HbA1c measurement. For example, early analyses recognized that diabetic patients with identical HbA1c values can have different mean glucose concentrations[13,14]. The regression analysis shown in Figure 1 for a cohort of our type 2 diabetic patients demonstrates that although the regression coefficients between HbA1c and mean glucose obtained either from CGM or concurrent SMBG measurements are similar (Kohnert *et al,* Unpublished); wide variations in the relationship among and within the patients can be seen. In a minority of patients such mismatch might partly be explained by unequal temporal distribution of glucose sampling, but more importantly, there are studies to provide evidence that this observation is due to differences in intracellular glycation rates[15]. It appears that glycation of hemoglobin is not simply a concentration-dependent process, and factors other than glucose are likely to be involved. Moreover, conditions that could interfere with HbA1c measurement, causing erroneous values, are high red cell turnover, hemolytic anemia, blood

transfusion, chronic renal or liver disease[16], and drug treatment. Under these circumstances, HbA1c cannot be used as a glucose control measure; and alternative markers should be considered. The most important limitation of HbA1c is its inability to predict hypoglycemia and to capture short-term changes of glycemia. Furthermore, we have previously shown that in well-controlled patients with type 2 diabetes, HbA1c is mainly determined by chronic sustained hyperglycemia; glycemic fluctuations go undetected[17]. However, this is critical for safe and timely adjustment of insulin administration and clinical decision making. Thus, there has been increasing interest in additional markers for better glycemic control over shorter timeframes. The markers in question, however, may have specific characteristics and are not equally suited for diabetes management.

Fasting glucose, postprandial glucose, and mean glucose

In contrast to HbA1c, estimation of glucose exposure for specific time periods overnight or 2 to 4 h postprandial may be useful in monitoring effects of food, exercise, or antidiabetic medications. Thus, fasting glucose (FPG) and postprandial glucose (PPG) provide an acute assessment of glycemia. However, in their original work on the relationship between FPG and PPG, Monnier *et al*[9] have shown that the relative contribution of these measures changes with increasing HbA1c values[3] and worsening of the metabolic situation is indicated by loss of postprandial glycemic control[18]. According to the ADA Standards of medical care in diabetes - 2014[19], FPG values of $\geqslant$ 7.0 mmol/L and 2-h plasma glucose (2hPG) of $\geqslant$ 11.1 mmol/L are considered criteria for the diagnosis of diabetes. Among a number of studies, which have examined the relationship of FPG or 2hPG to mortality, data from the Baltimore Longitudinal Study on Aging showed that FPG levels, exceeding 7.0 mmol/L increased the risk of mortality and the 2hPG added predictive power to that of FPG alone[20]. Impaired fasting glucose emerged also as independent predictor of cardiovascular mortality in the Australian Diabetes, Obesity and Lifestyle Study[21]. A recent meta-analysis suggested that reduction of FPG was related to a decrease of cardiovascular mortality with data on PPG pointing in the same direction[22].

Standl *et al*[23] have listed 14 long-term observational studies showing that elevated PPG levels increase the risk of cardiovascular disease or the occurrence of a cardiovascular event approximately threefold. By contrast, data from prospective studies on the association between PPG and cardiovascular risk in established diabetes are limited. The Diabetes Intervention study[24] has revealed the harmful link between PPG levels >10 mmol/L and increased risk of cardiovascular events and reported that reduction below this level decreased myocardial infarction and death in type 2 diabetes. Cavelot *et al*[25] confirmed this association in their follow-up study, demonstrating that PPG was a stronger predictor of cardiovascular events than fasting glucose. Data obtained form a study conducted by Esposito *et al*[26] showed that postmeal incremental glucose values > 2.78 mmol/L, found in two thirds of study participants, were correlated with carotid intima-media thickness. Further support for the concept of treating elevated PPG came from a post-hoc analysis of the HEART2D study[27]. Although all these studies could not clarify, whether PPG is a real marker of cardiovascular events or a surrogate of complex metabolic processes taking place in the postprandial phase[28], this measure appears to be helpful for assessing the meal-induced glucose excursion and efficacy of diabetes treatment. In order to reduce the risk of cardiovascular events, the ADA[19] and IDF[29] recommend PPG values $\leqslant$ 10.0 and $\leqslant$ 9.0 mmol/L, respectively.

When considering glucose exposure, mean glucose is the metric with which the quality of diabetes management can be judged by clinicians as well as patients at shorter intervals and more easily than with HbA1c. For this reason, an expert panel of diabetes specialists recommended mean glucose/median glucose of all readings as one of the helpful glucose metrics[30].

Fructosamin and glycated serum proteins

In recent years, fructosamin and serum glycated proteins with shorter half-lives (14-21 and 17-20 d, respectively) than hemoglobin have been evaluated as markers of glycemia. Fructosamin is formed by attachment of the molecule primarily to albumin *via* a nonenzymatic reaction. The fructosamin assay uses a colorimetric method, is rapid, inexpensive and specific, and can be applied to measure glycation of serum proteins, principally albumin[31]; however, there is little standardization of this test. Several studies showed good correlations between fructosamin and HbA1c and glycated albumin[32]. Glycated albumin (GA) is a ketoamine that is formed *via* nonenzymatic glycation and has been reported to be a useful marker of shorter-term glycemic control in diabetes[33]. It is a more rapidly responding indicator than hemoglobin, although the glycation rate for both proteins is comparable[34]. Various methods to quantify GA are available but have not been consistently standardized-most common are affinity chromatography and enzymatic assays. Two cross-sectional studies, a Japanese and an American one, involving diabetic patients on hemodialysis[35,36], suggested that GA is a better marker of glycemic control than HbA1c. The consistent finding of significantly lower % GA/HbA1c ratios in diabetic patients without nephropathy compared to those on dialysis indicates that HbA1c underestimates glycemic control under these circumstances. It is likely that factors such as reduced survival of red blood cells and transfusions contribute to lowering of HbA1c levels in diabetic patients on hemodialysis. GA has been found useful in neonatal and gestational diabetes to detect short-term changes in glycemia[37,38]. Since glycated albumin was shown to be an independent variable of maximum glucose levels, it appears to be a more sensitive marker than HbA1c for glycemic excursions, as they occur during postprandial

times[39]. This is important because postprandial glucose excursions are known risk factors for diabetic micro- and macrovascular diabetes complications. More recently, it was found that serum GA levels are higher in relation to HbA1c in diabetes patients with reduced basal pancreatic β-cell function[40]. If in the state of postprandial hyperglycemia, indicating postprandial β-cell dysfunction, serum concentrations were found to be increased, then GA could be a useful surrogate marker for cardiovascular risk[41]. This has not yet been confirmed by clinical trials, although the finding of elevated GA, but not HbA1c levels in patient with coronary artery stenosis points out such a relationship[42].

1,5- Anhydroglucitol

Another analyte, 1,5-anhydroglucitol (1,5-AG), has been suggested for use as intermediate marker of glycemia to complement HbA1c measurements[43]. It is a naturally occurring inert polyol, which represents a six-carbon chain monosaccharide with a structure similar to glucose. An automated assay named GlycoMark™ is commercially available. 1,5-AG competes with glucose for tubular re-absorption and can hence not be used as a marker for glycemic control in patients with impaired kidney function. Furthermore, it should be noted that glucose levels exceeding the renal threshold for glycosuria, *i.e.,* 10 mmol/L (180 mg/dL), lead to a rapid reduction in serum concentration of 1,5-AG[44]. Poor glycemic control, indicated by high HbA1c values (> 9.0%, > 75 mmol/mol), is therefore associated with lower not higher levels of 1,5-AG. Although this marker responds sensitively and rapidly to daily glucose excursions in patients with near or at goal HbA1c levels[45], it can not identify hypoglycemia. Dungan *et al*[46] have reported that 1,5-AG varied markedly in diabetes patients despite similar HbA1c and showed that this was mainly attributable to different postprandial glucose excursions. This makes 1,5-AG superior compared to HbA1c or GA (serum fructosamine) measurements as a marker for identifying postprandial hyperglycemia. Consequently, 1,5-AG has been used to evaluate drug strategies on postprandial glycemia. Studies, including exenatide[47], sitagliptin[48] or biphasic insulin[49], for example, support the usefulness of 1,5-AG as a marker to identify treatment effects on postprandial glycemic excursions that would have otherwise been missed. However, it must be emphasized that 1,5-AG is not able to determine glycemic variability.

Metrics of glycemic variability

Clinical observations in patients with type 1 and type 2 diabetes have revealed that glucose profiles can greatly differ even if patients are well-controlled. While in some patients small or moderate glucose excursions and rare hypoglycemia occur, there are marked postprandial increases with frequent hypoglycemic episodes in others. Such ups and downs in glucose levels over time, either measured within 24 h or from day to day at the same time point, reflect glycemic variability (GV) classified as within-day and between-day variability, respectively[50]. It was Monnier *et al*[51] who suggested that GV is one of the important components of dysglycemia in diabetes.

With the advent of CGM, quantification of GV gained considerable clinical importance[52]. Numerous indices for evaluation of various aspects of GV are currently available, which have been carefully characterized by Rodbard[53,54] and Cameron *et al*[55]. Although they can principally be calculated from frequently sampled SMBG data, *i.e.,* seven- or eight-point glucose profiles, it is advisable to use CGM datasets, because capturing relevant glucose peaks and nadirs requires sampling frequencies of 1-5 min. It is thus not unexpected that several studies found the magnitude of GV to depend on the sampling frequency[56,57]. Furthermore, it is very important to clearly differentiate between indices of GV and indices of the quality of glycemic control. Measures of GV quantify short-term changes in glycemia and reflect different and specific aspects of glycemic control but should not be interchanged. Validated indices such as mean amplitude of glycemic excursions (MAGE), mean of daily difference, continuous overall net glycemic action are often used in clinical research, but they are not easy to calculate. Several computer programs have recently been developed for better handling of sampled glucose data. We previously developed a computer program to calculate MAGE[58], and meanwhile, there is other software available, such as GlyCulator[59] and EasyGV (www.easygv.co.uk) for computing glycemic variability indices. In order to standardize measures of glycemia and glucose data reporting, an expert panel of diabetes specialists recommended for the ease of use, familiarity, and correlation with other factors of glycemic control, the following three measures of GV: SD around the mean glucose (SD), coefficient of variation (CV), and interquartile range (IQR)[30]. Especially, if CGM data are collected, IQR is the most reliable aggregate measure of GV, as the panel announced. Normative values for GV indices have been published by Hill *et al*[60] and Zhou *et al*[61].

In regard to the clinical relevance, it remains controversial whether GV is an independent causative or contributing factor to diabetes complications[62]. Nevertheless, there are a few studies in patients with type 1 diabetes to suggest GV to impact on the development of microvascular complications[63,64]. In an 11-year follow-up study, Bragd *et al*[65] found that GV measured by SD of blood glucose was a predictor of the prevalence of peripheral neuropathy. Moreover, Snell-Bergeron reported subclinical atherosclerosis to be associated with glucose levels and glucose SD in men with type 1 diabetes[66]. The potential importance of GV for the development of microvascular complications has been corroborated by Soupal *et al*[67] in a recent cross-sectional study of type 1 diabetes patients. This study showed significantly increased values for GV indices, such as SD, CV, and MAGE, for patients with microvascular complications as compared to those without complications. In this context, it should be

noted that analysis of data from the Diabetes Control and Complications Trial showed that long-term fluctuations in glycemia expressed as SDs of HbA1c independently relate to the development of retinopathy and nephropathy[68]. With respect to type 2 diabetes, there are more study data available than for type 1 diabetes, demonstrating close associations between GV and vascular complications[69]. In patients with well-controlled glycemia, Zhou *et al*[70] reported that increased MAGE is one of the risk factors for microalbuminuria. Vaduva *et al*[71] observed increased values for several GV indices in type 2 diabetic patients with chronic kidney disease compared to those without kidney damage; and Mirani *et al*[72] noticed glucose profiles with higher GV in insulin-treated diabetes patients on hemodialysis than in the hemodialysis-free intervals. One retrospective long-term follow-up study showed that fasting glucose variability was a risk factor for diabetic retinopathy independent of mean fasting glucose or HbA1c[73]. Regarding macrovascular complications, Chen *et al*[74] obtained data from a case-control study to suggest a significant association between GV and progression of atherosclerosis, as determined by measurement of carotid intima-media thickness. These latter data are consistent with the value of MAGE in predicting better than HbA1c major adverse cardiac events[75], coronary artery disease in newly diagnosed diabetes[76] and its severity in established type 2 diabetes[77]. A strong argument was presented for the role of GV by the recent analysis of the ADVANCE (Action in Diabetes and Vascular Disease: Preterax and Diamicron MR Controlled Evaluation) trial that revealed a clear association between SD of glucose and macro- as well as microvascular events in type 2 diabetes[78]. It should further be noted that MAGE, has been found by Rizzo *et al*[79] to be associated with impairment of cognitive function independent from the main markers of glycemia (HbA1c, FPG, PPG); and Penckofer *et al*[80] reported an impact of GV on mood and life quality in women with type 2 diabetes.

Finally, experimental findings and clinical observations suggesting that GV more than sustained chronic hyperglycemia induces increased oxidative stress[81] provide sure indications that GV is involved in the development of vascular disease. Because traditional measures of GV, with the exception of % CV, are closely correlated with mean glucose, it remains difficult to define an independent role for GV in the development of diabetes complications. Nevertheless, in clinical practice, minimizing GV is important to achieve acceptable glycemic stability without increasing the risk of hypoglycemia[82-84].

Metrics of glycemic risk

Essentially two indices, such as the average daily risk range (ADRR)[85] and the glycemic risk assessment diabetes equation (GRADE)[86] have been developed to grade the quality of glycemic control and to complement clinical assessment of diabetes treatment. These metrics are calculated by converting glucose values obtained from SMBG or CGM into risk scores, *i.e.,* they quantify the risk for glycemic extremes, both hyper- and hypoglycemia. They do not measure GV *per se*, rather its consequences. Nevertheless, ADRR scores correlate with several GV indices[60,87] and were further shown to correlate with patients' insulin sensitivity, epinephrine release[88], and weakly with basal β-cell function (HOMA%B)[89]. The ADRR includes the high blood glucose index and the low blood glucose index (LBGI), which quantify the risk for hyperglycemia and hypoglycemia. Among the advantages of ADRR that should be emphasized are the equal sensitivity to predict excessive hyperglycemic as well as hypoglycemic episodes and the possibility to use either SMBG or CGM data for its calculation[90]. On the other hand, ADRR has been considered as apparently less sensitive to therapeutic effects[87]. Nonetheless, with regard to our own research (Kohnert *et al,* unpublished data) we were able to differentiate between treatment modalities, as depicted in Figure 2. ADRR is usually reported as cutoff scores based on risk categories[90]. Even glucose meter software programs for automatic calculation are meanwhile available. Treatment studies that have used ADRR as outcome measure are still limited in number. Patton and coauthors[91] published a comprehensive review article on the use of ADRR in assessment research and treatment outcomes research, suggesting that adults and youths with diabetes could well benefit from monitoring their ADRR scores. However, as these authors stated, it is currently unknown to which extent ADRR is used in routine diabetes control.

GRADE has been introduced by Hill *et al*[86]. The GRADE score is an expression of the mean GRADE value derived from any glucose profile. The percentage of time spent in a specified range can be given as % $GRADE_{hypoglycemia}$, % $GRADE_{euglycemia}$, % $GRADE_{hyperglycemia}$. There have been only a few studies that have used GRADE scores, mainly in comparison with GV indices. One study has shown that GRADE was significantly improved in response to unmasking of CGM glucose values[87]; another study found GRADE scores to be reduced concomitant with lowering of GV after adjustment of therapy in patients with type 2 diabetes[84]. Although both ADRR and GRADE indicate increased glycemic risk, it should be noted that they are only moderately correlated with one another[87]. Nevertheless, as shown in Table 2, our data suggest that among the above metrics $GRADE_{hypoglycemia}$ and LBGI derived from CGM data are superior in estimating the risk of hypoglycemia (Kohnert *et al*, unpublished data).

Metrics of glucose dynamics

Regulation of glucose concentration is a complex process that is linked with several ultradian rhythms. Even though certain aspects of the failing glucoregulation observed in the development and progression of diabetes may be assessed by classical indices of GV, they do not include a time component[92]. The metrics of GV described above may thus give information about the extent of excursions, yet information about glucose dynamics is not

Table 2 Linear regression relating hypoglycemia as dependent variable with measures of glycemic control as independent variables in type 2 diabetes

Asymptomatic hypoglycemia	Measure	R^2	*P* value
Time (h/d) spent	GRADE$_{HYPO}$	0.734	< 0.001
< 3.9 mmol/L	LBGI	0.471	< 0.001
	% CV	0.293	< 0.001
	HbA1c	0.048	0.02

Data analyzed from 114 patients treated with diet and oral antidiabetic drugs. GRADE$_{HYPO}$: Glycemic risk assessment diabetes equation hypoglycemia; LBGI: Low blood glucose index; %CV: Percent coefficient of variation (Kohnert *et al*, Unpublished data).

sufficiently provided, *i.e.*, how the glucoregulatory system moves from one state to another over time. In other words, GV indices are not suitable to gain deeper insight into regulatory dynamics. Various analytical methods have been used for indicating the range of glycemic dynamics in nondiabetic and diabetic patients associated with typical disease conditions. Time-series analysis techniques provide an approach to discover changes in glucose dynamics. Thus, autocorrelation function has been applied to glucose time series analysis in nondiabetic and type 1 diabetic individuals[93], but is difficult to exploit in type 2 diabetes due to the largely nonstationary data sequence. Utilizing detrended fluctuation analysis (DFA), Churruca *et al*[94] and Yamamoto *et al*[95] observed a loss of glucose profile complexity, as detected by the short- and long-term scaling exponent α1 and α2, in the progression from normoglycemia to impaired glucose tolerance to overt diabetes. Ogata *et al*[96] have reported that increasing long-range DFA scaling exponents reflect abnormalities in glycemic control. Interestingly, they found that the MAGE was correlated only to the DFA long-range scaling exponent α2 in patients with diabetes. According to Khovanova *et al*[97], glucose profile dynamics can be defined by three complementary characteristics: nonstationarity (DFA exponent α), linear predictability (autocorrelation coefficient γ), and amplitude of variation (SD of glucose). Kovatchev *et al*[98] and Molnár *et al*[99] introduced the Poincaré plot time series analysis tool to acquire temporal glycemic variability from CGM data. The primary method defines short-term and long-term variability, corresponding to the length of the minor SD1 and major SD2 axes of the plot. In his recent work, Crenier[100] extended Poincaré plot quantification by introducing and validating new partial Poincaré plot metrics, *e.g.*, area and shape of the fitting ellipse calculated at specific time points. While the majority of these metrics closely correlated with classical indices of GV, the shape index did not, indicating that the Poincaré plot captures many types of variability. One may speculate that in order to solve the question of whether GV is an independent contributor to the development of diabetes complications, analysis at multiple time scales would provide a better approach than use of classical indices. Indeed, in a recent cross-sectional, observational study,

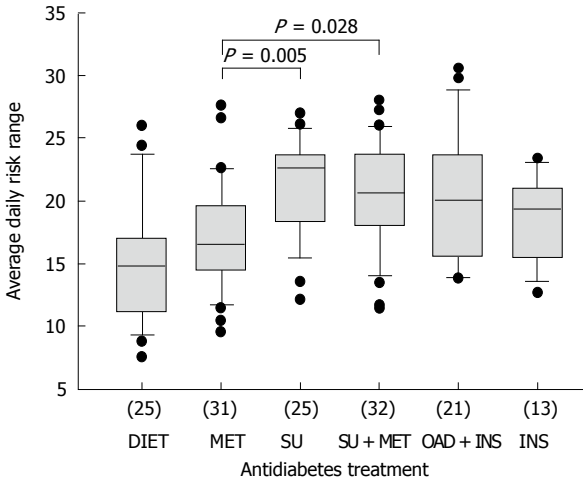

Figure 2 Differentiation between treatment groups of type 2 diabetic patients using the average daily risk range scores. Sample size for each group is given in parenthesis. Between-treatment group differences were evaluated by one-way analysis of variance and are statistically significant ($P < 0.001$). MET: Metformin; SU: Sulfonylurea; OAD: Oral antidiabetic drugs; INS: Insulin (Kohnert *et al*, Unpublished data).

Cui *et al*[101] introduced Multi-Scale glycemic variability for analysis of CGM data at multiple time scales. They identified five unique ultradian GV cycles that modulate glucose over time ranges of 0.5 to 12 h and showed that greater GV within these cycles was associated with detrimental changes in brain morphology and function.

Biomarkers and surrogate biomarkers for diabetes complications

It is agreed upon that chronic sustained hyperglycemia represents one of the today's most important surrogate biomarker for development of microvascular diabetes complications. In addition to markers of glycemia, several novel biomarkers have been identified, capable of predicting onset or progression of nephropathy in type 2 diabetes. In a recent systematic review, Hellemons *et al*[102] assessed the validity of such biomarkers and found, for example, that serum interleukin 18, urinary ceruloplasmin, immunoglobulin G, and transferrin were valid markers to predict onset of diabetic nephropathy. Vascular cell adhesion molecule 1, interleukin 6, von Willebrand factor, and intercellular adhesion molecule 1 were identified as markers for progression of nephropathy. Although a number of circulating (*e.g.*, high sensitive C-reactive protein, brain natriuretic peptide), genetic, and imaging biomarkers (*e.g.*, carotid intima-media thickness) are significantly related with cardiovascular risk, their predictive power for individuals is restricted. The relationship of hyperglycemia with macrovascular disease is not as clear as with microvascular complications. Since large clinical trials[6,7] failed to provide convincing evidence that HbA1c is a reliable surrogate, adequate markers for cardiovascular outcomes in diabetic individuals with longer disease duration are not yet available[103]. The uncertainty related to cardiovascular

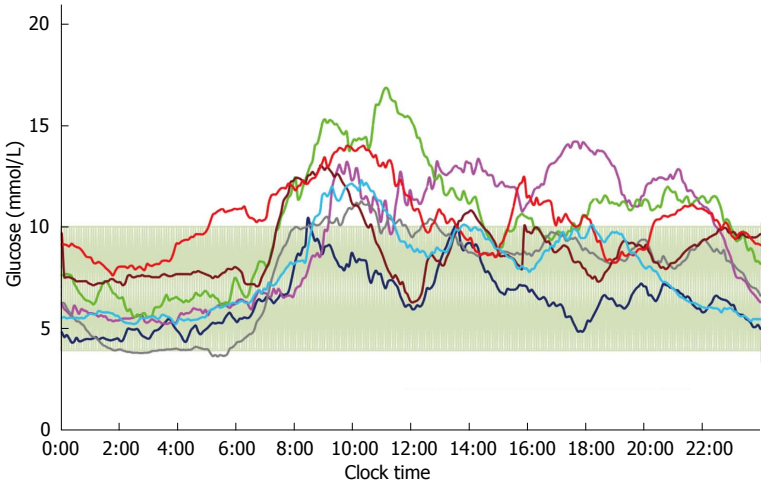

Figure 3 Continuous glucose monitoring traces from seven patients with an HbA1c value of 6.5% selected from the type 2 diabetes cohort treated with oral antidiabetes drugs. Average 24-h glucose profiles are shown. Shading indicates the glucose target range 3.9-10.0 mmol/L (modified from Kohnert *et al*, Bull Karaganda University 2013; 72: 6-15).

disease led to the release of the new recommendations on evaluating cardiovascular risk in drugs intended to treat type 2 diabetes[104] by the United Kingdom Food and Drug Administration. Given the complexity of diabetes, it is conceivable that no single biomarker can indicate the risk of complications or disease progression. New technologies, including metabolomics, proteomics, and genomics have the potential to unravel the pathogenesis of diabetes and put forward new concepts for the development of biomarkers beyond impaired glucose regulation.

GLUCOSE MONITORING

The development of hand-held blood glucose meters some decades ago made it possible for diabetes patients to monitor their own blood glucose levels at any time in a convenient way and enabled adjustment of therapy. With the universal availability of glucose meters, SMBG found broad application for management of glycemic control. However, this traditional monitoring usually measures single glucose values at a time point, which is determined by the user; it provides only a snapshot of the whole glucose picture and rapid changes occurring between single measurements escape detection. Introduction of the CGM technology presented a great step forward toward modern diabetes management, because it over comes limitations of traditional SMBG by producing glucose profiles instead of distinct measurements over several days, real-time glucose values, glucose trends and warnings when glucose values approach dangerously low or high levels. CGM recordings also provided evidence that diurnal glucose patterns may considerably differ in individual patients, even at identical HbA1c levels-a fact overlooked in the past. Figure 3 depicts individual average CGM profiles from a subsample of type 2 diabetes patients with identical HbA1c values. As can be seen, the profiles are quite different in that: (1) most of them exceed the target range (5%-23% glucose values above 10 mmol/L); (2) they show marked glycemic excursions (%CV 20.6-38.1); and (3) the glucose complexity long-range DFA scaling exponent $\alpha 2$ varies between 1.32 and 1.54. It is conceivable that frequent use of CGM and careful pattern analysis is able to improve glycemic control by uncovering such trouble points.

Clinical study outcomes and data obtained from every-day diabetes management have shown that the use of CGM can consistently improve glycemic control[105]. Notwithstanding that those with unstable diabetes who are prone to hypoglycemia and hypoglycemia unawareness will benefit most, the majority of diabetes patients can achieve their glucose targets when using CGM[106]. Two variants of CGM based on sensor technology are available: retrospective and real-time glucose monitoring[107,108]. While CGM systems such as CGMS Gold, Guardian T, Glucoday, and iPro2 were mainly designed as a tool for health care providers to collect glucose data over a sensing period of 3-7 d during which the data were masked to patients, provide real-time glucose monitors like Guardian RT, Dexcom Seven Plus, and Navigator real-time glucose values, trends, and alarms if glucose levels become high or low. The latter CGMs enable immediate therapeutic action, but require training experience for both health care practitioners and patients. However, all the above systems measure glucose subcutaneously, whereby the kinetics of the sensing process is defined by the physiology of the subcutaneous space. Glucose sensing in the peritoneal space, as recently shown, has the potential to optimize glucose monitoring because of faster intraperitoneal than subcutaneous kinetics[109].

Even though application of CGMs has convincingly demonstrated practical utility in diabetes management, *i.e.,* food response[110], reduction of glucose variability, time spent in hypo-/hyperglycemia, and improvement of HbA1c levels, this technology is still underutilized for a number of reasons[30]. One of the main problems is the lack of standardized metrics and a more user-friendly presentation of data. There are currently several well-established clinical and research measures that have shown to be useful in analyzing and characterizing CGM profiles. An expert panel of diabetes specialists identified time in range as one of the key metrics for guiding diabetes treatment[30]. This metric can be expressed either as "% of glucose readings" or "hours per day". As the

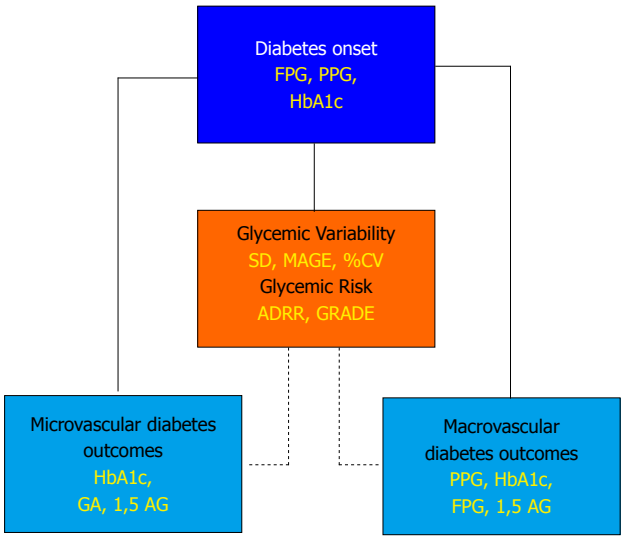

Figure 4 Glycemic Markers and Risk of Diabetic Complications. The solid lines show established relationships of the glycemic markers with microvascular and macrovascular diabetes complications; dotted lines represent possible relations with glycemic variability. FPG: Fasting plasma glucose; PPG: Postprandial plasma glucose; HbA1c: Hemoglobin A1c; GA: Glycated albumin; 1,5 AG: 1,5 Anhydroglucitol; SD: Standard deviation of plasma glucose values; MAGE: Mean amplitude of glycemic excursions; CV: Coefficient of variation; ADRR: Average daily risk range; GRADE: Glycemic risk assessment diabetes equation.

default target range, 3.9-10.0 mmol/L (70-180 mg/dL) was selected. Although this is not a "normal" range, it is commonly considered as acceptable in clinical practice. Individual targets closer to the physiological range can be defined, depending on age, comorbidities or patient compliance.

CONCLUSION

For the time being, HbA1c will remain the most important metric of long-term glycemic control, but may be supplanted by other parameters with advancing glucose monitoring technologies. Alternative metrics, such as GA and 1,5-AG can be clinically useful to assess medium- or short-term glycemic control, and in certain conditions that could interfere with HbA1c measurement. In view of the fact that many diabetes patients with apparently good glycemic control (HbA1c < 7%, < 53 mmol/mol) have high postmeal incremental glucose values, it seems warranted to integrate measurement of PPG into daily diabetes control. GV is one of the most important parameters that must not be neglected in order to optimize diabetes management. Since the known GV metrics are highly intercorrelated, any validated index can be used for evaluation of glucose fluctuations. MAGE and SD of glucose have been most commonly used; however, % CV is correlated to hypoglycemia and independent of mean glucose. ADRR as well as GRADE estimate the risk induced by high variability of glucose values and weigh low and high glucose equally. They can thus be helpful in patient care for assessments of glycemic quality. Based on our experience, we would recommend, in addition to the long-term measure HbA1c, mean glucose and PPG as shorter-term indicators, and ADRR or GRADE for the quality of glycemic control. We would further recommend SD around the mean glucose, MAGE, and %CV as metrics of GV. Since these measures do not consider a time component Poincaré plot metrics might attract more attention to quantify short-and long-term GV and their relationship to the development of diabetes complications. For practical reasons and according to specific needs, a combination of shorter and longer term glycemic markers should be used for assessment of diabetes control to predict vascular outcomes more precisely. Finally, the control of glucose concentration is incomplete without dynamic measurements. Because of the limited available data, the utility of current metrics of glucose dynamics can not yet be judged, but they have shown promising potential to provide deeper insight into the glucoregulatory system hitherto not achieved with currently used metrics.

Since this article brings into focus metrics of glycemic control, the schematic representation in Figure 4 depicts which of these metrics may be predictive of micro- and macrovascular outcomes in diabetes. Nevertheless, it remains unclear whether glycemic variability and/or changes in glucose dynamics are implicated, but to achieve optimal glycemic control one should be aware that other factors than simply high blood glucose levels are likely to contribute to complications of diabetes. The discovery of new markers as reliable surrogates for clinical outcomes rather than simply glycemic control will advance the ability to assess the risk of complications and target treatment of diabetes.

REFERENCES

1 **The Diabetes Control and Complication Trial Research Group.** The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 1993; **329:** 977-986 [PMID: 8366922 DOI: 10.1056/NEJM199309303291401]

2 **Nathan DM**, Cleary PA, Backlund JY, Genuth SM, Lachin JM, Orchard TJ, Raskin P, Zinman B, Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Study Research Group. Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. *N Engl J Med* 2005; **353**: 2643-2653 [PMID: 16371630 DOI: 10.1056/NEJMoa052187]

3 **Monnier L**, Lapinski H, Colette C. Contributions of fasting and postprandial plasma glucose increments to the overall diurnal hyperglycemia of type 2 diabetic patients: variations with increasing levels of HbA(1c). *Diabetes Care* 2003; **26**: 881-885 [PMID: 12610053 DOI: 10.2337/diacare.26.3.881]

4 **Temelkova-Kurktschiev TS**, Koehler C, Henkel E, Leonhardt W, Fuecker K, Hanefeld M. Postchallenge plasma glucose and glycemic spikes are more strongly associated with atherosclerosis than fasting glucose or HbA1c level. *Diabetes Care* 2000; **23**: 1830-1834 [PMID: 11128361]

5 **Raz I**, Wilson PW, Strojek K, Kowalska I, Bozikov V, Gitt AK, Jermendy G, Campaigne BN, Kerr L, Milicevic Z, Jacober SJ. Effects of prandial versus fasting glycemia on cardiovascular outcomes in type 2 diabetes: the HEART2D trial. *Diabetes Care* 2009; **32**: 381-386 [PMID: 19246588 DOI: 10.2337/dc08-1671]

6 **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, Action to Control Cardiovascular Risk in Diabetes Study Group. Effects of intensive glucose lowering in type 2 diabetes. *N Engl J Med* 2008; **358**: 2545-2559 [PMID: 18539917 DOI: 10.1056/NEJMoa0802743]

7 **Patel A**, MacMahon S, Chalmers J, Neal B, Billot L, Woodward M, Marre M, Cooper M, Glasziou P, Grobbee D, Hamet P, Harrap S, Heller S, Liu L, Mancia G, Mogensen CE, Pan C, Poulter N, Rodgers A, Williams B, Bompoint S, de Galan BE, Joshi R, Travert F. Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. *N Engl J Med* 2008; **358**: 2560-2572 [PMID: 18539916 DOI: 10.1056/NEJMoa0802987]

8 **Nathan DM**, McGee P, Steffes MW, Lachin JM, ADVANCE Collaborative Group. Relationship of glycated albumin to blood glucose and HbA1c values and to retinopathy, nephropathy, and cardiovascular outcomes in the DCCT/EDIC study. *Diabetes* 2014; **63**: 282-290 [PMID: 23990364 DOI: 10.2337/db13-0782]

9 **Khaw KT**, Wareham N. Glycated hemoglobin as a marker of cardiovascular risk. *Curr Opin Lipidol* 2006; **17**: 637-643 [PMID: 17095908 DOI: 10.1097/MOL.0b013e3280106b95]

10 **Elley CR**, Kenealy T, Robinson E, Drury PL. Glycated haemoglobin and cardiovascular outcomes in people with Type 2 diabetes: a large prospective cohort study. *Diabet Med* 2008; **25**: 1295-1301 [PMID: 19046219 DOI: 10.1111/j.1464-5491.2008.02581.x]

11 **Ma J**, Wang X, Wang Y, Zhao Y, Gao M, Li X. The relationship between glycated hemoglobin and complexity of coronary artery lesions among older patients with diabetes mellitus. *PLoS One* 2014; **9**: e91972 [PMID: 24658008 DOI: 10.1371/journal.pone.0091972]

12 **Di Angelantonio E**, Gao P, Khan H, Butterworth AS, Wormser D, Kaptoge S, Kondapally Seshasai SR, Thompson A, Sarwar N, Willeit P, Ridker PM, Barr EL, Khaw KT, Psaty BM, Brenner H, Balkau B, Dekker JM, Lawlor DA, Daimon M, Willeit J, Njølstad I, Nissinen A, Brunner EJ, Kuller LH, Price JF, Sundström J, Knuiman MW, Feskens EJ, Verschuren WM, Wald N, Bakker SJ, Whincup PH, Ford I, Goldbourt U, Gómez-de-la-Cámara A, Gallacher J, Simons LA, Rosengren A, Sutherland SE, Björkelund C, Blazer DG, Wassertheil-Smoller S, Onat A, Marín Ibañez A, Casiglia E, Jukema JW, Simpson LM, Giampaoli S, Nordestgaard BG, Selmer R, Wennberg P, Kauhanen J, Salonen JT, Dankner R, Barrett-Connor E, Kavousi M, Gudnason V, Evans D, Wallace RB, Cushman M, D'Agostino RB, Umans JG, Kiyohara Y, Nakagawa H, Sato S, Gillum RF, Folsom AR, van der Schouw YT, Moons KG, Griffin SJ, Sattar N, Wareham NJ, Selvin E, Thompson SG, Danesh J, Emerging Risk Factors Collaboration. Glycated hemoglobin measurement and prediction of cardiovascular disease. *JAMA* 2014; **311**: 1225-1233 [PMID: 24668104]

13 **Service FJ**, O'Brien PC. Influence of glycemic variables on hemoglobin A1c. *Endocr Pract* 2007; **13**: 350-354 [PMID: 17669710 DOI: 10.4158/EP.13.4.350]

14 **Kohnert KD**, Vogt L, Augstein P, Heinke P, Zander E, Peterson K, Freyse EJ, Salzsieder E. Relationships between glucose variability and conventional measures of glycemic control in continuously monitored patients with type 2 diabetes. *Horm Metab Res* 2009; **41**: 137-141 [PMID: 19214924 DOI: 10.1055/s-0028-1128143]

15 **Cohen RM**. A1C: does one size fit all? *Diabetes Care* 2007; **30**: 2756-2758 [PMID: 17901536 DOI: 10.2337/dc07-1301]

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 DOI: 10.1001/jama.295.14.1688]

17 **Kohnert KD**, Augstein P, Heinke P, Zander E, Peterson K, Freyse EJ, Salzsieder E. Chronic hyperglycemia but not glucose variability determines HbA1c levels in well-controlled patients with type 2 diabetes. *Diabetes Res Clin Pract* 2007; **77**: 420-426 [PMID: 17331614 DOI: 10.1016/j.diabres.2007.01.021]

18 **Monnier L**, Colette C, Dunseath GJ, Owens DR. The loss of postprandial glycemic control precedes stepwise deterioration of fasting with worsening diabetes. *Diabetes Care* 2007; **30**: 263-269 [PMID: 17259492 DOI: 10.2337/dc06-1612]

19 **American Diabetes Association.** Standards of medical care in diabetes-2014. *Diabetes Care* 2014; **37** Suppl1: S14-S80 [DOI: 10.2337/dc14-S014]

20 **Sorkin JD**, Muller DC, Fleg JL, Andres R. The relation of fasting and 2-h postchallenge plasma glucose concentrations to mortality: data from the Baltimore Longitudinal Study of Aging with a critical review of the literature. *Diabetes Care* 2005; **28**: 2626-2632 [PMID: 16249530 DOI: 10.2337/diacare28.11.2626]

21 **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]

22 **Monami M**, Adalsteinsson JE, Desideri CM, Ragghianti B, Dicembrini I, Mannucci E. Fasting and post-prandial glucose and diabetic complication. A meta-analysis. *Nutr Metab Cardiovasc Dis* 2013; **23**: 591-598 [PMID: 23711419 DOI: 10.1016/j.numecd.2013.03.007]

23 **Standl E**, Schnell O, Ceriello A. Postprandial hyperglycemia and glycemic variability: should we care? *Diabetes Care* 2011; **34** Suppl 2: S120-S127 [PMID: 21525442 DOI: 10.2337/dc11-s206]

24 **Hanefeld M**, Fischer S, Julius U, Schulze J, Schwanebeck U, Schmechel H, Ziegelasch HJ, Lindner J. Risk factors for myocardial infarction and death in newly detected NIDDM: the Diabetes Intervention Study, 11-year follow-up. *Diabetologia* 1996; **39**: 1577-1583 [PMID: 8960845 DOI: 10.1007/s001250050617]

25 **Cavalot F**, Pagliarino A, Valle M, Di Martino L, Bonomo K, Massucco P, Anfossi G, Trovati M. Postprandial blood glucose predicts cardiovascular events and all-cause mortality in type 2 diabetes in a 14-year follow-up: lessons from the San Luigi Gonzaga Diabetes Study. *Diabetes Care* 2011; **34**: 2237-2243 [PMID: 21949221 DOI: 10.2337/dc10-2414]

26 **Esposito K**, Ciotola M, Carleo D, Schisano B, Sardelli L, Di Tommaso D, Misso L, Saccomanno F, Ceriello A, Giugliano D. Post-meal glucose peaks at home associate with carotid intima-media thickness in type 2 diabetes. *J Clin Endocrinol Metab* 2008; **93**: 1345-1350 [PMID: 18198229 DOI: 10.1210/jc.2007-2000]

27 **Raz I**, Ceriello A, Wilson PW, Battioui C, Su EW, Kerr L, Jones CA, Milicevic Z, Jacober SJ. Post hoc subgroup analysis of the HEART2D trial demonstrates lower cardiovascular risk in older patients targeting postprandial versus fasting/premeal glycemia. *Diabetes Care* 2011; **34**: 1511-1513 [PMID: 21593301 DOI: 10.2337/dc10-2375]

28 **Avogaro A**. Postprandial glucose: marker or risk factor? *Diabetes Care* 2011; **34**: 2333-2335 [PMID: 21949226 DOI: 10.2337/dc11-1442]

29 **International Diabetes Federation.** Guideline for management of postmeal glucose in diabetes. Belgium: IDF Communications, 2011

30 **Bergenstal RM**, Ahmann AJ, Bailey T, Beck RW, Bissen J, Buckingham B, Deeb L, Dolin RH, Garg SK, Goland R, Hirsch IB, Klonoff DC, Kruger DF, Matfin G, Mazze RS, Olson BA, Parkin C, Peters A, Powers MA, Rodriguez H, Southerland P, Strock ES, Tamborlane W, Wesley DM. Recommendations for standardizing glucose reporting and analysis to optimize clinical decision making in diabetes: the Ambulatory Glucose

Profile (AGP). *Diabetes Technol Ther* 2013; **15**: 198-211 [PMID: 23448694 DOI: 10.1089/dia.20130051]
31 **Mittman N**, Desiraju B, Fazil I, Kapupara H, Chattopadhyay J, Jani CM, Avram MM. Serum fructosamine versus glycosylated hemoglobin as an index of glycemic control, hospitalization, and infection in diabetic hemodialysis patients. *Kidney Int Suppl* 2010; **78:** S41-S45 [PMID: 20671744 DOI: 10.1038/ki.2010.193]
32 **Negoro H**, Morley JE, Rosenthal MJ. Utility of serum fructosamine as a measure of glycemia in young and old diabetic and non-diabetic subjects. *Am J Med* 1988; **85**: 360-364 [PMID: 3414731 DOI: 10.1016/0002-9343(88)90587-6]
33 **Takahashi S**, Uchino H, Shimizu T, Kanazawa A, Tamura Y, Sakai K, Watada H, Hirose T, Kawamori R, Tanaka Y. Comparison of glycated albumin (GA) and glycated hemoglobin (HbA1c) in type 2 diabetic patients: usefulness of GA for evaluation of short-term changes in glycemic control. *Endocr J* 2007; **54**: 139-144 [PMID: 17159300 DOI: 10.3747/pdi.2008.00243]
34 **Cohen MP,** Hud E, Shea E. Rate of formation of glycated albumin revisited and clinical implications. *J Diabetes Metab* 2010; **1:** 102 [DOI: 10.4172/2155-6156.1000102]
35 **Inaba M**, Okuno S, Kumeda Y, Yamada S, Imanishi Y, Tabata T, Okamura M, Okada S, Yamakawa T, Ishimura E, Nishizawa Y. Glycated albumin is a better glycemic indicator than glycated hemoglobin values in hemodialysis patients with diabetes: effect of anemia and erythropoietin injection. *J Am Soc Nephrol* 2007; **18**: 896-903 [PMID: 17267743 DOI: 10.1681/ASN.200607772]
36 **Peacock TP**, Shihabi ZK, Bleyer AJ, Dolbare EL, Byers JR, Knovich MA, Calles-Escandon J, Russell GB, Freedman BI. Comparison of glycated albumin and hemoglobin A(1c) levels in diabetic subjects on hemodialysis. *Kidney Int* 2008; **73**: 1062-1068 [PMID: 18288102 DOI: 10.1038/ki.2008.25]
37 **Suzuki S**, Koga M. Glycemic control indicators in patients with neonatal diabetes mellitus. *World J Diabetes* 2014; **5**: 198-208 [PMID: 24748932 DOI: 10.4239/wjd.v5.i2.198]
38 **Seshiah V,** Balaji V, Srinivasan A, Balaji MS, Thiyagarajah A. Comparison of glycated albumin (GA) and glycosylated hemoglobin (A1C) in monitoring glycemic excursions during pregnancy. *Open J Obstet Gynecol* 2013; **3:** 47-50 [DOI: 10.4236/ojog.2013.31011]
39 **Yoshiuchi K,** Matsuhisa M, Katakami N, Nakatani Y, Sakamoto K, Matsuoka T, Umayahara Y, Kosugi K, Kaneto H, Yamasaki Y, Hori M. Glycated albumin is a better indicator for glucose excursion than glycated hemoglobin in type 1 and type 2 diabetes. *Endocr J* 2008; **55**: 503-507[PMID:18445997 DOI: 10.1057/endocrj.K07E-089]
40 **Koga M,** Murai J, Saito H, Kasayama S. Glycated albumin and glycated hemoglobin are influenced differently by endogenous insulin secretion in patients with type 2 diabetes. *Diabetes Care* 2010; **33**: 270-272 [PMID: 19846794 DOI: 10.2337/dc 09-1002]
41 **Shen Y**, Pu LJ, Lu L, Zhang Q, Zhang RY, Shen WF. Glycated albumin is superior to hemoglobin A1c for evaluating the presence and severity of coronary artery disease in type 2 diabetic patients. *Cardiology* 2012; **123**: 84-90 [PMID: 23018602 DOI: 10.1159/000342055]
42 **Pu LJ**, Lu L, Shen WF, Zhang Q, Zhang RY, Zhang JS, Hu J, Yang ZK, Ding FH, Chen QJ, Shen J, Fang DH, Lou S. Increased serum glycated albumin level is associated with the presence and severity of coronary artery disease in type 2 diabetic patients. *Circ J* 2007; **71**: 1067-1073 [PMID: 17587712 DOI: 10.1253/circj.711067]
43 **Dungan KM**. 1,5-anhydroglucitol (GlycoMark) as a marker of short-term glycemic control and glycemic excursions. *Expert Rev Mol Diagn* 2008; **8**: 9-19 [PMID: 18088226 DOI: 10.1586/14737159.8.1.9]
44 **Akanuma Y**, Morita M, Fukuzawa N, Yamanouchi T, Akanuma H. Urinary excretion of 1,5-anhydro-D-glucitol accompanying glucose excretion in diabetic patients. *Diabetologia* 1988; **31**: 831-835 [PMID: 3234638 DOI: 10.1007/BF00277486]
45 **Kishimoto M,** Yamasaki Y, Kubota M, Arai K, Morishima T, Kawamori R, Kamada T. 1,5 anhydro-d-glucitol evaluated daily glycemic excursions in well-controlled NIDDM. *Diabetes Care* 1995; **18:** 1156-1159 [DOI: 10.2337/diacare.18.81156]
46 **Dungan KM**, Buse JB, Largay J, Kelly MM, Button EA, Kato S, Wittlin S. 1,5-anhydroglucitol and postprandial hyperglycemia as measured by continuous glucose monitoring system in moderately controlled patients with diabetes. *Diabetes Care* 2006; **29**: 1214-1219 [PMID: 16731998 DOI: 10.2337/dc06-1910]
47 **Kazda C,** Bachmann O, Button E, Conlin T, Schroeder B, Guan X, Okerson T, Bhole D. Exenatide verbessert bei Patienten mit Typ-2-Diabetes die postprandiale Glucosekontrolle, gemessen an der Konzentration von 1,5-Anhydroglucitol. *Diabetologie und Stoffwechsel* 2008; **3:** A221 [DOI: 10.1055/s-2008-1076368]
48 **Kishimoto M**, Noda M. A pilot study of the efficacy of miglitol and sitagliptin for type 2 diabetes with a continuous glucose monitoring system and incretin-related markers. *Cardiovasc Diabetol* 2011; **10**: 115 [PMID: 22189184 DOI: 10.1186/1475-2840-10-115]
49 **Moses AC**, Raskin P, Khutoryansky N. Does serum 1,5-anhydroglucitol establish a relationship between improvements in HbA1c and postprandial glucose excursions? Supportive evidence utilizing the differential effects between biphasic insulin aspart 30 and insulin glargine. *Diabet Med* 2008; **25**: 200-205 [PMID: 18290862 DOI: 10.1111/j.1464-5491.2008.02384.x]
50 **Tylee TS,** Trence DL. Glycemic variability: looking beyond the A1C. *Diabetes Spectrum* 2012; **25**: 149-153 [DOI: 10.2337/diaspect.25.3.149]
51 **Monnier L**, Colette C, Owens DR. Glycemic variability: the third component of the dysglycemia in diabetes. Is it important? How to measure it? *J Diabetes Sci Technol* 2008; **2**: 1094-1100 [PMID: 19885298 DOI: 10.1177/193229680800200618]
52 **Kohnert KD,** Vogt L, Salzsieder E. Advances in understanding glucose variability and the role of continuous glucose monitoring. *European Endocrinol* 2010; **6**: 53-56
53 **Rodbard D**. Interpretation of continuous glucose monitoring data: glycemic variability and quality of glycemic control. *Diabetes Technol Ther* 2009; **11** Suppl 1: S55-S67 [PMID: 19469679 DOI: 10.1089/dia.2008.0132]
54 **Rodbard D**. Clinical interpretation of indices of quality of glycemic control and glycemic variability. *Postgrad Med* 2011; **123**: 107-118 [PMID: 21680995 DOI: 10.3810/pgm2011.07.2310]
55 **Cameron FJ**, Donath SM, Baghurst PA. Measuring glycaemic variation. *Curr Diabetes Rev* 2010; **6**: 17-26 [PMID: 20214595 DOI: 10.2174/157339910790442592]
56 **Baghurst PA**, Rodbard D, Cameron FJ. The minimum frequency of glucose measurements from which glycemic variation can be consistently assessed. *J Diabetes Sci Technol* 2010; **4**: 1382-1385 [PMID: 21129333 DOI: 10.1177/193229681000400612]
57 **Kohnert KD**, Heinke P, Fritzsche G, Vogt L, Augstein P, Salzsieder E. Evaluation of the mean absolute glucose change as a measure of glycemic variability using continuous glucose monitoring data. *Diabetes Technol Ther* 2013; **15**: 448-454 [PMID: 23550553 DOI: 10.1089/dia2012.0303]
58 **Fritzsche G**, Kohnert KD, Heinke P, Vogt L, Salzsieder E. The use of a computer program to calculate the mean amplitude of glycemic excursions. *Diabetes Technol Ther* 2011; **13**: 319-325 [PMID: 21291337 DOI: 10.1089/dia.2010.0108]
59 **Czerwoniuk D**, Fendler W, Walenciak L, Mlynarski W. GlyCulator: a glycemic variability calculation tool for continuous glucose monitoring data. *J Diabetes Sci Technol* 2011; **5**: 447-451 [PMID: 21527118 DOI: 10.1177/193229681100500236]
60 **Hill NR**, Oliver NS, Choudhary P, Levy JC, Hindmarsh P, Matthews DR. Normal reference range for mean tissue

glucose and glycemic variability derived from continuous glucose monitoring for subjects without diabetes in different ethnic groups. *Diabetes Technol Ther* 2011; **13**: 921-928 [PMID: 21714681 DOI: 10.1089/dia2010.0247]

61 **Zhou J**, Li H, Ran X, Yang W, Li Q, Peng Y, Li Y, Gao X, Luan X, Wang W, Jia W. Reference values for continuous glucose monitoring in Chinese subjects. *Diabetes Care* 2009; **32**: 1188-1193 [PMID: 19389816 DOI: 10.2337/dc09-0076]

62 **Kilpatrick ES**. Arguments for and against the role of glucose variability in the development of diabetes complications. *J Diabetes Sci Technol* 2009; **3**: 649-655 [PMID: 20144307 DOI: 10.1177/193229680900300405]

63 **Kilpatrick ES**, Rigby AS, Atkin SL. The effect of glucose variability on the risk of microvascular complications in type 1 diabetes. *Diabetes Care* 2006; **29**: 1486-1490 [PMID: 16801566 DOI: 10.2337/dc06-0293]

64 **Monnier L**, Colette C, Leiter L, Ceriello A, Hanefeld M, Owens D, Tajima N, Tuomiletho J, Davidson J, MD9 and on behalf of the PGR Group. The effect of glucose variability on the risk of microvascular complications in type 1 diabetes. *Diabetes Care* 2007; **30**: 185-186 [PMID: 17192364 DOI: 10.2337/dc06-1594]

65 **Bragd J**, Adamson U, Bäcklund LB, Lins PE, Moberg E, Oskarsson P. Can glycaemic varaibility, as calculated from blood glucose self-monitoring, predict the development of complications in type 1 diabetes over a decade? *Diabetes Metab* 2008; **34**: 612-616 [PMID: 18824382 DOI: 10.1016/j.diabet.200804.005]

66 **Snell-Bergeon JK**, Roman R, Rodbard D, Garg S, Maahs DM, Schauer IE, Bergman BC, Kinney GL, Rewers M. Glycaemic variability is associated with coronary artery calcium in men with Type 1 diabetes: the Coronary Artery Calcification in Type 1 Diabetes study. *Diabet Med* 2010; **27**: 1436-1442 [PMID: 21059097 DOI: 10.1111/j.1464-5491.2010.03127.x]

67 **Šoupal J**, Škrha J, Fajmon M, Horová E, Mráz M, Škrha J, Prázný M. Glycemic variability is higher in type 1 diabetes patients with microvascular complications irrespective of glycemic control. *Diabetes Technol Ther* 2014; **16**: 198-203 [PMID: 24401008 DOI: 10.1089/dia.2013.0205]

68 **Kilpatrick ES**, Rigby AS, Atkin SL. A1C variability and the risk of microvascular complications in type 1 diabetes: data from the Diabetes Control and Complications Trial. *Diabetes Care* 2008; **31**: 2198-2202 [PMID: 18650371 DOI: 10.2337/dc08-0864]

69 **Nalysnyk L**, Hernandez-Medina M, Krishnarajah G. Glycaemic variability and complications in patients with diabetes mellitus: evidence from a systematic review of the literature. *Diabetes Obes Metab* 2010; **12**: 288-298 [PMID: 20380649 DOI: 10.1111/j.1463-1326.2009.01160.x]

70 **Zhou J**, Jia WP, Ma XJ, Bao YQ, Lu W, Li M, Li Q, Hu C, Xiang KS. [Relationship between blood glucose variability and microalbuminuria in type 2 diabetic patients with well-controlled glycosylated hemoglobin]. *Zhonghua Yixue Zazhi* 2008; **88**: 2977-2981 [PMID: 19080075]

71 **Vaduva C**, Popa S, Mota M, Mota E. Analysis of chronic kidney disease – associated glycemic variability in patients with type 2 diabetes using continuous glucose monitoring system. *Rom J Diabetes Nutr Metab Dis* 2013; **20**: 315-322 [DOI: 10.2478/rjdnmd-2013-0030]

72 **Mirani M**, Berra C, Finazzi S, Calvetta A, Radaelli MG, Favareto F, Graziani G, Badalamenti S. Inter-day glycemic variability assessed by continuous glucose monitoring in insulin-treated type 2 diabetes patients on hemodialysis. *Diabetes Technol Ther* 2010; **12**: 749-753 [PMID: 20809678 DOI: 10.1089/dia.2010.0052]

73 **Takao T**, Ide T, Yanagisawa H, Kikuchi M, Kawazu S, Matsuyama Y. The effect of fasting plasma glucose variability on the risk of retinopathy in type 2 diabetic patients: Retrospective long-term follow-up. *Diabetes Res Clin Pract* 2010; **89**: 296-302 [PMID: 20416966 DOI: 10.1016/j.diabres.2010.03.027]

74 **Chen XM**, Zhang Y, Shen XP, Huang Q, Ma H, Huang YL, Zhang WQ, Wu HJ. Correlation between glucose fluctuations and carotid intima-media thickness in type 2 diabetes. *Diabetes Res Clin Pract* 2010; **90**: 95-99 [PMID: 20605247 DOI: 10.1016/j.diabres.2010.05.004]

75 **Su G**, Mi SH, Tao H, Li Z, Yang HX, Zheng H, Zhou Y, Tian L. Impact of admission glycemic variability, glucose, and glycosylated hemoglobin on major adverse cardiac events after acute myocardial infarction. *Diabetes Care* 2013; **36**: 1026-1032 [PMID: 23349547 DOI: 10.2337/dc12-0925]

76 **Mi SH**, Su G, Li Z, Yang HX, Zheng H, Tao H, Zhou Y, Tian L. Comparison of glycemic variability and glycated hemoglobin as risk factors of coronary artery disease in patients with undiagnosed diabetes. *Chin Med J* (Engl) 2012; **125**: 38-43 [PMID: 22340463]

77 **Su G**, Mi S, Tao H, Li Z, Yang H, Zheng H, Zhou Y, Ma C. Association of glycemic variability and the presence and severity of coronary artery disease in patients with type 2 diabetes. *Cardiovasc Diabetol* 2011; **10**: 19 [PMID: 21349201 DOI: 10.1186/1475-2840-10-19]

78 **Hirakawa Y**, Arima H, Zoungas S, Ninomiya T, Cooper M, Hamet P, Mancia G, Poulter N, Harrap S, Woodward M, Chalmers J. Impact of visit-to-visit glycemic variability on the risks of macrovascular and microvascular events and all-cause mortality in type 2 diabetes: the ADVANCE trial. *Diabetes Care* 2014; **37**: 2359-2365 [PMID: 24812434 DOI: 10.2337/dc14-0199]

79 **Rizzo MR**, Marfella R, Barbieri M, Boccardi V, Vestini F, Lettieri B, Canonico S, Paolisso G. Relationships between daily acute glucose fluctuations and cognitive performance among aged type 2 diabetic patients. *Diabetes Care* 2010; **33**: 2169-2174 [PMID: 20573753 DOI: 10.2337/dc10-0389]

80 **Penckofer S**, Quinn L, Byrn M, Ferrans C, Miller M, Strange P. Does glycemic variability impact mood and quality of life? *Diabetes Technol Ther* 2012; **14**: 303-310 [PMID: 22324383 DOI: 10.1089/dia.2011.0191]

81 **Monnier L**, Mas E, Ginet C, Michel F, Villon L, Cristol JP, Colette C. Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes. *JAMA* 2006; **295**: 1681-1687 [PMID: 16609090 DOI: 10.1001/jama.295.14.1681]

82 **Monnier L**, Wojtusciszyn A, Colette C, Owens D. The contribution of glucose variability to asymptomatic hypoglycemia in persons with type 2 diabetes. *Diabetes Technol Ther* 2011; **13**: 813-818 [PMID: 21561372 DOI: 10.1089/dia.2011.0049]

83 **Qu Y**, Jacober SJ, Zhang Q, Wolka LL, DeVries JH. Rate of hypoglycemia in insulin-treated patients with type 2 diabetes can be predicted from glycemic variability data. *Diabetes Technol Ther* 2012; **14**: 1008-1012 [PMID: 23101951 DOI: 10.1089/dia2012.0099]

84 **Kohnert KD**, Heinke P, Vogt L, Zander E, Fritzsche G, Augstein P, Salzsieder E. Reduced glucose variability is associated with improved quality of glycemic control in patients with type 2 diabetes: a 12-month observational study. *J Endocrinol Metab* 2011; **1**: 64-72

85 **Kovatchev BP**, Cox DJ. Assessment of risk for severe hypoglycemia among adults with IDDM: validation of the low blood glucose index. *Diabetes Care* 1998; **21**: 1870-1875 [PMID: 9802735 DOI: 10.2337/diacare.21.11.1870]

86 **Hill NR**, Hindmarsh PC, Stevens RJ, Stratton IM, Levy JC, Matthews DR. A method for assessing quality of control from glucose profiles. *Diabet Med* 2007; **24**: 753-758 [PMID: 17459094 DOI: 10.1111/j.1464.5491.2007.02119.x]

87 **Rodbard D**, Bailey T, Jovanovic L, Zisser H, Kaplan R, Garg SK. Improved quality of glycemic control and reduced glycemic variability with use of continuous glucose monitoring. *Diabetes Technol Ther* 2009; **11**: 717-723 [PMID: 19905888 DOI: 10.1089/dia.2009.0077]

88 **Pitsillides AN**, Anderson SM, Kovatchev B. Hypoglycemia risk and glucose variability indices derived from routine self-monitoring of blood glucose are related to laboratory measures of insulin sensitivity and epinephrine

counterregulation. *Diabetes Technol Ther* 2011; **13**: 11-17 [PMID: 21175266 DOI: 10.1089/dia.2010.0103]
89 **Park SA,** Ko SH, Lee SH, Cho JH, Moon SD, Jang SA, Song KH, Son HS, Yoon KH, Cha BY, Son HY, Ahn YB. Average Daily Risk Range-index of glycemic variability-related factor in type 2 diabetic inpatients. *Korean Diabetes J* 2009; **33**: 31-39 [DOI: 10.4093/kdj.2009.33.1.31]
90 **Kovatchev BP**, Otto E, Cox D, Gonder-Frederick L, Clarke W. Evaluation of a new measure of blood glucose variability in diabetes. *Diabetes Care* 2006; **29**: 2433-2438 [PMID: 17065680 DOI: 10.2337/dc06-1085]
91 **Patton SR**, Clements MA. Average daily risk range as a measure for clinical research and routine care. *J Diabetes Sci Technol* 2013; **7**: 1370-1375 [PMID: 24124966]
92 **Service FJ**. Glucose variability. *Diabetes* 2013; **62**: 1398-1404 [PMID: 23613565 DOI: 10.2337/db12-1396]
93 **Bremer T**, Gough DA. Is blood glucose predictable from previous values? A solicitation for data. *Diabetes* 1999; **48**: 445-451 [PMID: 10078542 DOI: 10.2337/diabetes.483.452]
94 **Churruca J,** Vigil L, Luna E, Ruiz-Galiana J, Varela M. The route to diabetes: Loss of complexity in the glycemic profile from health through the metabolic syndrome to type 2 diabetes. *Diabetes Metab Syndr Obes* 2008; **1**: 3-11 [PMID: 21437151 DOI: 102147/DMSO.S3812]
95 **Yamamoto N**, Kubo Y, Ishizawa K, Kim G, Moriya T, Yamanouchi T, Otsuka K. Detrended fluctuation analysis is considered to be useful as a new indicator for short-term glucose complexity. *Diabetes Technol Ther* 2010; **12**: 775-783 [PMID: 20809679 DOI: 10.1089/dia.2010.0059]
96 **Ogata H**, Tokuyama K, Nagasaka S, Ando A, Kusaka I, Sato N, Goto A, Ishibashi S, Kiyono K, Struzik ZR, Yamamoto Y. Long-range negative correlation of glucose dynamics in humans and its breakdown in diabetes mellitus. *Am J Physiol Regul Integr Comp Physiol* 2006; **291**: R1638-R1643 [PMID: 16873556 DOI: 10.1152/ajpregu.00241.2006]
97 **Khovanova NA**, Khovanov IA, Sbano L, Griffiths F, Holt TA. Characterisation of linear predictability and non-stationarity of subcutaneous glucose profiles. *Comput Methods Programs Biomed* 2013; **110**: 260-267 [PMID: 23253451 DOI: 10.1016/j.cmpb.2012.11.009]
98 **Kovatchev BP**, Clarke WL, Breton M, Brayman K, McCall A. Quantifying temporal glucose variability in diabetes via continuous glucose monitoring: mathematical methods and clinical application. *Diabetes Technol Ther* 2005; **7**: 849-862 [PMID: 16386091 DOI: 10.1089/dia.2005.7.849]
99 **Molnár GA**, Boros AG, Pótó L, Tamaskó M, Wittmann I. The Poincaré plot, but not the correlation R value, is a good marker of temporal variability of CGMS data. *Diabetes Technol Ther* 2008; **10**: 506-507 [PMID: 19049381 DOI: 10.1089/dia.2007.0301]
100 **Crenier L**. Poincaré plot quantification for assessing glucose variability from continuous glucose monitoring systems and a new risk marker for hypoglycemia: application to type 1 diabetes patients switching to continuous subcutaneous insulin infusion. *Diabetes Technol Ther* 2014; **16**: 247-254 [PMID: 24237387 DOI: 10.1089/dia.20130241]
101 **Cui X,** Abduljalil A, Manor BD, Peng CK, Novak V. Multi-scale variability: a link to gray matter atrophy and cognitive decline in type 2 diabetes. *Plos One* 2014; **9**: e86284 [DOI: 10.1371/journal.pone.0086284]
102 **Hellemons ME**, Kerschbaum J, Bakker SJ, Neuwirt H, Mayer B, Mayer G, de Zeeuw D, Lambers Heerspink HJ, Rudnicki M. Validity of biomarkers predicting onset or progression of nephropathy in patients with Type 2 diabetes: a systematic review. *Diabet Med* 2012; **29**: 567-577 [PMID: 21913962 DOI: 10.1111/j.1464-5491.2011.03437.x]
103 **Caveney EJ,** Cohen OJ. Diabetes and biomarkers. *J Diabetes Sci Technol* 2011; **5:** 192-197 [DOI: 10.1177/193229681100500127]
104 **Food and Drug Administration.** Guidance for Industry: Diabetes Mellitus-Evaluating cardiovascular risk in new antidiabetic therapies to treat type 2 diabetes. United States: Department of Health and Human Services, 2008
105 **Buckingham B**. Clinical overview of continuous glucose monitoring. *J Diabetes Sci Technol* 2008; **2**: 300-306 [PMID: 19885360 DOI: 10.1177/193229680800200223]
106 **Beck RW**, Hirsch IB, Laffel L, Tamborlane WV, Bode BW, Buckingham B, Chase P, Clemons R, Fiallo-Scharer R, Fox LA, Gilliam LK, Huang ES, Kollman C, Kowalski AJ, Lawrence JM, Lee J, Mauras N, O'Grady M, Ruedy KJ, Tansey M, Tsalikian E, Weinzimer SA, Wilson DM, Wolpert H, Wysocki T, Xing D, Juvenile Diabetes Research Foundation Continuous Glucose Monitoring Study Group. The effect of continuous glucose monitoring in well-controlled type 1 diabetes. *Diabetes Care* 2009; **32**: 1378-1383 [PMID: 19429875 DOI: 10.2337/dc09-0108]
107 **Blevins TC**. Professional continuous glucose monitoring in clinical practice 2010. *J Diabetes Sci Technol* 2010; **4**: 440-456 [PMID: 20307406]
108 **Mastrototaro J**, Welsh JB, Lee S. Practical considerations in the use of real-time continuous glucose monitoring alerts. *J Diabetes Sci Technol* 2010; **4**: 733-739 [PMID: 20513341 DOI: 10.1177/193229681000400329]
109 **Burnett DR**, Huyett LM, Zisser HC, Doyle FJ, Mensh BD. Glucose sensing in the peritoneal space offers faster kinetics than sensing in the subcutaneous space. *Diabetes* 2014; **63**: 2498-2505 [PMID: 24622798 DOI: 10.2337/db13-1649]
110 **Petersen K**, Chlup R, Jana Z, Kohnert KD, Kudlova P, Bartek J, Nakladalova M, Doubravova B, Seckar P. Influence of oral antidiabetic drugs on hyperglycemic response to foods in persons with type 2 diabetes mellitus as assessed by continuous glucose monitoring system: a pilot study. *J Diabetes Sci Technol* 2010; **4**: 983-992 [PMID: 20663465 DOI: 10.1177/193229681000400430]

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TOPIC HIGHLIGHT

WJD 5th Anniversary Special Issues (4): Diabetes-related complications

Causative anti-diabetic drugs and the underlying clinical factors for hypoglycemia in patients with diabetes

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Abstract

Recent clinical trials indicated that the intensive glycemic control do not reduce cardiovascular disease mortality among diabetic patients, challenging a significance of the strict glycemic control in diabetes management. Furthermore, retrospective analysis of the Action to Control Cardiovascular Risk in Diabetes study demonstrated a significant association between hypoglycemia and mortality. Here, we systematically reviewed the drug-induced hypoglycemia, and also the underlying clinical factors for hypoglycemia in patients with diabetes. The sulfonylurea use is significantly associated with severe hypoglycemia in patients with type 2 diabetes. The use of biguanide (approximately 45%-76%) and thiazolidinediones (approximately 15%-34%) are also highly associated with the development of severe hypoglycemia. In patients treated with insulin, the intensified insulin therapy is more frequently associated with severe hypoglycemia than the conventional insulin therapy and continuous subcutaneous insulin infusion. Among the underlying clinical factors for development of severe hypoglycemia, low socioeconomic status, aging, longer duration of diabetes, high HbA1c and low body mass index, comorbidities are precipitating factors for severe hypoglycemia. Poor cognitive and mental functions are also associated with severe hypoglycemia.

Key words: Comorbidity; Hypoglycemia; Insulin; Oral anti-diabetic drugs

Core tip: The use of sulfonylurea is significantly associated with severe hypoglycemia in patients with type 2 diabetes. Biguanide and thiazolidinediones use are also highly associated with severe hypoglycemia. The intensified insulin therapy is more frequently associated with severe hypoglycemia compared with other insulin therapies. Low socioeconomic status, aging, longer duration of diabetes, high HbA1c and low body mass index, comorbidities, poor cognitive and mental function are precipitating factors for severe hypoglycemia.

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INTRODUCTION

The Diabetes Controls and Complication Trial and the United Kingdom Prospective Diabetes Study lead us to consider the strict glycemic control to prevent micro- and macro-vascular complications[1,2]. Recent clinical trials such as Action to Control Cardiovascular Risk in Diabetes (ACCORD) presented that cardiovascular disease mortality did not decrease by the intensive glycemic control in diabetic patients[3-5], challenging a significance of the strict glycemic control in diabetes management.

In retrospective analysis of the ACCORD study, the annual mortality among patients in the intensive and standard glucose control arms were significantly higher in patients with severe hypoglycemia (2.8% and 3.7%, respectively) than those with no episodes (1.2% and 1.0%, respectively)[6].

Patients with diabetes treated with insulin and hypoglycemic drugs are at a greater risk of developing hypoglycemia than patients treated with only diet and exercise[7-9]. Drug-induced hypoglycemia causes substantial morbidity and mortality, and compromises physiological and behavioral defenses against subsequent hypoglycemia, and also precludes the maintenance of glyemic control[10-26].

Here we systematically reviewed drug-induced hypoglycemia, and the underlying clinical factors for the development in diabetic patients.

CAUSATIVE ANTI-DIABETIC DRUGS FOR HYPOGLYCEMIA

The list of published articles about the drug-induced hypoglycemia is shown in Table 1. Kim *et al*[27] analyzed subjects with severe hypoglycemia who were brought to the Emergency Departments (ED) between January 1, 2004 and December 30, 2009. Fifty three percent of subjects were treated by insulin. Among patients with severe hypoglycemia due to sulfonylurea (SU), the glimepiride use increased from 2004 to 2009, while the gliclazide use decreased. Among patients treated with insulin, the treatment by using long-acting insulin analogues and premixed insulin increased, while the treatment by neutral protamine Hagedorn (NPH)-insulin and regular insulin (RI) decreased. According to the accumulated data between 2004 and 2009, glimepiride (24.2%) and NPH/RI (38.3%) use were frequently associated with severe hypoglycemia.

A retrospective cohort study showed that severe hypoglycemia in patients with type 1 diabetes was almost due to insulin, and 42.3% and 51.1% of type 2 diabetic patients were due to SU and insulin, respectively[28]. Signorovitch *et al*[29] showed that the use of SU (38.2 %), biguanide (56.3%) and thiazolidinediones (TZD) (14.5%) were highly associated with the development of severe hypoglycemia. Although this study did not reveal whether monotherapy or combination therapy by using biguanide induced severe hypoglycemia, this study showed that the number of patients treated with biguanide was greater than those with SU. To understand the burden of severe hypoglycemia among new users of insulin and oral anti-diabetic drugs (OAD), Moisan *et al*[30] conducted an inception cohort study using the databases of the Quebec health insurance board and the Quebec registry of hospitalizations between January 1, 2000 and December 31, 2008. A total of 188659 new users of anti-diabetic treatment were included. A total of 3575 (1.9%) individuals had at least 1 hypoglycemia-related ED visit. This study also showed the greater use of metformin (45.0%) as compared with SU (32.1%).

Hsu *et al*[31] showed that the number of insulin and SU user was significantly greater in patients with severe hypoglycemia (24.2% for insulin, 67.8% for SU) than in patients without hypoglycemia (4.35% and 54.95%, respectively).

Holstein *et al*[32] compared the incidences of severe hypoglycemia between 2007-2010 and 1997-2000. Severe hypoglycemia among all emergency admissions significantly increased from 0.68% in 1997-2000 to 0.83% in 2007-2010, which was associated with the intensification of anti-hyperglycemic therapy. In type 1 diabetes, severe hypoglycemia increased from 11.5/100000 inhabitants to 23.4/100000 inhabitants for ten years, and also increased in type 2 diabetes from 18.5/100000 inhabitants to 32.6/100000 inhabitants. The number of drugs had increased in type 1 and type 2 diabetes. In patients with type 1 diabetes, the number of incidence of severe hypoglycemia due to the intensified insulin therapy (IIT) increased from 64 in 1997-2000 to 96 in 2007-2010, and severe hypoglycemia due to IIT (79.3%) was more frequent compared with the conventional (6.6%) or continuous subcutaneous insulin infusion (CSII) (13.2%), in 2007-2010. In type 2 diabetes, the frequency of IIT significantly increased in 2007-2010 as compared with those in 1997-2000. Severe hypoglycemia due to SU monotherapy increased from 45 cases to 67 cases. Severe hypoglycemia due to glimepiride (n = 65) occurred fourfold more frequently than severe hypoglycemia due to glibenclamide (n = 16). Ha *et al*[33] also reported that glimepiride was the most frequently prescribed drug in patients with severe hypoglycemia in South Korea.

In the survey by Geller *et al*[34], in an estimated 22.9% of ED visits for insulin-related hypoglycemia, more than 1 type of insulin product was documented. Long-acting (32.9%) and rapid-acting (26.4%) products were the most commonly documented insulin product types. Metformin and SU were the most commonly documented concomitant OAD, identified in 50.9% (95%CI: 47.6%-54.2%) and 39.2% (95%CI: 34.8%-43.6%),

Table 1 Published articles about the drug-induced hypoglycemia in patients with diabetes

Ref.	Subjects	Year	Nation	Setting	OAD	Insulin	Combination
Kim *et al*[27]	Type 2 (*n* = 298)	2004-2009	South Korea	The Emergency Department of two general hospitals	Glimepiride (24.2%) Gliclazide (5.4%) Glibenclamide (8.4%)	NPH/RI (38.3%) Premixed (11.1%) Glargine/Detemir (13.1%)	
Tsujimoto *et al*[28]	Type 1 (*n* = 85)	2006-2012	Japan	Retrospective cohort study in one medical center		Insulin (100%)	
	Type 2 (*n* = 305)				SU (42.3%) Others (6.6%)	Insulin (51.1%)	
Signorovitch *et al*[29]	Type 2 not treated with insulin (*n* = 5582)	1998-2010	United States	US-based employer claims database	SU (38.2%) Biguanides (56.3%) a-GI (0.9%) Sitagliptin (1.0%) Incretin mimetics (0.5%) TZD (14.9%)		
Moisan *et al*[30]	Not determined (*n* = 3575)	2000-2008	Canada	Inception cohort study using the database of the Quebec health insurance board and the Quebec registry of hospitalizations	SU (32.1%) Metformin (45.0%) SU + Metformin (12.3%) Others (2.1%)	Insulin (8.5%)	
Hsu *et al*[31]	Type 2 (*n* = 500)	1998-2009	Taiwan	A nationwide population-based study using the National Health Insurance Research Database	SU (67.8%) Others (61.4%)	Insulin (24.2%)	
Holstein *et al*[32]	Type 1 (*n* = 92)	1997-2000	German	A longitudinal population-based study		Conventional (27.2%) Intensified (69.6%) CSII (3.3%)	
	Type 1 (*n* = 121)	2007-2010				Conventional (6.6%) Intensified (79.3%) CSII (13.2%)	
	Type 2 (*n* = 148)	1997-2000			SU (30.4%)	Conventional (52.7%) Intensified (0%) CSII (0%)	SU + Insulin (16.9%)
	Type 2 (*n* = 225)	2007-2010			SU (29.8%) Metformin (0.9%)	Conventional (40.8%) Intensified (21.8%) CSII (0%)	SU + Insulin (6.7%)
Ha *et al*[33]	Not determined (*n* = 320)	2006-2009	South Korea	Retrospective analysis of hypoglycemic patients presented to emergency room of Uijeongbu St. Mary's Hospital	Glimepiride (29.7%) Glibenclamide (4.7%) Gliclazide (4.7%) Gliquidone (1.3%) Glipizide (0.9%) Others (24.7%)	Insulin (29.1%)	SU + Insulin (5.0%)
Geller *et al*[34]	Not determined (*n* = 8100)	2007-2011	United States	Nationally representative public health surveillance of adverse drug events among insulin-treated patients seeking emergency department care		Insulin (83.4%)	Insulin + Biguanide (8.5%) SU (6.6%) TZD (3.6%) DPP-4 inhibitors (1.3%) GLP-1 analogues (0.2%) Others (0.9%)
Ben-Ami *et al*[35]	Type 1 and 2 (*n* = 99)	1986-1992	Israel	Retrospective analysis of the medical record in Rambam Medical Center	Glyburide (51.5%) Glyburide + Metformin (10.2%)	Insulin (23.2%)	Insulin + Glyburide (13.1%) Insulin + Metformin (2.0%)
Quilliam *et al*[36]	Type 2 (*n* = 536581)	2004-2008	United States	Retrospective cohort designed to assess the rate and costs of hypoglycemia among working-age patients with type 2 diabetes in the MarketScan database	SU (42.3%) Metformin (75.7%) TZD (33.3%) Other oral agents (4.4%)	Insulin (6.0%) Other injectable agents (2.7%)	

Parsaik *et al*[37]	Type 1 (*n* = 210)	2003-2009	United States	Population-based study		Simple insulin (10.0%) MDI (67.0%) CSII(18.0%)	OAD + Insulin (1.0%)
	Type 2 (*n* = 503)				OAD (23.0%)	Simple insulin (27.0%) MDI (37.0%) CSII (1.0%)	OAD + Insulin (11.0%)

a-GI: a-glucosidase inhibitors; CSII: Continuous subcutaneous insulin infusion; DPP-4: Dipeptidyl peptidase-4; GLP-1: Glucagon-like peptide-1; MDI: Multiple daily insulin injection; NPH: Neutral protamine Hagedorn; OAD: Oral anti-diabetic drug; RI: Regular insulin; SU: Sulfonylurea; TZD: Thiazolidinediones.

respectively, of estimated ED visits for insulin-related hypoglycemia.

Ben-Ami *et al*[35] found that the glyburide use as mono-therapy (51.5%) and as combination therapy with metformin was the most frequently used drug in patients with hypoglycemic coma. Quilliam *et al*[36] estimated the rate and costs of hypoglycemia in patients with type 2 diabetes, by using a retrospective cohort design to assess the rate and costs of hypoglycemia among working-age patients in the 2004-2008 MarketScan database. The use of SU (42.3%), metformin (75.7%) and TZD (33.3%) were highly associated with the development of hypoglycemia. In the study among patients with type 1 diabetes by Parsaik *et al*[37], multiple daily insulin injection (MDI) (67.0%) was more frequently associated with severe hypoglycemia as compared with simple insulin (10.0%) and CSII (18.0%). In type 2 diabetes, MDI was also more frequently associated with severe hypoglycemia than simple insulin (27.0%), CSII (1.0%) and combination therapy with OAD (11.0%).

UNDERLYING CLINICAL FACTORS FOR HYPOGLYCEMIA

According to "Evaluation and Management of Adult Hypoglycemia Disorders: An Endocrine Society Clinical Practice Guideline", the causes of hypoglycemia in ill or medicated adult individuals include hypoglycemia due to anti-diabetic drugs (insulin or insulin secretagogue), alcohol and drugs other than anti-diabetic agents and alcohol; critical illness (hepatic, renal and heart failure), sepsis and inanition; deficiency of cortisol, glucagon and epinephrine; non-islet cell tumor[38]. These can also be the causes of hypoglycemia in diabetic patients. Conventional risk factors include excessive anti-diabetic drugs doses, ill-timed, or of the wrong type; decreased exogenous glucose delivery; increased glucose utilization; decreased endogenous glucose production; increased insulin sensitivity; decreased insulin clearance[38].

Hypoglycemia occurs due to relative or absolute insulin excess and compromised physiological defenses against decrease in plasma glucose[38-42]. The physiological defenses against decrease in plasma glucose include: reduction of insulin secretion; enhancement of glucagon and epinephrine secretion[39,43,44], which are compromised in patients with type 1 diabetes and also patients with long duration of type 2 diabetes[39,40,45,46]. Defective glucose counter-regulation is associated with the risk of severe hypoglycemia[47,48].

The list of published articles about the underlying clinical factors for hypoglycemia is shown in Table 2. Yaffe *et al*[49] reported that black race and low education level were significantly associated with severe hypoglycemia. Punthakee *et al*[50] also reported that significant associations of race and education level with severe hypoglycemia. Leese *et al*[51] indicated older age, a longer duration of diabetes, and a higher HbA1c as underlying clinical factors for hypoglycemic patients, which was also reported by Punthakee *et al*[50]. Yaffe *et al*[49] also suggested a significant association between severe hypoglycemia and a higher HbA1c. A lower body mass index (BMI) was also associated with the development of severe hypoglycemia[50,51].

Punthakee *et al*[50] studied the association between severe hypoglycemia and cognitive function, and showed poor cognitive function is associated with severe hypoglycemia in type 2 diabetic patients. Yaffe *et al*[49], Hsu *et al*[31] and Signorovitch *et al*[29] also reported a significant association between mental disorders and severe hypoglycemia. Neurological disorders such as stroke and epilepsy which influence mental and cognitive functions were also associated with development of severe hypoglycemia[29,31,50].

Heart, liver and renal functions affect pharmacokinetics and clearance of insulin and OAD. Liver cirrhosis, renal disease including diabetic nephropathy, heart diseases including cardiovascular diseases are significantly associated with severe hypoglycemia[29,31,50]. Hsu *et al*[31] performed a nationwide cohort study, and suggested that comorbidities such as hypertension and renal disease are associated with hypoglycemic episodes. Signorovitch *et al*[29] also indicated a significant associations of hypoglycemia with comorbidities such as mental disorders and stroke. In their study, patients with hypoglycemia showed a higher Charlson comorbidity index than those without hypoglycemia.

Neuropathy is also associated with hypoglycemia[50]. In neuropathy, especially, hypoglycemia-associated autonomic failure (HAAF) is significantly associated with the development of severe hypoglycemia[46,52]. In patients with HAAF, in the absence of reduction of insulin secretion and enhancement of glucagon secretion, the defective glucose counter-regulation by epinephrine induces hypoglycemia unawareness by reducing the sympathetic neural activity and neurogenic symptoms[39,40,45]. According to "Evaluation and Management of Adult Hypoglycemia Disorders: An Endocrine Society Clinical Practice

Table 2 Published articles about the underlying clinical factors for the development of hypoglycemia in patients with diabetes

Ref.	Clinical factors	Hypoglycemia	No hypoglycemia	*P* value
Yaffe et al[49]	Black race/ethnicity (%)	72.1	44.9	< 0.01
	Education (< high school education) (%)	36.1	24.0	0.04
	Glycated hemoglobin level (%)	8.0	7.2	< 0.01
	Prevalent diabetes mellitus (%)	85.2	47.9	< 0.01
	MMSE score [mean (SD)]	89.6 (5.7)	91.5 (5.2)	< 0.01
Hsu *et al*[31]	Hypertension (%)	63.6	51.2	< 0.0001
	Liver cirrhosis (%)	3.0	1.3	0.0074
	Renal disease (%)	17.4	5.2	< 0.0001
	Mental disease (%)	21.4	12.5	< 0.0001
	Cancer (%)	8.0	2.4	< 0.0001
	Stroke (%)	15.0	4.0	< 0.0001
	Heart disease (%)	13.2	3.6	< 0.0001
Leese *et al*[51]	Age (mean, yr)			
	Type 1 treated with insulin	37.7	32.8	0.009
	Type 2 treated with insulin	66.6	63.2	0.038
	Diabetes duration (mean, years)			
	Type 1 treated with insulin	20.7	16.7	0.013
	BMI (mean, kg/m^2)			
	Type 2 treated with insulin	26.7	30.1	< 0.001
Signorovitch *et al*[29]	Mental disorders (%)	15.2	11.4	< 0.001
	Neurological disorders (%)	17.2	10.7	< 0.001
	Cardiovascular disorders (%)	60.4	59.0	0.05
	Renal disorders (%)	16.5	12.3	< 0.001
	Epilepsy (%)	1.2	0.7	< 0.001
	Stroke (%)	4.9	2.9	< 0.001
	CCI [mean (SD)]	1.42 (1.70)	1.3	< 0.001
Punthakee *et al*[50]	Age [yr, mean (SD)]	63.91 (6.41)	62.41 (5.77)	0.002
	Female (%)	55.6	46.1	0.019
	Race			< 0.0001
	Non-Hispanic white (%)	60.0	70.9	
	African American (%)	30.0	15.4	
	Hispanic (%)	6.3	7.1	
	Others (%)	3.8	6.6	
	Education			0.01
	Less than high school (%)	16.3	12.8	
	High school graduate (%)	35.0	25.2	
	Some college (%)	26.9	35.1	
	College graduate (%)	21.9	26.9	
	BMI [mean (SD), kg/m^2]	32.08 (5.64)	33.03 (5.33)	0.029
	Diabetes duration [mean (SD) of years]	14.13 (8.74)	10.18 (7.22)	< 0.0001
	HbA1c (%)	8.46 (1.06)	8.27 (1.05)	0.021
	History of stroke (%)	11.3	4.6	0.0002
	History of cardiovascular disease (%)	41.9	28.4	0.0003
	Neuropathy score [mean (SD)]	0.53 (0.50)	0.45 (0.50)	0.049
	UACR (mg/mmol)			< 0.0001
	< 30 (%)	58.8	72.4	
	30-300 (%)	27.5	21.9	
	> 300 (%)	13.8	5.7	
	DSST score [mean (SD)]	46.45 (17.01)	52.89 (15.76)	< 0.0001
	RAVLT score [mean (SD)]	6.90 (2.72)	7.55 (2.53)	0.002
	Stroop score [mean (SD)]	37.69 (22.02)	31.66 (16.25)	< 0.0001
	MMSE score [mean (SD)]	26.83 (2.80)	27.45 (2.49)	0.002

BMI: Body mass index; CCI: Charlson comorbidity index; DSST: Digit Symbol Substitution Test; MMSE: Mini-Mental Status Exam; RAVLT: Rey Auditory Verbal Learning Test; UACR: Urinary albumin creatinine ratio.

Guideline", risk factors for HAAF include absolute deficiency of endogenous insulin secretion; a history of severe hypoglycemia, and hypoglycemia unawareness[38].

CONCLUSION

The use of SU is significantly associated with severe hypoglycemia in patients with type 2 diabetes. Especially, the glimepiride-induced severe hypoglycemia (approximately 20%-30%) occurred more frequently as compared with other SU. The use of biguanide (approximately 45%-76%) and TZD (approximately 15%-34%) are also highly associated with the development of severe hypoglycemia. The study that investigated insulin product types and

Table 3 Summary of the underlying clinical factors for the development of hypoglycemia in patients with diabetes

1 Socioeconomic status (education, race)
2 Aging
3 State of diabetes (duration, HbA1c, body mass index)
4 Cognitive and mental function
5 Comorbidity
6 Failure of organ which influence on clearance of insulin and oral anti-diabetic drugs (Heart, liver, renal failure)
7 Hypoglycemia-associated autonomic failure

hypoglycemia is very limited. In one study in Korea, NPH/RI was more frequently associated with severe hypoglycemia as compared with premixed insulin and glargine/detemir. In diabetic patients treated with insulin, IIT is more frequently associated with severe hypoglycemia compared with conventional insulin therapy and CSII.

Summary of the underlying clinical factors for hypoglycemia is shown in Table 3. Low socioeconomic status, aging, longer duration of diabetes, high HbA1c and low BMI are precipitating factors for severe hypoglycemia. Poor cognitive and mental functions are also associated with the development of severe hypoglycemia. Comorbidities including heart, liver, renal failures are likely to induce severe hypoglycemia. We should also pay attention to HAAF which leads to very serious hypoglycemia.

REFERENCES

1 **The Diabetes Control and Complications Trial Research Group.** The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group. *N Engl J Med* 1993; **329**: 977-986 [PMID: 8366922 DOI: 10.1056/NEJM199309303291401]

2 **UK Prospective Diabetes Study (UKPDS) Group.** Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. *Lancet* 1998; **352**: 837-853 [PMID: 9742976 DOI: 10.1016/S0140-6736(98)07019-6]

3 **Duckworth W**, Abraira C, Moritz T, Reda D, Emanuele N, Reaven PD, Zieve FJ, Marks J, Davis SN, Hayward R, Warren SR, Goldman S, McCarren M, Vitek ME, Henderson WG, Huang GD. Glucose control and vascular complications in veterans with type 2 diabetes. *N Engl J Med* 2009; **360**: 129-139 [PMID: 19092145 DOI: 10.1056/NEJMoa0808431]

4 **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]

5 **Patel A**, MacMahon S, Chalmers J, Neal B, Billot L, Woodward M, Marre M, Cooper M, Glasziou P, Grobbee D, Hamet P, Harrap S, Heller S, Liu L, Mancia G, Mogensen CE, Pan C, Poulter N, Rodgers A, Williams B, Bompoint S, de Galan BE, Joshi R, Travert F. Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. *N Engl J Med* 2008; **358**: 2560-2572 [PMID: 18539916 DOI: 10.1056/NEJMoa0802987]

6 **Bonds DE**, Miller ME, Bergenstal RM, Buse JB, Byington RP, Cutler JA, Dudl RJ, Ismail-Beigi F, Kimel AR, Hoogwerf B, Horowitz KR, Savage PJ, Seaquist ER, Simmons DL, Sivitz WI, Speril-Hillen JM, Sweeney ME. The association between symptomatic, severe hypoglycaemia and mortality in type 2 diabetes: retrospective epidemiological analysis of the ACCORD study. *BMJ* 2010; **340**: b4909 [PMID: 20061358 DOI: 10.1136/bmj.b4909]

7 **Gale EA**, Tattersall RB. Unrecognised nocturnal hypoglycaemia in insulin-treated diabetics. *Lancet* 1979; **1**: 1049-1052 [PMID: 86775 DOI: 10.1016/S0140-6736(79)92950-7]

8 **Unger RH**. Nocturnal hypoglycemia in aggressively controlled diabetes. *N Engl J Med* 1982; **306**: 1294 [PMID: 7040970 DOI: 10.1056/NEJM198205273062113]

9 **Pramming S**, Thorsteinsson B, Bendtson I, Rønn B, Binder C. Nocturnal hypoglycaemia in patients receiving conventional treatment with insulin. *Br Med J* (Clin Res Ed) 1985; **291**: 376-379 [PMID: 3926200 DOI: 10.1136/bmj.291.6492.376]

10 **Cryer PE**. The barrier of hypoglycemia in diabetes. *Diabetes* 2008; **57**: 3169-3176 [PMID: 19033403 DOI: 10.2337/db08-1084]

11 **Harris EL**. Adverse reactions to oral antidiabetic agents. *Br Med J* 1971; **3**: 29-30 [PMID: 5091891 DOI: 10.1136/bmj.3.5765.29]

12 **Seltzer HS**. Drug-induced hypoglycemia. A review based on 473 cases. *Diabetes* 1972; **21**: 955-966 [PMID: 4626706 DOI: 10.2337/diab.21.9.955]

13 **Deckert T**, Poulsen JE, Larsen M. Prognosis of diabetics with diabetes onset before the age of thirty-one. I. Survival, causes of death, and complications. *Diabetologia* 1978; **14**: 363-370 [PMID: 669100]

14 **Goldstein DE**, England JD, Hess R, Rawlings SS, Walker B. A prospective study of symptomatic hypoglycemia in young diabetic patients. *Diabetes Care* 1981; **4**: 601-605 [PMID: 6751735 DOI: 10.2337/diacare.4.6.601]

15 **Salans LB**. NIH plans study of diabetes control and complications. *N Engl J Med* 1982; **307**: 1527-1528 [PMID: 7144825]

16 **Goldgewicht C**, Slama G, Papoz L, Tchobroutsky G. Hypoglycaemic reactions in 172 Type 1 (insulin-dependent) diabetic patients. *Diabetologia* 1983; **24**: 95-99 [PMID: 6341141 DOI: 10.1007/BF00297389]

17 **Asplund K**, Wiholm BE, Lithner F. Glibenclamide-associated hypoglycaemia: a report on 57 cases. *Diabetologia* 1983; **24**: 412-417 [PMID: 6411511 DOI: 10.1007/BF00257338]

18 **Casparie AF**, Elving LD. Severe hypoglycemia in diabetic patients: frequency, causes, prevention. *Diabetes Care* 1985; **8**: 141-145 [PMID: 3888563 DOI: 10.2337/diacare.8.2.141]

19 **Wallis WE**, Donaldson I, Scott RS, Wilson J. Hypoglycemia masquerading as cerebrovascular disease (hypoglycemic hemiplegia). *Ann Neurol* 1985; **18**: 510-512 [PMID: 4073844 DOI: 10.1002/ana.410180415]

20 **Malouf R**, Brust JC. Hypoglycemia: causes, neurological manifestations, and outcome. *Ann Neurol* 1985; **17**: 421-430 [PMID: 4004166 DOI: 10.1002/ana.410170502]

21 **Nesto RW**, Phillips RT. Asymptomatic myocardial ischemia in diabetic patients. *Am J Med* 1986; **80**: 40-47 [PMID: 3706356 DOI: 10.1016/0002-9343(86)90451-1]

22 **Jennings AM**, Wilson RM, Ward JD. Symptomatic hypoglycemia in NIDDM patients treated with oral hypogly-cemic agents. *Diabetes Care* 1989; **12**: 203-208 [PMID: 2702912 DOI: 10.2337/diacare.12.3.203]

23 **Seltzer HS**. Drug-induced hypoglycemia. A review of 1418 cases. *Endocrinol Metab Clin North Am* 1989; **18**: 163-183 [PMID: 2645125]

24 **Hepburn DA**, Steel JM, Frier BM. Hypoglycemic convulsions cause serious musculoskeletal injuries in patients with IDDM. *Diabetes Care* 1989; **12**: 32-34 [PMID: 2653747 DOI: 10.2337/diacare.12.1.32]

25 **Pladziewicz DS**, Nesto RW. Hypoglycemia-induced silent myocardial ischemia. *Am J Cardiol* 1989; **63**: 1531-1532 [PMID: 2658533 DOI: 10.1016/0002-9149(89)90025-8]

26 **Patrick AW**, Campbell IW. Fatal hypoglycaemia in insulin-treated diabetes mellitus: clinical features and neuropathological changes. *Diabet Med* 1990; **7**: 349-354 [PMID: 2140089 DOI:

10.1111/j.1464-5491.1990.tb01403.x]
27 **Kim JT**, Oh TJ, Lee YA, Bae JH, Kim HJ, Jung HS, Cho YM, Park KS, Lim S, Jang HC, Lee HK. Increasing trend in the number of severe hypoglycemia patients in Korea. *Diabetes Metab J* 2011; **35**: 166-172 [PMID: 21738899 DOI: 10.4093/dmj.2011.35.2.166]
28 **Tsujimoto T**, Yamamoto-Honda R, Kajio H, Kishimoto M, Noto H, Hachiya R, Kimura A, Kakei M, Noda M. Vital signs, QT prolongation, and newly diagnosed cardiovascular disease during severe hypoglycemia in type 1 and type 2 diabetic patients. *Diabetes Care* 2014; **37**: 217-225 [PMID: 23939540 DOI: 10.2337/dc13-0701]
29 **Signorovitch JE**, Macaulay D, Diener M, Yan Y, Wu EQ, Gruenberger JB, Frier BM. Hypoglycaemia and accident risk in people with type 2 diabetes mellitus treated with non-insulin antidiabetes drugs. *Diabetes Obes Metab* 2013; **15**: 335-341 [PMID: 23121373 DOI: 10.1111/dom.12031]
30 **Moisan J**, Breton MC, Villeneuve J, Grégoire JP. Hypoglycemia-related emergency department visits and hypoglycemia-related hospitalizations among new users of antidiabetes treatments. *Can J Diabetes* 2013; **37**: 143-149 [PMID: 24070836 DOI: 10.1016/j.jcjd.2013.02.039]
31 **Hsu PF**, Sung SH, Cheng HM, Yeh JS, Liu WL, Chan WL, Chen CH, Chou P, Chuang SY. Association of clinical symptomatic hypoglycemia with cardiovascular events and total mortality in type 2 diabetes: a nationwide population-based study. *Diabetes Care* 2013; **36**: 894-900 [PMID: 23223349 DOI: 10.2337/dc12-0916]
32 **Holstein A**, Patzer OM, Machalke K, Holstein JD, Stumvoll M, Kovacs P. Substantial increase in incidence of severe hypoglycemia between 1997-2000 and 2007-2010: a German longitudinal population-based study. *Diabetes Care* 2012; **35**: 972-975 [PMID: 22410817 DOI: 10.2337/dc11-1470]
33 **Ha WC**, Oh SJ, Kim JH, Lee JM, Chang SA, Sohn TS, Son HS. Severe hypoglycemia is a serious complication and becoming an economic burden in diabetes. *Diabetes Metab J* 2012; **36**: 280-284 [PMID: 22950059 DOI: 10.4093/dmj.2012.36.4.280]
34 **Geller AI**, Shehab N, Lovegrove MC, Kegler SR, Weidenbach KN, Ryan GJ, Budnitz DS. National estimates of insulin-related hypoglycemia and errors leading to emergency department visits and hospitalizations. *JAMA Intern Med* 2014; **174**: 678-686 [PMID: 24615164 DOI: 10.1001/jamainternmed.2014.136]
35 **Ben-Ami H**, Nagachandran P, Mendelson A, Edoute Y. Drug-induced hypoglycemic coma in 102 diabetic patients. *Arch Intern Med* 1999; **159**: 281-284 [PMID: 9989540 DOI: 10.1001/archinte.159.3.281]
36 **Quilliam BJ**, Simeone JC, Ozbay AB, Kogut SJ. The incidence and costs of hypoglycemia in type 2 diabetes. *Am J Manag Care* 2011; **17**: 673-680 [PMID: 22106460]
37 **Parsaik AK**, Carter RE, Pattan V, Myers LA, Kumar H, Smith SA, Russi CS, Levine JA, Basu A, Kudva YC. Population-based study of severe hypoglycemia requiring emergency medical service assistance reveals unique findings. *J Diabetes Sci Technol* 2012; **6**: 65-73 [PMID: 22401324 DOI: 10.1177/193229681200600109]
38 **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]
39 **Cryer P.** Glucose homeostasis and hypoglycemia. In: Kronenberg H, Melmed S, Polonsky K, Larsen P, editors. Williams textbook of endocrinology, 11th ed. Philadelphia: Saunders, an imprint of Elsevier, Inc., 2008: 1503-1533
40 **Cryer PE**. Diverse causes of hypoglycemia-associated autonomic failure in diabetes. *N Engl J Med* 2004; **350**: 2272-2279 [PMID: 15163777 DOI: 10.1056/NEJMra031354]
41 **Cryer PE**. Hypoglycaemia: the limiting factor in the glycaemic management of Type I and Type II diabetes. *Diabetologia* 2002; **45**: 937-948 [PMID: 12136392 DOI: 10.1007/s00125-002-0822-9]
42 **Cryer PE**, Davis SN, Shamoon H. Hypoglycemia in diabetes. *Diabetes Care* 2003; **26**: 1902-1912 [PMID: 12766131 DOI: 10.2337/diacare.26.6.1902]
43 **Cryer PE**. Hypoglycemia, functional brain failure, and brain death. *J Clin Invest* 2007; **117**: 868-870 [PMID: 17404614 DOI: 10.1172/JCI31669]
44 Cryer P. The prevention and correction of hypoglycemia. In: Jefferson L, Cherrington A, Goodman H, editors. Handbook of physiology; Section 7, the endocrine system. Volume II. The endocrine pancreas and regulation of metabolism. New York: Oxford University Press, 2001: 1057-1092
45 **Dagogo-Jack SE**, Craft S, Cryer PE. Hypoglycemia-associated autonomic failure in insulin-dependent diabetes mellitus. Recent antecedent hypoglycemia reduces autonomic responses to, symptoms of, and defense against subsequent hypoglycemia. *J Clin Invest* 1993; **91**: 819-828 [PMID: 8450063 DOI: 10.1172/JCI116302]
46 **Segel SA**, Paramore DS, Cryer PE. Hypoglycemia-associated autonomic failure in advanced type 2 diabetes. *Diabetes* 2002; **51**: 724-733 [PMID: 11872673 DOI: 10.2337/diabetes.51.3.724]
47 **White NH**, Skor DA, Cryer PE, Levandoski LA, Bier DM, Santiago JV. Identification of type I diabetic patients at increased risk for hypoglycemia during intensive therapy. *N Engl J Med* 1983; **308**: 485-491 [PMID: 6337335 DOI: 10.1056/NEJM198303033080903]
48 **Bolli GB**, De Feo P, De Cosmo S, Perriello G, Ventura MM, Benedetti MM, Santeusanio F, Gerich JE, Brunetti P. A reliable and reproducible test for adequate glucose counterregulation in type I diabetes mellitus. *Diabetes* 1984; **33**: 732-737 [PMID: 6378698 DOI: 10.2337/diabetes.33.8.732]
49 **Yaffe K**, Falvey CM, Hamilton N, Harris TB, Simonsick EM, Strotmeyer ES, Shorr RI, Metti A, Schwartz AV. Association between hypoglycemia and dementia in a biracial cohort of older adults with diabetes mellitus. *JAMA Intern Med* 2013; **173**: 1300-1306 [PMID: 23753199 DOI: 10.1001/jamainternmed.2013.6176]
50 **Punthakee Z**, Miller ME, Launer LJ, Williamson JD, Lazar RM, Cukierman-Yaffee T, Seaquist ER, Ismail-Beigi F, Sullivan MD, Lovato LC, Bergenstal RM, Gerstein HC. Poor cognitive function and risk of severe hypoglycemia in type 2 diabetes: post hoc epidemiologic analysis of the ACCORD trial. *Diabetes Care* 2012; **35**: 787-793 [PMID: 22374637 DOI: 10.2337/dc11-1855]
51 **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]
52 **Davis MR**, Mellman M, Shamoon H. Further defects in counterregulatory responses induced by recurrent hypoglycemia in IDDM. *Diabetes* 1992; **41**: 1335-1340 [PMID: 1397708 DOI: 10.2337/diabetes.41.10.1335]

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TOPIC HIGHLIGHT

WJD 5th Anniversary Special Issues (4): Diabetes-related complications

Literature review on the management of diabetic foot ulcer

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Abstract

Diabetic foot ulcer (DFU) is the most costly and devastating complication of diabetes mellitus, which affect 15% of diabetic patients during their lifetime. Based on National Institute for Health and Clinical Excellence strategies, early effective management of DFU can reduce the severity of complications such as preventable amputations and possible mortality, and also can improve overall quality of life. The management of DFU should be optimized by using a multidisciplinary team, due to a holistic approach to wound management is required. Based on studies, blood sugar control, wound debridement, advanced dressings and offloading modalities should always be a part of DFU management. Furthermore, surgery to heal chronic ulcer and prevent recurrence should be considered as an essential component of management in some cases. Also, hyperbaric oxygen therapy, electrical stimulation, negative pressure wound therapy, bio-engineered skin and growth factors could be used as adjunct therapies for rapid healing of DFU. So, it's suggested that with appropriate patient education encourages them to regular foot care in order to prevent DFU and its complications.

Key words: Diabetes mellitus; Wound management; Diabetic foot ulcer; Amputation; Foot care

Core tip: Diabetic foot ulcer (DFU) is the most common complication of diabetes mellitus that usually fail to heal, and leading to lower limb amputation. Early effective management of DFU as follows: education, blood sugar control, wound debridement, advanced dressing, offloading, advance therapies and in some cases surgery, can reduce the severity of complications, and also cam improve overall quality of life of patients especially by using a multidisciplinary team approach.

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INTRODUCTION

Diabetes mellitus (DM) is one of the main problems in health systems and a global public health threat that has increased dramatically over the past 2 decades[1,2]. According to epidemiological studies, the number of patients with DM increased from about 30 million cases in 1985, 177 million in 2000, 285 million in 2010, and estimated if the situation continues, more than 360 million people by 2030 will have DM[3,4].

Patients with DM are prone to multiple complications such as diabetic foot ulcer (DFU). DFU is a common complication of DM that has shown an increasing trend over previous decades[5-7]. In total, it is estimated that 15% of patients with diabetes will suffer from DFU during their lifetime[8]. Although accurate figures are difficult to obtain for the prevalence of DFU, the prevalence of this complication ranges from 4%-27%[9-11].

To date, DFU is considered as a major source of morbidity and a leading cause of hospitalization in patients with diabetes[1,5,12,13]. It is estimated that approximately 20% of hospital admissions among patients with DM are the result of DFU[14]. Indeed, DFU can lead to infection, gangrene, amputation, and even death if necessary care is not provided[14]. On the other hand, once DFU has developed, there is an increased risk of ulcer progression that may ultimately lead to amputation. Overall, the rate of lower limb amputation in patients with DM is 15 times higher than patients without diabetes[8]. It is estimated that approximately 50%-70% of all lower limb amputations are due to DFU[8]. In addition, it is reported that every 30 s one leg is amputated due to DFU in worldwide[9]. Furthermore, DFU is responsible for substantial emotional and physical distress as well as productivity and financial losses that lower the quality of life[15]. The previous literature indicates that healing of a single ulcer costs approximately $$17500 (1998 United States Dollars). In cases where lower extremity amputation is required, health care is even more expensive at $30000-33500[16]. These costs do not represent the total economic burden, because indirect costs related to losses of productivity, preventive efforts, rehabilitation, and home care should be considered. When all this is considered, 7%-20% of the total expenditure on diabetes in North America and Europe might be attributable to DFU[17].

ETIOLOGY OF DFU

Recent studies have indicated multiple risk factors associated with the development of DFU[18-21]. These risk factors are as follows: gender (male), duration of diabetes longer than 10 years, advanced age of patients, high Body Mass Index, and other comorbidities such as retinopathy, diabetic peripheral neuropathy, peripheral vascular disease, glycated hemoglobin level (HbA_1C), foot deformity, high plantar pressure, infections, and inappropriate foot self-care habits[1,12,20-22] (Figure 1).

Although the literature has identified a number of diabetes related risk factors that contribute to lower-extremity ulceration and amputation, to date most DFU has been caused by ischemic, neuropathic or combined neuroischemic abnormalities[6,17] (Figure 2). Pure ischemic ulcers probably represent only 10% of DFU and 90% are caused by neuropathy, alone or with ischemia. In recent years, the incidence of neuroischemic problems has increased and neuroischemic ulcers are the most common ulcers seen in most United Kingdom diabetic foot clinics now[23].

In total, the most common pathway to develop foot problems in patients with diabetes is peripheral sensorimotor and autonomic neuropathy that leads to high foot pressure, foot deformities, and gait instability, which increases the risks of developing ulcers[24-26]. Today, numerous investigations have shown that elevated plantar pressures are associated with foot ulceration[27-29]. Additionally, it has been demonstrated that foot deformities and gait instability increases plantar pressure, which can result in foot ulceration[24,30].

MANGMENT OF DFU

Unfortunately, often patients are in denial of their disease and fail to take ownership of their illness along with the necessary steps to prevent complication and to deal with the many challenges associated with the management of DFU. However, numerous studies have shown that proper management of DFU can greatly reduce, delay, or prevent complications such as infection, gangrene, amputation, and even death[6,31,32].

The primary management goals for DFU are to obtain wound closure as expeditiously as possible[33,34]. As diabetes is a multi-organ systemic disease, all comorbidities that affect wound healing must be managed by a multidisciplinary team for optimal outcomes with DFU[35-38]. Based on National Institute for Health and Clinical Excellence strategies, the management of DFU should be done immediately with a multidisciplinary team that consists of a general practitioner, a nurse, an educator, an orthotic specialist, a podiatrist, and consultations with other specialists such as vascular surgeons, infectious disease specialists, dermatologists, endocrinologists, dieticians, and orthopedic specialists[39]. Today, numerous studies have shown that a multidisciplinary team can reduce amputation rates, lower costs, and leads to better quality of life for patients with DFU[39-41]. The American Diabetes Association has concluded that a preventive care team, defined as a multidisciplinary team, can decrease the risks associated with DFU and amputation by 50%-85%[42]. It's suggested that with applying this approach take appropriate strategies for management of DFU to consequently reduce the severity of complications, improve overall quality of life, and increase the life expectancy of patients[36]. In this article, we review available evidence on the management of DFU as follows: education, blood sugar control, wound debridement, advanced dressing,

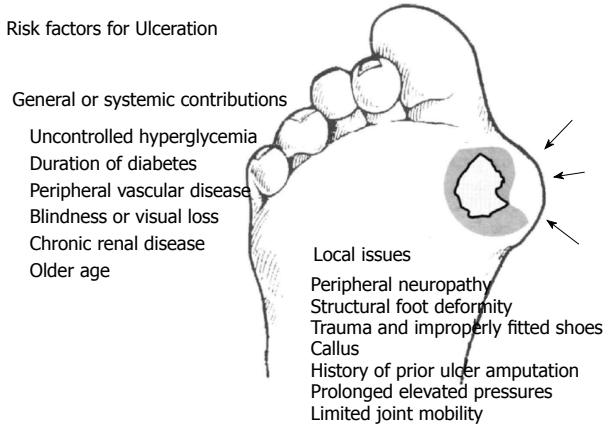

Figure 1 The risk factors for diabetic foot ulcer. Ulcers may be distinguished by general or systemic considerations *vs* those localized to the foot and its pathology. (Data adapted from Frykberg *et al*[18]).

offloading, surgery, and advanced therapies that are used clinically.

RESEARCH

In this review article, we searched for articles published between March 1, 1980 and July 28, 2014 in the following five electronic databases: PubMed, Science Direct, Embase, Web of Science, and Scopus, for both English and non-English language articles with the following keywords: "diabetic foot ulcer", "amputations", "wound management", "debridement", "advanced dressings", "offloading modalities", "hyperbaric oxygen therapy", "electrical stimulation", "negative pressure wound therapy", "bio-engineered skin", "growth factors", and "foot care" as the medical subject heading (MeSH). Study designs that were included were randomized controlled trials (RCTs), case-control studies, cohort studies, prospective and retrospective uncontrolled studies, cross-sectional studies, and review studies. Case reports and case series were excluded. We searched bibliographies for all retrieved and relevant publications to identify other studies.

Education

It has been shown that up to 50% of DFU cases can be prevented by effective education. In fact, educating patients on foot self-management is considered the cornerstone to prevent DFU[12,43-45].

Patient education programs need to emphasize patient responsibility for their own health and well-being. The ultimate aim of foot care education for people with diabetes is to prevent foot ulcers and amputation. Currently, a wide range and combinations of patient educational interventions have been evaluated for the prevention of DFU that vary from brief education to intensive education including demonstration and hands-on teaching[46]. Patients with DFU should be educated about risk factors and the importance of foot care, including the need for self-inspection, monitoring foot temperature, appropriate daily foot hygiene, use of proper footwear, and blood sugar control[47]. However, education is better when combined with other care strategies, because previous reviews on patient education has suggested that when these methods were combined with a comprehensive approach, these methods can reduce the frequency and morbidity of the limb threatening complications caused by DFU[48].

Blood sugar control

In patients with DFU, glucose control is the most important metabolic factor. In fact, it is reported inadequate control of blood sugar is the primary cause of DFU[6,49,50].

The best indicator of glucose control over a period of time is HbA_1C level. This test measures the average blood sugar concentration over a 90-d span of the average red blood cell in peripheral circulation. The higher the HbA_1C level, the more glycosylation of hemoglobin in red blood cells will occur. Studies have shown that blood glucose levels > 11.1 mmol/L (equivalent to > 310 mg/mL or an HbA_1C level of > 12) is associated with decreased neutrophil function, including leukocyte chemotaxis[50]. Indeed, a greater elevation of blood glucose level has been associated with a higher potential for suppressing inflammatory responses and decreasing host response to an infection[6]. Pomposelli *et al*[51] has indicated that a single blood glucose level > 220 mg/dL on the first postoperative day was a sensitive (87.5%) predictor of postoperative infection. Furthermore, the authors found that patients with blood glucose values > 220 mg/dL had infection rates that were 2.7 times higher than for patients with lower blood glucose values (31.3% *vs* 11.5%, respectively)[51]. In addition, it's indicated that a 1% mean reduction in HbA_1C was associated with a 25% reduction in micro vascular complications, including neuropathy[47]. Investigations have found that poor glucose control accelerated the manifestation of Peripheral Arterial Disease (PAD). It has been shown that for every 1% increase in HbA_1C, there is an increase of 25%-28% in the relative risk of PAD, which is a primary cause of DFU[52]. However, to date, no RCT has been performed to determine whether improved glucose control has benefits after a foot ulcer has developed.

Debridement

Debridement is the removal of necrotic and senescent tissues as well as foreign and infected materials from a wound, which is considered as the first and the most important therapeutic step leading to wound closure and a decrease in the possibility of limb amputation in patients with DFU[53-56]. Debridement seems to decrease bacterial counts and stimulates production of local growth factors. This method also reduces pressure, evaluates the wound bed, and facilitates wound drainage[32,57].

There are different kinds of debridement including surgical, enzymatic, autolytic, mechanical, and biological[58] (Table 1). Among these methods, surgical debridement has

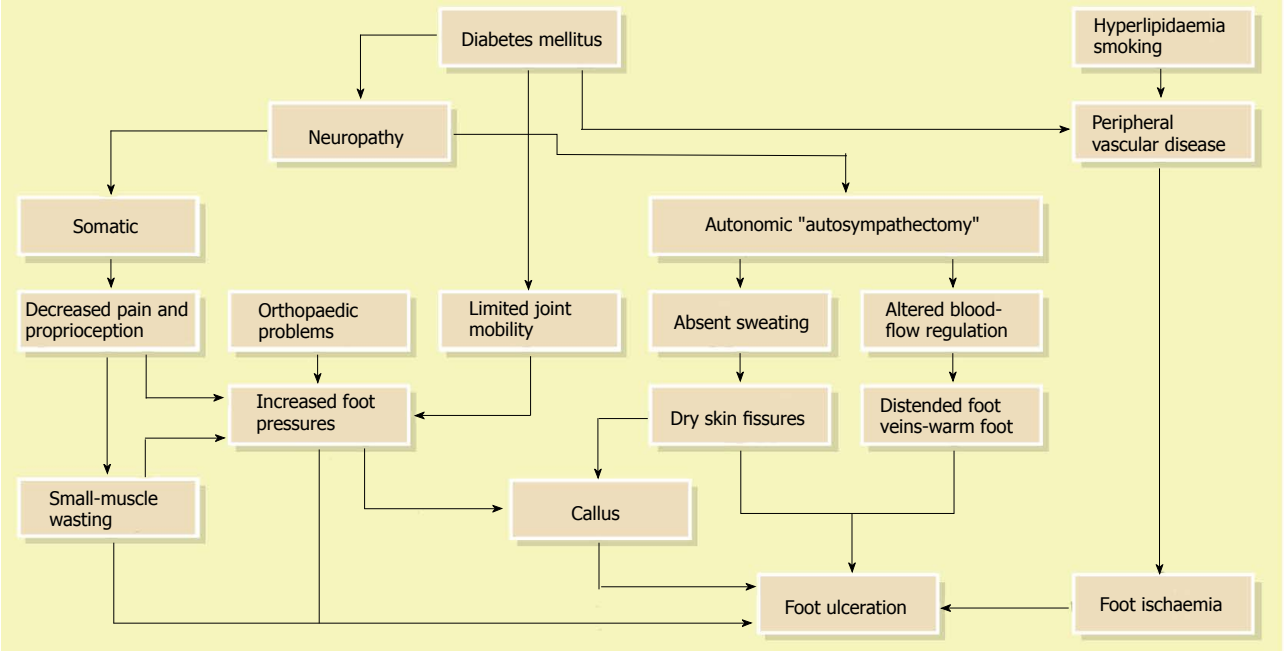

Figure 2 Etiology of diabetic foot ulcer. (Data adapted from Boulton *et al*[17]).

Table 1 Different kind of debridement for patients with diabetic foot ulcer

Method	Explanation	Advantages	Disadvantages
Surgical or Sharp	Callus and all nonviable soft tissues and bone remove from the open wound with a scalpel, tissue nippers, curettes, and curved scissors. Excision of necrotic tissues should extend as deeply and proximally as necessary until healthy, bleeding soft tissues and bone are encountered[59]	Only requires sterile scissors or a scalpel, so is cost-effective[55]	Requires a certain amount of skill to prevent enlarging the wound[55]
Mechanical	This method includes wet to dry dressings, high pressure irrigation, pulsed lavage and hydrotherapy[76], and commonly used to clean wounds prior to surgical or sharp debridement[76]	Allows removal of hardened necrosis	It is not discriminating and may remove granulating tissue It may be painful for the patients[55]
Autolytic	This method occurs naturally in a healthy, moist wound environment when arterial perfusion and venous drainage are maintained[18]	It's cost-effective[55] It is suitable for an extremely painful wound[18]	It's time consuming and may require an equivocal time for treatment[18]
Enzymatic	The only formulation available in the United Kingdom contains Streptokinase and Streptodornase (Varidase Topical® Wyeth Laboratories). This enzyme aggressively digests the proteins fibrin, collagen and elastin, which are commonly found in the necrotic exudate of a wound[77,78]	They can be applied directly into the necrotic area[55]	Streptokinase can be systemically absorbed and is therefore contraindicated in patients at risk of an MI It's expensive[55]
Biological	Sterile maggots of the green bottle fly (Lucilia sericata) are placed directly into the affected area and held in place by a close net dressing. The larvae have a ferocious appetite for necrotic material while actively avoiding newly formed healthy tissue[79,80]	They discriminate between the necrotic and the granulating tissue[79]	There may be a reluctance to use this treatment by patients and clinicians It's expensive[79,80]

been shown to be more effective in DFU healing[54,59-62]. Surgical or sharp debridement involves cutting away dead and infected tissues followed by daily application of saline moistened cotton gauze[53]. The main purpose of this type of debridement is to turn a chronic ulcer into an acute one. Surgical debridement should be repeated as often as needed if new necrotic tissue continues to form[63]. It has been reported that regular (weekly) sharp debridement is associated with the rapid healing of ulcers than for less frequent debridement[59,64-66]. In a retrospective cohort study, Wilcox *et al*[66] indicated that frequent debridement healed more wounds in a shorter time ($P < 0.001$). In fact, the more frequent the debridement, the better the healing outcome.

The method of debridement depends on characteristics, preferences, and practitioner level of expertise[54]. When surgical or sharp debridement is not indicated, then other types of debridement could be used.

An older debridement type that is categorized as biological debridement is maggot debridement therapy (MDT), which is also known as maggot therapy or larval therapy. In this method, sterile and live forms of the Lucilia sericata larvae are applied to the wound to achieve debridement, disinfection, and ultimately wound

Table 2 Common offloading techniques

Technique	Casting techniques	Footwear related techniques	Surgical offloading techniques	Other techniques
Examples	TCC (Figure 3)	Shoes or half shoes (Figure 7)	ATL	Bed rest
	iTCC (Figure 5)	Sandals	Liquid silicone injections/tissue augmentation	Crutches/Canes/Wheelchairs
	RCW (Figure 4)	Insoles	Callus debridement	Bracing (patella tendon bearing, ankle-foot orthoses)
	Scotch-cast boots (Figure 6)	In-shoe orthoses	Metatarsal head resection osteotomy/ arthroplasty/os ectomy/ exostectomy	Walkers
	Windowed casts	Socks	External fixation	Offloading dressings
	Custom splints			Felted foam/padding
				Plugs

Data adapted from Armstrong *et al*[82]. TCC: Total contact cast; iTCC: Instant TCC; RCW: Removable cast walkers; ATL: Achilles tendon lengthening.

healing[67-69]. Indeed, larvae secrete a powerful autolytic enzyme that liquefies necrotic tissues, stimulates the healing processes, and destroys bacterial biofilms[70-72]. This technique is indicated for open wounds and ulcers that contain gangrenous or necrotic tissues with or without infection[72]. To date, paucity of RCTs show efficacy of this method with DFU; however, some of retrospective[71,73]; and prospective[74] studies have shown MDT as a clinically effective treatment for DFU. These studies reported that MDT can significantly diminish wound odor and bacterial count, including *Methicillin-Resistant Staphylococcus Aurous*, prevent hospital admission, and decrease the number of outpatient visits among patients with DFU[71,73-75].

Despite the advantages of debridement, adequate debridement must always precede the application of topical wound healing agents, dressings, or wound closure procedures, which may be expensive.

Offloading

The use of offloading techniques, commonly known as pressure modulation, is considered the most important component for the management of neuropathic ulcers in patients with diabetes[81,82]. Recent studies have provided evidence indicating that proper offloading promotes DFU healing[83-85].

Although many offloading modalities are currently in use (Table 2), only a few studies describe the frequency and rate of wound healing with some of the methods frequently used clinically. The choice of these methods is determined by patient physical characteristics and abilities to comply with the treatment along with the location and severity of the ulcer[82].

The most effective offloading technique for the treatment of neuropathic DFU is total contact casts (TCC)[82,86,87]. TCC is minimally padded and molded carefully to the shape of the foot with a heel for walking (Figure 3). The cast is designed to relieve pressure from the ulcer and distribute pressure over the entire surface of the foot; thus, protecting the site of the wound[82]. Mueller *et al*[87] conducted an RCT that showed TCC healed a higher percentage of plantar ulcers at a faster rate when compared with the standard treatment. In addition, a histologic examination of ulcer specimens has shown that patients treated with TCC before debridement had better healing as indicated by angiogenesis with the formation of granulation tissue than for patients treated with debridement alone as indicated by a predominance of inflammatory elements[88]. The contributory factors to the efficacy of TCC treatment are likely to be due to pressure redistribution and offloading from the ulcer area. In addition, the patient is unable to remove the cast, which thereby forces compliance, reduces activity levels, and consequently improves wound healing[84]. However, the frequency of side effects referred to in the literature and minimal patient acceptance make this approach inappropriate for wide applications[89,90]. Fife *et al*[91] has shown that TCC is vastly underutilized for DFU wound care in the United States. Based on this study, only 16% of patients with DFU used TCC as their offloading modalities. The main disadvantage of TCC was the need for expertise in its application. Most centers do not have a physician or cast technician available with adequate training or experience to safely apply TCC. In addition, improper cast application can cause skin irritation and in some cases even frank ulceration. Also, the expense of time and materials (the device should be replaced weekly), limitations on daily activities (*e.g.*, bathing), and the potential of a rigid cast to injure the insensate neuropathic foot are considered other disadvantages. Furthermore, TCC does not allow daily assessment of the foot or wound, which is often contraindicative in cases of soft tissue or bone infections[36,32,83]. In some cases, it is suggested to use other kinds of offloading techniques such as a removable cast walker (RCW) or Instant TCC (iTCC).

An RCW is cast-like device that is easily removable to allow for self-inspection of the wound and application of topical therapies that require frequent administration[82,90] (Figure 4). The application of this method allows for bathing and comfortable sleep. In addition, because RCW is removable, they can be used for infected wounds as well as for superficial ulcers[82]. However, in a study that compared the effectiveness of TCC, RCW, and half-shoe, this method did not show equivalent healing time (mean healing time: 33.5, 50.4, and 61.1 d, respectively), and a

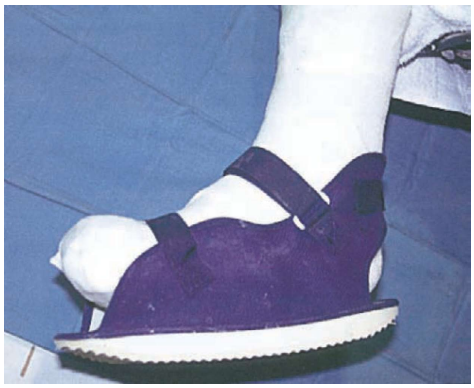

Figure 3 Total contact cast for patients with diabetic foot ulcer. (Data adapted from Armstrong *et al*[82]).

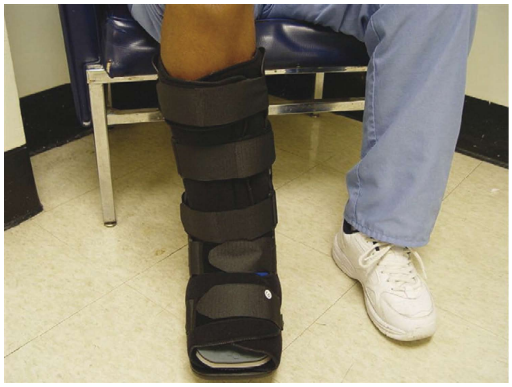

Figure 4 Removable cast walker (DH Walker) for patients with diabetic foot ulcer. (Data adapted from Rathur *et al*[86]).

significantly higher proportion of people with DFU were healed after 12 wk wearing a TCC compared with the two other widely used offloading modalities[81].

iTCC, which involves simply wrapping a RCW with a single layer of cohesive bandage, Elastoplast or casting tape (Figure 5), is another offloading technique that is shown to be more effective than TCC [92] and RCW [93]. This technique forces the patient to adhere to advice to immobilize the foot while allowing for ease of application and examination of the ulcer as needed. A preliminary randomized trial of TCC *vs* iTCC (Figure 6) in the management of plantar neuropathic foot ulcers has confirmed equivalent efficacy of the two devices and that iTCC is cheaper, quicker to apply, and has fewer adverse effects than traditional TCC[93]. As this device does not require a skilled technician to apply it, it could revolutionize the future management of plantar neuropathic ulcers. It has been suggested that iTCC will dramatically change the treatment of non-ischemic, neuropathic, diabetic plantar ulcers, and has the potential to replace TCC as the gold standard for offloading plantar neuropathic ulcers[92].

Regardless of the modality selected, patients should return to an unmodified shoe until complete healing of the ulcer has occurred (Figure 7). Furthermore, any shoe that resulted in the formation of an ulcer should not be worn again[94].

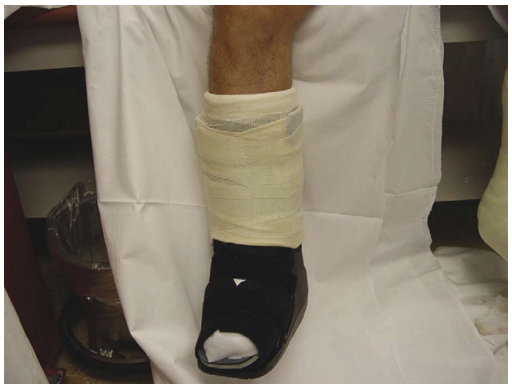

Figure 5 Instant total contact cast for patients with diabetic foot ulcers. The removable cast walker shown in Figure 5 has now been rendered irremovable by the application of bands of casting. (Data adapted from Rathur *et al*[86]).

Advanced dressing

A major breakthrough for DFU management over the last decades was the demonstration of novel dressings[13,95]. Ideally, dressings should confer moisture balance, protease sequestration, growth factor stimulation, antimicrobial activity, oxygen permeability, and the capacity to promote autolytic debridement that facilitates the production of granulation tissues and the re-epithelialization process. In addition, it should have a prolonged time of action, high efficiency, and improved sustained drug release in the case of medicated therapies[95,96]. Hence, no single dressing fulfills all the requirements of a diabetic patient with a foot ulcer. The choice of dressing is largely determined by the causes of DFU, wound location, depth, amount of scar or slough, exudates, condition of wound margins, presence of infection and pain, need for adhesiveness, and conformability of the dressing[13].

Wound dressing can be categorized as passive, active, or interactive[97]. Passive dressings are used as protective functions and for acute wounds because they absorb reasonable amounts of exudates and ensure good protection. Active and interactive dressings are capable of modifying the physiology of a wound by stimulating cellular activity and growth factors release. In addition, they are normally used for chronic wounds because they adapt to wounds easily and maintain a moist environment that can stimulate the healing process[95,98]. The main categories of dressings used for DFU are as follows: films, hydrogels, hydrocolloids, alginates, foams, and silver-impregnated (Table 3).

Today, all dressings are commonly used in clinical practice, while the efficacy of these products has been a challenge for researchers and clinicians, and there are controversial results regarding their use[36,99]. However, dressings are used based on DFU characteristics (Figure 8), hydrogels have been found to be the most popular choice of dressing for all DFU types[96]. Some studies dealing with the incorporation of these products show great potential in the treatment of DFU[100,101]. However, these findings do not represent a practical option since the application of these compounds is expensive and

Table 3 Classification of advanced wound dressings used for diabetic foot ulcers healing

Type	Example	Explanation	Advantages	Disadvantages
Hydrocolloids	Duoderm (Convatec) Granuflex (Convatec) Comfeel (Coloplast)	These kind of dressings usually composed of a hydrocolloid matrix bonded onto a vapor permeable film or foam backing. When in contact with the wound surface this matrix forms a gel to provide a moist environment[102]	Absorbent Can be left for several days Aid autolysis[96]	Concerns about use for infected wounds May cause maceration Unpleasant odor[96]
Hydrogels	Aquaform (Maersk Medical) Intrasite Gel (Smith and Nephew) Aquaflo (Covidien)	These dressings consist of cross-linked insoluable polymers (*i.e.*, starch or carboxymethylcellulose) and up to 96% water. These dressings are designed to absorb wound exudate or rehydrate a wound depending on the wound moisture levels. They are supplied in either flat sheets, an amorphous hydrogel or as beads[96]	Absorbent Donate liquid Aid autolysis[96]	Concerns about use for infected wounds May cause maceration using for highly exudative wounds[96]
Foams	Allevyn (Smith and Nephew) Cavicare (Smith and Nephew) Biatain (Coloplast) Tegaderm (3M)	These dressings normally contain hydrophilic polyurethane foam and are designed to absorb wound exudate and maintain a moist wound surface[103]	Highly absorbent and protective Manipulate easily[96] Can be left up to seven days Thermal insulation[96]	Occasional dermatitis with adhesive[96] Bulky[6] May macerate surrounding skin[6]
Films	Tegaderm (3M) Opsite (Smith and Nephew)	Film dressings often form part of the construction of other dressings such as hydrocolloids, foams, hydrogel sheets and composite dressings, which are made up of several materials with the film being used as the outer layer[107,108]	Cheap Manipulate easily Permeable to water vapor and oxygen but not to water microorganisms[95]	May need wetting before removal[96] Aren't suitable for infected wounds[107,108] Nonabsorbent If fluid collects under film it must be drained or the film replaced[6]
Alginates	Calcium Alginate Dressing (Smith and Nephew Inc., Australia) Kaltostat (ConvaTec) Sorbalgon (Hartman United States, Inc.l) Medihoney (Derma Sciences Inc., Canada)	The alginate forms a gel when in contact with the wound surface which can be lifted off with dressing removal or rinsed away with sterile saline. Bonding to a secondary viscose pad increases absorbency[104]	Highly absorbent Bacteriostatic Hemostatic Useful in cavities[96]	May need wetting before removal[96]
Silver-impregnated	Acticoat (Smith and Nephew) Urgosorb Silver (Urgo)	These dressing used to treat infected wounds as silver ions are thought to have antimicrobial properties[109]	Antiseptic Absorbent[96] Reduce odor Improved pain-related symptoms Decrease wound exudates Have a prolonged dressing wear time[112]	High cost[96]

Figure 6 Scotch-cast boot for off-loading pressure from the foot of a diabetic patient with foot ulcer. (Data adapted from Armstrong *et al*[82]).

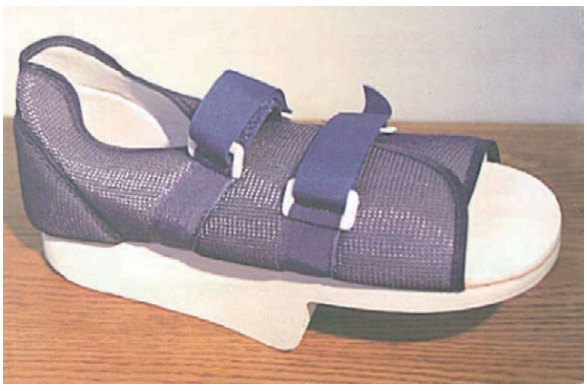

Figure 7 Half shoe for off-loading pressure from the foot of a diabetic patient with foot ulcer. (Data adapted from Armstrong *et al*[82]).

difficult to regulate[102-105]. Nevertheless, they have longer wear times, greater absorbency, may be less painful, and

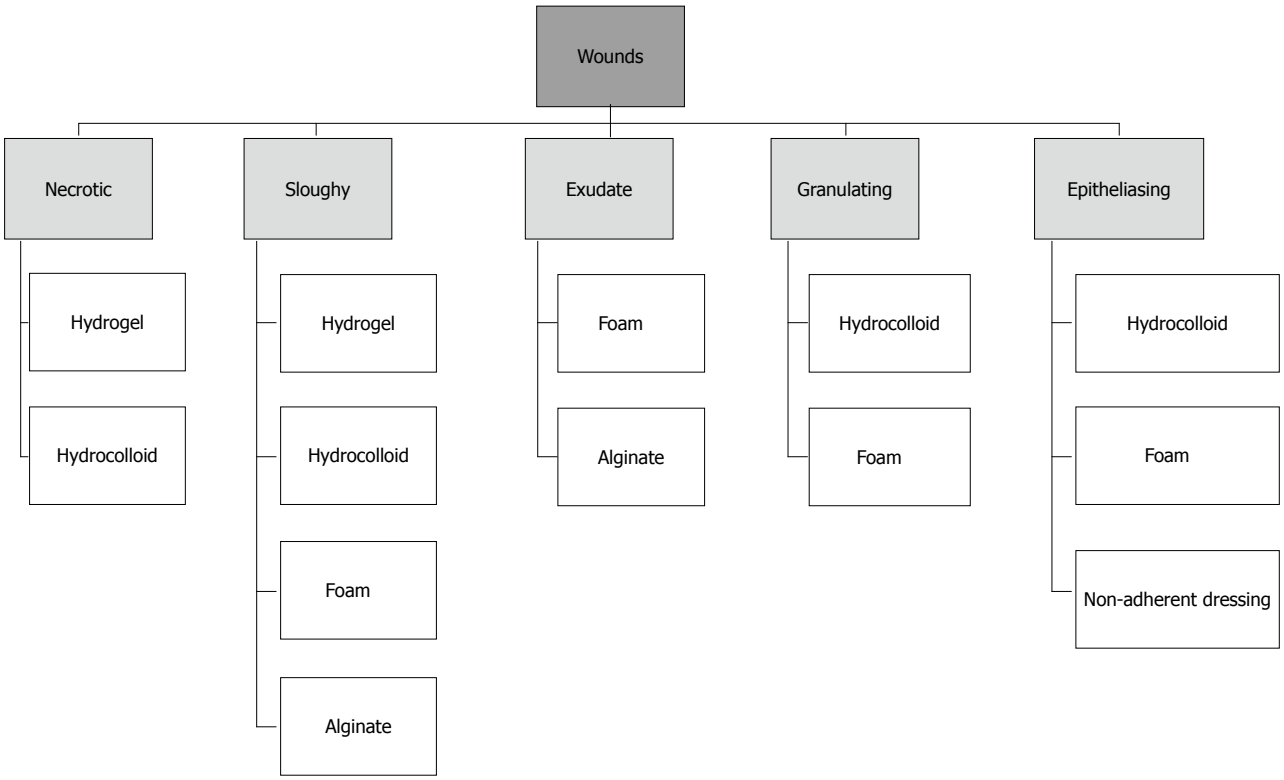

Figure 8 Classification of the different advanced dressing types usually used in diabetic foot ulcer treatment. (Data adapted from Moura *et al*[95]).

are typically less traumatic when removed. Moreover, in certain patients, they are cost effective because of the lowered frequency of dressing changes and not requiring extensive nursing time[106].

Surgery

Diabetic foot surgery plays an essential role in the prevention and management of DFU[110], and has been on the increase over the past 2 decades[111,112]. Although surgical interventions for patients with DFU are not without risk, the selective correction of persistent foot ulcers can improve outcomes[113].

In general, surgery for DFU healing includes non-vascular foot surgery, vascular foot surgery, and in some cases amputation. Nonvascular foot surgery is divided into elective, prophylactic, curative, and emergent surgeries that aim to correct deformities that increase plantar pressure[114] (Table 4). Today, a few studies have reported long-term outcomes for diabetic foot surgery in RCTs [60,115,116]. In one study conducted by Mueller *et al*[115], subjects were randomized into two groups of Achilles Tendon-Lengthening (ATL) group, who received treatment of ATL and TCC, and a group who received TCC only. Their results showed that all ulcers healed in the ATL group and the risk for ulcer recurrence was 75% less at seven months and 52% less at two years than for the TCC group[115].

Vascular foot surgery such as bypass grafts from femoral to pedal arteries and peripheral angioplasty to improve blood flow for an ischemic foot have been recently developed[117]. While studies have shown that these procedures help to heal ischemic ulcers[118-120], no RCT has been shown to reduce DFU.

While the primary goal of DFU management focuses on limb salvage, in some cases amputation may offer a better functional outcome, although this is often not clearly defined[41]. This decision is individualized and multifactorial to match patient lifestyle, medical, physical, and psychological comorbidities[121]. In general, amputation is considered as an urgent or curative surgery and should be the last resort after all other salvage techniques have been explored, and the patient must be in agreement[122]. Indications for an amputation include the removal of infected or gangrenous tissues, control of infection, and creation of a functional foot or stump that can accommodate footwear or prosthesis[123].

ADVANCED THERAPIES

Hyperbaric oxygen therapy

Hyperbaric oxygen therapy (HBOT) has shown promise in the treatment of serious cases of non-healing DFU, which are resistant to other therapeutic methods[124-127]. HBOT involves intermittent administration of 100% oxygen, usually in daily sessions[128]. During each session, patients breathed pure oxygen at 1.4-3.0 absolute atmospheres during 3 periods of 30 min (overall 90 min) intercalated by 5 min intervals in a hyperbaric chamber[124,129] (Figure 9).

Today, RCTs have reported beneficial effects from HBOT in numerous studies[130-134]. A recent double-blind RCT conducted by Löndahl *et al*[134] demonstrated a significantly improved outcome in the intervention

Table 4 Different types of nonvascular diabetic foot surgery

Type	Explanation
Elective	The main goal of this surgery is to relieve the pain associated with particular deformities such as hammertoes, bunions, and bone spurs in patients without peripheral sensory neuropathy and at low risk for ulceration
Prophylactic	These procedures are indicated to prevent ulceration from occurring or recurring in patients with neuropathy, including those with a past history of ulceration (but without active ulceration)
Curative	These procedures are performed to effect healing of a non-healing ulcer or a chronically recurring ulcer when offloading and standard wound care techniques are not effective. These include multiple surgical procedures aimed at removing areas of chronically increased peak pressure as well as procedures for resecting infected bone or joints as an alternative to partial foot amputation
Emergent	These procedures are performed to arrest or limit progression of acute infection

Data adapted from Frykberg *et al*[18].

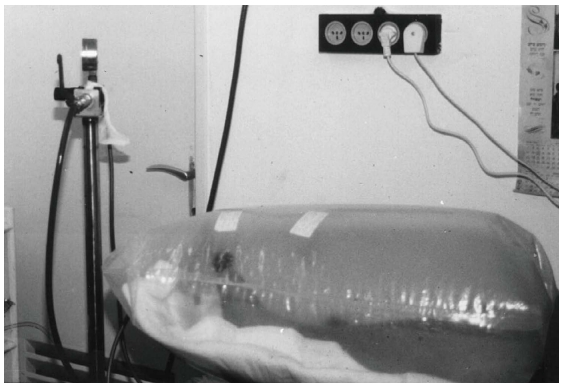

Figure 9 The polyethylene hyperbaric chamber. Oxygen in a concentration of 100% was pumped into the bag through a regular car wheel valve. The open end of the bag was sealed by an elastic bandage to the leg above the knee. Oxygen was allowed to leak around the bandage, and the pressure in the chamber was kept to between 20 and 30 mmHg (1.02-1.03 atm) above atmospheric pressure. (Data adapted from Landau[127]).

group as the treated patients were more likely to heal within 12 mo [25.48 (52%) *vs* 12.42 (29%); $P = 0.03$]. In addition, Kranke *et al*[135], in a systematic review, revealed that treatment with HBOT resulted in a significantly higher proportion of healed DFU when compared with treatment without HBO (relative risk, 5.20; 95%CI: 1.25-21.66; $P = 0.02$). However, in another systematic review conducted by O'Reilly *et al*[136], no significant effects on amputation rates were found in the RCT evidence and in the high quality studies, no difference was found between HBOT group compared to standard wound care group.

The exact mechanism of HBOT remains poorly understood. Some studies have reported that HBOT improved wound tissue hypoxia, enhanced perfusion, reduced edema, down regulated inflammatory cytokines, and promoted fibroblast proliferation, collagen production, and angiogenesis[137-140]. In addition, it was demonstrated that HBOT stimulated vasculogenic stem cell mobilization from bone marrow and recruited them to the skin wound[139].

Despite reports of increased healing rates and decreased amputation rates with using HBOT, adjuvant use of this method in DFU remains a controversial issue. HBOT does not substitute for antibiotic therapy, local humid therapy, or surgical wound debridement. Furthermore, HBOT is available in only a minority of communities as it is expensive [a full course of treatment in the United States typically costs $50000 (Medicare) to $200000 (private pay)] and is time-consuming (an average of 60 total hours in the chamber)[5,6].

Electrical stimulation

Electrical stimulation (ES) has been reported as a perfect adjunctive therapy for DFU healing in recent literature. Currently, there is a substantial body of work that supports the effectiveness of ES for DFU healing[141-144]. In a randomized, double-blind, placebo-controlled trial study conducted by Peters *et al*[141] on 40 patients with DFU, significant differences in number of healed ulcers (65% in treatment group *vs* 35% in control group) were found at 12 wk.

Based on the literature review, it is suggested that ES could improve common deficiencies that have been associated with faulty wound healing in DFU, such as poor blood flow, infection, and deficient cellular responses[141,145]. This therapy is a safe, inexpensive, and a simple intervention to improve wound healings in patients with DFU[145,146].

Negative pressure wound therapy

Negative pressure wound therapy (NPWT) is a non-invasive wound closure system that uses controlled, localized negative pressure to help heal chronic and acute wounds. This system uses latex-free and sterile polyurethane or polyvinyl alcohol foam dressing that is fitted at the bedside to the appropriate size for every wound, and then covered with an adhesive drape to create an airtight seal. Most commonly, 80-125 mmHg of negative pressure is used, either continuously or in cycles. The fluid suctioned from the wound is collected into a container in the control unit[147,148] (Figure 10).

It seems that NPWT removes edema and chronic exudate, reduces bacterial colonization, enhances formation of new blood vessels, increases cellular proliferation, and improves wound oxygenation as the result of applied mechanical force[149-151].

This method has been advocated by numerous RCTs as a safe and effective adjunctive modality in the treatment of DFU. Studies have shown that wound

Table 5 Brief description of commonly used bioengineered tissue products

Type	Explanation	Use	RCT studies
Apligraf (Advanced Biohealing Inc., La Jolla, CA)	A bilayered living-skin construct containing an outer layer of live allogeneic human keratinocytes and a second layer of live allogeneic fibroblasts on type 1 collagen dispersed in a dermal layer matrix. Both cell layers are grown from infant fore skin and looks and feels like human skin[164,165]	It's used for full-thickness neuropathic DFU of greater than 3 wk duration, resistant to standard therapy (also without tendon, muscle, capsule, or bone exposure) and is contraindicated in infected ulcers[167]	Veves *et al*[168] Falanga *et al*[169] Edmonds[170] Steinberg *et al*[171]
Dermagraft (Organogenesis Inc, Canton, Mass)	An allogeneic living-dermis equivalent and includes neonatal fibroblasts from human fore skin cultured on a polyglactin scaffold[164,165]	It's used for DFU of greater than 6 wk duration, full thickness in depth but without tendon, muscle, joint, or bone exposure and is contraindicated in infected ulcers[164,167]	Marston *et al*[172] Gentzkow *et al*[173]
Oasis (Cook Biotech, West Lafayette, IN)	An acellular biomaterial derived from porcine small intestine submucosa, contains numerous crucial dermal components including collagen, glycosaminoglycans (hyaluronic acid), proteoglycans, fibronectin, and bioactive growth factors such as fibroblast growth factor-2, transforming growth factor β1, and VEGF[164,165]	It's used for full-thickness DFU[174]	Niezgoda *et al*[174]

DFU: Diabetic foot ulcer; VEGF: Vascular endothelial growth factor.

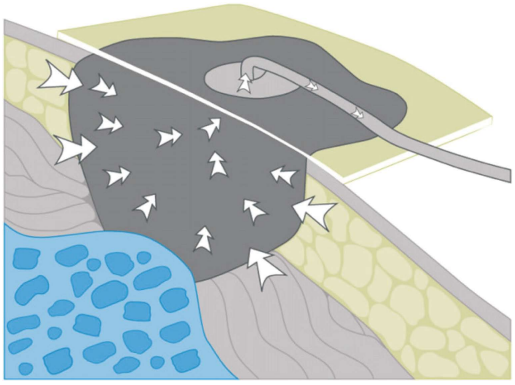

Figure 10 Schematic drawing of the negative pressure wound treatment. (Data adapted from Vikatmaa *et al*[147]).

healing with this approach results in a higher proportion of healed wounds, faster time for wound closure, a more rapid and robust granulation tissue response, and a potential trend towards reduced risk for a second amputation than for the control treatment[148,152-156]. In addition, meta-analysis studies have indicated that NPWT significantly reduces healing times and increases the number of healed wounds[147,157,158].

While the evidence for NPWT in DFU patients is promising, this method does not replace surgical wound debridement to improve blood circulation in all DFU patients. Investigations have shown that when NPWT is initiated, there must be no significant infection or gangrene in the wound[147,158]. Also, RCTs have shown significantly higher mean material expenses for wounds treated with NPWT when compared to conventional therapy (moist gauze) in the management of full-thickness wounds requiring surgical closure[159,160].

Bioengineered skin

Bio-engineered skin (BES) has been used during the last decades as a new therapeutic method to treat DFU[161-164]. This method replaces the degraded and destructive milieu of extra cellular matrix (ECM) with the introduction of a new ground substance matrix with cellular components to start a new healing trajectory[165]. Currently, three kinds of BES products approved in the United States are available to use for DFU including Derma graft (Advanced Bio healing Inc., La Jolla, CA), Apligraf (Organogenesis Inc., Canton, Mass), and, more recently, Oasis (Cook Biotech, West Lafayette, IN)[164,166]; and numerous RCT studies shown their efficacy in DFUs healing (Table 5).

BES product cells are seeded into the scaffolds and cultured *in vitro*. *In vitro* incubation establishes the cells and allows the cell-secreted ECM and growth factors to accumulate in the scaffold. The cells within live cell scaffolds are believed to accelerate DFU healing by actively secreting growth factors during the repair process[164,165]. In addition, it seems that BES can provide the cellular substrate and molecular components necessary to accelerate wound healing and angiogenesis. They act as biologic dressings and as delivery systems for growth factors and ECM components through the activity of live human fibroblasts contained in the dermal elements[162,163,170].

Despite the advantages of BES, they cannot be used in isolation to treat DFU. Peripheral ischemia, which is one of the pathological characteristics of DFU, is a critical contributing factor that affects BES transplantation. Hence, surgical revascularization and decompression as well as wound bed preparation are considered as essential prerequisites for BES applications. In addition, this method needs control of the infection[77,175]. Therefore, the above-mentioned points may result in high long-term costs and cause major concern for use of this treatment[176].

Growth factors

DFU has demonstrated the benefits from growth factors (GFs) such as platelet derived growth factor (PDGF), fibroblast growth factor, vascular endothelial growth factor, insulin-like growth factors (IGF1, IGF2), epidermal growth factor, and transforming growth factor b[177]. Among the aforementioned GFs, only recombinant human PDGF

(rhPDGF) (Becaplermin or Regranex), which is a hydrogel that contains 0.01% of PDGF-BB (rhPDGF-BB), has demonstrated increased healing rates when compared with controls in a number of clinical trials[178-181] and has shown sufficient DFU repair efficacy to earn Food and Drug Administration (FDA) approval[182]. In one randomized placebo controlled trial involving patients with full thickness DFU, Becaplermin demonstrated a 43% increase in complete closure *vs* placebo gel (50% *vs* 35%)[183]. In another randomized placebo-controlled trial, Sibbald *et al*[184] demonstrated that patients with infection-free chronic foot ulcers treated with the best clinical care and once-daily applications of 100 μg/g Becaplermin gel had a significantly greater chance of 100% ulcer closure by 20 wk than those receiving the best clinical care plus placebo (vehicle gel) alone.

GFs have been shown to stimulate chemotaxis and mitogenesis of neutrophils, fibroblasts, monocytes, and other components that form the cellular basis of wound healing[178,185]. Despite FDA approval and other reviewed studies, the clinical use of Becaplermin remains limited because of its high cost[186] and uncertain patient-specific clinical benefits[187,188]. Some studies have indicated that endogenous PDGF stimulates tumor infiltrating fibroblasts found in human melanoma cells and is overexpressed at all stages of human astrocytoma growth[164]. So, it would be biologically possible that topical administration of recombinant PDGF could promote cancer.

CONCLUSION

Foot ulcers in patients with diabetes is common, and frequently leads to lower limb amputation unless a prompt, rational, multidisciplinary approach to therapy is taken. The main components of management that can ensure successful and rapid healing of DFU include education, blood sugar control, wound debridement, advanced dressing, offloading, surgery, and advanced therapies, which are used clinically. These approaches should be used whenever feasible to reduce high morbidity and risk of serious complications resulting from foot ulcers.

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REFERENCES

1 **Shahbazian H**, Yazdanpanah L, Latifi SM. Risk assessment of patients with diabetes for foot ulcers according to risk classification consensus of International Working Group on Diabetic Foot (IWGDF). *Pak J Med Sci* 2013; **29**: 730-734 [PMID: 24353617 DOI: 10.12669/pjms.293.3473]

2 **Ramachandran A**, Snehalatha C, Shetty AS, Nanditha A. Trends in prevalence of diabetes in Asian countries. *World J Diabetes* 2012; **3**: 110-117 [PMID: 22737281 DOI: 10.4239/wjd.v3.i6.110]

3 **Shaw JE**, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Res Clin Pract* 2010; **87**: 4-14 [PMID: 19896746 DOI: 10.1016/j.diabres.2009.10.007]

4 **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]

5 **Aalaa M**, Malazy OT, Sanjari M, Peimani M, Mohajeri-Tehrani M. Nurses' role in diabetic foot prevention and care; a review. *J Diabetes Metab Disord* 2012; **11**: 24 [PMID: 23497582 DOI: 10.1186/2251-6581-11-24]

6 **Alavi A**, Sibbald RG, Mayer D, Goodman L, Botros M, Armstrong DG, Woo K, Boeni T, Ayello EA, Kirsner RS. Diabetic foot ulcers: Part II. Management. *J Am Acad Dermatol* 2014; **70**: 21.e1-2124; quiz 21.e1-2124 [PMID: 24355276 DOI: 10.1016/j.jaad.2013.07.048]

7 **Cavanagh PR**, Lipsky BA, Bradbury AW, Botek G. Treatment for diabetic foot ulcers. *Lancet* 2005; **366**: 1725-1735 [PMID: 16291067 DOI: 10.1016/S0140-6736(05)67699-4]

8 **Leone S**, Pascale R, Vitale M, Esposito S. [Epidemiology of diabetic foot]. *Infez Med* 2012; **20** Suppl 1: 8-13 [PMID: 22982692]

9 **Richard JL**, Schuldiner S. [Epidemiology of diabetic foot problems]. *Rev Med Interne* 2008; **29** Suppl 2: S222-S230 [PMID: 18822247 DOI: 10.1016/S0248-8663(08)73949-3]

10 **Nather A**, Bee CS, Huak CY, Chew JL, Lin CB, Neo S, Sim EY. Epidemiology of diabetic foot problems and predictive factors for limb loss. *J Diabetes Complications* 2008; **22**: 77-82 [PMID: 18280436 DOI: 10.1016/j.jdiacomp.2007.04.004]

11 **Bakri FG**, Allan AH, Khader YS, Younes NA, Ajlouni KM. Prevalence of Diabetic Foot Ulcer and its Associated Risk Factors among Diabetic Patients in Jordan. *J Med J* 2012; **46**: 118-125

12 **Iraj B**, Khorvash F, Ebneshahidi A, Askari G. Prevention of diabetic foot ulcer. *Int J Prev Med* 2013; **4**: 373-376 [PMID: 23626896]

13 **Fard AS**, Esmaelzadeh M, Larijani B. Assessment and treatment of diabetic foot ulcer. *Int J Clin Pract* 2007; **61**: 1931-1938 [PMID: 17935551 DOI: 10.1111/j.1742-1241.2007.01534.x]

14 **Snyder RJ**, Hanft JR. Diabetic foot ulcers--effects on QOL, costs, and mortality and the role of standard wound care and advanced-care therapies. *Ostomy Wound Manage* 2009; **55**: 28-38 [PMID: 19934461]

15 **Vileikyte L**. Diabetic foot ulcers: a quality of life issue. *Diabetes Metab Res Rev* 2001; **17**: 246-249 [PMID: 11544609 DOI: 10.1002/dmrr.216]

16 **Ragnarson Tennvall G**, Apelqvist J. Health-economic consequences of diabetic foot lesions. *Clin Infect Dis* 2004; **39** Suppl 2: S132-S139 [PMID: 15306992 DOI: 10.1086/383275]

17 **Boulton AJ**, Vileikyte L, Ragnarson-Tennvall G, Apelqvist J. The global burden of diabetic foot disease. *Lancet* 2005; **366**: 1719-1724 [PMID: 16291066 DOI: 10.1016/j.mpmed.2010.08.011]

18 **Frykberg RG**, Zgonis T, Armstrong DG, Driver VR, Giurini JM, Kravitz SR, Landsman AS, Lavery LA, Moore JC, Schuberth JM, Wukich DK, Andersen C, Vanore JV. Diabetic foot disorders. A clinical practice guideline (2006 revision). *J Foot Ankle Surg* 2006; **45**: S1-66 [PMID: 17280936 DOI: 10.1016/S1067-2516(07)60001-5]

19 **Bortoletto MS**, de Andrade SM, Matsuo T, Haddad Mdo C, González AD, Silva AM. Risk factors for foot ulcers--a cross sectional survey from a primary care setting in Brazil. *Prim Care Diabetes* 2014; **8**: 71-76 [PMID: 23639609 DOI: 10.1016/j.pcd.2013.04.003]

20 **Waaijman R**, de Haart M, Arts ML, Wever D, Verlouw AJ, Nollet F, Bus SA. Risk factors for plantar foot ulcer recurrence in neuropathic diabetic patients. *Diabetes Care* 2014; **37**: 1697-1705 [PMID: 24705610 DOI: 10.2337/dc13-2470]

21 **Monteiro-Soares M**, Boyko EJ, Ribeiro J, Ribeiro I, Dinis-Ribeiro M. Predictive factors for diabetic foot ulceration: a

systematic review. *Diabetes Metab Res Rev* 2012; **28**: 574-600 [PMID: 22730196 DOI: 10.1002/dmrr.2319]
22 **McEwen LN**, Ylitalo KR, Herman WH, Wrobel JS. Prevalence and risk factors for diabetes-related foot complications in Translating Research Into Action for Diabetes (TRIAD). *J Diabetes Complications* 2013; **27**: 588-592 [PMID: 24035357 DOI: 10.1016/j.jdiacomp.2013.08.003]
23 **Prompers L**, Huijberts M, Apelqvist J, Jude E, Piaggesi A, Bakker K, Edmonds M, Holstein P, Jirkovska A, Mauricio D, Ragnarson Tennvall G, Reike H, Spraul M, Uccioli L, Urbancic V, Van Acker K, van Baal J, van Merode F, Schaper N. High prevalence of ischaemia, infection and serious comorbidity in patients with diabetic foot disease in Europe. Baseline results from the Eurodiale study. *Diabetologia* 2007; **50**: 18-25 [PMID: 17093942 DOI: 10.1007/s00125-006-0491-1]
24 **Formosa C**, Gatt A, Chockalingam N. Diabetic foot complications in Malta: prevalence of risk factors. *Foot* (Edinb) 2012; **22**: 294-297 [PMID: 22981100 DOI: 10.1016/j.foot.2012.08.008]
25 **Malgrange D.** [Physiopathology of the diabetic foot]. *Rev Med Interne* 2008; **29** Suppl 2: S231-S237 [PMID: 18822248 DOI: 10.1016/S0248-8663(08)73950-X]
26 **Sawacha Z**, Gabriella G, Cristoferi G, Guiotto A, Avogaro A, Cobelli C. Diabetic gait and posture abnormalities: a biomechanical investigation through three dimensional gait analysis. *Clin Biomech* (Bristol, Avon) 2009; **24**: 722-728 [PMID: 19699564 DOI: 10.1016/j.clinbiomech.2009.07.007]
27 **Ledoux WR**, Shofer JB, Cowley MS, Ahroni JH, Cohen V, Boyko EJ. Diabetic foot ulcer incidence in relation to plantar pressure magnitude and measurement location. *J Diabetes Complications* 2013; **27**: 621-626 [PMID: 24012295 DOI: 10.1016/j.jdiacomp.2013.07.004]
28 **Amemiya A**, Noguchi H, Oe M, Ohashi Y, Ueki K, Kadowaki T, Mori T, Sanada H. Elevated plantar pressure in diabetic patients and its relationship with their gait features. *Gait Posture* 2014; **40**: 408-414 [PMID: 24974127 DOI: 10.1016/j.gaitpost.2014.05.063]
29 **Fernando ME**, Crowther RG, Pappas E, Lazzarini PA, Cunningham M, Sangla KS, Buttner P, Golledge J. Plantar pressure in diabetic peripheral neuropathy patients with active foot ulceration, previous ulceration and no history of ulceration: a meta-analysis of observational studies. *PLoS One* 2014; **9**: e99050 [PMID: 24915443 DOI: 10.1371/journal.pone.0099050]
30 **Bacarin TA**, Sacco IC, Hennig EM. Plantar pressure distribution patterns during gait in diabetic neuropathy patients with a history of foot ulcers. *Clinics* (Sao Paulo) 2009; **64**: 113-120 [PMID: 19219316 DOI: 10.1590/S1807-59322009000200008]
31 **Schaper NC**, Apelqvist J, Bakker K. The international consensus and practical guidelines on the management and prevention of the diabetic foot. *Curr Diab Rep* 2003; **3**: 475-479 [PMID: 14611743 DOI: 10.1007/s11892-003-0010-4]
32 **DiPreta JA**. Outpatient assessment and management of the diabetic foot. *Med Clin North Am* 2014; **98**: 353-373 [PMID: 24559880 DOI: 10.1016/j.mcna.2013.10.010]
33 **Markowitz JS**, Gutterman EM, Magee G, Margolis DJ. Risk of amputation in patients with diabetic foot ulcers: a claims-based study. *Wound Repair Regen* 2006; **14**: 11-17 [PMID: 16476067 DOI: 10.1111/j.1524-475X.2005.00083.x]
34 **Patout CA**, Birke JA, Horswell R, Williams D, Cerise FP. Effectiveness of a comprehensive diabetes lower-extremity amputation prevention program in a predominantly low-income African-American population. *Diabetes Care* 2000; **23**: 1339-1342 [PMID: 10977029 DOI: 10.2337/diacare.23.9.1339]
35 **Driver VR**, Madsen J, Goodman RA. Reducing amputation rates in patients with diabetes at a military medical center: the limb preservation service model. *Diabetes Care* 2005; **28**: 248-253 [PMID: 15677774 DOI: 10.2337/diacare.28.2.248]
36 **Frykberg RG**. Diabetic foot ulcers: pathogenesis and management. *Am Fam Physician* 2002; **66**: 1655-1662 [PMID: 12449264]
37 **Sumpio BE**, Aruny J, Blume PA. The multidisciplinary approach to limb salvage. *Acta Chir Belg* 2004; **104**: 647-653 [PMID: 15663269]
38 **Wraight PR**, Lawrence SM, Campbell DA, Colman PG. Creation of a multidisciplinary, evidence based, clinical guideline for the assessment, investigation and management of acute diabetes related foot complications. *Diabet Med* 2005; **22**: 127-136 [PMID: 15660728 DOI: 10.1111/j.1464-5491.2004.01363.x]
39 **Malekian Ragheb S**, Naderi Beni M. Management of a diabetic foot ulcer by specialist nurses in Iran. *Wounds International* 2013; **4**: 20-23
40 **Aydin K**, Isildak M, Karakaya J, Gürlek A. Change in amputation predictors in diabetic foot disease: effect of multidisciplinary approach. *Endocrine* 2010; **38**: 87-92 [PMID: 20960107 DOI: 10.1007/s12020-010-9355-z]
41 **Lepäntalo M**, Apelqvist J, Setacci C, Ricco JB, de Donato G, Becker F, Robert-Ebadi H, Cao P, Eckstein HH, De Rango P, Diehm N, Schmidli J, Teraa M, Moll FL, Dick F, Davies AH. Chapter V: Diabetic foot. *Eur J Vasc Endovasc Surg* 2011; **42** Suppl 2: S60-S74 [PMID: 22172474 DOI: 10.1016/S1078-5884(11)60012-9]
42 **Seaman S**. The role of the nurse specialist in the care of patients with diabetic foot ulcers. *Foot Ankle Int* 2005; **26**: 19-26 [PMID: 15680114]
43 **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* 2005; **28** Suppl 1: S72-S79 [PMID: 15618119 DOI: 10.2337/diacare.28.suppl_1.S72]
44 **Malone JM**, Snyder M, Anderson G, Bernhard VM, Holloway GA, Bunt TJ. Prevention of amputation by diabetic education. *Am J Surg* 1989; **158**: 520-523; discussion 523-524 [PMID: 2589581]
45 **Annersten Gershater M**, E Pilhammar E, Apelqvist J, Alm-Roijer C. Patient education for the prevention of diabetic foot ulcers: Interim analysis of a randomised controlled trial due to morbidity and mortality of participants. *EDN* 2011; **8**: 102-107 [DOI: 10.1002/edn.189]
46 **Dorresteijn JA**, Kriegsman DM, Assendelft WJ, Valk GD. Patient education for preventing diabetic foot ulceration. *Cochrane Database Syst Rev* 2012; **10**: CD001488 [PMID: 23076893 DOI: 10.1002/14651858.CD001488]
47 **American Diabetes Association**. Standards of medical care in diabetes--2006. *Diabetes Care* 2006; **29** Suppl 1: S4-42 [PMID: 16373931]
48 **Faglia E**, Favales F, Morabito A. New ulceration, new major amputation, and survival rates in diabetic subjects hospitalized for foot ulceration from 1990 to 1993: a 6.5-year follow-up. *Diabetes Care* 2001; **24**: 78-83 [PMID: 11194246 DOI: 10.2337/diacare.24.1.78]
49 **Bowering CK**. Diabetic foot ulcers. Pathophysiology, assessment, and therapy. *Can Fam Physician* 2001; **47**: 1007-1016 [PMID: 11398715]
50 **McMurry JF**. Wound healing with diabetes mellitus. Better glucose control for better wound healing in diabetes. *Surg Clin North Am* 1984; **64**: 769-778 [PMID: 6433493]
51 **Pomposelli JJ**, Baxter JK, Babineau TJ, Pomfret EA, Driscoll DF, Forse RA, Bistrian BR. Early postoperative glucose control predicts nosocomial infection rate in diabetic patients. *JPEN J Parenter Enteral Nutr* 1998; **22**: 77-81 [PMID: 9527963 DOI: 10.1177/014860719802200277]
52 Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). UK Prospective Diabetes Study (UKPDS) Group. *Lancet* 1998; **352**: 854-865 [PMID: 9742977 DOI: 10.1016/S0140-6736(98)07037-8]
53 **Tallis A**, Motley TA, Wunderlich RP, Dickerson JE,

Waycaster C, Slade HB. Clinical and economic assessment of diabetic foot ulcer debridement with collagenase: results of a randomized controlled study. *Clin Ther* 2013; **35**: 1805-1820 [PMID: 24145042 DOI: 10.1016/j.clinthera.2013.09.013]
54 **Lebrun E**, Tomic-Canic M, Kirsner RS. The role of surgical debridement in healing of diabetic foot ulcers. *Wound Repair Regen* 2010; **18**: 433-438 [PMID: 20840517 DOI: 10.1111/j.1524-475X.2010.00619.x]
55 **Edwards J**, Stapley S. Debridement of diabetic foot ulcers. *Cochrane Database Syst Rev* 2010; **(1)**: CD003556 [PMID: 20091547 DOI: 10.1002/14651858.CD003556]
56 **Brem H**, Sheehan P, Boulton AJ. Protocol for treatment of diabetic foot ulcers. *Am J Surg* 2004; **187**: 1S-10S [PMID: 15147985 DOI: 10.1016/S0002-9610(03)00299-X]
57 **Enoch S**, Harding K. Wound bed preparation: the science behind the removal of barrier to healing. *Wounds* 2003; **15**: 213-229
58 **Jain AC**. A New Classification (Grading System) of Debridement in Diabetic Lower Limbs-an Improvization and Standardization in Practice of Diabetic Lower Limb Salvage around the World. *Medicine Science* 2014; **3**: 991-1001 [DOI: 10.5455/medscience.2013.02.8093]
59 **Steed DL**, Donohoe D, Webster MW, Lindsley L. Effect of extensive debridement and treatment on the healing of diabetic foot ulcers. Diabetic Ulcer Study Group. *J Am Coll Surg* 1996; **183**: 61-64 [PMID: 8673309]
60 **Piaggesi A**, Schipani E, Campi F, Romanelli M, Baccetti F, Arvia C, Navalesi R. Conservative surgical approach versus non-surgical management for diabetic neuropathic foot ulcers: a randomized trial. *Diabet Med* 1998; **15**: 412-417 [PMID: 9609364]
61 **Saap LJ**, Falanga V. Debridement performance index and its correlation with complete closure of diabetic foot ulcers. *Wound Repair Regen* 2002; **10**: 354-359 [PMID: 12453138 DOI: 10.1046/j.1524-475X.2002.10603.x]
62 **Cardinal M**, Eisenbud DE, Armstrong DG, Zelen C, Driver V, Attinger C, Phillips T, Harding K. Serial surgical debridement: a retrospective study on clinical outcomes in chronic lower extremity wounds. *Wound Repair Regen* 2009; **17**: 306-311 [PMID: 19660037 DOI: 10.1111/j.1524-475X.2009.00485.x]
63 **Attinger CE**, Bulan E, Blume PA. Surgical débridement. The key to successful wound healing and reconstruction. *Clin Podiatr Med Surg* 2000; **17**: 599-630 [PMID: 11070797]
64 **Falanga V**. Wound healing and its impairment in the diabetic foot. *Lancet* 2005; **366**: 1736-1743 [PMID: 16291068 DOI: 10.1016/S0140-6736(05)67700-8]
65 **Warriner RA**, Wilcox JR, Carter MJ, Stewart DG. More frequent visits to wound care clinics result in faster times to close diabetic foot and venous leg ulcers. *Adv Skin Wound Care* 2012; **25**: 494-501 [PMID: 23080236 DOI: 10.1097/01.ASW.0000422629.03053.06]
66 **Wilcox JR**, Carter MJ, Covington S. Frequency of debridements and time to heal: a retrospective cohort study of 312744 wounds. *JAMA Dermatol* 2013; **149**: 1050-1058 [PMID: 23884238 DOI: 10.1001/jamadermatol.2013.4960]
67 **Sherman RA**. Maggot therapy takes us back to the future of wound care: new and improved maggot therapy for the 21st century. *J Diabetes Sci Technol* 2009; **3**: 336-344 [PMID: 20144365]
68 **Armstrong DG**, Mossel J, Short B, Nixon BP, Knowles EA, Boulton AJ. Maggot debridement therapy: a primer. *J Am Podiatr Med Assoc* 2002; **92**: 398-401 [PMID: 12122127]
69 **Mumcuoglu KY**. Clinical applications for maggots in wound care. *Am J Clin Dermatol* 2001; **2**: 219-227 [PMID: 11705249]
70 **Sherman RA**. Maggot therapy for foot and leg wounds. *Int J Low Extrem Wounds* 2002; **1**: 135-142 [PMID: 15871964 DOI: 10.1177/1534734602001002009]
71 **Sherman RA**. Maggot therapy for treating diabetic foot ulcers unresponsive to conventional therapy. *Diabetes Care* 2003; **26**: 446-451 [PMID: 12547878 DOI: 10.2337/diacare.26.2.446]
72 **van Veen LJ**. Maggot debridement therapy: a case study. *J Wound Ostomy Continence Nurs* 2008; **35**: 432-436 [PMID: 18635997 DOI: 10.1097/01.WON.0000326667.62884.51]
73 **Armstrong DG**, Salas P, Short B, Martin BR, Kimbriel HR, Nixon BP, Boulton AJ. Maggot therapy in "lower-extremity hospice" wound care: fewer amputations and more antibiotic-free days. *J Am Podiatr Med Assoc* 2005; **95**: 254-257 [PMID: 15901812]
74 **Paul AG**, Ahmad NW, Lee HL, Ariff AM, Saranum M, Naicker AS, Osman Z. Maggot debridement therapy with Lucilia cuprina: a comparison with conventional debridement in diabetic foot ulcers. *Int Wound J* 2009; **6**: 39-46 [PMID: 19291114 DOI: 10.1111/j.1742-481X.2008.00564.x]
75 **Scott RG**, Loehne HB. 5 questions--and answers--about pulsed lavage. *Adv Skin Wound Care* 2000; **13**: 133-134 [PMID: 11075009]
76 **Schultz GS**, Sibbald RG, Falanga V, Ayello EA, Dowsett C, Harding K, Romanelli M, Stacey MC, Teot L, Vanscheidt W. Wound bed preparation: a systematic approach to wound management. *Wound Repair Regen* 2003; **11** Suppl 1: S1-28 [PMID: 12654015 DOI: 10.1046/j.1524-475X.11.s2.1.x]
77 **Ramundo J**, Gray M. Enzymatic wound debridement. *J Wound Ostomy Continence Nurs* 2008; **35**: 273-280 [PMID: 18496083 DOI: 10.1097/01.WON.0000319125.21854.78]
78 **Langer V**, Bhandari PS, Rajagopalan S, Mukherjee MK. Enzymatic debridement of large burn wounds with papain-urea: Is it safe? *Med J Armed Forces India* 2013; **69**: 144-150 [PMID: 24600088 DOI: 10.1016/j.mjafi.2012.09.001]
79 **Jarczyk G**, Jackowski M, Szpila K, Boszek G, Kapelaty S. Use of Lucilia sericata blowfly maggots in the treatment of diabetic feet threatened with amputation. *Acta Angiologica* 2008; **14**: 42-55
80 **Bowling FL**, Salgami EV, Boulton AJ. Larval therapy: a novel treatment in eliminating methicillin-resistant Staphylococcus aureus from diabetic foot ulcers. *Diabetes Care* 2007; **30**: 370-371 [PMID: 17259512 DOI: 10.2337/dc06-2348]
81 **Armstrong DG**, Nguyen HC, Lavery LA, van Schie CH, Boulton AJ, Harkless LB. Off-loading the diabetic foot wound: a randomized clinical trial. *Diabetes Care* 2001; **24**: 1019-1022 [PMID: 11375363 DOI: 10.2337/diacare.24.6.1019]
82 **Armstrong DG**, Lavery LA, Nixon BP, Boulton AJ. It's not what you put on, but what you take off: techniques for debriding and off-loading the diabetic foot wound. *Clin Infect Dis* 2004; **39** Suppl 2: S92-S99 [PMID: 15306986 DOI: 10.1086/383269]
83 **Cavanagh PR**, Bus SA. Off-loading the diabetic foot for ulcer prevention and healing. *J Vasc Surg* 2010; **52**: 37S-43S [PMID: 20804932 DOI: 10.1016/j.jvs.2010.06.007]
84 **Boulton AJ**. Pressure and the diabetic foot: clinical science and offloading techniques. *Am J Surg* 2004; **187**: 17S-24S [PMID: 15147987 DOI: 10.1016/S0002-9610(03)00297-6]
85 **Rathur HM**, Boulton AJ. Pathogenesis of foot ulcers and the need for offloading. *Horm Metab Res* 2005; **37** Suppl 1: 61-68 [PMID: 15918113 DOI: 10.1055/s-2005-861398]
86 **Rathur HM**, Boulton AJ. The diabetic foot. *Clin Dermatol* 2007; **25**: 109-120 [PMID: 17276208 DOI: 10.1016/j.clindermatol.2006.09.015]
87 **Mueller MJ**, Diamond JE, Sinacore DR, Delitto A, Blair VP, Drury DA, Rose SJ. Total contact casting in treatment of diabetic plantar ulcers. Controlled clinical trial. *Diabetes Care* 1989; **12**: 384-388 [PMID: 2659299]
88 **Piaggesi A**, Viacava P, Rizzo L, Naccarato G, Baccetti F, Romanelli M, Zampa V, Del Prato S. Semiquantitative analysis of the histopathological features of the neuropathic foot ulcer: effects of pressure relief. *Diabetes Care* 2003; **26**: 3123-3128 [PMID: 14578249 DOI: 10.2337/diacare.26.11.3123]
89 **Prompers L**, Huijberts M, Apelqvist J, Jude E, Piaggesi A,

Bakker K, Edmonds M, Holstein P, Jirkovska A, Mauricio D, Tennvall GR, Reike H, Spraul M, Uccioli L, Urbancic V, Van Acker K, Van Baal J, Van Merode F, Schaper N. Delivery of care to diabetic patients with foot ulcers in daily practice: results of the Eurodiale Study, a prospective cohort study. *Diabet Med* 2008; **25**: 700-707 [PMID: 18544108 DOI: 10.1111/j.1464-5491.2008.02445.x.]

90 **Wu SC**, Jensen JL, Weber AK, Robinson DE, Armstrong DG. Use of pressure offloading devices in diabetic foot ulcers: do we practice what we preach? *Diabetes Care* 2008; **31**: 2118-2119 [PMID: 18694976 DOI: 10.2337/dc08-0771]

91 **Fife CE**, Carter MJ, Walker D, Thomson B, Eckert KA. Diabetic foot ulcer off-loading: The gap between evidence and practice. Data from the US Wound Registry. *Adv Skin Wound Care* 2014; **27**: 310-316 [PMID: 24932951 DOI: 10.1097/01.ASW.0000450831.65667.89]

92 **Katz IA**, Harlan A, Miranda-Palma B, Prieto-Sanchez L, Armstrong DG, Bowker JH, Mizel MS, Boulton AJ. A randomized trial of two irremovable off-loading devices in the management of plantar neuropathic diabetic foot ulcers. *Diabetes Care* 2005; **28**: 555-559 [PMID: 15735187 DOI: 10.2337/diacare.28.3.555]

93 **Armstrong DG**, Lavery LA, Wu S, Boulton AJ. Evaluation of removable and irremovable cast walkers in the healing of diabetic foot wounds: a randomized controlled trial. *Diabetes Care* 2005; **28**: 551-554 [PMID: 15735186 DOI: 10.2337/diacare.28.3.551]

94 **Singh N**, Armstrong DG, Lipsky BA. Preventing foot ulcers in patients with diabetes. *JAMA* 2005; **293**: 217-228 [PMID: 15644549 DOI: 10.1001/jama.293.2.217]

95 **Moura LI**, Dias AM, Carvalho E, de Sousa HC. Recent advances on the development of wound dressings for diabetic foot ulcer treatment--a review. *Acta Biomater* 2013; **9**: 7093-7114 [PMID: 23542233 DOI: 10.1016/j.actbio.2013.03.033]

96 **Hilton JR**, Williams DT, Beuker B, Miller DR, Harding KG. Wound dressings in diabetic foot disease. *Clin Infect Dis* 2004; **39** Suppl 2: S100-S103 [PMID: 15306987 DOI: 10.1086/383270]

97 **Hansson C**. Interactive wound dressings. A practical guide to their use in older patients. *Drugs Aging* 1997; **11**: 271-284 [PMID: 9342557]

98 **Dinh T**, Pham H, Veves A. Emerging treatments in diabetic wound care. *Wounds* 2002; **14**: 2-10

99 **Mason J**, O'Keeffe C, Hutchinson A, McIntosh A, Young R, Booth A. A systematic review of foot ulcer in patients with Type 2 diabetes mellitus. II: treatment. *Diabet Med* 1999; **16**: 889-909 [PMID: 10588519 DOI: 10.1046/j.1464-5491.1999.00137.x]

100 **Veves A**, Sheehan P, Pham HT. A randomized, controlled trial of Promogran (a collagen/oxidized regenerated cellulose dressing) vs standard treatment in the management of diabetic foot ulcers. *Arch Surg* 2002; **137**: 822-827 [PMID: 12093340 DOI: 10.1001/archsurg.137.7.822]

101 **Jude EB**, Apelqvist J, Spraul M, Martini J. Prospective randomized controlled study of Hydrofiber dressing containing ionic silver or calcium alginate dressings in non-ischaemic diabetic foot ulcers. *Diabet Med* 2007; **24**: 280-288 [PMID: 17305788 DOI: 10.1111/j.1464-5491.2007.02079.x]

102 **Dumville JC**, Deshpande S, O'Meara S, Speak K. Hydrocolloid dressings for healing diabetic foot ulcers. *Cochrane Database Syst Rev* 2013; **8**: CD009099 [PMID: 23922167 DOI: 10.1002/14651858]

103 **Dumville JC**, Deshpande S, O'Meara S, Speak K. Foam dressings for healing diabetic foot ulcers. *Cochrane Database Syst Rev* 2011; **(9)**: CD009111 [PMID: 21901731 DOI: 10.1002/14651858.CD009111]

104 **Dumville JC**, O'Meara S, Deshpande S, Speak K. Alginate dressings for healing diabetic foot ulcers. *Cochrane Database Syst Rev* 2012; **2**: CD009110 [PMID: 22336860]

105 **Bergin SM**, Wraight P. Silver based wound dressings and topical agents for treating diabetic foot ulcers. *Cochrane Database Syst Rev* 2006; **(1)**: CD005082 [PMID: 16437516]

106 **Lo SF**, Chang CJ, Hu WY, Hayter M, Chang YT. The effectiveness of silver-releasing dressings in the management of non-healing chronic wounds: a meta-analysis. *J Clin Nurs* 2009; **18**: 716-728 [PMID: 19239539 DOI: 10.1111/j.1365-2702.2008.02534.x]

107 **Thomas DR**, Goode PS, LaMaster K, Tennyson T, Parnell LK. A comparison of an opaque foam dressing versus a transparent film dressing in the management of skin tears in institutionalized subjects. *Ostomy Wound Manage* 1999; **45**: 22-24, 27-28 [PMID: 10655859]

108 **Fletcher J**. Using film dressings. *Nurs Times* 2003; **99**: 57 [PMID: 12861644]

109 **Carter MJ**, Tingley-Kelley K, Warriner RA. Silver treatments and silver-impregnated dressings for the healing of leg wounds and ulcers: a systematic review and meta-analysis. *J Am Acad Dermatol* 2010; **63**: 668-679 [PMID: 20471135 DOI: 10.1016/j.jaad.2009.09.007]

110 **Capobianco CM**, Stapleton JJ, Zgonis T. Soft tissue reconstruction pyramid in the diabetic foot. *Foot Ankle Spec* 2010; **3**: 241-248 [PMID: 20610846 DOI: 10.1177/1938640010375113]

111 **Blume PA**, Paragas LK, Sumpio BE, Attinger CE. Single-stage surgical treatment of noninfected diabetic foot ulcers. *Plast Reconstr Surg* 2002; **109**: 601-609 [PMID: 11818842]

112 **Armstrong DG**, Lavery LA, Stern S, Harkless LB. Is prophylactic diabetic foot surgery dangerous? *J Foot Ankle Surg* 1996; **35**: 585-589 [PMID: 8986899]

113 **Hinchliffe RJ**, Valk GD, Apelqvist J, Armstrong DG, Bakker K, Game FL, Hartemann-Heurtier A, Löndahl M, Price PE, van Houtum WH, Jeffcoate WJ. A systematic review of the effectiveness of interventions to enhance the healing of chronic ulcers of the foot in diabetes. *Diabetes Metab Res Rev* 2008; **24** Suppl 1: S119-S144 [PMID: 18442185 DOI: 10.1002/dmrr.825]

114 **Armstrong DG**, Frykberg RG. Classifying diabetic foot surgery: toward a rational definition. *Diabet Med* 2003; **20**: 329-331 [PMID: 12675649 DOI: 10.1046/j.1464-5491.2003.00933.x]

115 **Mueller MJ**, Sinacore DR, Hastings MK, Strube MJ, Johnson JE. Effect of Achilles tendon lengthening on neuropathic plantar ulcers. A randomized clinical trial. *J Bone Joint Surg Am* 2003; **85-A**: 1436-1445 [PMID: 12925622]

116 **Lin SS**, Lee TH, Wapner KL. Plantar forefoot ulceration with equinus deformity of the ankle in diabetic patients: the effect of tendo-Achilles lengthening and total contact casting. *Orthopedics* 1996; **19**: 465-475 [PMID: 8727341]

117 **Lepäntalo M**, Biancari F, Tukiainen E. Never amputate without consultation of a vascular surgeon. *Diabetes Metab Res Rev* 2000; **16** Suppl 1: S27-S32 [PMID: 11054884]

118 **Sumpio BE**, Lee T, Blume PA. Vascular evaluation and arterial reconstruction of the diabetic foot. *Clin Podiatr Med Surg* 2003; **20**: 689-708 [PMID: 14636033 DOI: 10.1016/S0891-8422(03)00088-0]

119 **Faglia E**, Mantero M, Caminiti M, Caravaggi C, De Giglio R, Pritelli C, Clerici G, Fratino P, De Cata P, Dalla Paola L, Mariani G, Poli M, Settembrini PG, Sciangula L, Morabito A, Graziani L. Extensive use of peripheral angioplasty, particularly infrapopliteal, in the treatment of ischaemic diabetic foot ulcers: clinical results of a multicentric study of 221 consecutive diabetic subjects. *J Intern Med* 2002; **252**: 225-232 [PMID: 12270002 DOI: 10.1046/j.1365-2796.2002.01015.x]

120 **van Baal JG**. Surgical treatment of the infected diabetic foot. *Clin Infect Dis* 2004; **39** Suppl 2: S123-S128 [PMID: 15306990 DOI: 10.1086/383273]

121 **Attinger CE**, Brown BJ. Amputation and ambulation in

diabetic patients: function is the goal. *Diabetes Metab Res Rev* 2012; **28** Suppl 1: 93-96 [PMID: 22271731 DOI: 10.1002/dmrr.2236]
122 **Frykberg RG**, Armstrong DG, Giurini J, Edwards A, Kravette M, Kravitz S, Ross C, Stavosky J, Stuck R, Vanore J. Diabetic foot disorders: a clinical practice guideline. American College of Foot and Ankle Surgeons. *J Foot Ankle Surg* 2000; **39**: S1-60 [PMID: 11280471]
123 **Abou-Zamzam AM**, Gomez NR, Molkara A, Banta JE, Teruya TH, Killeen JD, Bianchi C. A prospective analysis of critical limb ischemia: factors leading to major primary amputation versus revascularization. *Ann Vasc Surg* 2007; **21**: 458-463 [PMID: 17499967 DOI: 10.1016/j.avsg.2006.12.006]
124 **Oliveira N**, Rosa P, Borges L, Dias E, Oliveira F, Cássio I. Treatment of diabetic foot complications with hyperbaric oxygen therapy: a retrospective experience. *Foot Ankle Surg* 2014; **20**: 140-143 [PMID: 24796835 DOI: 10.1016/j.fas.2014.02.004]
125 **Strauss MB**. Hyperbaric oxygen as an intervention for managing wound hypoxia: its role and usefulness in diabetic foot wounds. *Foot Ankle Int* 2005; **26**: 15-18 [PMID: 15680113]
126 **Cianci P**. Advances in the treatment of the diabetic foot: Is there a role for adjunctive hyperbaric oxygen therapy? *Wound Repair Regen* 2004; **12**: 2-10 [PMID: 14974958 DOI: 10.1111/j.1067-1927.2004.012104.x]
127 **Landau Z**. Topical hyperbaric oxygen and low energy laser for the treatment of diabetic foot ulcers. *Arch Orthop Trauma Surg* 1998; **117**: 156-158 [PMID: 9521521]
128 **Barnes RC**. Point: hyperbaric oxygen is beneficial for diabetic foot wounds. *Clin Infect Dis* 2006; **43**: 188-192 [PMID: 16779745]
129 **Thackham JA**, McElwain DL, Long RJ. The use of hyperbaric oxygen therapy to treat chronic wounds: A review. *Wound Repair Regen* 2008; **16**: 321-330 [PMID: 18471250 DOI: 10.1111/j.1524-475X.2008.00372.x]
130 **Abidia A**, Laden G, Kuhan G, Johnson BF, Wilkinson AR, Renwick PM, Masson EA, McCollum PT. The role of hyperbaric oxygen therapy in ischaemic diabetic lower extremity ulcers: a double-blind randomised-controlled trial. *Eur J Vasc Endovasc Surg* 2003; **25**: 513-518 [PMID: 12787692 DOI: 10.1053/ejvs.2002.1911]
131 **Ma L**, Li P, Shi Z, Hou T, Chen X, Du J. A prospective, randomized, controlled study of hyperbaric oxygen therapy: effects on healing and oxidative stress of ulcer tissue in patients with a diabetic foot ulcer. *Ostomy Wound Manage* 2013; **59**: 18-24 [PMID: 23475448]
132 **Kessler L**, Bilbault P, Ortéga F, Grasso C, Passemard R, Stephan D, Pinget M, Schneider F. Hyperbaric oxygenation accelerates the healing rate of nonischemic chronic diabetic foot ulcers: a prospective randomized study. *Diabetes Care* 2003; **26**: 2378-2382 [PMID: 12882865 DOI: 10.2337/diacare.26.8.2378]
133 **Löndahl M**, Katzman P, Nilsson A, Hammarlund C. Hyperbaric oxygen therapy facilitates healing of chronic foot ulcers in patients with diabetes. *Diabetes Care* 2010; **33**: 998-1003 [PMID: 20427683 DOI: 10.2337/dc09-1754]
134 **Löndahl M**. Hyperbaric oxygen therapy as treatment of diabetic foot ulcers. *Diabetes Metab Res Rev* 2012; **28** Suppl 1: 78-84 [PMID: 22271728 DOI: 10.1002/dmrr.2256]
135 **Kranke P**, Bennett MH, Martyn-St James M, Schnabel A, Debus SE. Hyperbaric oxygen therapy for chronic wounds. *Cochrane Database Syst Rev* 2012; **4**: CD004123 [PMID: 22513920 DOI: 10.1002/14651858.CD004123.pub3]
136 **O'Reilly D**, Pasricha A, Campbell K, Burke N, Assasi N, Bowen JM, Tarride JE, Goeree R. Hyperbaric oxygen therapy for diabetic ulcers: systematic review and meta-analysis. *Int J Technol Assess Health Care* 2013; **29**: 269-281 [PMID: 23863187 DOI: 10.1017/S0266462313000263]
137 **Gill AL**, Bell CN. Hyperbaric oxygen: its uses, mechanisms of action and outcomes. *QJM* 2004; **97**: 385-395 [PMID: 15208426 DOI: 10.1093/qjmed/hch074]
138 **Al-Waili NS**, Butler GJ. Effects of hyperbaric oxygen on inflammatory response to wound and trauma: possible mechanism of action. *ScientificWorldJournal* 2006; **6**: 425-441 [PMID: 16604253]
139 **Thom SR**. Hyperbaric oxygen: its mechanisms and efficacy. *Plast Reconstr Surg* 2011; **127** Suppl 1: 131S-141S [PMID: 21200283 DOI: 10.1097/PRS.0b013e3181fbe2bf]
140 **Niinikoski JH**. Clinical hyperbaric oxygen therapy, wound perfusion, and transcutaneous oximetry. *World J Surg* 2004; **28**: 307-311 [PMID: 14961187]
141 **Peters EJ**, Lavery LA, Armstrong DG, Fleischli JG. Electric stimulation as an adjunct to heal diabetic foot ulcers: a randomized clinical trial. *Arch Phys Med Rehabil* 2001; **82**: 721-725 [PMID: 11387573 DOI: 10.1053/apmr.2001.23780]
142 **Petrofsky JS**, Lawson D, Berk L, Suh H. Enhanced healing of diabetic foot ulcers using local heat and electrical stimulation for 30 min three times per week. *J Diabetes* 2010; **2**: 41-46 [PMID: 20923474 DOI: 10.1111/j.1753-0407.2009.00058.x]
143 **Lundeberg TC**, Eriksson SV, Malm M. Electrical nerve stimulation improves healing of diabetic ulcers. *Ann Plast Surg* 1992; **29**: 328-331 [PMID: 1466529]
144 **Baker LL**, Chambers R, DeMuth SK, Villar F. Effects of electrical stimulation on wound healing in patients with diabetic ulcers. *Diabetes Care* 1997; **20**: 405-412 [PMID: 9051395 DOI: 10.2337/diacare.20.3.405]
145 **Thakral G**, Lafontaine J, Najafi B, Talal TK, Kim P, Lavery LA. Electrical stimulation to accelerate wound healing. *Diabet Foot Ankle* 2013; **4** [PMID: 24049559 DOI: 10.3402/dfa.v4i0.22081]
146 **Barnes R**, Shahin Y, Gohil R, Chetter I. Electrical stimulation vs. standard care for chronic ulcer healing: a systematic review and meta-analysis of randomised controlled trials. *Eur J Clin Invest* 2014; **44**: 429-440 [PMID: 24456185 DOI: 10.1016/j.ejvs.2008.06.010]
147 **Vikatmaa P**, Juutilainen V, Kuukasjärvi P, Malmivaara A. Negative pressure wound therapy: a systematic review on effectiveness and safety. *Eur J Vasc Endovasc Surg* 2008; **36**: 438-448 [PMID: 18675559]
148 **Armstrong DG**, Lavery LA. Negative pressure wound therapy after partial diabetic foot amputation: a multicentre, randomised controlled trial. *Lancet* 2005; **366**: 1704-1710 [PMID: 16291063 DOI: 10.1016/S0140-6736(05)67695-7]
149 **DeFranzo AJ**, Argenta LC, Marks MW, Molnar JA, David LR, Webb LX, Ward WG, Teasdall RG. The use of vacuum-assisted closure therapy for the treatment of lower-extremity wounds with exposed bone. *Plast Reconstr Surg* 2001; **108**: 1184-1191 [PMID: 11604617 DOI: 10.1016/j.cpm.2007.03.011]
150 **Espensen EH**, Nixon BP, Lavery LA, Armstrong DG. Use of subatmospheric (VAC) therapy to improve bioengineered tissue grafting in diabetic foot wounds. *J Am Podiatr Med Assoc* 2002; **92**: 395-397 [PMID: 12122126]
151 **Venturi ML**, Attinger CE, Mesbahi AN, Hess CL, Graw KS. Mechanisms and clinical applications of the vacuum-assisted closure (VAC) Device: a review. *Am J Clin Dermatol* 2005; **6**: 185-194 [PMID: 15943495]
152 **Eginton MT**, Brown KR, Seabrook GR, Towne JB, Cambria RA. A prospective randomized evaluation of negative-pressure wound dressings for diabetic foot wounds. *Ann Vasc Surg* 2003; **17**: 645-649 [PMID: 14534844]
153 **Eto¨z A**, O¨zgenel Y, O¨ zcan M. The use of negative pressure wound therapy on diabetic foot ulcers: a preliminary controlled trial. *Wounds* 2004; **16**: 264-269
154 **McCallon SK**, Knight CA, Valiulus JP, Cunningham MW, McCulloch JM, Farinas LP. Vacuum-assisted closure versus saline-moistened gauze in the healing of postoperative diabetic foot wounds. *Ostomy Wound Manage* 2000; **46**: 28-32, 34 [PMID: 11189545]
155 **Blume PA**, Walters J, Payne W, Ayala J, Lantis J. Comparison

of negative pressure wound therapy using vacuum-assisted closure with advanced moist wound therapy in the treatment of diabetic foot ulcers: a multicenter randomized controlled trial. *Diabetes Care* 2008; **31**: 631-636 [PMID: 18162494 DOI: 10.2337/dc07-2196]

156 **Akbari A**, Moodi H, Ghiasi F, Sagheb HM, Rashidi H. Effects of vacuum-compression therapy on healing of diabetic foot ulcers: randomized controlled trial. *J Rehabil Res Dev* 2007; **44**: 631-636 [PMID: 17943674 DOI: 10.1682/JRRD.2007.01.0002]

157 **Sadat U**, Chang G, Noorani A, Walsh SR, Hayes PD, Varty K. Efficacy of TNP on lower limb wounds: a meta-analysis. *J Wound Care* 2008; **17**: 45-48 [PMID: 18210955]

158 **Ubbink DT**, Westerbos SJ, Nelson EA, Vermeulen H. A systematic review of topical negative pressure therapy for acute and chronic wounds. *Br J Surg* 2008; **95**: 685-692 [PMID: 18446777 DOI: 10.1002/bjs.6238]

159 **Mouës CM**, van den Bemd GJ, Meerding WJ, Hovius SE. An economic evaluation of the use of TNP on full-thickness wounds. *J Wound Care* 2005; **14**: 224-227 [PMID: 15909439]

160 **Braakenburg A**, Obdeijn MC, Feitz R, van Rooij IA, van Griethuysen AJ, Klinkenbijl JH. The clinical efficacy and cost effectiveness of the vacuum-assisted closure technique in the management of acute and chronic wounds: a randomized controlled trial. *Plast Reconstr Surg* 2006; **118**: 390-397; discussion 398-400 [PMID: 16874208]

161 **Kim PJ**, Heilala M, Steinberg JS, Weinraub GM. Bioengineered alternative tissues and hyperbaric oxygen in lower extremity wound healing. *Clin Podiatr Med Surg* 2007; **24**: 529-46, x [PMID: 17613390]

162 **Teng YJ**, Li YP, Wang JW, Yang KH, Zhang YC, Wang YJ, Tian JH, Ma B, Wang JM, Yan X. Bioengineered skin in diabetic foot ulcers. *Diabetes Obes Metab* 2010; **12**: 307-315 [PMID: 20380651 DOI: 10.1111/j.1463-1326.2009.01164.x]

163 **Bello YM**, Falabella AF, Eaglstein WH. Tissue-engineered skin. Current status in wound healing. *Am J Clin Dermatol* 2001; **2**: 305-313 [PMID: 11721649]

164 **Richmond NA**, Vivas AC, Kirsner RS. Topical and biologic therapies for diabetic foot ulcers. *Med Clin North Am* 2013; **97**: 883-898 [PMID: 23992899 DOI: 10.1016/j.mcna.2013.03.014]

165 **Futrega K**, King M, Lott WB, Doran MR. Treating the whole not the hole: necessary coupling of technologies for diabetic foot ulcer treatment. *Trends Mol Med* 2014; **20**: 137-142 [PMID: 24485902 DOI: 10.1016/j.molmed.2013.12.004]

166 **Kirsner RS**, Warriner R, Michela M, Stasik L, Freeman K. Advanced biological therapies for diabetic foot ulcers. *Arch Dermatol* 2010; **146**: 857-862 [PMID: 20713816 DOI: 10.1001/archdermatol.2010.164]

167 **O'Loughlin A**, McIntosh C, Dinneen SF, O'Brien T. Review paper: basic concepts to novel therapies: a review of the diabetic foot. *Int J Low Extrem Wounds* 2010; **9**: 90-102 [PMID: 20483808 DOI: 10.1177/1534734610371600]

168 **Veves A**, Falanga V, Armstrong DG, Sabolinski ML. Graftskin, a human skin equivalent, is effective in the management of noninfected neuropathic diabetic foot ulcers: a prospective randomized multicenter clinical trial. *Diabetes Care* 2001; **24**: 290-295 [PMID: 11213881 DOI: 10.2337/diacare.24.2.290]

169 **Falanga V**, Sabolinski M. A bilayered living skin construct (APLIGRAF) accelerates complete closure of hard-to-heal venous ulcers. *Wound Repair Regen* 1999; **7**: 201-207 [PMID: 10781211 DOI: 10.1046/j.1524-475X.1999.00201.x]

170 **Edmonds M**. Apligraf in the treatment of neuropathic diabetic foot ulcers. *Int J Low Extrem Wounds* 2009; **8**: 11-18 [PMID: 19189997 DOI: 10.1177/1534734609331597]

171 **Steinberg JS**, Edmonds M, Hurley DP, King WN. Confirmatory data from EU study supports Apligraf for the treatment of neuropathic diabetic foot ulcers. *J Am Podiatr Med Assoc* 2010; **100**: 73-77 [PMID: 20093548]

172 **Marston WA**, Hanft J, Norwood P, Pollak R. The efficacy and safety of Dermagraft in improving the healing of chronic diabetic foot ulcers: results of a prospective randomized trial. *Diabetes Care* 2003; **26**: 1701-1705 [PMID: 12766097 DOI: 10.2337/diacare.26.6.1701]

173 **Gentzkow GD**, Iwasaki SD, Hershon KS, Mengel M, Prendergast JJ, Ricotta JJ, Steed DP, Lipkin S. Use of dermagraft, a cultured human dermis, to treat diabetic foot ulcers. *Diabetes Care* 1996; **19**: 350-354 [PMID: 8729158]

174 **Niezgoda JA**, Van Gils CC, Frykberg RG, Hodde JP. Randomized clinical trial comparing OASIS Wound Matrix to Regranex Gel for diabetic ulcers. *Adv Skin Wound Care* 2005; **18**: 258-266 [PMID: 15942317]

175 **Lorenzi G**, Crippa M, Rossi G, Ferrari S, Terzi A, Motolese A. Open and endovascular revascularization combined with regenerative dermal skin graft in the treatment of ischemic ulcers. *Ital J Vasc Endovasc Surg* 2005; **12**: 61-64

176 **Dinh TL**, Veves A. The efficacy of Apligraf in the treatment of diabetic foot ulcers. *Plast Reconstr Surg* 2006; **117**: 152S-157S; discussion 158S-159S [PMID: 16799383]

177 **Papanas N**, Maltezos E. Becaplermin gel in the treatment of diabetic neuropathic foot ulcers. *Clin Interv Aging* 2008; **3**: 233-240 [PMID: 18686746]

178 **Bennett SP**, Griffiths GD, Schor AM, Leese GP, Schor SL. Growth factors in the treatment of diabetic foot ulcers. *Br J Surg* 2003; **90**: 133-146 [PMID: 12555288 DOI: 10.1002/bjs.4019]

179 **Wieman TJ**. Clinical efficacy of becaplermin (rhPDGF-BB) gel. Becaplermin Gel Studies Group. *Am J Surg* 1998; **176**: 74S-79S [PMID: 9777976]

180 **Knighton DR**, Ciresi KF, Fiegel VD, Austin LL, Butler EL. Classification and treatment of chronic nonhealing wounds. Successful treatment with autologous platelet-derived wound healing factors (PDWHF). *Ann Surg* 1986; **204**: 322-330 [PMID: 3753059 DOI: 10.1097/00000658-198609000-00011]

181 **Steed DL**. Clinical evaluation of recombinant human platelet-derived growth factor for the treatment of lower extremity ulcers. *Plast Reconstr Surg* 2006; **117**: 143S-149S; discussion 150S-151S [PMID: 16799381]

182 **Barrientos S**, Stojadinovic O, Golinko MS, Brem H, Tomic-Canic M. Growth factors and cytokines in wound healing. *Wound Repair Regen* 2008; **16**: 585-601 [PMID: 19128254 DOI: 10.1111/j.1524-475X.2008.00410.x]

183 **Wieman TJ**, Smiell JM, Su Y. Efficacy and safety of a topical gel formulation of recombinant human platelet-derived growth factor-BB (becaplermin) in patients with chronic neuropathic diabetic ulcers. A phase III randomized placebo-controlled double-blind study. *Diabetes Care* 1998; **21**: 822-827 [PMID: 9589248 DOI: 10.2337/diacare.21.5.822]

184 **Sibbald RG**, Torrance G, Hux M, Attard C, Milkovich N. Cost-effectiveness of becaplermin for nonhealing neuropathic diabetic foot ulcers. *Ostomy Wound Manage* 2003; **49**: 76-84 [PMID: 14652415]

185 **Hogge J**, Krasner D, Nguyen H, Harkless LB, Armstrong DG. The potential benefits of advanced therapeutic modalities in the treatment of diabetic foot wounds. *J Am Podiatr Med Assoc* 2000; **90**: 57-65 [PMID: 10697968 DOI: 10.7547/87507315-90-2-57]

186 **Lantis JC**, Boone D, Gendics C, Todd G. Analysis of patient cost for recombinant human platelet-derived growth factor therapy as the first-line treatment of the insured patient with a diabetic foot ulcer. *Adv Skin Wound Care* 2009; **22**: 167-171 [PMID: 19325276 DOI: 10.1097/01.ASW.0000305466.25177.a8]

187 **Fang RC**, Galiano RD. A review of becaplermin gel in the treatment of diabetic neuropathic foot ulcers. *Biologics* 2008; **2**: 1-12 [PMID: 19707423]

188 **Smiell JM**, Wieman TJ, Steed DL, Perry BH, Sampson AR, Schwab BH. Efficacy and safety of becaplermin (recombinant human platelet-derived growth factor-BB) in patients with

nonhealing, lower extremity diabetic ulcers: a combined analysis of four randomized studies. *Wound Repair Regen* 1999; **7**: 335-346 [PMID: 10564562 DOI: 10.1046/j.1524-475X.1999.00335.x]

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TOPIC HIGHLIGHT

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Pathogenesis of diabetic cerebral vascular disease complication

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Abstract

Diabetes mellitus is one of the most potent independent risk factors for the development of diabetic cerebral vascular disease (CVD). Many evidences suggested that hyperglycemia caused excess free fatty acids, the loss of endothelium-derived nitric oxide, insulin resistance, the prothrombotic state, endothelial dysfunction, the abnormal release of endothelial vasoactivators, vascular smooth muscle dysfunction, oxidative stress, and the downregulation of miRs participated in vessel generation and recovery as well as the balance of endotheliocytes. In turn, these abnormalities, mainly *via* phosphatidylinositol 3 kinase, mitogen-activated protein kinase, polyol, hexosamine, protein kinase C activation, and increased generation of advanced glycosylation end products pathway, play an important role in inducing diabetic CVD complication. A deeper comprehension of pathogenesis producing diabetic CVD could offer base for developing new therapeutic ways preventing diabetic CVD complications, therefore, in the paper we mainly reviewed present information about the possible pathogenesis of diabetic CVD complication.

Key words: Complication; Diabetes mellitus; Cerebral vascular disease; Pathway; Pathogenesis

Core tip: A better understanding pathogenesis of diabetic cerebral vascular disease (CVD) could provide the basis for developing novel therapeutic strategies against diabetic CVD complication. Our article highlights the pathogenesis as some promising options to prevent CVD complications in diabetes, including metabolic and vascular changes and main pathways are involved in diabetic CVD complication.

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INTRODUCTION

Diabetic mellitus (DM) is a chronic disease leading to a

fault of insulin due to pancreas dysfunction, which causes hyperglycemia with metabolic imbalances in carbohydrate, fat and protein[1]. Morbidities of DM significantly elevate in late decades, which are primarily due to alter in life style, an elevation in the incidence of obesity and longevity. Current projections estimate that the number of people with DM will nearly double by 2025[2,3].

About 100 million populations suffer from DM in the world[4], Among them, five to ten percent are type 1 DM of insulin dependence and ninety to ninety-five percent are type 2 DM (T2MD) of non insulin dependence. The current evidences demonstrated that morbidities of T2MD would increase owing to life styles leading to obesity[5]. T2DM is a very common disease, which has an asymptomatic period between the actual onset of diabetic hyperglycemia and clinical diagnosis. This stage has been evaluated to sustain at the fewest 4-7 years, and 30%-50% patients of T2DM are still unknown. This leads to the development of chronic complications of diabetes, which remain the chief problems in diabetic care, and which cause a lack of fitness to work, disability, and premature death[6,7].

Among the chronic complications of diabetes, the vasculopathy is the first serious complication. The vasculopathy related to DM was traditionally divided into two major parts. Firstly, diabetic complications associated micrangium including retina, nephridium and neural system lesion; secondly, the atherothrombotic complications related to macro-arteries like myocardial infarction, hypertension, peripheral artery lesion[8].

DM is one of the well known risk factor for cerebrovascular accident[9,10]. Prolonged untreated DM contributes to micrangium lesion, hypoxic and ischemic damages of tissues, which elevates the danger of apoplexy and aggravates cerebral lesion caused by blood insufficiency[10,11]. Its incidence in DM patients is 2-6 times more than non DM[12-14] and ultimately its complications and subsequent prevalence is higher yet. Patients demonstrate a progressive atherosclerosis in cerebral arteries and increased a vascular reaction to vascular constrictors, a deregulated reaction to vascular dilators and damaged automatic regulation of brain blood stream. Changed endothelium function of small arteries and a damaged vascular motor function of resistance vessel can lead to change mediation of local blood stream and deficient perfusion of tissue in diabetic patients[10,15,16].

Many studies[8,17-19] stress the strong link between the cerebral vascular disease (CVD) complications and DM and describe a close association between CVD microvascular complications and DM, suggest that excess free fatty acids (FFAs), the loss of endothelium-derived nitric oxide (NO), insulin resistance, the prothrombotic state, endothelial dysfunction, the abnormal release of endothelial vasoactivators, vascular smooth muscle (VSM) dysfunction, oxidative stress and the miRs downregulation participated in vessel generation, vessel recovery as well as endothelium balance generate diabetic CVD complications by these major mechanisms of phosphatidylinositol 3 kinase, mitogen-activated protein kinase, polyol, hexosamine, generation of advanced glycosylation end products (AGEs) and protein kinase C (PKC) pathways activation[20,21]. The aim of this review is to review the possible pathogenesis of diabetic CVD complication.

SUPERFLUOUS FREE FATTY ACIDS

DM facilitates lipolysis, reduces uptaking of skeletal muscle, which result in superfluous concentrations of FFAs. Moreover, elevates the flux of FFAs into liver causes the stimulation of triglycerides synthesis, assembly and secretion of very low-density lipoprotein (VLDL) particles. Hypertriglyceridemia and the decreased high-density lipoprotein (HDL) cholesterin as the transportation of cholesterin from HDL to VLDL have been determined to be strongly relative to atherosclerosis. It also is likely that FFAs generation promotes reactive oxygen species (ROS) and PKC. PKC elevation and phosphatidylinositol 3 kinase (PI3-K) downregulation may cause endothelial impairment.

Recycling concentrations of FFAs rise in DM because of the superfluous releasion derived from adipose tissue as well as the reducing uptake of skeletal muscle[22-24]. FFAs can damage endothelial function *via* a series of mechanisms such as elevating oxygen-mediated free radicals generation, PKC activation and dyslipidemia aggravation[25-27]. FFAs levels increase activates PKC, reduces insulin receptor substrate-1 associated PI3-K activity[25,28]. Increased triglyceride levels decreases HDL through facilitating cholesterin transportation from HDL to VLDL[29]. These disturbances alter LDL configuration, elevating the quantity of the more LDL of small density contributing to atherosclerosis[30,31]. Hypertriglyceridemia and decreased HDL are suggested to be relative to endothelial dysfunction[21,32-34].

THE LOSS OF ENDOTHELIUM-DERIVED NITRIC OXIDE

Endothelial and vascular smooth muscle cells (VSMCs) dysfunction and an inclination of thrombus formation result in atherogenesis as well as the relative complications. Because endothelial cells (ECs) mediate the vascular function and structure, they take on an important anatomical location on interaction of circulatory blood and vascular wall. In normal conditions, ECs active substances, synthesize and release vascular activators to preserve vessel balance, to ensure a normal blood stream and nutritious transportation while avoiding thrombus formation as well as white blood cell permeation[35]. One of key molecules produced by ECs is NO, it is generated by an endothelial NO synthase (eNOS) *via* a 5 electrons oxidation of the guanidine nitrogen terminal of L-arginine[36].

The NO biologic availability is a vital element in vessel abnormality, which results in vascular dilation activated guanylyl cyclase in VSMCs[36]. Furthermore, NO prevents vascellum from internal lesion like atherogenesis-mediated

molecule signal that stops platelet and leukocyte interacting with vessel wall and inhibits VSMCs proliferation and migration[37,38]. Contrarily, ECs reduction-mediated NO induces elevated pro-inflammatory transcription factor nuclear factor kappa B (NFκB) activity which causes a leukocyte adhesion molecules expression, chemokines as well as cytokines generation[39]. The effects facilitate mono cells and VSMCs to migrate into the internal membrane and macrophage foam cells formation, producing an early morphologic alteration of atherogenesis[39-43]. Disorder of endothelium function such as damaged endothelium dependent and NO-derived relaxation is identified in cell and animal studies of DM[21,44-47].

INSULIN RESISTANCE

Insulin resistance is another vital pathogenesis that exerts a major effect on the diabetic CVD complication. Insulin exerts effects by two pathways including PI3-K and mitogen-activated protein kinase (MAPK). Insulin signal producing by PI3-K has effects of anti-proliferative and anti-coagulant, the effects activated by MAPK have a proatherogenic function. On base of insulin resistance, although the first pathway is damaged, the second pathway maintains intact. Therefore, the decrease endothelial dependent vasodilatation as well as increase mitosis effects is a key result[48,49].

Insulin resistance also critically takes part in vascular dysfunction in patients with T2DM[50]. In fact, the reduction of PI3-K/Akt pathway causes eNOS depression, decreases NO generation[51]. Combining with decreasing NO synthesis, intracellular oxidization of stored FFAs produces ROS contributing to vascular inflammation, AGEs synthesis, inhibited PGI2 synthase activity, and PKC activation[51,52].

Rised ROS concentrations closely related with insulin resistance remove NO generation, generate peroxynitrite accompanying with a more decrease of NO biologic availability. Decreased cell concentrations of NO activate pro-inflammatory pathways promoted by increasing cytokine generation. In fact, TNF-α and IL-1 facilitate NFκB activity and adhesion molecules expression. TNF-α also induces C reactive protein expression which lowers the regulation of eNOS and elevates adhesion molecules and endothelin-1 (ET-1) generation[50,53].

Adipokines associated with vasculopathy are leptin, adipocyte fatty acid-binding protein, interleukins, lipocalin-2 and pigment epithelium- derived factor, which could produce disorders of vessel function through increasing proliferation and migration of smooth muscle cells (SMCs), eNOS depression, and NFκB signaling activation accompanied with adhesion molecule expression and atherogenesis[54].

PROTHROMBOTIC STATE

Damaged fibrinolysis, as a result of enhancing generation of PAI-1 and excessive activity of platelet result from of glycoprotein Ⅱb/Ⅲa superfluous expression and excessive production of thromboxane A2 in DM. Furthermore, DM rises concentrations of Ⅶ, Ⅷ factor as well as thrombin-antithrombin compounds[48,55-57]. Haemostasis chaos elevates the risk of cerebral thrombosis.

Abnormality of platelet function as well as elevation of both glycoprotein Ib and Ⅱb/Ⅲa expression in DM augment interaction between platelet-von Willebrand factor (vWF) and platelet-fibrin[58]. The internal-cellular platelet glucose level responses the external-cellular circumstance, is relative to increasing superoxide anion (O^{2-}) generation as well as PKC activity, reduced platelet mediated NO[59]. High blood glucose more alters hematoblastic functions through damaging calcium balance, thus changes platelet activation and aggregation such as platelet construct and mediators release[60]. In DM, plasm coagulate factors Ⅶ, thrombin and impairment-dependent coagulate elements such as tissue factor (TF) elevated, and endogenic anti-coagulate factors like thrombomodulin as well as protein C reduced[61-63]. In addition, the generation of plasminogen activator inhibitor-1 (PAI-1), a fibrinolysis inhibitor elevated[64-66]. Therefore, a inclination of hematoblastic activating and aggregating accompanied with coagulate propensity is associated with a danger of thrombus formation complicated plaque burst.

Diabetic CVD largely results from an abnormality of elements participated in activation of coagulate factors and hematoblast[67]. Insulin resistance, high blood glucose involve in the nosogenesis of the prothrombotic status[68]. Insulin resistance rises PAI-1 and fibrinogen, decreases levels of tissue plasminogen activator. Hyperinsulinemia elevates TF expression in monocytes of T2DM contributing to increase TF procoagulant activity and thrombin production[69]. Low level of inflammation also leads TF expression in vessel endothelial cells of DM patients, and results in atherothrombosis[68,69].

Platelet hyperreactivity is one of major relations in elements leading to DM prothrombotic status[70]. A lot of mechanisms leaded to platelet dysfunction affect the adhesive, activated and aggregative stages of hematoblast induced thrombus formation. High blood glucose changes hematoblast calcium ion homeostasis contributing to a abnormality of cellular constructure, elevates the production of pro-aggregant elements[58]. Furthermore, the increase production of glycoproteins Ib and Ⅱb/Ⅲa in DM subjects contributes to thrombosis through interacting with vWF and fibrin.

In DM, the elevation of glucose levels contributes to the activation of PKC, down-regulates the production of platelet derived NO, rises the formation of O^{2-}[59], and also triggers the disorder of calcium homeostasis in platelets[60]. The abnormal calcium regulation may significantly lead to disordered activity, because the intraplatelet calcium mediates a shape change, secretion, aggregation and thromboxane formation of platelet. Their disorders may cause by decreasing endothelial generation of the antiaggregants NO and prostacyclin, increasing generation of fibrinogen, and increasing generation of platelet

activators like thrombin and vWF[58]. In general, diabetic disorders elevate the activation of platelet and depress internal depressors of platelet activity.

DM increases a blood coagulability which makes it more likely that atherosclerotic plaque rupture or erosion will lead to the thrombotic occlusion of artery. T2DM has damaged fibrinolytic capacity owing to increasing levels of PAI-1 in atherogenetic damage and nonateromatous artery[71]. DM elevates the TF (a forceful procoagulant) expression and plasm coagulate elements like factor Ⅶ, reduces contents of internal anti-coagulate factors like antithrombin Ⅲ and protein C[62,63,72]. A number of these disorders are associated with the occurrence of hyperglycemia and proinsulin split products[73]. Therefore, DM increases a tendency of coagulation accompanying with damaging fibrinolysis, facilitates the formation and persistence of thrombi.

ENDOTHELIAL DYSFUNCTION

The endothelium is an organ composing of a mono cell layer arraying the intimal surface of the vasculature, serving as a paclose between blood and tissues. The normal paracrine and autocrine functions of endothelial cells include the synthesis of a series of substances that moderate vascular relaxation, mediate local inflammation, depress leucocyte migration and affect platelet activation.

Endothelial dysfunction consists of many abnormalities, encompassing changed vasomotor activity, VSMC dysfunction, excess generation of inflammatory cytokines and chemokines, damaged platelet function and abnormal coagulation, which contribute to elevating vasoconstriction, inflammation and thrombosis[74].

The endothelium in DM is more frail in producing atherosclerotic plaques compared with the endothelium of non DM. A series of mechanisms may lead to the elevated risk of generating atherosclerotic plaques in DM. The abnormal cluster of hyperglycemia, increased FFAs and insulin resistance in DM, targeting the endothelial cell, causing oxidative stress and endothelial dysfunction[75]. The endothelial dysfunction leads to a defective endothelium dependent vasorelaxation and vasoconstriction, a migration of monocytes, a VSMCs transport into internal membrane as well as a generation of macrophage foam cells, which trigger an atherogenesis production. As far as ET-1, besides a vascular constrictive function, which has the function of proinflammation, mitogenesis and proliferation yet[48,76].

Hyperglycemia, elevated FFAs as well as insulin resistance exert through a usual pathogenesis characterized by rising ROS generation (Especially O^{2-}), subsequently result in damaging endothelial dependent NO induced relaxation. Elevated ET-1 generation and VSMCs proliferation also lead to endothelium dysfunction. Hypertriglyceridemia, consequent atherogenesis and platelet hyperactivity as well as diminished fibrinolysis and hypercoagulability are features of vessel circumstances in DM. In general, an initial and progressive atherogenesis, endothelium dysfunction as well as elevated thrombus production are extremely susceptible to generating thrombotic occlusive events at brain circle for this type of DM[20].

Mechanisms of the endothelial dysfunction encompass elevating polyol pathway flux, changed cell redox status, increasing generation of diacylglycerol, specific PKC isoforms activation, and exacerbated non-enzymatic production of AGEs. A lot of pathways promote the generation of oxidative and nitrosative mediated oxidants and free radicals like O^{2-} and peroxynitrite, exerting a important effect in the mechanism of the DM-relative endothelial dysfunction. The cellular sources of ROS like O^{2-} are diverse and encompass AGEs, NADH, NADPH oxidases, mitochondrial respiratory chain, xanthine oxidase, arachidonic acid cascade, and microsomal enzymes[77-82]. The oxygen and nitrogen stress mediated by hyperglycemia results in DNA-lesion as well as succedent poly (ADP-ribose) polymerase (PARP) activation[83]. The endothelial dysfunction progression was related to a concurrent NAD^+ and NADPH loss in vascular systems, PARP depression inversed their alterations. Endothelium dysfunction in DM is relied on a PARP-derived, inverse cell NADPH insufficient[83,84].

A mono layer of ECs is seated in the internal membrane of all vascellum, which offers a metabolically active interactive spot between blood and vessels regulating blood influx, nutritious transport, coagulation and thrombosis, and leukocyte diapedesis[85]. ECs synthetize a lot of key bioactive substances, such as ROS, prostaglandin, endothelin as well as angiotensin Ⅱ, which modulates vascular effect and structure. Moreover, it diminishes platelet activation, inhibits inflammation by decreasing leukocyte adhesion to endothelium and migrating into the vascular wall, and reduces VSMCs proliferation and migration[38,86,87]. In general, these characters prevent atherogenesis and protect the vascular vessel.

DM damages endothelium dependent vasodilation prior to the generation of atheroma[88,89]. Many of fundamental mechanisms lead to the lower bioavailability of vasoactivators in DM. Hyperglycemia diminishes generation of NO by inhibiting the activation of eNOS synthase and elevating the generation of ROS, particularly O^{2-}, in endothelial and VSMCs[78].

Insulin resistance contributes to excessive release of FFAs from adipose tissue[90], activating the signal enzyme PKC, depressing PI3-K, and increasing the generation of ROS-mechanisms[27]. Generation of peroxynitrite decreases synthetizing the vessel dilatory and antiplatelet prostanoid prostacyclin[91]. The increased levels of FFAs in DM trigger the production of oxidized low-density lipoproteins (Ox-LDL), including vital initiating events for atherosclerosis. Ox-LDL can impair ECs and increase adhesion molecules expression like P-selectin[92] and chemotactic factors like monocyte chemoattractant protein-1, macrophage colony stimulating factor[93,94] and thus lead to endothelial dysfunction in DM[78,95].

ABNORMAL RELEASE OF ENDOTHELIAL VASOACTIVATORS

Hyperglycemia rises the cyclooxygenase-2 mRNA expression in DM. Endothelin could be especially associated with the pathophysiology of vasculopathy in DM, because endothelin triggers inflammatory reaction, results in VSMCs contraction and growth[95]. The abnormalities of endothelium associated factors or vasoactivators generation consisting of vascular oxidative stress[96], inflammatory factors, NO, prostanoids (prostacyclin), ET-1, angiotensin Ⅱ (ANG-Ⅱ), tissue-type plasminogen activator (t-PA), PAI-1, vWF, adhesion molecules such as vascular cell adhesion molecule (VCAM) leukocyte adhesion molecules, intercellular adhesion molecule (ICAM) as well as cytokines[97]. These vascular activated factors contribute to elevating vascular tone, resulting in microvascular and macrovascular impairment and apoptosis of microvascular cells, consequently contributing to DM associated vascular complications[97]. In lots of pathological conditions, the abnormal balance of these regulatory mediators causes the onset and process of vascular endothelial dysfunction[98]. Vascular endothelial dysfunction elevates effects of leukocyte, smooth muscle proliferation, vascular constriction, damaged coagulating, vessel inflammation, thrombus generation, and atherogenesis, these mechanisms are the base of later DM complications like retinopathy, nephropathy, vasculopathy as well as neuropathy[99]. ET-1 is a forceful vasoconstrictor generated by ECs. The generation and the level of ET-1 in plasma elevated in DM patients, and it is reported a positive correlation between plasm ET-1 concentrations and the micro-vessel lesion of DM. Therefore, ET-1 could exert a possible key effect on endothelial dysfunction by a disorder between ECs mediated vascular dilator and vascular constrictor factors on mechanisms of the vessel complication in DM[100]. Besides its direct vasoconstrictor functions, elevated levels of ET-1 may lead to endothelial dysfunction *via* generating a series of vascular active substances consisting of ROS, NO and inflammatory factors[101-103].

CGRP is a key mediator of ET-1 vasoconstriction. The elevation of CGRP expression leads an abnormal balance in the CGRP/ET-1 ratio, inducing abnormal vascular constriction to result in topical endothelial dysfunction as well as vessel impairment[104,105]. VCAM-1 is one of key ECs receptors, mediating leukocyte adhesion to the vascular ECs. Current studied results have highly proposed that VCAM-1 might exert a key effect on mechanisms of atherosclerosis on account of VCAM-1 effects on leukocyte adhesion and transmigration is key as well as its expression is upregulated in the initial phases of neogenetic atheroma plaques[106]. Moreover, VCAM-1expression is upregulated by pro-inflammatory stimuli like TNF-α and IL-1β, is least partially mediated by NFκB[107]. The expression of VCAM-1 is upregulated in vessel stress circumstances like insulin resistance and chronic high blood glucose yet. In the circumstance, the elevation in the activity of VCAM-1 expression is found yet[108,109]. Besides binding leukocytes, VCAM-1 engagement leads to leukocyte transendothelial migration (TEM) *via* inducing gap formation between cells in the endothelial monolayer, facilitating TEM[110]. The gap formation is moderated by VCAM-1[111,112]. VCAM-1 accumulation elevates the internal cellular free calcium level yet[111]. Therefore, the expression of VCAM-1 rises the permeability of vascular endothelium as well as the TEM of leukocyte, leads to the impairment of vessel and abnormal endothelial function, as well as mediates atherogenesis production. The ICAM-1 expression in ECs is risen in atherogenesis and in the animal model of atherogenesis[113,114]. In normal conditions, ICAM-1 is presented in low concentrations in ECs, however ICAM-1 is significantly elevated while stimulating by pro-inflammatory factors such as the pro-inflammatory cytokines TNF-α, IL-1β as well as interferon-γ[114]. Soluble ICAM-1 may mediate leukocyte adhesion, migration. Promoting leukocyte to attach to the ECs surface isn't the only effect of ICAM-1. Effect of ICAM-1 mediates signal in ECs, rises the IL-8 generation, facilitates the ICAM-1 and c-fos expression. It is likely that pro-inflammatory IL-8 and c-fos activated by ICAM-1 facilitates a positive feedback loop, contributing to excessive ICAM-1 and VCAM-1 expression and thus initiating the persistent recruitment of leukocytes to regions of atherosclerosis, facilitating the progress of atherogenesis through producing pro-inflammatory stimuli through indirect or direct endothelial dysfunction[115-117].

P-selectin is mediated by pro-inflammatory stimuli stored in internal cellular vesicles of ECs combined with the plasm membrane being responsible to many stimuli like ischemia and chronic high blood glucose yet[118]. On some conditions, P-selectin translocation to the endothelial cell surface is modulated by a ROS-dependent mechanism. P-selectin accumulation rises cytosol free Ca^{2+}, mediates changes of cell morphology, facilitating endothelial dysfunction to ultimately generate vessel impairment[119-121].

VCAM-1, ICAM-1, P-selectin exert a key effect on vascular integrity and permeability through endothelial dysfunction[122]. Increased concentrations of soluble EAM could be one of the common causes for the pathogenesis of between atherogenetic CVD and endothelial dysfunction[122]. The adhesive molecule expression in ECs facilitates the adhesion and transportation of leukocytes into the sub-endothelial space, consequently contributing to abnormal endothelial function and sub-endothelial structure alteration[123]. Elevated vessel permeability owing to structure changes can then decrease insulin transportation to insulin sensitive peripheral tissues, ultimately form insulin resistance. In addition, insulin resistance may directly contribute to endothelial dysfunction[124]. The investigation in non-diabetic subjects have proposed that lightly damaged glucose tolerance in the normal glycemic range may facilitate the process of endothelial dysfunction through side effects of oxidative stress, generation of AGEs, and

increased concentrations of FFAs[125].

Vascular endothelial dysfunction may be prior to the development of insulin resistance, which results from a decrease of insulin sensitivity, generates a vicious cycle[126-128]. Our studied results provided the further evidences that endothelial dysfunction exerts a causal role in the pathogenesis of CVD in T2DM, and also highlights new insights into the possible clinical value of endothelial function in CVD of T2DM. The pathophysiologic mechanisms of CVD in T2DM could be relative to an abnormal expressive balance of ET-1, CGRP, VCAM-1, ICAM-1 and P-selectin, causing endothelial dysfunction *via* a series of chemical factors like ROS, NO and inflammatory factors. Alternatively, we speculated that emotion, cerebral splanchno-motor and neuroendocrine center could participate in the mechanisms of CVD in T2MD through changes of ET-1, CGRP, VCAM-1, ICAM-1 and P-selectin expression, but further researches need to be warranted[129].

VASCULAR SMOOTH MUSCLE DYSFUNCTION

The changes in vascular homeostasis owing to the dysfunction of ECs and SMCs are the primary characters of diabetic vascular diseases, which are favor of a pro-inflammatory or thrombotic status, finally contributing to artery thrombus formation. The diabetic affect on vascular function isn't confined to ECs. The abnormal regulation of VSM function is accelerated through damaging the sympathetic nervous system function[130]. DM rises PKC activity, NFκB and oxygen free radicals generation in VSM, which is similar to the effects in ECs[131]. Furthermore, DM elevates the migration of VSMCs into early atherosclerotic impairment, replicating and generating external cellular matrix key process in later impairment production[132]. VSMCs apoptosis during atherogenetic impairment is rised yet, so that DM patients are apt to have fewer VSMCs in impairment, increasing the tendency of plaque rupture. In DM, the cytokine generation reduces VSM synthesis of collagen and elevates generation of matrix metalloproteinases, producing an elevated propensity for plaque destabilization and rupture[133].

DM promotes the VSMCs atherogenic activity. Hyperglycemia stimulates PKC, receptor for AGEs and NFκB in VSMCs, which is similar in ECs. Promotion of these systems increases generation of O^{2-}, which leading to the oxidant gathering circumstances[27]. VSMCs are indispensable in the progression of atherosclerosis. In case the formation of the macrophage abundant fatty streak, VSMCs in the middle layer of the arteries migrate into the early intimal impairment, replicate and generate a complicate extracellular matrix vital process in the development formed atherosclerotic plaque. VSMCs heighten the atheroma by way of the collagen source, which makes it less possibly to rupture and results in thrombosis. In fact, the impairments that have disrupted and resulted in fatal thrombosis are inclined to have few VSMCs[134]. Hyperglycemic lipid modifications of LDL may partially modulate the risen migration and the following apoptosis of VSMCs in atherosclerosis impairment. LDL that has suffered nonenzymatic glycation promotes VSMCs migration, while oxidized glycated LDL can promote apoptosis of VSMCs[135]. Therefore, DM changes VSMCs function through facilitating atherosclerotic lesion formation, plaque instability[68].

OXIDATIVE STRESS

The generation of ROS is severely controlled in normal cells, but excessive generation at the condition of metabolized disorder contributes to cell lesions. O^{2-} and NO are correspondingly nonvalent, however, while the both are combined they produce highly active peroxynitrites that impairs and diminishes protein and lipid. Moreover, O^{2-} and NO can impair the iron sulfur center of enzyme and other protein, releasing iron atom and thus depressing enzyme and protein activity. There are a number of key proteins that are highly sensitive to the type of inhibition such as complexes Ⅰ-Ⅲ in the electron transfer chain, aconitase in the trichloroacetic acid cycle as well as biotin synthase[136,137].

The production of lipid, protein and nucleic acid compounds participates in lots of complicated chain reactions in ways of a series of biological substrates containing reactive methylene groups. Intermediate productions in these chain reactions can have very strongly oxidative effects, thus cell lesion can be comprehensive[138,139]. Lipids locate in plasm, mitochondria and endoplasmic reticulum membranes are main attacked objects of ROS and peroxidation. Terminal productions of lipid peroxidation like lipid peroxides can be toxic to cells, require to be resolved by glutathione. In the same way, proteins and nucleic acids can be suffered by peroxidation and nitrosylation. The terminal products aren't commonly directly toxic to cells, the gather of inactive proteins can excessively increase the ability of cells to recycle them, DNA impairment is known to promote the pathogenesis of apoptosis.

Diabetic CVD complications are majorly owing to a prolonged exposure of hyperglycemia[140]. The early trigger high glucose levels change vessel function is the disorder between NO bio-availability and gather of ROS, contributing to endothelial dysfunction. In fact, high blood glucose mediated production of O^{2-} inactivates NO to generate peroxynitrite ($ONOO^-$), a forceful oxidant, it easily pass through phospholipid membranes, causes substrate nitration[21]. Hyperglycemia mediated ROS generation promotes a series of cellular mechanisms such as polyol and hexosamine flux, AGEs, PKC activation and NFκB induced vascular inflammation[52,141]. One of the major resources of ROS in the condition of high blood glucose is represented by PKC and its downstream subjects. The circumstance of high blood glucose promotes a chronic increase of

diacyglycerol concentrations in ECs accompanying the following membrane translocation of conventional and nonconventional PKC isoforms. In case activated, PKC would be responsible for different structure and functions alterations in vascular systems such as changes in permeability, inflammation, angiogenesis, growth, external cell matrix expansion and apoptosis of cells[142]. A key result of PKC activation is ROS production. Hyperglycemia mediated the PKC activation rises superoxide generation by NADPH oxidase in vascular ECs[27]. PKC also contributes to elevated generation of ET-1, which is favor of vasoconstricting and platelet aggregating[142].

In the vascular wall, the generation of PKC-depended ROS takes part in the atherogenetic progression *via* promoting vessel inflammation yet[52,143]. ROS contributes to upregulation and nuclear translocation of NFκB subunit p65, thus, the transcription of pro-inflammatory genes encodes for monocyte chemoattractant protein-1 (MCP-1), selectins, VCAM-1, and ICAM-1. The latter event promotes the adhesion of monocytes to vascular endothelial cells, rolls and exudes in the subendothelium accompanying the following production of foam cells. The production of IL-1 and TNF-α derived from a active macrophage keeps elevation of adhesion molecule through augmenting NFκB signal in the endothelial cell and also stimulates SMCs growth and proliferation[144].

Endothelial dysfunction in DM is the subsequence of damaged NO availability and the risen synthesis of vascular constrictor and prostanoid[144]. The up-regulation of PKC induced cyclooxygenase-2 (COX-2) is related to an elevation of thromboxane A2 as well as a downregulation of prostacyclin (PGI2) release[145]. The data speculate that PKC is upstream signaling molecules which affect vessel balance at the condition of hyperglycemia[145]. Production of AGEs contributes to cell disorders by triggering of a AGEs receptor (RAGE) activation[146,147]. AGE-RAGE signal conversely promotes ROS-sensitive biochemical pathways like the hexosamine flux[52]. At the circumstance of hyperglycemia, an elevated flux of fructose-6-phosphate promotes a series of reactions leading different glycosilated patterns being responsible for down-regulation of enzymes involved in vessel balance. Especially, OglcNAcylation at the Akt site of eNOS protein contributes to decreasing eNOS activities and ECs disorders[52,148]. Furthermore, transcription factors glycosylation results in upregulation of inflammation (TGFα, TGFβ1) and prothrombus genes (PAI-1)[148,149]. Hyperglycemia mediated ROS generation promotes the polyol pathway flux participated in vessel redox stress yet[141,150]. Therefore, hyperactivation of the pathway is relative to elevated atherogenetic damages in a DM mouse[151,152].

ABNORMALITY OF MIRS PARTICIPATE IN ANGIOGENESIS, VASCULAR REPAIR AND ENDOTHELIAL HOMEOSTASIS

MicroRNAs (miRs) are a currently found one type of small no coding RNAs known as important effects on mechanisms of high blood glucose mediated vessel lesion[153,154]. The small no coded RNAs mediate many aspects of DM vasculopathy through moderating gene expression at the posttranscriptional time. DM shows a obvious abnormality of miRs participated in angiogenesis, vascular repair and endothelial homeostasis[155]. When ECs are exposed to prolonged hyperglycemia, miR-320 is largely expressed and triggers a series of angiogenic factors and their receptors such as vascular endothelial growth factor and insulin like growth factor-1 (IGF-1). Increased expression of the miR is relative to decreasing cellular proliferation and migration. When the miR decrease recoveries the characters and elevates IGF-1 expression, facilitating angiogenesis and vascular repair[156].

High blood glucose also elevates the miR-221 expression, a mediator of angiogenesis for c-kit receptor is associated with migrating as well as homing of endothelial progenitor cells (EPCs)[157]. miR-221 and 222 were identified to induced AGE mediated vessel lesion yet[157]. In fact, the decrease of miR-222 expression in human ECs exposed to hyperglycemia and in a DM mouse results in AGE associated ECs disorders through targeted, cyclin depended kinase proteins participated in cellular cycle depression (P27KIP1 and P57KIP2)[157]. A current research revealed that miR-503 severely participated in high blood glucose mediated endothelial dysfunction in a DM mouse, is elevated in muscles of ischemia limbs in DM patients[158]. The pernicious function of miR-503 at the condition of DM has been identified by the interaction with CCNE and cdc25A, which are key mediators of cell cycle process influencing the migration and proliferation of ECs. It is interesting that miR-503 depression can actualize the normalization of post-ischemic novel vascularization and blood stream repairs in a DM mouse. The studied results offer a base to predict protective effects on regulating miR-503 expression against DM vasculopathy.

Assay of plasm miR demonstrated a largely decrease of miR-126 in a cohort of DM patients[155]. Current studied results propose that down-regulation of miR-126 expression is in part responsible for damaging vascular recovery capacities in DM[159,160]. The expression of miR-126 decreases in EPCs isolated from DM and transfection using anti-miR-126 diminished the proliferation and migration of EPCs[159,160]. By comparison, the recovered expression of miR-126 facilitated EPCs associated a recovery capacity and depressed apoptosis. The miR-126 effect in EPCs is regulated by Spred-1, an inhibitor of Ras/ERK signal pathway, a key mediator of cellular cycles. In general, the findings provide further evidences that miRs promotes a series of complicated signaling network through triggering the genes expression participated in the differentiation, migration as well as survival of cell[161].

In summary, many factors affect the diabetic CVD complication, which are summaried in the Figure 1 of diagrammatic sketch. Such factors will promote optimal understanding of the pathogenesis of the diabetic CVD complication and lead to the identification of the specific preventive therapy. Ultimately, the knowledge

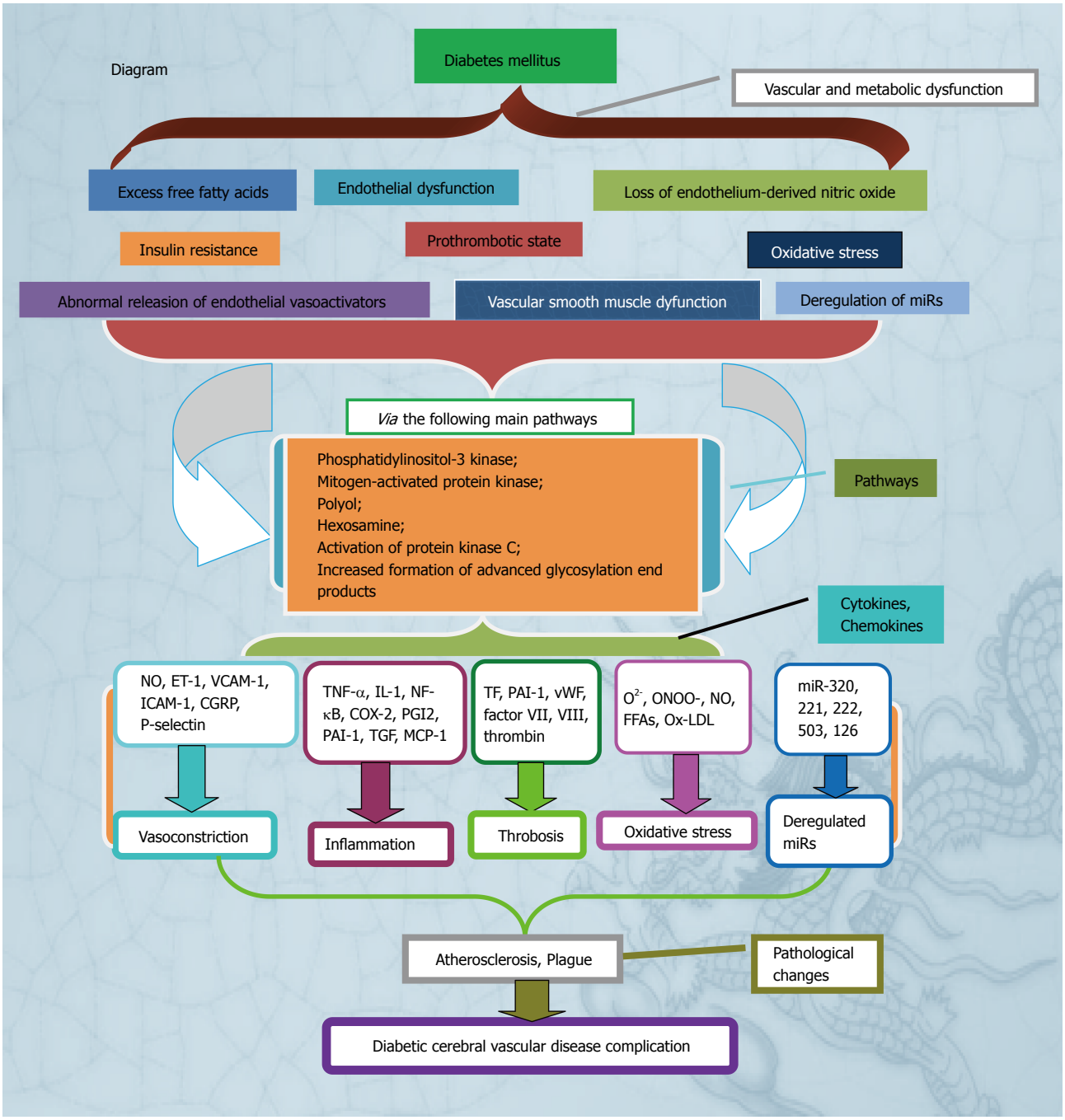

Figure 1 Diagram of pathogenesis of diabetic cerebral vascular disease complication. NO: Endothelium-derived nitric oxide; ET-1: Endothelin-1; VCAM: Vascular cell adhesion molecule; ICAM: Intercellular adhesion molecule; TNF: Tumor necrosis factor; IL: Interleukin; NF-κB: Nuclear factor-κB; COX-2: Cyclooxygenase-2; PGI2: Prostacyclin: PAI-1: Prothrombus genes; TGF-β: Transforming growth factor beta; MCP-1: Monocyte chemoattractant protein-1; TF: Tissue factor; Ox-LDL: Oxidized low-density lipoproteins; FFAs: Free fatty acids.

gained from these previous studies can be used to obtain the potential drug for preventing the diabetic CVD complication.

REFERENCES

1 **Bucala R**. Diabetes, aging, and their tissue complications. *J Clin Invest* 2014; **124**: 1887-1888 [PMID: 24789881 DOI: 10.1172/JCI75224]

2 **Arinzon Z**, Shabat S, Shuval I, Peisakh A, Berner Y. Prevalence of diabetes mellitus in elderly patients received enteral nutrition long-term care service. *Arch Gerontol Geriatr* 2008; **47**: 383-393 [PMID: 17950479]

3 **Bethel MA**, Sloan FA, Belsky D, Feinglos MN. Longitudinal incidence and prevalence of adverse outcomes of diabetes mellitus in elderly patients. *Arch Intern Med* 2007; **167**: 921-927 [PMID: 17502533]

4 **Amos AF**, McCarty DJ, Zimmet P. The rising global burden of diabetes and its complications: estimates and projections to the year 2010. *Diabet Med* 1997; **14** Suppl 5: S1-85 [PMID: 9450510]

5 **Mokdad AH**, Bowman BA, Ford ES, Vinicor F, Marks JS, Koplan JP. The continuing epidemics of obesity and diabetes

in the United States. *JAMA* 2001; **286**: 1195-1200 [PMID: 11559264]
6 **Piechowski-Jozwiak B**, Maulaz A, Bogousslavsky J. Secondary prevention of stroke with antiplatelet agents in patients with diabetes mellitus. *Cerebrovasc Dis* 2005; **20** Suppl 1: 15-23 [PMID: 16276081]
7 **Spijkerman AM**, Dekker JM, Nijpels G, Adriaanse MC, Kostense PJ, Ruwaard D, Stehouwer CD, Bouter LM, Heine RJ. Microvascular complications at time of diagnosis of type 2 diabetes are similar among diabetic patients detected by targeted screening and patients newly diagnosed in general practice: the hoorn screening study. *Diabetes Care* 2003; **26**: 2604-2608 [PMID: 12941726]
8 **Krentz AJ**, Clough G, Byrne CD. Interactions between microvascular and macrovascular disease in diabetes: pathophysiology and therapeutic implications. *Diabetes Obes Metab* 2007; **9**: 781-791 [PMID: 17924862]
9 **Harada S**, Fujita-Hamabe W, Tokuyama S. Ischemic stroke and glucose intolerance: a review of the evidence and exploration of novel therapeutic targets. *J Pharmacol Sci* 2012; **118**: 1-13 [PMID: 22188858]
10 **Fülesdi B**, Limburg M, Bereczki D, Michels RP, Neuwirth G, Legemate D, Valikovics A, Csiba L. Impairment of cerebrovascular reactivity in long-term type 1 diabetes. *Diabetes* 1997; **46**: 1840-1845 [PMID: 9356034]
11 **Unfirer S**, Kibel A, Drenjancevic-Peric I. The effect of hyperbaric oxygen therapy on blood vessel function in diabetes mellitus. *Med Hypotheses* 2008; **71**: 776-780 [PMID: 18722723 DOI: 10.1016/j.mehy.2008.06.016]
12 **Kleiser B**, Widder B. Course of carotid artery occlusions with impaired cerebrovascular reactivity. *Stroke* 1992; **23**: 171-174 [PMID: 1561643]
13 **Larsen FS**, Olsen KS, Hansen BA, Paulson OB, Knudsen GM. Transcranial Doppler is valid for determination of the lower limit of cerebral blood flow autoregulation. *Stroke* 1994; **25**: 1985-1988 [PMID: 7916502]
14 **Lipsitz LA**, Mukai S, Hamner J, Gagnon M, Babikian V. Dynamic regulation of middle cerebral artery blood flow velocity in aging and hypertension. *Stroke* 2000; **31**: 1897-1903 [PMID: 10926954]
15 **Sena CM**, Pereira AM, Seiça R. Endothelial dysfunction - a major mediator of diabetic vascular disease. *Biochim Biophys Acta* 2013; **1832**: 2216-2231 [PMID: 23994612 DOI: 10.1016/j.bbadis.2013.08.006]
16 **Moghaddasi M**, Mamarabadi M, Habibi AH. A comparison of cerebral vasomotor reactivity in diabetic and nondiabetic Iranian patients. *J Res Med Sci* 2010; **15**: 50-53 [PMID: 21526058]
17 **Konig M**, Lamos EM, Stein SA, Davis SN. An insight into the recent diabetes trials: what is the best approach to prevent macrovascular and microvascular complications? *Curr Diabetes Rev* 2013; **9**: 371-381 [PMID: 23865412]
18 **Tandon N**, Ali MK, Narayan KM. Pharmacologic prevention of microvascular and macrovascular complications in diabetes mellitus: implications of the results of recent clinical trials in type 2 diabetes. *Am J Cardiovasc Drugs* 2012; **12**: 7-22 [PMID: 22217193 DOI: 10.2165/11594650-]
19 **Sosale A**, Prasanna Kumar KM, Sadikot SM, Nigam A, Bajaj S, Zargar AH, Singh SK. Chronic complications in newly diagnosed patients with Type 2 diabetes mellitus in India. *Indian J Endocrinol Metab* 2014; **18**: 355-360 [PMID: 24944931 DOI: 10.4103/2230-8210.131184]
20 **Muntean C**, Mitrea A, Mota M, Tudorica V. Type 2 diabetes and its implications in cerebrovascular disease. *Rom J Diabetes Nutr Metab Dis* 2012; **19**: 81-88 [DOI: 10.2478/v10255-012-0011-7]
21 **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]
22 **Boden G**. Free fatty acids, insulin resistance, and type 2 diabetes mellitus. *Proc Assoc Am Physicians* 1999; **111**: 241-248 [PMID: 10354364]
23 **Fujimoto WY**. The importance of insulin resistance in the pathogenesis of type 2 diabetes mellitus. *Am J Med* 2000; **108** Suppl 6a: 9S-14S [PMID: 10764845]
24 **Kelley DE**, Simoneau JA. Impaired free fatty acid utilization by skeletal muscle in non-insulin-dependent diabetes mellitus. *J Clin Invest* 1994; **94**: 2349-2356 [PMID: 7989591]
25 **Dresner A**, Laurent D, Marcucci M, Griffin ME, Dufour S, Cline GW, Slezak LA, Andersen DK, Hundal RS, Rothman DL, Petersen KF, Shulman GI. Effects of free fatty acids on glucose transport and IRS-1-associated phosphatidylinositol 3-kinase activity. *J Clin Invest* 1999; **103**: 253-259 [PMID: 9916137]
26 **Dichtl W**, Nilsson L, Goncalves I, Ares MP, Banfi C, Calara F, Hamsten A, Eriksson P, Nilsson J. Very low-density lipoprotein activates nuclear factor-kappaB in endothelial cells. *Circ Res* 1999; **84**: 1085-1094 [PMID: 10325246]
27 **Inoguchi T**, Li P, Umeda F, Yu HY, Kakimoto M, Imamura M, Aoki T, Etoh T, Hashimoto T, Naruse M, Sano H, Utsumi H, Nawata H. High glucose level and free fatty acid stimulate reactive oxygen species production through protein kinase C--dependent activation of NAD(P)H oxidase in cultured vascular cells. *Diabetes* 2000; **49**: 1939-1945 [PMID: 11078463]
28 **Griffin ME**, Marcucci MJ, Cline GW, Bell K, Barucci N, Lee D, Goodyear LJ, Kraegen EW, White MF, Shulman GI. Free fatty acid-induced insulin resistance is associated with activation of protein kinase C theta and alterations in the insulin signaling cascade. *Diabetes* 1999; **48**: 1270-1274 [PMID: 10342815]
29 **Sniderman AD**, Scantlebury T, Cianflone K. Hypertriglyceridemic hyperapob: the unappreciated atherogenic dyslipoproteinemia in type 2 diabetes mellitus. *Ann Intern Med* 2001; **135**: 447-459 [PMID: 11560458]
30 **Sniderman A**, Thomas D, Marpole D, Teng B. Low density lipoprotein. A metabolic pathway for return of cholesterol to the splanchnic bed. *J Clin Invest* 1978; **61**: 867-873 [PMID: 207724]
31 **Dimitriadis E**, Griffin M, Owens D, Johnson A, Collins P, Tomkin GH. Oxidation of low-density lipoprotein in NIDDM: its relationship to fatty acid composition. *Diabetologia* 1995; **38**: 1300-1306 [PMID: 8582539]
32 **de Man FH**, Weverling-Rijnsburger AW, van der Laarse A, Smelt AH, Jukema JW, Blauw GJ. Not acute but chronic hypertriglyceridemia is associated with impaired endothelium-dependent vasodilation: reversal after lipid-lowering therapy by atorvastatin. *Arterioscler Thromb Vasc Biol* 2000; **20**: 744-750 [PMID: 10712400]
33 **Kuhn FE**, Mohler ER, Satler LF, Reagan K, Lu DY, Rackley CE. Effects of high-density lipoprotein on acetylcholine-induced coronary vasoreactivity. *Am J Cardiol* 1991; **68**: 1425-1430 [PMID: 1746422]
34 **Koya D**, King GL. Protein kinase C activation and the development of diabetic complications. *Diabetes* 1998; **47**: 859-866 [PMID: 9604860]
35 **Kinlay S**, Libby P, Ganz P. Endothelial function and coronary artery disease. *Curr Opin Lipidol* 2001; **12**: 383-389 [PMID: 11507322]
36 **Moncada S**, Higgs A. The L-arginine-nitric oxide pathway. *N Engl J Med* 1993; **329**: 2002-2012 [PMID: 7504210]
37 **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]
38 **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]
39 **Zeiher AM**, Fisslthaler B, Schray-Utz B, Busse R. Nitric oxide

modulates the expression of monocyte chemoattractant protein 1 in cultured human endothelial cells. *Circ Res* 1995; **76**: 980-986 [PMID: 7758169]
40 **Libby P**. Changing concepts of atherogenesis. *J Intern Med* 2000; **247**: 349-358 [PMID: 10762452]
41 **Nomura S**, Shouzu A, Omoto S, Nishikawa M, Fukuhara S. Significance of chemokines and activated platelets in patients with diabetes. *Clin Exp Immunol* 2000; **121**: 437-443 [PMID: 10971508]
42 **Mohamed AK**, Bierhaus A, Schiekofer S, Tritschler H, Ziegler R, Nawroth PP. The role of oxidative stress and NF-kappaB activation in late diabetic complications. *Biofactors* 1999; **10**: 157-167 [PMID: 10609877]
43 **Collins T**, Cybulsky MI. NF-kappaB: pivotal mediator or innocent bystander in atherogenesis? *J Clin Invest* 2001; **107**: 255-264 [PMID: 11160146]
44 **Tesfamariam B**, Brown ML, Deykin D, Cohen RA. Elevated glucose promotes generation of endothelium-derived vasoconstrictor prostanoids in rabbit aorta. *J Clin Invest* 1990; **85**: 929-932 [PMID: 2312734]
45 **Bohlen HG**, Lash JM. Topical hyperglycemia rapidly suppresses EDRF-mediated vasodilation of normal rat arterioles. *Am J Physiol* 1993; **265**: H219-H225 [PMID: 8342636]
46 **Meraji S**, Jayakody L, Senaratne MP, Thomson AB, Kappagoda T. Endothelium-dependent relaxation in aorta of BB rat. *Diabetes* 1987; **36**: 978-981 [PMID: 3596063]
47 **Pieper GM**, Meier DA, Hager SR. Endothelial dysfunction in a model of hyperglycemia and hyperinsulinemia. *Am J Physiol* 1995; **269**: H845-H850 [PMID: 7573526]
48 **Holt R**, Cockram C, Flyvbjerg A, Goldstein B J. Textbook of Diabetes. 4th ed. USA: Wiley-Blackwell, 2010
49 **DeFronzo RA**. Insulin resistance, lipotoxicity, type 2 diabetes and atherosclerosis: the missing links. The Claude Bernard Lecture 2009. *Diabetologia* 2010; **53**: 1270-1287 [PMID: 20361178 DOI: 10.1007/s00125-010-1684-1]
50 **Kim JA**, Montagnani M, Koh KK, Quon MJ. Reciprocal relationships between insulin resistance and endothelial dysfunction: molecular and pathophysiological mechanisms. *Circulation* 2006; **113**: 1888-1904 [PMID: 16618833]
51 **Du X**, Edelstein D, Obici S, Higham N, Zou MH, Brownlee M. Insulin resistance reduces arterial prostacyclin synthase and eNOS activities by increasing endothelial fatty acid oxidation. *J Clin Invest* 2006; **116**: 1071-1080 [PMID: 16528409]
52 **Giacco F**, Brownlee M. Oxidative stress and diabetic complications. *Circ Res* 2010; **107**: 1058-1070 [PMID: 21030723 DOI: 10.1161/CIRCRESAHA.110.223545]
53 **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]
54 **Li ZY**, Wang P, Miao CY. Adipokines in inflammation, insulin resistance and cardiovascular disease. *Clin Exp Pharmacol Physiol* 2011; **38**: 888-896 [PMID: 21910745 DOI: 10.1111/j.1440-1681.2011.05602.x]
55 **Collinson DJ**, Rea R, Donnelly R. Vascular risk: diabetes. *Vasc Med* 2004; **9**: 307-310 [PMID: 15678624]
56 **Novak V**, Zhao P, Manor B, Sejdic E, Alsop D, Abduljalil A, Roberson PK, Munshi M, Novak P. Adhesion molecules, altered vasoreactivity, and brain atrophy in type 2 diabetes. *Diabetes Care* 2011; **34**: 2438-2441 [PMID: 21926285 DOI: 10.2337/dc11-0969]
57 **Reagan LP**. Diabetes as a chronic metabolic stressor: causes, consequences and clinical complications. *Exp Neurol* 2012; **233**: 68-78 [PMID: 21320489 DOI: 10.1016/j.expneurol.2011.02.004]
58 **Vinik AI**, Erbas T, Park TS, Nolan R, Pittenger GL. Platelet dysfunction in type 2 diabetes. *Diabetes Care* 2001; **24**: 1476-1485 [PMID: 11473089]
59 **Assert R**, Scherk G, Bumbure A, Pirags V, Schatz H, Pfeiffer AF. Regulation of protein kinase C by short term hyperglycaemia in human platelets in vivo and in vitro. *Diabetologia* 2001; **44**: 188-195 [PMID: 11270675]
60 **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]
61 **Hafer-Macko CE**, Ivey FM, Gyure KA, Sorkin JD, Macko RF. Thrombomodulin deficiency in human diabetic nerve microvasculature. *Diabetes* 2002; **51**: 1957-1963 [PMID: 12031986]
62 **Ceriello A**, Giacomello R, Stel G, Motz E, Taboga C, Tonutti L, Pirisi M, Falleti E, Bartoli E. Hyperglycemia-induced thrombin formation in diabetes. The possible role of oxidative stress. *Diabetes* 1995; **44**: 924-928 [PMID: 7621998]
63 **Ceriello A**, Giugliano D, Quatraro A, Marchi E, Barbanti M, Lefèbvre P. Evidence for a hyperglycaemia-dependent decrease of antithrombin III-thrombin complex formation in humans. *Diabetologia* 1990; **33**: 163-167 [PMID: 2184068]
64 **Ren S**, Lee H, Hu L, Lu L, Shen GX. Impact of diabetes-associated lipoproteins on generation of fibrinolytic regulators from vascular endothelial cells. *J Clin Endocrinol Metab* 2002; **87**: 286-291 [PMID: 11788661]
65 **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]
66 **Pandolfi A**, Cetrullo D, Polishuck R, Alberta MM, Calafiore A, Pellegrini G, Vitacolonna E, Capani F, Consoli A. Plasminogen activator inhibitor type 1 is increased in the arterial wall of type II diabetic subjects. *Arterioscler Thromb Vasc Biol* 2001; **21**: 1378-1382 [PMID: 11498469]
67 **Vazzana N**, Ranalli P, Cuccurullo C, Davì G. Diabetes mellitus and thrombosis. *Thromb Res* 2012; **129**: 371-377 [PMID: 22197180 DOI: 10.1016/j.thromres.2011.11.052]
68 **Beckman JA**, Creager MA, Libby P. Diabetes and atherosclerosis: epidemiology, pathophysiology, and management. *JAMA* 2002; **287**: 2570-2581 [PMID: 12020339]
69 **Boden G**, Rao AK. Effects of hyperglycemia and hyperinsulinemia on the tissue factor pathway of blood coagulation. *Curr Diab Rep* 2007; **7**: 223-227 [PMID: 17547839]
70 **Linden MD**, Tran H, Woods R, Tonkin A. High platelet reactivity and antiplatelet therapy resistance. *Semin Thromb Hemost* 2012; **38**: 200-212 [PMID: 22422334 DOI: 10.1055/s-0032-1301417]
71 **Carr ME**. Diabetes mellitus: a hypercoagulable state. *J Diabetes Complications* 2001; **15**: 44-54 [PMID: 11259926]
72 **Ceriello A**, Giugliano D, Quatraro A, Dello Russo P, Torella R. Blood glucose may condition factor VII levels in diabetic and normal subjects. *Diabetologia* 1988; **31**: 889-891 [PMID: 3240844]
73 **Nordt TK**, Bode C. Impaired endogenous fibrinolysis in diabetes mellitus: mechanisms and therapeutic approaches. *Semin Thromb Hemost* 2000; **26**: 495-501 [PMID: 11129405]
74 **Snell-Bergeon JK**, Wadwa RP. Hypoglycemia, diabetes, and cardiovascular disease. *Diabetes Technol Ther* 2012; **14** Suppl 1: S51-S58 [PMID: 22650225 DOI: 10.1089/dia.2012.0031]
75 **Aljada A**. Endothelium, inflammation, and diabetes. *Metab Syndr Relat Disord* 2003; **1**: 3-21 [PMID: 18370622 DOI: 10.1089/154041903321648225]
76 **Avogaro A**, Albiero M, Menegazzo L, de Kreutzenberg S, Fadini GP. Endothelial dysfunction in diabetes: the role of reparatory mechanisms. *Diabetes Care* 2011; **34** Suppl 2: S285-S290 [PMID: 21525470 DOI: 10.2337/dc11-s239]
77 **Giugliano D**, Ceriello A, Paolisso G. Oxidative stress and diabetic vascular complications. *Diabetes Care* 1996; **19**: 257-267 [PMID: 8742574]
78 **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]
79 **Nishikawa T**, Edelstein D, Brownlee M. The missing link: a single unifying mechanism for diabetic complications. *Kidney*

Int Suppl 2000; **77**: S26-S30 [PMID: 10997687]
80 **Brownlee M**. Biochemistry and molecular cell biology of diabetic complications. *Nature* 2001; **414**: 813-820 [PMID: 11742414]
81 **Guzik TJ**, Mussa S, Gastaldi D, Sadowski J, Ratnatunga C, Pillai R, Channon KM. Mechanisms of increased vascular superoxide production in human diabetes mellitus: role of NAD(P)H oxidase and endothelial nitric oxide synthase. *Circulation* 2002; **105**: 1656-1662 [PMID: 11940543]
82 **Ceriello A**. New insights on oxidative stress and diabetic complications may lead to a "causal" antioxidant therapy. *Diabetes Care* 2003; **26**: 1589-1596 [PMID: 12716823]
83 **Garcia Soriano F L**, Jagtap P, Szabó E, Mabley JG, Liaudet L, Marton A, Hoyt DG, Murthy KG, Salzman AL, Southan GJ, Szabó C. Diabetic endothelial dysfunction: the role of poly(ADP-ribose) polymerase activation. *Nat Med* 2001; **7**: 108-113 [PMID: 11135624]
84 **Soriano FG**, Pacher P, Mabley J, Liaudet L, Szabó C. Rapid reversal of the diabetic endothelial dysfunction by pharmacological inhibition of poly(ADP-ribose) polymerase. *Circ Res* 2001; **89**: 684-691 [PMID: 11597991]
85 **Cines DB**, Pollak ES, Buck CA, Loscalzo J, Zimmerman GA, McEver RP, Pober JS, Wick TM, Konkle BA, Schwartz BS, Barnathan ES, McCrae KR, Hug BA, Schmidt AM, Stern DM. Endothelial cells in physiology and in the pathophysiology of vascular disorders. *Blood* 1998; **91**: 3527-3561 [PMID: 9572988]
86 **Verma S**, Anderson TJ. The ten most commonly asked questions about endothelial function in cardiology. *Cardiol Rev* 2001; **9**: 250-252 [PMID: 11569467]
87 **Kubes P**, Suzuki M, Granger DN. Nitric oxide: an endogenous modulator of leukocyte adhesion. *Proc Natl Acad Sci USA* 1991; **88**: 4651-4655 [PMID: 1675786]
88 **Williams SB**, Cusco JA, Roddy MA, Johnstone MT, Creager MA. Impaired nitric oxide-mediated vasodilation in patients with non-insulin-dependent diabetes mellitus. *J Am Coll Cardiol* 1996; **27**: 567-574 [PMID: 8606266]
89 **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]
90 **Hennes MM**, O'Shaughnessy IM, Kelly TM, LaBelle P, Egan BM, Kissebah AH. Insulin-resistant lipolysis in abdominally obese hypertensive individuals. Role of the renin-angiotensin system. *Hypertension* 1996; **28**: 120-126 [PMID: 8675251]
91 **Zou M**, Yesilkaya A, Ullrich V. Peroxynitrite inactivates prostacyclin synthase by heme-thiolate-catalyzed tyrosine nitration. *Drug Metab Rev* 1999; **31**: 343-349 [PMID: 10335439]
92 **Vora DK**, Fang ZT, Liva SM, Tyner TR, Parhami F, Watson AD, Drake TA, Territo MC, Berliner JA. Induction of P-selectin by oxidized lipoproteins. Separate effects on synthesis and surface expression. *Circ Res* 1997; **80**: 810-818 [PMID: 9168783]
93 **Cushing SD**, Berliner JA, Valente AJ, Territo MC, Navab M, Parhami F, Gerrity R, Schwartz CJ, Fogelman AM. Minimally modified low density lipoprotein induces monocyte chemotactic protein 1 in human endothelial cells and smooth muscle cells. *Proc Natl Acad Sci USA* 1990; **87**: 5134-5138 [PMID: 1695010]
94 **Rajavashisth TB**, Andalibi A, Territo MC, Berliner JA, Navab M, Fogelman AM, Lusis AJ. Induction of endothelial cell expression of granulocyte and macrophage colony-stimulating factors by modified low-density lipoproteins. *Nature* 1990; **344**: 254-257 [PMID: 1690354]
95 **Hopfner RL**, Gopalakrishnan V. Endothelin: emerging role in diabetic vascular complications. *Diabetologia* 1999; **42**: 1383-1394 [PMID: 10651255]
96 **Cohen RA**, Tong X. Vascular oxidative stress: the common link in hypertensive and diabetic vascular disease. *J Cardiovasc Pharmacol* 2010; **55**: 308-316 [PMID: 20422735]
97 **van den Oever IA**, Raterman HG, Nurmohamed MT, Simsek S. Endothelial dysfunction, inflammation, and apoptosis in diabetes mellitus. *Mediators Inflamm* 2010; **2010**: 792393 [PMID: 20634940]
98 **Tan KC**, Chow WS, Ai VH, Lam KS. Effects of angiotensin II receptor antagonist on endothelial vasomotor function and urinary albumin excretion in type 2 diabetic patients with microalbuminuria. *Diabetes Metab Res Rev* 2002; **18**: 71-76 [PMID: 11921421]
99 **Verma S**, Anderson TJ. Fundamentals of endothelial function for the clinical cardiologist. *Circulation* 2002; **105**: 546-549 [PMID: 11827916]
100 **Wedgwood S**, McMullan DM, Bekker JM, Fineman JR, Black SM. Role for endothelin-1-induced superoxide and peroxynitrite production in rebound pulmonary hypertension associated with inhaled nitric oxide therapy. *Circ Res* 2001; **89**: 357-364 [PMID: 11509453]
101 **Romero M**, Jiménez R, Sánchez M, López-Sepúlveda R, Zarzuelo MJ, O'Valle F, Zarzuelo A, Pérez-Vizcaíno F, Duarte J. Quercetin inhibits vascular superoxide production induced by endothelin-1: Role of NADPH oxidase, uncoupled eNOS and PKC. *Atherosclerosis* 2009; **202**: 58-67 [PMID: 18436224 DOI: 10.1016/j.atherosclerosis.2008.03.007]
102 **Brain SD**, Williams TJ, Tippins JR, Morris HR, MacIntyre I. Calcitonin gene-related peptide is a potent vasodilator. *Nature* 1985; **313**: 54-56 [PMID: 3917554]
103 **McCulloch J**, Uddman R, Kingman TA, Edvinsson L. Calcitonin gene-related peptide: functional role in cerebrovascular regulation. *Proc Natl Acad Sci USA* 1986; **83**: 5731-5735 [PMID: 3488550]
104 **Cybulsky MI**, Iiyama K, Li H, Zhu S, Chen M, Iiyama M, Davis V, Gutierrez-Ramos JC, Connelly PW, Milstone DS. A major role for VCAM-1, but not ICAM-1, in early atherosclerosis. *J Clin Invest* 2001; **107**: 1255-1262 [PMID: 11375415]
105 **Iademarco MF**, McQuillan JJ, Rosen GD, Dean DC. Characterization of the promoter for vascular cell adhesion molecule-1 (VCAM-1). *J Biol Chem* 1992; **267**: 16323-16329 [PMID: 1379595]
106 **van Buul JD**, Voermans C, van den Berg V, Anthony EC, Mul FP, van Wetering S, van der Schoot CE, Hordijk PL. Migration of human hematopoietic progenitor cells across bone marrow endothelium is regulated by vascular endothelial cadherin. *J Immunol* 2002; **168**: 588-596 [PMID: 11777950]
107 **van Wetering S**, van den Berk N, van Buul JD, Mul FP, Lommerse I, Mous R, ten Klooster JP, Zwaginga JJ, Hordijk PL. VCAM-1-mediated Rac signaling controls endothelial cell-cell contacts and leukocyte transmigration. *Am J Physiol Cell Physiol* 2003; **285**: C343-C352 [PMID: 12700137]
108 **Madonna R**, Pandolfi A, Massaro M, Consoli A, De Caterina R. Insulin enhances vascular cell adhesion molecule-1 expression in human cultured endothelial cells through a pro-atherogenic pathway mediated by p38 mitogen-activated protein-kinase. *Diabetologia* 2004; **47**: 532-536 [PMID: 14762656]
109 **Okouchi M**, Okayama N, Shimizu M, Omi H, Fukutomi T, Itoh M. High insulin exacerbates neutrophil-endothelial cell adhesion through endothelial surface expression of intercellular adhesion molecule-1 via activation of protein kinase C and mitogen-activated protein kinase. *Diabetologia* 2002; **45**: 556-559 [PMID: 12032633]
110 **Rahman A**, Fazal F. Hug tightly and say goodbye: role of endothelial ICAM-1 in leukocyte transmigration. *Antioxid Redox Signal* 2009; **11**: 823-839 [PMID: 18808323 DOI: 10.1089/ARS.2008.2204]
111 **Doran AC**, Meller N, McNamara CA. Role of smooth muscle cells in the initiation and early progression of atherosclerosis. *Arterioscler Thromb Vasc Biol* 2008; **28**: 812-819 [PMID: 18276911 DOI: 10.1161/ATVBAHA.107.159327]
112 **Galkina E**, Ley K. Vascular adhesion molecules in atherosclerosis. *Arterioscler Thromb Vasc Biol* 2007; **27**: 2292-2301 [PMID: 17673705]
113 **Pi X**, Lockyer P, Dyer LA, Schisler JC, Russell B, Carey S,

Sweet DT, Chen Z, Tzima E, Willis MS, Homeister JW, Moser M, Patterson C. Bmper inhibits endothelial expression of inflammatory adhesion molecules and protects against atherosclerosis. *Arterioscler Thromb Vasc Biol* 2012; **32**: 2214-2222 [PMID: 22772758 DOI: 10.1161/ATVBAHA.112.252015]

114 **Lawson C**, Wolf S. ICAM-1 signaling in endothelial cells. *Pharmacol Rep* 2009; **61**: 22-32 [PMID: 19307690]

115 **Deem TL**, Cook-Mills JM. Vascular cell adhesion molecule 1 (VCAM-1) activation of endothelial cell matrix metalloproteinases: role of reactive oxygen species. *Blood* 2004; **104**: 2385-2393 [PMID: 15265790]

116 **Setiadi H**, McEver RP. Signal-dependent distribution of cell surface P-selectin in clathrin-coated pits affects leukocyte rolling under flow. *J Cell Biol* 2003; **163**: 1385-1395 [PMID: 14676308]

117 **Yang SX**, Yan J, Deshpande SS, Irani K, Lowenstein CJ. Rac1 regulates the release of Weibel-Palade Bodies in human aortic endothelial cells. *Chin Med J (Engl)* 2004; **117**: 1143-1150 [PMID: 15361285]

118 **Panés J**, Kurose I, Rodriguez-Vaca D, Anderson DC, Miyasaka M, Tso P, Granger DN. Diabetes exacerbates inflammatory responses to ischemia-reperfusion. *Circulation* 1996; **93**: 161-167 [PMID: 8616923]

119 **Kaplanski G**, Farnarier C, Benoliel AM, Foa C, Kaplanski S, Bongrand P. A novel role for E- and P-selectins: shape control of endothelial cell monolayers. *J Cell Sci* 1994; **107** (Pt 9): 2449-2457 [PMID: 7531200]

120 **Pasceri V**, Willerson JT, Yeh ET. Direct proinflammatory effect of C-reactive protein on human endothelial cells. *Circulation* 2000; **102**: 2165-2168 [PMID: 11056086]

121 **Rodriguez CJ**, Miyake Y, Grahame-Clarke C, Di Tullio MR, Sciacca RR, Boden-Albala B, Sacco RL, Homma S. Relation of plasma glucose and endothelial function in a population-based multiethnic sample of subjects without diabetes mellitus. *Am J Cardiol* 2005; **96**: 1273-1277 [PMID: 16253596]

122 **Blankenberg S**, Barbaux S, Tiret L. Adhesion molecules and atherosclerosis. *Atherosclerosis* 2003; **170**: 191-203 [PMID: 14612198]

123 **Simionescu M**. Implications of early structural-functional changes in the endothelium for vascular disease. *Arterioscler Thromb Vasc Biol* 2007; **27**: 266-274 [PMID: 17138941]

124 **Raghavan VA**. Insulin resistance and atherosclerosis. *Heart Fail Clin* 2012; **8**: 575-587 [PMID: 22999241 DOI: 10.1016/j.hfc.2012.06.014]

125 **Rask-Madsen C**, King GL. Mechanisms of Disease: endothelial dysfunction in insulin resistance and diabetes. *Nat Clin Pract Endocrinol Metab* 2007; **3**: 46-56 [PMID: 17179929]

126 **Libby P**, Ridker PM, Maseri A. Inflammation and atherosclerosis. *Circulation* 2002; **105**: 1135-1143 [PMID: 11877368]

127 **Perez del Villar C**, Garcia Alonso CJ, Feldstein CA, Juncos LA, Romero JC. Role of endothelin in the pathogenesis of hypertension. *Mayo Clin Proc* 2005; **80**: 84-96 [PMID: 15667034]

128 **Wedgwood S**, Dettman RW, Black SM. ET-1 stimulates pulmonary arterial smooth muscle cell proliferation via induction of reactive oxygen species. *Am J Physiol Lung Cell Mol Physiol* 2001; **281**: L1058-L1067 [PMID: 11597896]

129 **Xu R**, Yang R, Hu H, Xi Q, Wan H, Wu Y. Diabetes alters the expression of partial vasoactivators in cerebral vascular disease susceptible regions of the diabetic rat. *Diabetol Metab Syndr* 2013; **5**: 63 [PMID: 24499567]

130 **McDaid EA**, Monaghan B, Parker AI, Hayes JR, Allen JA. Peripheral autonomic impairment in patients newly diagnosed with type II diabetes. *Diabetes Care* 1994; **17**: 1422-1427 [PMID: 7882811]

131 **Hattori Y**, Hattori S, Sato N, Kasai K. High-glucose-induced nuclear factor kappaB activation in vascular smooth muscle cells. *Cardiovasc Res* 2000; **46**: 188-197 [PMID: 10727667]

132 **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]

133 **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]

134 **Libby P**. Current concepts of the pathogenesis of the acute coronary syndromes. *Circulation* 2001; **104**: 365-372 [PMID: 11457759]

135 **Taguchi S**, Oinuma T, Yamada T. A comparative study of cultured smooth muscle cell proliferation and injury, utilizing glycated low density lipoproteins with slight oxidation, auto-oxidation, or extensive oxidation. *J Atheroscler Thromb* 2000; **7**: 132-137 [PMID: 11480453]

136 **Brown GC**, Borutaite V. Nitric oxide, cytochrome c and mitochondria. *Biochem Soc Symp* 1999; **66**: 17-25 [PMID: 10989653]

137 **Andersson U**, Leighton B, Young ME, Blomstrand E, Newsholme EA. Inactivation of aconitase and oxoglutarate dehydrogenase in skeletal muscle in vitro by superoxide anions and/or nitric oxide. *Biochem Biophys Res Commun* 1998; **249**: 512-516 [PMID: 9712727]

138 **Beckman KB**, Ames BN. Endogenous oxidative damage of mtDNA. *Mutat Res* 1999; **424**: 51-58 [PMID: 10064849]

139 **Requena JR**, Fu MX, Ahmed MU, Jenkins AJ, Lyons TJ, Thorpe SR. Lipoxidation products as biomarkers of oxidative damage to proteins during lipid peroxidation reactions. *Nephrol Dial Transplant* 1996; **11** Suppl 5: 48-53 [PMID: 9044307]

140 **DeFronzo RA**, Ferrannini E. Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. *Diabetes Care* 1991; **14**: 173-194 [PMID: 2044434]

141 **Nishikawa T**, Edelstein D, Du XL, Yamagishi S, Matsumura T, Kaneda Y, Yorek MA, Beebe D, Oates PJ, Hammes HP, Giardino I, Brownlee M. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature* 2000; **404**: 787-790 [PMID: 10783895]

142 **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]

143 **Kouroedov A**, Eto M, Joch H, Volpe M, Lüscher TF, Cosentino F. Selective inhibition of protein kinase Cbeta2 prevents acute effects of high glucose on vascular cell adhesion molecule-1 expression in human endothelial cells. *Circulation* 2004; **110**: 91-96 [PMID: 15210597]

144 **Hink U**, Li H, Mollnau H, Oelze M, Matheis E, Hartmann M, Skatchkov M, Thaiss F, Stahl RA, Warnholtz A, Meinertz T, Griendling K, Harrison DG, Forstermann U, Munzel T. Mechanisms underlying endothelial dysfunction in diabetes mellitus. *Circ Res* 2001; **88**: E14-E22 [PMID: 11157681]

145 **Cosentino F**, Eto M, De Paolis P, van der Loo B, Bachschmid M, Ullrich V, Kouroedov A, Delli Gatti C, Joch H, Volpe M, Lüscher TF. High glucose causes upregulation of cyclooxygenase-2 and alters prostanoid profile in human endothelial cells: role of protein kinase C and reactive oxygen species. *Circulation* 2003; **107**: 1017-1023 [PMID: 12600916]

146 **Yan SF**, Ramasamy R, Schmidt AM. The RAGE axis: a fundamental mechanism signaling danger to the vulnerable vasculature. *Circ Res* 2010; **106**: 842-853 [PMID: 20299674 DOI: 10.1161/CIRCRESAHA.109.212217]

147 **Bierhaus A**, Humpert PM, Morcos M, Wendt T, Chavakis T, Arnold B, Stern DM, Nawroth PP. Understanding RAGE, the receptor for advanced glycation end products. *J Mol Med* (Berl) 2005; **83**: 876-886 [PMID: 16133426]

148 **Fülöp N**, Marchase RB, Chatham JC. Role of protein O-linked N-acetyl-glucosamine in mediating cell function and survival in the cardiovascular system. *Cardiovasc Res* 2007; **73**: 288-297 [PMID: 16970929]

149 **Buse MG**. Hexosamines, insulin resistance, and the complications of diabetes: current status. *Am J Physiol Endocrinol*

Metab 2006; **290**: E1-E8 [PMID: 16339923]
150 **Lee AY**, Chung SS. Contributions of polyol pathway to oxidative stress in diabetic cataract. *FASEB J* 1999; **13**: 23-30 [PMID: 9872926]
151 **Vikramadithyan RK**, Hu Y, Noh HL, Liang CP, Hallam K, Tall AR, Ramasamy R, Goldberg IJ. Human aldose reductase expression accelerates diabetic atherosclerosis in transgenic mice. *J Clin Invest* 2005; **115**: 2434-2443 [PMID: 16127462]
152 **Vincent AM**, Russell JW, Low P, Feldman EL. Oxidative stress in the pathogenesis of diabetic neuropathy. *Endocr Rev* 2004; **25**: 612-628 [PMID: 15294884]
153 **Shantikumar S**, Caporali A, Emanueli C. Role of microRNAs in diabetes and its cardiovascular complications. *Cardiovasc Res* 2012; **93**: 583-593 [PMID: 22065734 DOI: 10.1093/cvr/cvr300]
154 **Zampetaki A**, Mayr M. MicroRNAs in vascular and metabolic disease. *Circ Res* 2012; **110**: 508-522 [PMID: 22302757 DOI: 10.1161/CIRCRESAHA.111.247445]
155 **Zampetaki A**, Kiechl S, Drozdov I, Willeit P, Mayr U, Prokopi M, Mayr A, Weger S, Oberhollenzer F, Bonora E, Shah A, Willeit J, Mayr M. Plasma microRNA profiling reveals loss of endothelial miR-126 and other microRNAs in type 2 diabetes. *Circ Res* 2010; **107**: 810-817 [PMID: 20651284 DOI: 10.1161/CIRCRESAHA.110.226357]
156 **Wang XH**, Qian RZ, Zhang W, Chen SF, Jin HM, Hu RM. MicroRNA-320 expression in myocardial microvascular endothelial cells and its relationship with insulin-like growth factor-1 in type 2 diabetic rats. *Clin Exp Pharmacol Physiol* 2009; **36**: 181-188 [PMID: 18986336 DOI: 10.1111/j.1440-1681.2008.05057.x]
157 **Togliatto G**, Trombetta A, Dentelli P, Rosso A, Brizzi MF. MIR221/MIR222-driven post-transcriptional regulation of P27KIP1 and P57KIP2 is crucial for high-glucose- and AGE-mediated vascular cell damage. *Diabetologia* 2011; **54**: 1930-1940 [PMID: 21461636 DOI: 10.1007/s00125-011-2125 -5]
158 **Caporali A**, Meloni M, Völlenkle C, Bonci D, Sala-Newby GB, Addis R, Spinetti G, Losa S, Masson R, Baker AH, Agami R, le Sage C, Condorelli G, Madeddu P, Martelli F, Emanueli C. Deregulation of microRNA-503 contributes to diabetes mellitus-induced impairment of endothelial function and reparative angiogenesis after limb ischemia. *Circulation* 2011; **123**: 282-291 [PMID: 21220732 DOI: 10.1161/CIRCULATIONAHA.110.952325]
159 **Meng S**, Cao JT, Zhang B, Zhou Q, Shen CX, Wang CQ. Downregulation of microRNA-126 in endothelial progenitor cells from diabetes patients, impairs their functional properties, via target gene Spred-1. *J Mol Cell Cardiol* 2012; **53**: 64-72 [PMID: 22525256 DOI: 10.1016/j.yjmcc.2012.04.003]
160 **Wang DE**. MicroRNA Regulation and its Biological Significance in Personalized Medicine and Aging. *Curr Genomics* 2009; **10**: 143 [PMID: 19881907 DOI: 10.2174/138920209788185216]
161 **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]

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REVIEW

Type 1 diabetes and polyglandular autoimmune syndrome: A review

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Abstract

Type 1 diabetes (T1D) is an autoimmune disorder caused by inflammatory destruction of the pancreatic tissue. The etiopathogenesis and characteristics of the pathologic process of pancreatic destruction are well described. In addition, the putative susceptibility genes for T1D as a monoglandular disease and the relation to polyglandular autoimmune syndrome (PAS) have also been well explored. The incidence of T1D has steadily increased in most parts of the world, especially in industrialized nations. T1D is frequently associated with autoimmune endocrine and non-endocrine diseases and patients with T1D are at a higher risk for developing several glandular autoimmune diseases. Familial clustering is observed, which suggests that there is a genetic predisposition. Various hypotheses pertaining to viral- and bacterial-induced pancreatic autoimmunity have been proposed, however a definitive delineation of the autoimmune pathomechanism is still lacking. In patients with PAS, pancreatic and endocrine autoantigens either colocalize on one antigen-presenting cell or are expressed on two/various target cells sharing a common amino acid, which facilitates binding to and activation of T cells. The most prevalent PAS phenotype is the adult type 3 variant or PAS type Ⅲ, which encompasses T1D and autoimmune thyroid disease. This review discusses the findings of recent studies showing noticeable differences in the genetic background and clinical phenotype of T1D either as an isolated autoimmune endocrinopathy or within the scope of polyglandular autoimmune syndrome.

Key words: Autoimmune thyroid disease; Polyglandular autoimmune syndrome; Addison's disease; Susceptibility genes; Type 1 diabetes

Core tip: Type 1 diabetes (T1D) occurs in conjunction with several autoimmune endocrine and non-endocrine diseases. Recent studies have revealed noticeable differences in the genetic background and clinical phenotype of T1D either as an isolated autoimmune endocrinopathy or within the scope of polyglandular autoimmune syndrome. These findings are relevant for diagnostic and therapeutic procedures in daily practice as well as for the general understanding of endocrine autoimmunity.

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INTRODUCTION

Type 1 diabetes (T1D) is an endocrine disorder characterized by autoimmune destruction of insulin-producing pancreatic β-cells, which subsequently reduces insulin production and induces metabolic dysregulation[1-4]. Although T1D onset was once thought to be restricted to children and adolescents, it can occur at any age, with the highest rate of incidence below the age of 30 years[5-7]. Approximately 50 T1D susceptibility genes have been identified to date. These genes also carry a potential risk for various autoimmune diseases occurring simultaneously or within a narrow time interval and might explain to some extent why additional endocrine autoimmune diseases are comorbid in one third of all T1D patients[8-12]. These associated autoimmune disorders are either glandular diseases [*e.g.*, Addison's disease or autoimmune thyroid disease (AITD)] that lead to polyglandular autoimmune syndrome (PAS) or non-glandular autoimmune diseases (*e.g.,* rheumatoid arthritis or celiac disease)[13-15]. The variation in these comorbidities may hold the key to understanding the pathogenesis of autoimmune diseases, but also simultaneously complicates the diagnosis and treatment of T1D and is therefore of interest to both scientists and clinicians.

ISOLATED T1D

Approximately 5%-10% of all newly diagnosed patients with diabetes mellitus (nearly 400 million subjects worldwide) have T1D (20-40 million, accordingly)[5,16]. This number may be even higher as 5%-15% of all adults with type 2 diabetes are positive for pancreatic islet autoantibodies[17,18]. The age-adjusted incidence ranges from 0.1:100000/year (*e.g.,* China) to 40.9:100000/year (Finland), while the highest incidence rates are found in North American and European populations. Large studies confirmed a continuing rise of T1D incidence in Europe from 1989 through 2008 by approximately 3%-4% per year, which is higher than the average annual increase of 2.8%[19]. There is a subtle gender bias, where males have the highest incidence between 10-14 years of age and females have the highest incidence between the ages of 5 and 9 years[5,20,21]. The initial onset of T1D occurs primarily between the ages of 8 and 14 years, in close proximity to the start of puberty[22].

Clinical spectrum and diagnosis

Clinical symptoms, caused by the high glucose levels from T1D, develop quickly and range from chronic fatigue, weight loss, polydipsia and polyuria to symptoms of diabetic ketoacidosis (*e.g.,* nausea, acute abdomen or even coma). The diagnosis and differential diagnosis rely mainly on typical history and signs as well as measuring organ-specific autoantibodies directed against pancreatic islet cells, insulin, glutamate decarboxylase (GAD) and tyrosine phosphatase, which are positive in 95% of the cases at T1D onset. These pancreas autoantibodies may appear months or years before the clinical manifestation with various sensitivity, specificity and predictive relevance[23,24]. Positive titers of islet cell autoantibodies (ICAs), glutamic acid decarboxylase autoantibodies (GADAs), insulinoma-associated protein 2 autoantibodies (IA2As), insulin autoantibodies (IAAs) and the recently discovered zinc transporter 8 autoantibodies (ZnT8As) are important serologic diagnostic parameters. An early presence of autoantibodies is associated with a greater risk for T1D. The first antibodies to appear in young children are IAAs with a peak under the age of five years; a valid titer can only be measured before initiation of insulin therapy[25]. While titers of ICAs, ZnT8As and IAAs have been reported to decline after the onset of T1D, GADAs persist for years in the sera of diabetic patients independent of inflammatory β-cell destruction[26]. Therefore, measurement of GADAs is preferred in adults with late onset diabetes mellitus. ZnT8As can be found in about a fourth of T1D patients seronegative for ICAs, GADAs, IA2As and IAAs, and in approximately one third of patients with autoimmune disorders associated with T1D (Table 1)[27,28]. Considering the prevalence of organ-specific autoantibodies and their role in diagnosis of T1D, an autoimmune component in the disease manifestation seems undeniable.

Pathogenesis

Inflammatory infiltrates predominantly consisting of $CD4^+$ and $CD8^+$ lymphocytes and macrophages in the pancreatic tissue of patients with recent onset of T1D make an autoimmune etiology most likely[3,29-31]. In addition to direct killing of β-cells by natural killer cells, with a subsequent expression and presentation of autoantigens and a loss of peripheral immunologic tolerance, recently detected β-cell regeneration in children with T1D and β-cell persistence in older patients highlight a more complex pathogenesis that includes the involvement of cytokines, regulatory T cells and hormones[31-34]. Several studies have confirmed a higher cumulative risk for T1D in family members (Table 2). According to twin studies, the genetic predisposition and environmental effects might contribute 80% and 20%, respectively, to the clinical phenotype of T1D[35,36]. Further studies focusing on serologic and genetic characteristics of these patients revealed a multitude of susceptibility genes, antigens, serologic markers and environmental risk factors.

Genetics

Familial clustering (λs) imparts a relative risk (RR) for siblings of T1D-affected patients compared to the general

Table 1 Characteristics of the relevant autoantibodies in type 1 diabetes[125-134]

	Antigen	Sensitivity	Specificity	Percent at onset	Annotation
ICA	Islet cells	70%	99%	70%-90%	Single positivity similar predictive;
GADA	Glutamic acid decarboxylase (65 kDa)	65%-75%	99%	70%-80%	in combination ⩾ 3 increasing risk to
IA2A	Tyrosine phosphatase-related islet antigen 2	50%-90%	99%	50%-70%	approximately 90%; age independent
IAA	Proinsulin/insulin	74%	99%	30%-50%	Inverse correlation with age; measurement prior to insulin therapy required
ZnT8	C terminal domain of the zinc transporter 8	65%-75%	99%	60%-80%	Declines rapidly after onset of T1D

IAA: Insulin autoantibody; IA2A: Tyrosine phosphatase-related islet antigen 2 autoantibodies; ICA: Islet cell autoantibodies; GADA: Glutamic acid decarboxylase autoantibodies; T1D: Type 1 diabetes; ZnT8: Zinc transporter 8.

Table 2 Involvement of family members of patients with type 1 diabetes[5,27,135]

Affected family member	Presence of T1D
First degree relative (general)	5%-6%
Mother	2%
Father	7%
Monozygotic twin	30%-50%
Dizygotic twin	6%-10%

T1D: Type 1 diabetes.

population, amounting to RR = 15[37]. Several affected sibling pair linkage studies showed the importance of genetic predisposition and the association of T1D with polymorphisms in the specific human leukocyte antigen (HLA) loci on chromosome 6p21.3[38,39]. HLA class Ⅱ loci are assumed to be responsible for 40%-50% genetic risk[40,41]. HLA-DR3 or -DR4, which can be detected in approximately 95% of Caucasian Anglo-Saxon patients with T1D, partly reflect the distribution of the incidence among different countries and ethnicities in their genotype frequencies. Several studies graded the susceptibility of HLA class Ⅱ genotypes[42-45] as follows: the highest risk was found in DR3/4 heterozygotes, followed by DR4 homozygotes, DR3 homozygotes and DR4 heterozygotes combined with another DR allele[27]. Furthermore, many non-HLA polymorphisms that appear to make a smaller contribution to the manifestation of T1D have been identified[46]. Nevertheless, a concordance rate lower than 50% in monozygotic twins, a manifestation of T1D in 10% of the carriers of high-risk genes and a 15-fold difference in the disease incidence among European Caucasians indicates that genetics alone cannot explain disease onset[8,47,48]. In contrast, an increase in patients with low-risk or protective HLA genotypes emphasizes the importance of environmental factors such as viral infections, nutrition and chemicals or epigenetics, respectively[49-52].

ASSOCIATION OF T1D WITH OTHER ENDOCRINOPATHIES

Additional or associated autoimmune glandular and non-glandular diseases in patients with T1D have been described and frequently involve organ-specific as well as systemic autoimmunity. The following autoimmune diseases are listed in the order of their frequency: autoimmune thyroid diseases (AITD, 15%-30%), autoimmune type A gastritis (15%), pernicious anemia (10%), celiac disease (4%-9%), vitiligo (1%-7%), rheumatoid arthritis (1.2%), systemic lupus erythematosus (1.15%), autoimmune adrenal failure or Addison's disease (0.5%) and multiple sclerosis (0.2%) (Table 3)[53-60]. In addition to a common environment, many overlapping risk factors for T1D and other autoimmune diseases have been identified. While a role for HLA class Ⅰ -recognizing CD8 T cells has been known to affect T1D and celiac disease, recent studies also showed a joint susceptibility for these diseases in HLA class Ⅱ[61]. HLA-DQ2 can be found in 90% of patients with celiac disease and in 55% of patients with T1D, while HLA-DQ8 is present in approximately 10% and 70%, respectively[62]. In patients with HLA-DQ2-DQ8 heterozygosity, a transdimer (DQ2α/8β) binds a gliadin peptide and T1D-specific antigens, which implicates both gluten and the gut microbiome as additional factors or triggers for autoimmune diseases, respectively[63-66]. Because the co-occurrence of non-glandular immunopathies such as autoimmune gastritis and pernicious anemia may lead to an atypical clinical presentation and additional discomfort, early and regular screening for serologic parameters (*e.g.*, parietal cell antibodies) and red blood cell count is recommended[67].

The manifestation of additional glandular autoimmune diseases in association with T1D has recently become of particular interest for research on the common pathogenesis of general autoimmunity. PAS characterized by a combination of at least two autoimmune endocrinopathies can be classified into a juvenile form (PAS type Ⅰ) and an adult form, which is then subdivided according to the specific constellation of autoimmune glandular diseases (PAS types Ⅱ-Ⅳ)[68-70].

T1D WITHIN THE SCOPE OF JUVENILE PAS TYPE I

PAS type Ⅰ, also known as Whitaker's syndrome, autoimmune polyendocrinopathy-candidiasis-ectodermal-

Table 3 Prevalence of associated autoimmunity in patients with type 1 diabetes[15,55,56,60,97,100,136-143]

Associated disease	Patients with type 1 diabetes		General population	
	Prevalence of organ-specific Abs	Overt disease	Prevalence of organ-specific Abs	Overt disease
Type 1 diabetes	ICA in 85%-90%	100%	ICA in 1%-3 %	0.1%-1.0%
Hashimoto's thyroiditis	TPO Abs in 15%-30%	10%-20%	TPO Abs in 2%-10%	0.5%-9.0%
Graves' disease	TSH-R Abs in 1%-18%	3%-6%	TSH-R Abs in 1%-2%	0.1%-2.0%
Addison's disease	21-OH Abs in 0.7%-2.0%	0.5%-0.8%	21-OH Abs in 0.6%	0.005%-0.140%
Autoimmune hypophysitis and/or hypopituitarism	Pituitary Abs in 3.6%	0.4%-0.9%	Pituitary Abs in 0.5%	0.24%-0.80%
Autoimmune type A gastritis and pernicious anemia	Gastric parietal cell Abs in 13%-25%	5%-10% (2%-6%)	Gastric parietal cell Abs in 2.5%-12.0%	2% (0.15%-1.00%)
Celiac disease	Transglutaminase Abs in 8%-12%	1%-9%	Transglutaminase Abs in 0.5%-1.0%	0.50%

Abs: Antibodies; ICA: Islet cell antibodies; TPO: Thyroperoxidase; TSH-R: Thyrotropin receptor antibodies; 21-OH: 21 Hydroxylase.

dystrophy or multiple endocrine deficiency autoimmune candidiasis syndrome, is a hereditary disorder with disease manifestation that occurs in a characteristic order at an early age. Mucocutaneous candidiasis is typically the first of the three major components to occur, typically prior to five years of age. Before the age of ten years, hypoparathyroidism becomes apparent and precedes Addison's disease, which is usually the last disorder to appear (in many cases before the age of 15 years). By definition, at least two of these major components must be present for PAS type Ⅰ. Additional disorders were described that occurred prior to the fifth decade[71,72]. T1D was found in 12%-33% of all patients with PAS type Ⅰ[72,73]. Several studies have suggested that a young age of clinical onset correlates with the manifestation of multiple concomitant autoimmune diseases[74,75]. As a monogenetic disease with autosomal recessive inheritance caused by mutations in the autoimmune regulatory gene on chromosome 21, the prevalence of PAS Ⅰ varies highly between ethnicities ranging from 1:6500 in Iranian Jews to 1:10000000 in the Japanese population, with a female/male ratio of 0.8-2.4[76-78].

Screening for the co-occurrence of T1D in patients with PAS Ⅰ is less effective. This is because the positive predictive value of ICAs and GAD65 autoantibodies is only 27%, whereas 18%-28% of PAS type Ⅰ patients without T1D have islet cell autoantibodies present[73,79]. This peculiarity led to the hypothesis that the detected autoantibody epitopes differ from those in patients with isolated T1D and that a limited, subclinical autoimmune reaction within the pancreas may exist without causing an overt clinical manifestation[73,80]. A novel β-cell antigen, initially identified as a 51 kDa protein, was found to be aromatic-L-amino-acid decarboxylase[81-83]. Though no correlation of T1D manifestation in PAS and anti-aromatic-L-amino-acid decarboxylase autoantibodies has been found yet, its high prevalence in PAS type Ⅰ-associated diseases (*e.g.,* vitiligo and autoimmune hepatitis) warrants further research on its role in disease pathogenesis of autoimmune disorders[82]. Similar to isolated T1D, the combination of autoantibodies in polyendocrinopathies has been suggested to provide a higher predictive value than any isolated autoantibody.

T1D WITHIN THE SCOPE OF THE ADULT PAS (TYPES Ⅱ-Ⅳ)

T1D is the most frequent disorder of the PAS and is often the first disease to appear. The exact immunopathogenesis has not been fully elucidated, but several studies provide evidence for common immunologic mechanisms induced by environmental factors in a background with genetic polymorphisms[84-86]. The frequent finding of combined manifestations of autoimmune glandular diseases led to a sub-classification for adult PAS as follows[68,87]: (1) PAS type Ⅱ: Addison's disease in combination with at least one additional autoimmune endocrinopathy (*e.g.,* T1D); (2) PAS type Ⅲ: autoimmune thyroid disease in combination with T1D but excluding Addison's disease; (3) PAS type Ⅳ: combination of at least two autoimmune endocrinopathies but excluding PAS types Ⅰ-Ⅲ.

Clinical spectrum and diagnosis of T1D within PAS types Ⅱ-Ⅳ

In approximately 40%-50% of patients with Addison's disease, additional autoimmune glandular diseases occur and become overt as PAS type Ⅱ. Of these, T1D is apparent in 12%-24%[88-90]. Autoantibodies directed against the adrenal cortex are found in 0.7%-3.0% of T1D patients. Although T1D often develops before Addison's disease, GAD65 antibodies are detected in 5%-7% of patients with Addison's disease but without T1D, thus a thorough follow-up should be performed in islet cell antibody-positive patients. The concomitant presence of Addison's disease and T1D leads to frequent hypoglycemia due to decreased gluconeogenesis and increased insulin sensitivity. Thus, autoimmune-induced adrenal failure should be considered in patients with T1D suffering from unexplained recurrent hypoglycemia and fatigue, whereas insulin therapy combined with cortisol substitution warrants close monitoring during treatment of T1D patients with adrenal failure.

PAS type Ⅲ is the most frequent subtype of poly-glandular autoimmune diseases, containing 41% of the possible endocrine component combinations[68]. The co-occurrence of autoimmune-induced hypothyroidism (generally caused by chronic lymphocytic Hashimoto's

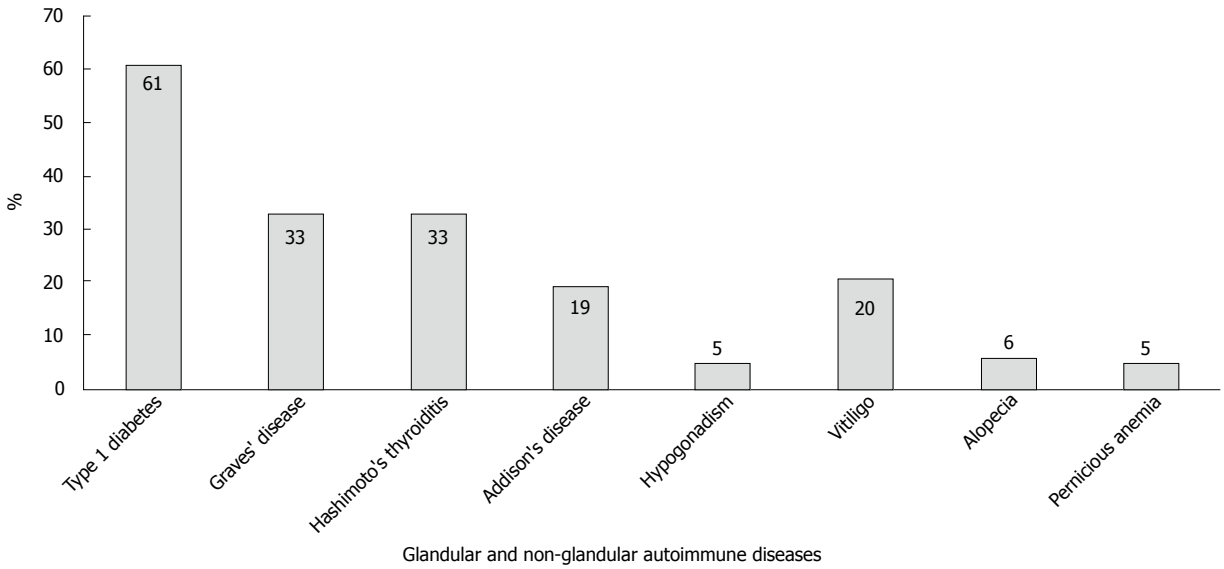

Figure 1 Endocrine and non-endocrine autoimmune diseases in patients with polyglandular autoimmune syndrome. The prevalence of glandular (dark grey) and non-glandular (light grey) autoimmune diseases in the 151 patients with adult polyglandular autoimmune syndrome (PAS) followed at the Johannes Gutenberg University Medical Center.

thyroiditis) and T1D is often accompanied by hypoglycemia due to increased insulin sensitivity. Hypothyroidism leads to a reduction in glucose resorption in the duodenum and glucose release from the liver. Because patients exhibit a decreased appetite and intake of calories, the risk for hypoglycemia is significantly enhanced[91-93]. During the hypothyroid phase, the insulin dosage should be carefully evaluated and a reduction by approximately 20%-25% for 3-4 wk is recommended. After substitution with levothyroxine, the baseline insulin dosage may be administered again, after the patient becomes biochemically euthyroid. In hypothyroid children, chronic hypoglycemia and decreased food intake frequently lead to growth disorders. Either anti-thyroid peroxidase and/or antithyroglobulin autoantibodies are present in 19%-24% of T1D patients, whereas hypothyroidism (subclinical with normal free thyroid hormone levels but pathologically increased baseline serum thyroid-stimulating hormone) is observed in 10%-20% of patients[12,94-96]. In comparison, subclinical and overt hyperthyroidism occur less frequently (3% and 6%, respectively)[97]. Overt hyperthyroidism is accompanied in 50% of the cases by glucose intolerance and in 3% of the cases by overt diabetes. The impaired glucose tolerance is due to decreased insulin sensitivity and decreased hepatic storage of glycogen, whereas both secretion of glucagon and intestinal glucose absorption are enhanced. Thus, hyperthyroidism increases glucose resorption and hepatic glucose release leading to hyperglycemia. In T1D patients, this leads to insulin resistance and an increased release of fatty acids causing ketoacidosis[53,91,98]. T1D usually manifests at a very young age. Moreover, in 60% of PAS type Ⅲ patients, Graves' hyperthyroidism may occur prior to T1D, as has been reported in Japanese populations, usually within a time period of less than ten years[99]. Onset of T1D in patients with Graves' disease and Hashimoto's thyroiditis occurred at a mean age of 34 years in 0.78% and 1.17% of cases, respectively[100]. ZnT8As, and especially GADAs, are observed more frequently in PAS type Ⅲ than in isolated T1D, while IA2As may indicate a slow onset of T1D[99]. In addition, in patients with T1D and PAS type Ⅲ, gastric parietal cell and adrenocortical autoantibodies have been observed in 16.8% and 5.1% of cases, respectively[96].

PAS type Ⅳ is a very heterogeneous and less well-defined group of polyglandular autoimmune diseases. It is frequently incorrectly published that this syndrome is defined as the combination of a monoglandular autoimmune disease (*e.g.,* T1D) with a non-glandular autoimmune disease (*e.g.,* autoimmune gastritis or celiac disease). In PAS type Ⅳ, pituitary antibodies have been detected in 3.6% of T1D patients, and clinically overt pituitary failure was noted in 0.9%[101]. Aside from PAS type Ⅰ, the combinations of T1D with autoimmune hypopituitarism or hypergonadotrophic hypogonadism as rare forms of PAS type Ⅳ have an estimated prevalence of < 1% and are rarely described in the literature[102,103].

Our own findings

In a screening of 471 consecutive T1D patients that were followed at the endocrine outpatient clinic at the Johannes Gutenberg University Medical Center, multiple glandular involvement and PAS type Ⅲ were found in 27% (n = 127) and 10%, respectively[104]. Subsequent prospective screening of 15000 consecutive patients with monoglandular autoimmune disease (*e.g.,* T1D) revealed a high prevalence (1%) of patients with the adult PAS types Ⅱ-Ⅳ, with a female bias of 75%. Figure 1 shows the various spectrums of autoimmune diseases registered in our PAS cohort. Significant male and female biases were noted for T1D and Hashimoto's thyroiditis, respectively. T1D manifested early (mean: 27.5 years), whereas other component diseases appeared later, ranging from an age

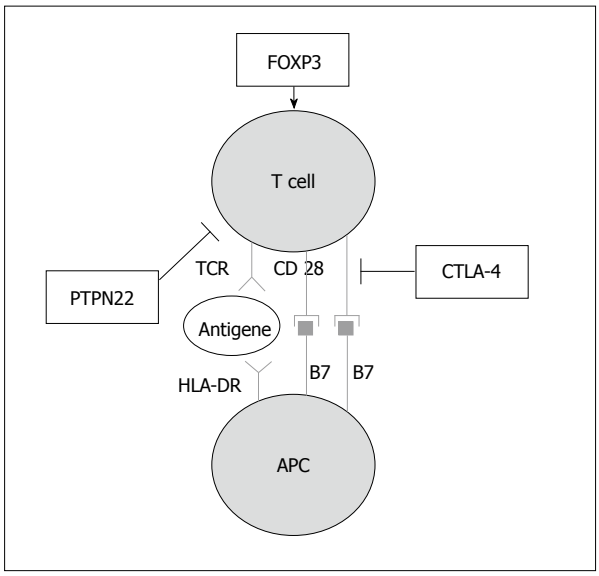

Figure 2 Immunologic synapse. This schematic depicts T cell activation and how it is influenced by expression of common susceptibility genes. Shared susceptibility genes for autoimmune thyroid disease and type 1 diabetes are involved in the immunological synapse. HLA-DR molecules present autoantigens to T cells, CTLA-4 expression suppresses T cell activation, PTPN22 expression negatively influences the T cell receptor (TCR) signaling pathway and FOXP3 expression regulates the differentiation of regulatory T cells (modified according to ref.[124]). APC: Antigen presenting cell; CTLA-4: Cytotoxic T lymphocyte antigen 4; HLA: Human leukocyte antigen; PTPN22: Protein tyrosine phosphatase non-receptor type 22; FOXP3: Forkhead box protein P3.

of 36.5-40.5 years. T1D was also the first component disease of adult PAS in half of the patients (48.3%), whereas Graves' disease (19.2%), Hashimoto's thyroiditis (17.2%), Addison's disease (14.6%) and vitiligo (12.6%) were less likely to be the first component disease. The predominant frequency of the coexistence of T1D and AITD was confirmed in our large collective. The time interval between manifestations of the first and second endocrinopathies varied considerably, with the longest time intervals between T1D and AITD, and a short time interval between Addison's disease and AITD[105].

GENETICS OF THE ADULT PAS TYPES Ⅱ-Ⅳ

Unlike the 1:1 gender ratio of isolated T1D and PAS type Ⅰ, there is a clear female bias of 3:1 in adult PAS, with a prevalence of 1:20000[13,104,106]. The incidence of adult PAS is approximately 1:100000/year and has a peak in the third or fourth decade of life. For a majority of the glandular autoimmune disorders, common susceptibility genes have been identified, including polymorphisms in protein tyrosine phosphatase non-receptor type 22, cytotoxic T lymphocyte antigen 4 (CTLA-4), MHC class I polypeptide-related sequence A, and HLA (Table 4, Figure 2). Thus, the association of endocrine autoimmune diseases is primarily due to a common genetic predisposition. The HLA class Ⅱ haplotypes

Table 4 Odds ratio of susceptibility genes for autoimmune endocrinopathies[117,144-160]

	T1D	HT	GD	AD
HLA-DR3	3.5	3.7	2-4	5
MICA	1.6	2.5	2	7
PTPN22	1.8	1.6	1.6	1.5
CTLA-4	1.5	5	1.5	1.8

AD: Addison's disease; CTLA-4: Cytotoxic T lymphocyte antigen 4; GD: Graves' disease; HLA: Human leukocyte antigen; HT: Hashimoto's thyroiditis; MICA: MHC class I polypeptide-related sequence A; PTPN22: Protein tyrosine phosphatase non-receptor type 22; T1D: Type 1 diabetes.

DRB1*03-DQA1*0501-DQB1*0201 and DRB1*04-DQA1-0301-DQB1*0302 have been reported to be associated with isolated T1D as well as with T1D within the scope of adult PAS[10,107]. This joint susceptibility for both T1D and AITD has been demonstrated in both Caucasians and in Asians[108-112]. CTLA-4 A/G49 single nucleotide polymorphisms (SNP) confer susceptibility to PAS type Ⅲ[113,114]. In particular, the CTLA-4 SNP rs3087243 (+ 6230 G > A) variant seems to predispose patients to a combined manifestation of T1D and Graves' disease[115]. The 1858 C→T substitution in the protein tyrosine phosphatase non-receptor type 22 gene is associated with AITD, isolated T1D and PAS type Ⅲ and the G1,123C SNP is associated with T1D and AITD in Asians[116-119]. Additionally, a SNP in the forkhead box P3 (*FOXP3*) gene on the arm of the X chromosome has been associated with increased susceptibility to PAS type Ⅲ in Caucasians[113]. A mutation in *FOXP3* has also been shown to be the susceptibility gene in the extremely rare immunodysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome[120]. Typically, T1D is associated with severe enteropathy, hypothyroidism and autoimmune skin diseases such as psoriasis, neurodermitis and psoriasis vulgaris[121]. There is a large variability in the organs affected by the additional autoimmune diseases in the severe IPEX syndrome and many patients die in infancy. Because *FOXP3* plays an important role in the function of regulatory T cells, a recent study suggested a similar CD25-correlated pathogenesis in isolated T1D and T1D within the context of the IPEX syndrome (Table 5)[122,123].

CONCLUSION

In isolation as a monoglandular disease, or within the larger context of PAS, the manifestation of T1D justifies an extensive serologic and functional screening for additional autoimmune glandular and gastrointestinal diseases both in patients with T1D of recent onset as well as every two years during patient follow-up (Figure 3). In particular, in families with clustering of T1D patients or in families of patients with PAS, the risk for associated autoimmune diseases and endocrine or autoimmune involvement of the first-degree relatives is significantly

Table 5 Polyglandular autoimmune syndromes[13,68,78,161-166]

	PAS Type Ⅰ	PAS Type Ⅱ-Ⅳ	IPEX
Onset	Childhood	Adulthood	Infancy
Incidence	< 1:100000/yr	1–2:100000/yr	Extremely rare
Male/Female ratio	3:04	1:03	Male >> Female
Genetics	Monogenetic (AIRE)	Polygenetic	X-linked (FOXP3)
Autoantibodies	Anti-interferon-α/ω antibodies 100%, additional Abs	Organ-specific Abs depending on the autoimmune components	ANA (42%) SSA (25%) TG Abs (25%)
Prevalence of T1D	2%-33%	40%-60%	80%
Additional autoimmune endocrine components	Hypoparathyroidism (80%-85%) Addison's disease (60%-70%) Hypogonadism (12%) Autoimmune thyroid disease (10%)	Autoimmune thyroid disease (70%-75%) Addison's disease (40%-50%) Hypoparathyroidism (0%-5%) Hypogonadism (0%-3%) Hypopituitarism (0%-2%)	Autoimmune thyroid disease (25%)
Concomitant non-endocrine diseases	Mucocutaneous candidiasis (70%-80%); autoimmune hepatitis; autoimmune gastritis; alopecia areata; vitiligo; keratoconjunctivitis	Autoimmune gastritis; pernicious anemia; neurodermitis; alopecia areata; myasthenia gravis; systemic lupus erythematosus; rheumatoid arthritis; autoimmune hepatitis	Malabsorption; autoimmune skin diseases; multiple sclerosis

Abs: Antibodies; AIRE: Autoimmune regulatory gene; ANA: Anti-nuclear antibodies; FOXP3: Forkhead box protein P3; IPEX: Immunodysregulation, polyendocrinopathy, enteropathy, X-linked syndrome; PAS: Polyglandular autoimmune syndrome; TG: Transglutaminase; T1D: Type 1 diabetes.

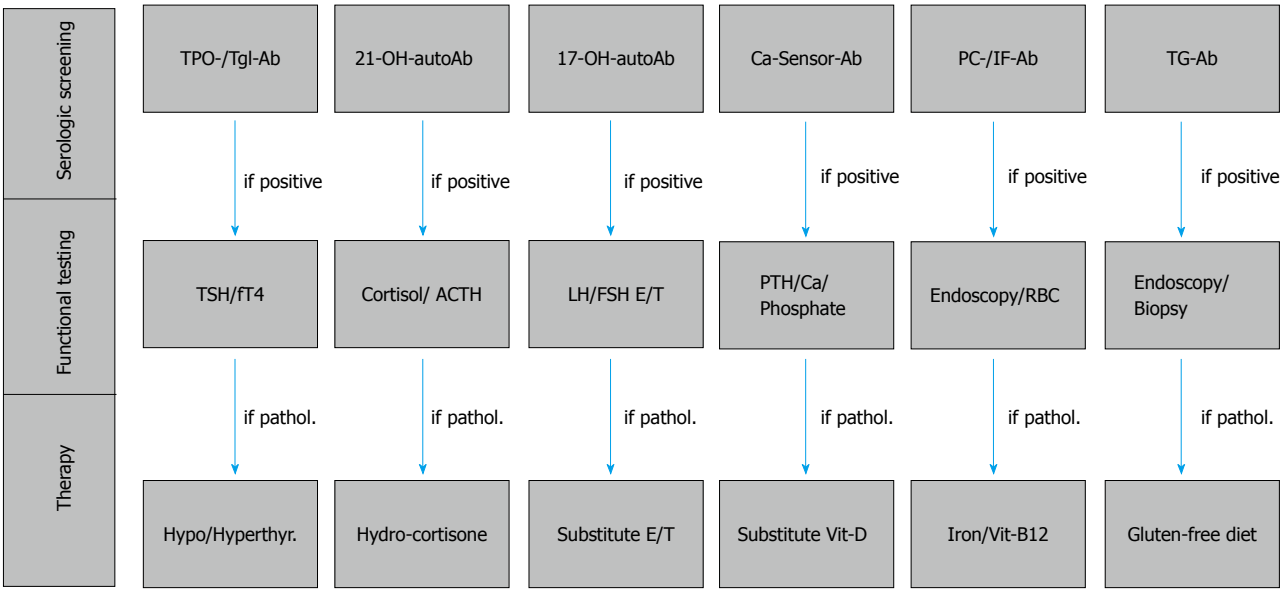

Figure 3 Serologic and functional screening in patients with type 1 diabetes. The serologic and functional screening for associated autoimmune diseases in patients with type 1 diabetes (T1D) performed at the onset of T1D and during follow-up appointments every two years. After diagnosis of thyroid dysfunction, adrenal failure, primary hypogonadism, hypoparathyroidism, type A autoimmune gastritis with or without pernicious anemia and celiac disease, substitution proceeds with levothyroxine, hydrocortisone, estradiol or testosterone, vitamin D, iron tablets and vitamin B12 intramuscularly, with a strict gluten-free diet. In contrast, hyperthyroidism due to the autoimmune Graves' disease will be managed first with the administration of anti-thyroid drugs (*e.g.*, methimazole). Ab: Antibody; ACTH: Adrenocorticotropic hormone; Ca: Calcium; Ca-Sensor: Calcium-sensing receptor; E: Estradiol; FSH: Follicle-stimulating hormone; fT4: Free thyroxine; Hypo: Hypothyroidism; Hyperthyr: Hyperthyroidism; IF: Intrinsic factor; LH: Luteinizing hormone; PC: Parietal cell; PTH: Parathyroid hormone; RBC: Red blood cell count; T: Total testosterone; TG: Transglutaminase/deaminated anti-gliadin; Tgl: Thyroglobulin; TPO: Thyroid peroxidase; TSH: Thyrotropin; Vit: Vitamin; 17-OH: 17-hydroxylase; 21-OH: 21-hydroxylase.

high. Within a few years, approximately one third of T1D patients will develop thyroid autoantibodies and thyroid dysfunction leading to PAS type Ⅲ. Furthermore, in subjects with either monoglandular T1D or the relatively rare autoimmune adrenal failure, organ-specific autoantibody screening and functional testing will help identify both patients at risk for developing PAS, as well as subclinical PAS that may already be present. Clinicians should pay particular attention to autoimmune endocrinopathies, (*e.g.,* Addison's disease or AITD), which are associated with T1D and strongly impact the patients' treatment with insulin. Thus, adrenal 21-hydroxylase autoantibodies should be assayed in all patients with T1D and GAD antibodies should be examined in all patients

with Addison's disease for early identification of subjects with a preclinical manifestation of a PAS. In conclusion, management of T1D within the context of PAS requires professional oversight and intervention provided in specialized centers for autoimmune endocrine and metabolic disorders.

REFERENCES

1 **Atkinson MA**. The pathogenesis and natural history of type 1 diabetes. *Cold Spring Harb Perspect Med* 2012; **2**: pii [PMID: 23125199 DOI: 10.1101/cshperspect.a007641]
2 **Bluestone JA**, Herold K, Eisenbarth G. Genetics, pathogenesis and clinical interventions in type 1 diabetes. *Nature* 2010; **464**: 1293-1300 [PMID: 20432533]
3 **Gillespie KM**. Type 1 diabetes: pathogenesis and prevention. *CMAJ* 2006; **175**: 165-170 [PMID: 16847277 DOI: 10.1503/cmaj.060244]
4 **Todd JA**. Etiology of type 1 diabetes. *Immunity* 2010; **32**: 457-467 [PMID: 20412756 DOI: 10.1016/j.immuni.2010.04.001]
5 **Tuomilehto J**. The emerging global epidemic of type 1 diabetes. *Curr Diab Rep* 2013; **13**: 795-804 [PMID: 24072479 DOI: 10.1007/s11892-013-0433-5]
6 **DIAMOND Project Group**. Incidence and trends of childhood Type 1 diabetes worldwide 1990-1999. *Diabet Med* 2006; **23**: 857-866 [PMID: 16911623 DOI: 10.1111/j.1464-5491.2006.01925.x]
7 **Cerna M**, Kolostova K, Novota P, Romzova M, Cejkova P, Pinterova D, Pruhova S, Treslova L, Andel M. Autoimmune diabetes mellitus with adult onset and type 1 diabetes mellitus in children have different genetic predispositions. *Ann N Y Acad Sci* 2007; **1110**: 140-150 [PMID: 17911429 DOI: 10.1196/annals.1423.016]
8 **Xie Z**, Chang C, Zhou Z. Molecular mechanisms in autoimmune type 1 diabetes: a critical review. *Clin Rev Allergy Immunol* 2014; **47**: 174-192 [PMID: 24752371 DOI: 10.1007/s12016-014-8422-2]
9 **Morahan G**. Insights into type 1 diabetes provided by genetic analyses. *Curr Opin Endocrinol Diabetes Obes* 2012; **19**: 263-270 [PMID: 22732486 DOI: 10.1097/MED.0b013e328355b7fe]
10 **Weinstock C**, Matheis N, Barkia S, Haager MC, Janson A, Marković A, Bux J, Kahaly GJ. Autoimmune polyglandular syndrome type 2 shows the same HLA class II pattern as type 1 diabetes. *Tissue Antigens* 2011; **77**: 317-324 [PMID: 21388354 DOI: 10.1111/j.1399-0039.2011.01634.x]
11 **Steck AK**, Rewers MJ. Genetics of type 1 diabetes. *Clin Chem* 2011; **57**: 176-185 [PMID: 21205883 DOI: 10.1373/clinchem.2010.148221]
12 **Triolo TM**, Armstrong TK, McFann K, Yu L, Rewers MJ, Klingensmith GJ, Eisenbarth GS, Barker JM. Additional autoimmune disease found in 33% of patients at type 1 diabetes onset. *Diabetes Care* 2011; **34**: 1211-1213 [PMID: 21430083 DOI: 10.2337/dc10-1756]
13 **Hansen MP**, Kahaly GJ. [Autoimmune polyglandular syndromes]. *Dtsch Med Wochenschr* 2013; **138**: 319-326; quiz 327-328 [PMID: 23393002 DOI: 10.1055/s-0032-1327355]
14 **Fröhlich-Reiterer EE**, Hofer S, Kaspers S, Herbst A, Kordonouri O, Schwarz HP, Schober E, Grabert M, Holl RW. Screening frequency for celiac disease and autoimmune thyroiditis in children and adolescents with type 1 diabetes mellitus--data from a German/Austrian multicentre survey. *Pediatr Diabetes* 2008; **9**: 546-553 [PMID: 18713134 DOI: 10.1111/j.1399-5448.2008.00435.x]
15 **Van den Driessche A**, Eenkhoorn V, Van Gaal L, De Block C. Type 1 diabetes and autoimmune polyglandular syndrome: a clinical review. *Neth J Med* 2009; **67**: 376-387 [PMID: 20009114]
16 **International Diabetes Federation**. IDF Diabetes Atlas teB. Belgium: International Diabetes Federation, 2013. Available from: URL: http: //www.idf.org/diabetesatlas
17 **Tuomi T**. Type 1 and type 2 diabetes: what do they have in common? *Diabetes* 2005; **54** Suppl 2: S40-S45 [PMID: 16306339]
18 **Atkinson MA**, Eisenbarth GS, Michels AW. Type 1 diabetes. *Lancet* 2014; **383**: 69-82 [PMID: 23890997 DOI: 10.1016/S0140-6736(13)60591-7]
19 **Patterson CC**, Gyürüs E, Rosenbauer J, Cinek O, Neu A, Schober E, Parslow RC, Joner G, Svensson J, Castell C, Bingley PJ, Schoenle E, Jarosz-Chobot P, Urbonaité B, Rothe U, Krzisnik C, Ionescu-Tirgoviste C, Weets I, Kocova M, Stipancic G, Samardzic M, de Beaufort CE, Green A, Dahlquist GG, Soltész G. Trends in childhood type 1 diabetes incidence in Europe during 1989-2008: evidence of non-uniformity over time in rates of increase. *Diabetologia* 2012; **55**: 2142-2147 [PMID: 22638547 DOI: 10.1007/s00125-012-2571-8]
20 **Harjutsalo V**, Sjöberg L, Tuomilehto J. Time trends in the incidence of type 1 diabetes in Finnish children: a cohort study. *Lancet* 2008; **371**: 1777-1782 [PMID: 18502302 DOI: 10.1016/s0140-6736(08)60765-5]
21 **Patterson CC**, Dahlquist GG, Gyürüs E, Green A, Soltész G. Incidence trends for childhood type 1 diabetes in Europe during 1989-2003 and predicted new cases 2005-20: a multicentre prospective registration study. *Lancet* 2009; **373**: 2027-2033 [PMID: 19481249 DOI: 10.1016/s0140-6736(09)60568-7]
22 **Pundziute-Lyckå A**, Dahlquist G, Nyström L, Arnqvist H, Björk E, Blohmé G, Bolinder J, Eriksson JW, Sundkvist G, Ostman J. The incidence of Type I diabetes has not increased but shifted to a younger age at diagnosis in the 0-34 years group in Sweden 1983-1998. *Diabetologia* 2002; **45**: 783-791 [PMID: 12107721 DOI: 10.1007/s00125-002-0845-2]
23 **Notkins AL**, Lernmark A. Autoimmune type 1 diabetes: resolved and unresolved issues. *J Clin Invest* 2001; **108**: 1247-1252 [PMID: 11696564 DOI: 10.1172/jci14257]
24 **Kimpimäki T**, Kulmala P, Savola K, Kupila A, Korhonen S, Simell T, Ilonen J, Simell O, Knip M. Natural history of beta-cell autoimmunity in young children with increased genetic susceptibility to type 1 diabetes recruited from the general population. *J Clin Endocrinol Metab* 2002; **87**: 4572-4579 [PMID: 12364437]
25 **Hoppu S**, Ronkainen MS, Kimpimäki T, Simell S, Korhonen S, Ilonen J, Simell O, Knip M. Insulin autoantibody isotypes during the prediabetic process in young children with increased genetic risk of type 1 diabetes. *Pediatr Res* 2004; **55**: 236-242 [PMID: 14605243 DOI: 10.1203/01.pdr.0000100905.41131.3f]
26 **Schmidli RS**, DeAizpurua HJ, Harrison LC, Colman PG. Antibodies to glutamic acid decarboxylase in at-risk and clinical insulin-dependent diabetic subjects: relationship to age, sex and islet cell antibody status, and temporal profile. *J Autoimmun* 1994; **7**: 55-66 [PMID: 8198702 DOI: 10.1006/jaut.1994.1005]
27 **Gan MJ**, Albanese-O'Neill A, Haller MJ. Type 1 diabetes: current concepts in epidemiology, pathophysiology, clinical care, and research. *Curr Probl Pediatr Adolesc Health Care* 2012; **42**: 269-291 [PMID: 23046732 DOI: 10.1016/j.cppeds.2012.07.002]
28 **Wenzlau JM**, Juhl K, Yu L, Moua O, Sarkar SA, Gottlieb P, Rewers M, Eisenbarth GS, Jensen J, Davidson HW, Hutton JC. The cation efflux transporter ZnT8 (Slc30A8) is a major autoantigen in human type 1 diabetes. *Proc Natl Acad Sci USA* 2007; **104**: 17040-17045 [PMID: 17942684 DOI: 10.1073/pnas.0705894104]
29 **Foulis AK**, McGill M, Farquharson MA. Insulitis in type 1 (insulin-dependent) diabetes mellitus in man--macrophages, lymphocytes, and interferon-gamma containing cells. *J Pathol* 1991; **165**: 97-103 [PMID: 1744803 DOI: 10.1002/path.1711650203]
30 **Santamaria P**. The long and winding road to understanding and conquering type 1 diabetes. *Immunity* 2010; **32**: 437-445 [PMID: 20412754 DOI: 10.1016/j.immuni.2010.04.003]

31 **Li M**, Song LJ, Qin XY. Advances in the cellular immunological pathogenesis of type 1 diabetes. *J Cell Mol Med* 2014; **18**: 749-758 [PMID: 24629100 DOI: 10.1111/jcmm.12270]

32 **Cutolo M**, Paolino S, Sulli A, Smith V, Pizzorni C, Seriolo B. Vitamin D, steroid hormones, and autoimmunity. *Ann N Y Acad Sci* 2014; **1317**: 39-46 [PMID: 24739090 DOI: 10.1111/nyas.12432]

33 **Keenan HA**, Sun JK, Levine J, Doria A, Aiello LP, Eisenbarth G, Bonner-Weir S, King GL. Residual insulin production and pancreatic ß-cell turnover after 50 years of diabetes: Joslin Medalist Study. *Diabetes* 2010; **59**: 2846-2853 [PMID: 20699420 DOI: 10.2337/db10-0676]

34 **Gianani R**. Beta cell regeneration in human pancreas. *Semin Immunopathol* 2011; **33**: 23-27 [PMID: 21188381 DOI: 10.1007/s00281-010-0235-7]

35 **Kyvik KO**, Green A, Beck-Nielsen H. Concordance rates of insulin dependent diabetes mellitus: a population based study of young Danish twins. *BMJ* 1995; **311**: 913-917 [PMID: 7580548 DOI: 10.1136/bmj.311.7010.913]

36 **Hyttinen V**, Kaprio J, Kinnunen L, Koskenvuo M, Tuomilehto J. Genetic liability of type 1 diabetes and the onset age among 22,650 young Finnish twin pairs: a nationwide follow-up study. *Diabetes* 2003; **52**: 1052-1055 [PMID: 12663480 DOI: 10.2337/diabetes.52.4.1052]

37 **Risch N**. Assessing the role of HLA-linked and unlinked determinants of disease. *Am J Hum Genet* 1987; **40**: 1-14 [PMID: 3468804]

38 **Pociot F**, McDermott MF. Genetics of type 1 diabetes mellitus. *Genes Immun* 2002; **3**: 235-249 [PMID: 12140742 DOI: 10.1038/sj.gene.6363875]

39 **Singal DP**, Blajchman MA. Histocompatibility (HL-A) antigens, lymphocytotoxic antibodies and tissue antibodies in patients with diabetes mellitus. *Diabetes* 1973; **22**: 429-432 [PMID: 4541338]

40 **Buzzetti R**, Quattrocchi CC, Nisticò L. Dissecting the genetics of type 1 diabetes: relevance for familial clustering and differences in incidence. *Diabetes Metab Rev* 1998; **14**: 111-128 [PMID: 9679666]

41 **Cudworth AG**, Wolf E. The genetics of Type 1 (insulin-dependent) diabetes. *Curr Probl Clin Biochem* 1983; **12**: 45-64 [PMID: 6418443]

42 **Nguyen C**, Varney MD, Harrison LC, Morahan G. Definition of high-risk type 1 diabetes HLA-DR and HLA-DQ types using only three single nucleotide polymorphisms. *Diabetes* 2013; **62**: 2135-2140 [PMID: 23378606 DOI: 10.2337/db12-1398]

43 **Kockum I**, Sanjeevi CB, Eastman S, Landin-Olsson M, Dahlquist G, Lernmark A. Complex interaction between HLA DR and DQ in conferring risk for childhood type 1 diabetes. *Eur J Immunogenet* 1999; **26**: 361-372 [PMID: 10553503 DOI: 10.1046/j.1365-2370.1999.00173.x]

44 **Platz P**, Jakobsen BK, Morling N, Ryder LP, Svejgaard A, Thomsen M, Christy M, Kromann H, Benn J, Nerup J, Green A, Hauge M. HLA-D and -DR antigens in genetic analysis of insulin dependent diabetes mellitus. *Diabetologia* 1981; **21**: 108-115 [PMID: 6167481 DOI: 10.1007/BF00251276]

45 **Undlien DE**, Lie BA, Thorsby E. HLA complex genes in type 1 diabetes and other autoimmune diseases. Which genes are involved? *Trends Genet* 2001; **17**: 93-100 [PMID: 11173119 DOI: 10.1016/S0168-9525(00)02180-6]

46 **Steck AK**, Bugawan TL, Valdes AM, Emery LM, Blair A, Norris JM, Redondo MJ, Babu SR, Erlich HA, Eisenbarth GS, Rewers MJ. Association of non-HLA genes with type 1 diabetes autoimmunity. *Diabetes* 2005; **54**: 2482-2486 [PMID: 16046318 DOI: 10.2337/diabetes.54.8.2482]

47 **Knip M**, Simell O. Environmental triggers of type 1 diabetes. *Cold Spring Harb Perspect Med* 2012; **2**: a007690 [PMID: 22762021 DOI: 10.1101/cshperspect.a007690]

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

49 **Afonso G**, Mallone R. Infectious triggers in type 1 diabetes: is there a case for epitope mimicry? *Diabetes Obes Metab* 2013; **15** Suppl 3: 82-88 [PMID: 24003924 DOI: 10.1111/dom.12166]

50 **Luopajärvi K**, Savilahti E, Virtanen SM, Ilonen J, Knip M, Akerblom HK, Vaarala O. Enhanced levels of cow's milk antibodies in infancy in children who develop type 1 diabetes later in childhood. *Pediatr Diabetes* 2008; **9**: 434-441 [PMID: 18503496 DOI: 10.1111/j.1399-5448.2008.00413.x]

51 **Howard SG**, Lee DH. What is the role of human contamination by environmental chemicals in the development of type 1 diabetes? *J Epidemiol Community Health* 2012; **66**: 479-481 [PMID: 21502091 DOI: 10.1136/jech.2011.133694]

52 **MacFarlane AJ**, Strom A, Scott FW. Epigenetics: deciphering how environmental factors may modify autoimmune type 1 diabetes. *Mamm Genome* 2009; **20**: 624-632 [PMID: 19697079 DOI: 10.1007/s00335-009-9213-6]

53 **Perros P**, McCrimmon RJ, Shaw G, Frier BM. Frequency of thyroid dysfunction in diabetic patients: value of annual screening. *Diabet Med* 1995; **12**: 622-627 [PMID: 7554786 DOI: 10.1111/j.1464-5491.1995.tb00553.x]

54 **Barera G**, Bonfanti R, Viscardi M, Bazzigaluppi E, Calori G, Meschi F, Bianchi C, Chiumello G. Occurrence of celiac disease after onset of type 1 diabetes: a 6-year prospective longitudinal study. *Pediatrics* 2002; **109**: 833-838 [PMID: 11986443 DOI: 10.1542/peds.109.5.833]

55 **Barker JM**. Clinical review: Type 1 diabetes-associated autoimmunity: natural history, genetic associations, and screening. *J Clin Endocrinol Metab* 2006; **91**: 1210-1217 [PMID: 16403820 DOI: 10.1210/jc.2005-1679]

56 **Barker JM**, Yu J, Yu L, Wang J, Miao D, Bao F, Hoffenberg E, Nelson JC, Gottlieb PA, Rewers M, Eisenbarth GS. Autoantibody "subspecificity" in type 1 diabetes: risk for organ-specific autoimmunity clusters in distinct groups. *Diabetes Care* 2005; **28**: 850-855 [PMID: 15793184]

57 **Liao KP**, Gunnarsson M, Källberg H, Ding B, Plenge RM, Padyukov L, Karlson EW, Klareskog L, Askling J, Alfredsson L. Specific association of type 1 diabetes mellitus with anti-cyclic citrullinated peptide-positive rheumatoid arthritis. *Arthritis Rheum* 2009; **60**: 653-660 [PMID: 19248096 DOI: 10.1002/art.24362]

58 **Van Hattem S**, Bootsma AH, Thio HB. Skin manifestations of diabetes. *Cleve Clin J Med* 2008; **75**: 772, 774, 776-777 passim [PMID: 19068958]

59 **Nielsen NM**, Westergaard T, Frisch M, Rostgaard K, Wohlfahrt J, Koch-Henriksen N, Melbye M, Hjalgrim H. Type 1 diabetes and multiple sclerosis: A Danish population-based cohort study. *Arch Neurol* 2006; **63**: 1001-1004 [PMID: 16831970 DOI: 10.1001/archneur.63.7.1001]

60 **Kota SK**, Meher LK, Jammula S, Kota SK, Modi KD. Clinical profile of coexisting conditions in type 1 diabetes mellitus patients. *Diabetes Metab Syndr* 2012; **6**: 70-76 [PMID: 23153973 DOI: 10.1016/j.dsx.2012.08.006]

61 **Mazzarella G**, Stefanile R, Camarca A, Giliberti P, Cosentini E, Marano C, Iaquinto G, Giardullo N, Auricchio S, Sette A, Troncone R, Gianfrani C. Gliadin activates HLA class I-restricted CD8+ T cells in celiac disease intestinal mucosa and induces the enterocyte apoptosis. *Gastroenterology* 2008; **134**: 1017-1027 [PMID: 18395083 DOI: 10.1053/j.gastro.2008.01.008]

62 **Hermann R**, Turpeinen H, Laine AP, Veijola R, Knip M, Simell O, Sipilä I, Akerblom HK, Ilonen J. HLA DR-DQ-encoded genetic determinants of childhood-onset type 1 diabetes in Finland: an analysis of 622 nuclear families. *Tissue Antigens* 2003; **62**: 162-169 [PMID: 12889996]

63 **Kooy-Winkelaar Y**, van Lummel M, Moustakas AK, Schweizer J, Mearin ML, Mulder CJ, Roep BO, Drijfhout JW, Papadopoulos GK, van Bergen J, Koning F. Gluten-specific T cells cross-react between HLA-DQ8 and the HLA-DQ2α/DQ8β transdimer. *J Immunol* 2011; **187**: 5123-5129 [PMID:

22013116 DOI: 10.4049/jimmunol.1101179]
64 **Troncone R**, Discepolo V. Celiac disease and autoimmunity. *J Pediatr Gastroenterol Nutr* 2014; **59** Suppl 1: S9-S11 [PMID: 24979198 DOI: 10.1097/01.mpg.0000450394.30780.ea]
65 **Antvorskov JC**, Fundova P, Buschard K, Funda DP. Impact of dietary gluten on regulatory T cells and Th17 cells in BALB/c mice. *PLoS One* 2012; **7**: e33315 [PMID: 22428018 DOI: 10.1371/journal.pone.0033315]
66 **Cohn A**, Sofia AM, Kupfer SS. Type 1 diabetes and celiac disease: clinical overlap and new insights into disease pathogenesis. *Curr Diab Rep* 2014; **14**: 517 [PMID: 24952108 DOI: 10.1007/s11892-014-0517-x]
67 **Alonso N**, Soldevila B, Sanmartí A, Pujol-Borrell R, Martínez-Cáceres E. Regulatory T cells in diabetes and gastritis. *Autoimmun Rev* 2009; **8**: 659-662 [PMID: 19393198 DOI: 10.1016/j.autrev.2009.02.014]
68 **Kahaly GJ**. Polyglandular autoimmune syndromes. *Eur J Endocrinol* 2009; **161**: 11-20 [PMID: 19411300 DOI: 10.1530/eje-09-0044]
69 **Neufeld M**, Maclaren N, Blizzard R. Autoimmune polyglandular syndromes. *Pediatr Ann* 1980; **9**: 154-162 [PMID: 6990358]
70 **Betterle C**, Zanchetta R. Update on autoimmune polyendocrine syndromes (APS). *Acta Biomed* 2003; **74**: 9-33 [PMID: 12817789]
71 **Betterle C**, Greggio NA, Volpato M. Clinical review 93: Autoimmune polyglandular syndrome type 1. *J Clin Endocrinol Metab* 1998; **83**: 1049-1055 [PMID: 9543115 DOI: 10.1210/jcem.83.4.4682]
72 **Ahonen P**, Myllärniemi S, Sipilä I, Perheentupa J. Clinical variation of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) in a series of 68 patients. *N Engl J Med* 1990; **322**: 1829-1836 [PMID: 2348835 DOI: 10.1056/nejm199006283222601]
73 **Tuomi T**, Björses P, Falorni A, Partanen J, Perheentupa J, Lernmark A, Miettinen A. Antibodies to glutamic acid decarboxylase and insulin-dependent diabetes in patients with autoimmune polyendocrine syndrome type I. *J Clin Endocrinol Metab* 1996; **81**: 1488-1494 [PMID: 8636356 DOI: 10.1210/jcem.81.4.8636356]
74 **Perheentupa J**, Miettinen A. Type 1 autoimmune polyglandular disease. *Ann Med Interne* (Paris) 1999; **150**: 313-325 [PMID: 10519019]
75 **Neufeld M**, Maclaren NK, Blizzard RM. Two types of autoimmune Addison's disease associated with different polyglandular autoimmune (PGA) syndromes. *Medicine* (Baltimore) 1981; **60**: 355-362 [PMID: 7024719 DOI: 10.1097/00005792-198109000-00003]
76 **Zlotogora J**, Shapiro MS. Polyglandular autoimmune syndrome type I among Iranian Jews. *J Med Genet* 1992; **29**: 824-826 [PMID: 1453436 DOI: 10.1136/jmg.29.11.824]
77 **Sato K**, Nakajima K, Imamura H, Deguchi T, Horinouchi S, Yamazaki K, Yamada E, Kanaji Y, Takano K. A novel missense mutation of AIRE gene in a patient with autoimmune polyendocrinopathy, candidiasis and ectodermal dystrophy (APECED), accompanied with progressive muscular atrophy: case report and review of the literature in Japan. *Endocr J* 2002; **49**: 625-633 [PMID: 12625412 DOI: 10.1507/endocrj.49.625]
78 **Betterle C**, Dal Pra C, Mantero F, Zanchetta R. Autoimmune adrenal insufficiency and autoimmune polyendocrine syndromes: autoantibodies, autoantigens, and their applicability in diagnosis and disease prediction. *Endocr Rev* 2002; **23**: 327-364 [PMID: 12050123 DOI: 10.1210/edrv.23.3.0466]
79 **Gylling M**, Tuomi T, Björses P, Kontiainen S, Partanen J, Christie MR, Knip M, Perheentupa J, Miettinen A. ss-cell autoantibodies, human leukocyte antigen II alleles, and type 1 diabetes in autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. *J Clin Endocrinol Metab* 2000; **85**: 4434-4440 [PMID: 11134089 DOI: 10.1210/jcem.85.12.7120]
80 **Björk E**, Velloso LA, Kämpe O, Karlsson FA. GAD autoantibodies in IDDM, stiff-man syndrome, and autoimmune polyendocrine syndrome type I recognize different epitopes. *Diabetes* 1994; **43**: 161-165 [PMID: 7505244]
81 **Velloso LA**, Winqvist O, Gustafsson J, Kämpe O, Karlsson FA. Autoantibodies against a novel 51 kDa islet antigen and glutamate decarboxylase isoforms in autoimmune polyendocrine syndrome type I. *Diabetologia* 1994; **37**: 61-69 [PMID: 8150232]
82 **Husebye ES**, Gebre-Medhin G, Tuomi T, Perheentupa J, Landin-Olsson M, Gustafsson J, Rorsman F, Kämpe O. Autoantibodies against aromatic L-amino acid decarboxylase in autoimmune polyendocrine syndrome type I. *J Clin Endocrinol Metab* 1997; **82**: 147-150 [PMID: 8989249 DOI: 10.1210/jcem.82.1.3647]
83 **Rorsman F**, Husebye ES, Winqvist O, Björk E, Karlsson FA, Kämpe O. Aromatic-L-amino-acid decarboxylase, a pyridoxal phosphate-dependent enzyme, is a beta-cell autoantigen. *Proc Natl Acad Sci USA* 1995; **92**: 8626-8629 [PMID: 7567987]
84 **Anaya JM**. Common mechanisms of autoimmune diseases (the autoimmune tautology). *Autoimmun Rev* 2012; **11**: 781-784 [PMID: 22353402 DOI: 10.1016/j.autrev.2012.02.002]
85 **Miller FW**. Environmental agents and autoimmune diseases. *Adv Exp Med Biol* 2011; **711**: 61-81 [PMID: 21627043]
86 **Selmi C**. Autoimmunity in 2011. *Clin Rev Allergy Immunol* 2012; **43**: 194-206 [PMID: 22733376 DOI: 10.1007/s12016-012-8330-2]
87 **Anaya JM**. The diagnosis and clinical significance of polyautoimmunity. *Autoimmun Rev* 2014; **13**: 423-426 [PMID: 24424171 DOI: 10.1016/j.autrev.2014.01.049]
88 **Cutolo M**. Autoimmune polyendocrine syndromes. *Autoimmun Rev* 2014; **13**: 85-89 [PMID: 24055063 DOI: 10.1016/j.autrev.2013.07.006]
89 **Erichsen MM**, Løvås K, Skinningsrud B, Wolff AB, Undlien DE, Svartberg J, Fougner KJ, Berg TJ, Bollerslev J, Mella B, Carlson JA, Erlich H, Husebye ES. Clinical, immunological, and genetic features of autoimmune primary adrenal insufficiency: observations from a Norwegian registry. *J Clin Endocrinol Metab* 2009; **94**: 4882-4890 [PMID: 19858318 DOI: 10.1210/jc.2009-1368]
90 **Betterle C**, Morlin L. Autoimmune Addison's disease. *Endocr Dev* 2011; **20**: 161-172 [PMID: 21164269 DOI: 10.1159/000321239]
91 **Brenta G**. Diabetes and thyroid disorders. *Br J Diabetes Vasc Dis* 2010; **10**: 172-177 [DOI: 10.1177/1474651410371321]
92 **Duntas LH**, Orgiazzi J, Brabant G. The interface between thyroid and diabetes mellitus. *Clin Endocrinol* (Oxf) 2011; **75**: 1-9 [PMID: 21521298 DOI: 10.1111/j.1365-2265.2011.04029.x]
93 **Kadiyala R**, Peter R, Okosieme OE. Thyroid dysfunction in patients with diabetes: clinical implications and screening strategies. *Int J Clin Pract* 2010; **64**: 1130-1139 [PMID: 20642711 DOI: 10.1111/j.1742-1241.2010.02376.x]
94 **Kordonouri O**, Klinghammer A, Lang EB, Grüters-Kieslich A, Grabert M, Holl RW. Thyroid autoimmunity in children and adolescents with type 1 diabetes: a multicenter survey. *Diabetes Care* 2002; **25**: 1346-1350 [PMID: 12145233]
95 **Denzer C**, Karges B, Näke A, Rosenbauer J, Schober E, Schwab KO, Holl RW. Subclinical hypothyroidism and dyslipidemia in children and adolescents with type 1 diabetes mellitus. *Eur J Endocrinol* 2013; **168**: 601-608 [PMID: 23384709 DOI: 10.1530/eje-12-0703]
96 **Riley WJ**, Maclaren NK, Lezotte DC, Spillar RP, Rosenbloom AL. Thyroid autoimmunity in insulin-dependent diabetes mellitus: the case for routine screening. *J Pediatr* 1981; **99**: 350-354 [PMID: 7264787]
97 **Roldán MB**, Alonso M, Barrio R. Thyroid autoimmunity in children and adolescents with Type 1 diabetes mellitus. *Diabetes Nutr Metab* 1999; **12**: 27-31 [PMID: 10517303]
98 **Lambadiari V**, Mitrou P, Maratou E, Raptis AE, Tountas N, Raptis SA, Dimitriadis G. Thyroid hormones are positively associated with insulin resistance early in the development of type 2 diabetes. *Endocrine* 2011; **39**: 28-32 [PMID: 21072691 DOI: 10.1007/s12020-010-9408-3]

99 **Horie I**, Kawasaki E, Ando T, Kuwahara H, Abiru N, Usa T, Yamasaki H, Ejima E, Kawakami A. Clinical and genetic characteristics of autoimmune polyglandular syndrome type 3 variant in the Japanese population. *J Clin Endocrinol Metab* 2012; **97**: E1043-E1050 [PMID: 22466347 DOI: 10.1210/jc.2011-3109]

100 **Boelaert K**, Newby PR, Simmonds MJ, Holder RL, Carr-Smith JD, Heward JM, Manji N, Allahabadia A, Armitage M, Chatterjee KV, Lazarus JH, Pearce SH, Vaidya B, Gough SC, Franklyn JA. Prevalence and relative risk of other autoimmune diseases in subjects with autoimmune thyroid disease. *Am J Med* 2010; **123**: 183.e1-183.e9 [PMID: 20103030 DOI: 10.1016/j.amjmed.2009.06.030]

101 **Lupi I**, Raffaelli V, Di Cianni G, Caturegli P, Manetti L, Ciccarone AM, Bogazzi F, Mariotti S, Del Prato S, Martino E. Pituitary autoimmunity in patients with diabetes mellitus and other endocrine disorders. *J Endocrinol Invest* 2013; **36**: 127-131 [PMID: 23481612 DOI: 10.1007/BF03346747]

102 **Yoshioka K**, Ohsawa A, Yoshida T, Yokoh S. Insulin-dependent diabetes mellitus associated with Graves' disease and idiopathic hypoparathyroidism. *J Endocrinol Invest* 1993; **16**: 643-646 [PMID: 8258654 DOI: 10.1007/BF03347687]

103 **Shapiro MS**, Zamir R, Weiss E, Radnay J, Shenkman L. The polyglandular deficiency syndrome: a new variant in Persian Jews. *J Endocrinol Invest* 1987; **10**: 1-7 [PMID: 3496374 DOI: 10.1007/BF03347139]

104 **Förster G**, Krummenauer F, Kühn I, Beyer J, Kahaly G. [Polyglandular autoimmune syndrome type II: epidemiology and forms of manifestation]. *Dtsch Med Wochenschr* 1999; **124**: 1476-1481 [PMID: 10629665 DOI: 10.1055/s-2008-1035684]

105 **Dittmar M**, Kahaly GJ. Polyglandular autoimmune syndromes: immunogenetics and long-term follow-up. *J Clin Endocrinol Metab* 2003; **88**: 2983-2992 [PMID: 12843130 DOI: 10.1210/jc.2002-021845]

106 **Schatz DA**, Winter WE. Autoimmune polyglandular syndrome. II: Clinical syndrome and treatment. *Endocrinol Metab Clin North Am* 2002; **31**: 339-352 [PMID: 12092454]

107 **Santamaria P**, Barbosa JJ, Lindstrom AL, Lemke TA, Goetz FC, Rich SS. HLA-DQB1-associated susceptibility that distinguishes Hashimoto's thyroiditis from Graves' disease in type I diabetic patients. *J Clin Endocrinol Metab* 1994; **78**: 878-883 [PMID: 8157715 DOI: 10.1210/jcem.78.4.8157715]

108 **Huang W**, Connor E, Rosa TD, Muir A, Schatz D, Silverstein J, Crockett S, She JX, Maclaren NK. Although DR3-DQB1*0201 may be associated with multiple component diseases of the autoimmune polyglandular syndromes, the human leukocyte antigen DR4-DQB1*0302 haplotype is implicated only in beta-cell autoimmunity. *J Clin Endocrinol Metab* 1996; **81**: 2559-2563 [PMID: 8675578 DOI: 10.1210/jcem.81.7.8675578]

109 **Chikuba N**, Akazawa S, Yamaguchi Y, Kawasaki E, Takino H, Yoshimoto M, Ohe N, Yamashita K, Yano A, Nagataki S. Immunogenetic heterogeneity in type 1 (insulin-dependent) diabetes among Japanese--class II antigen and autoimmune thyroid disease. *Diabetes Res Clin Pract* 1995; **27**: 31-37 [PMID: 7781492 DOI: 10.1016/0168-8227(94)01025-U]

110 **Chuang LM**, Wu HP, Chang CC, Tsai WY, Chang HM, Tai TY, Lin BJ. HLA DRB1/DQA1/DQB1 haplotype determines thyroid autoimmunity in patients with insulin-dependent diabetes mellitus. *Clin Endocrinol* (Oxf) 1996; **45**: 631-636 [PMID: 8977762 DOI: 10.1046/j.1365-2265.1996.00857.x]

111 **Kim EY**, Shin CH, Yang SW. Polymorphisms of HLA class II predispose children and adolescents with type 1 diabetes mellitus to autoimmune thyroid disease. *Autoimmunity* 2003; **36**: 177-181 [PMID: 12911285 DOI: 10.1080/0891693031000101279]

112 **Golden B**, Levin L, Ban Y, Concepcion E, Greenberg DA, Tomer Y. Genetic analysis of families with autoimmune diabetes and thyroiditis: evidence for common and unique genes. *J Clin Endocrinol Metab* 2005; **90**: 4904-4911 [PMID: 15928253 DOI: 10.1210/jc.2004-2236]

113 **Villano MJ**, Huber AK, Greenberg DA, Golden BK, Concepcion E, Tomer Y. Autoimmune thyroiditis and diabetes: dissecting the joint genetic susceptibility in a large cohort of multiplex families. *J Clin Endocrinol Metab* 2009; **94**: 1458-1466 [PMID: 19141582 DOI: 10.1210/jc.2008-2193]

114 **Dultz G**, Matheis N, Dittmar M, Bender K, Kahaly GJ. CTLA-4 CT60 polymorphism in thyroid and polyglandular autoimmunity. *Horm Metab Res* 2009; **41**: 426-429 [PMID: 19530270]

115 **Awata T**, Kawasaki E, Tanaka S, Ikegami H, Maruyama T, Shimada A, Nakanishi K, Kobayashi T, Iizuka H, Uga M, Kawabata Y, Kanazawa Y, Kurihara S, Osaki M, Katayama S. Association of type 1 diabetes with two Loci on 12q13 and 16p13 and the influence coexisting thyroid autoimmunity in Japanese. *J Clin Endocrinol Metab* 2009; **94**: 231-235 [PMID: 18940880 DOI: 10.1210/jc.2008-0718]

116 **Dultz G**, Matheis N, Dittmar M, Röhrig B, Bender K, Kahaly GJ. The protein tyrosine phosphatase non-receptor type 22 C1858T polymorphism is a joint susceptibility locus for immunthyroiditis and autoimmune diabetes. *Thyroid* 2009; **19**: 143-148 [PMID: 19090780 DOI: 10.1089/thy.2008.0301]

117 **Smyth D**, Cooper JD, Collins JE, Heward JM, Franklyn JA, Howson JM, Vella A, Nutland S, Rance HE, Maier L, Barratt BJ, Guja C, Ionescu-Tîrgoviste C, Savage DA, Dunger DB, Widmer B, Strachan DP, Ring SM, Walker N, Clayton DG, Twells RC, Gough SC, Todd JA. Replication of an association between the lymphoid tyrosine phosphatase locus (LYP/PTPN22) with type 1 diabetes, and evidence for its role as a general autoimmunity locus. *Diabetes* 2004; **53**: 3020-3023 [PMID: 15504986]

118 **Kawasaki E**, Awata T, Ikegami H, Kobayashi T, Maruyama T, Nakanishi K, Shimada A, Uga M, Kurihara S, Kawabata Y, Tanaka S, Kanazawa Y, Lee I, Eguchi K. Systematic search for single nucleotide polymorphisms in a lymphoid tyrosine phosphatase gene (PTPN22): association between a promoter polymorphism and type 1 diabetes in Asian populations. *Am J Med Genet A* 2006; **140**: 586-593 [PMID: 16470599 DOI: 10.1002/ajmg.a.31124]

119 **Bottini N**, Musumeci L, Alonso A, Rahmouni S, Nika K, Rostamkhani M, MacMurray J, Meloni GF, Lucarelli P, Pellecchia M, Eisenbarth GS, Comings D, Mustelin T. A functional variant of lymphoid tyrosine phosphatase is associated with type I diabetes. *Nat Genet* 2004; **36**: 337-338 [PMID: 15004560 DOI: 10.1038/ng1323]

120 **van der Vliet HJ**, Nieuwenhuis EE. IPEX as a result of mutations in FOXP3. *Clin Dev Immunol* 2007; **2007**: 89017 [PMID: 18317533 DOI: 10.1155/2007/89017]

121 **Wildin RS**, Smyk-Pearson S, Filipovich AH. Clinical and molecular features of the immunodysregulation, polyendocrinopathy, enteropathy, X linked (IPEX) syndrome. *J Med Genet* 2002; **39**: 537-545 [PMID: 12161590]

122 **Long SA**, Cerosaletti K, Bollyky PL, Tatum M, Shilling H, Zhang S, Zhang ZY, Pihoker C, Sanda S, Greenbaum C, Buckner JH. Defects in IL-2R signaling contribute to diminished maintenance of FOXP3 expression in CD4(+)CD25(+) regulatory T-cells of type 1 diabetic subjects. *Diabetes* 2010; **59**: 407-415 [PMID: 19875613 DOI: 10.2337/db09-0694]

123 **Husebye ES**, Anderson MS. Autoimmune polyendocrine syndromes: clues to type 1 diabetes pathogenesis. *Immunity* 2010; **32**: 479-487 [PMID: 20412758 DOI: 10.1016/j.immuni.2010.03.016]

124 **Tomer Y**, Menconi F. Type 1 diabetes and autoimmune thyroiditis: the genetic connection. *Thyroid* 2009; **19**: 99-102 [PMID: 19191741 DOI: 10.1089/thy.2008.1565]

125 **Bingley PJ**, Christie MR, Bonifacio E, Bonfanti R, Shattock M, Fonte MT, Bottazzo GF, Gale EA. Combined analysis of autoantibodies improves prediction of IDDM in islet cell antibody-positive relatives. *Diabetes* 1994; **43**: 1304-1310 [PMID: 7926304]

126 **Deja G**, Myrda A, Jarosz-Chobot P, Siekiera U. The assessment of autoimmunological status and prevalence of

different forms of celiac disease among children with type 1 diabetes mellitus and celiac disease. *Mediators Inflamm* 2008; **2008**: 285989 [PMID: 18437226 DOI: 10.1155/2008/285989]
127 **Irvine WJ**, McCallum CJ, Gray RS, Campbell CJ, Duncan LJ, Farquhar JW, Vaughan H, Morris PJ. Pancreatic islet-cell antibodies in diabetes mellitus correlated with the duration and type of diabetes, coexistent autoimmune disease, and HLA type. *Diabetes* 1977; **26**: 138-147 [PMID: 320073]
128 **Kawasaki E**. ZnT8 and type 1 diabetes. *Endocr J* 2012; **59**: 531-537 [PMID: 22447136]
129 **Orban T**, Sosenko JM, Cuthbertson D, Krischer JP, Skyler JS, Jackson R, Yu L, Palmer JP, Schatz D, Eisenbarth G. Pancreatic islet autoantibodies as predictors of type 1 diabetes in the Diabetes Prevention Trial-Type 1. *Diabetes Care* 2009; **32**: 2269-2274 [PMID: 19741189 DOI: 10.2337/dc09-0934]
130 **Petruzelkova L**, Ananieva-Jordanova R, Vcelakova J, Vesely Z, Stechova K, Lebl J, Dusatkova P, Sumnik Z, Coles R, Powell M, Furmaniak J, Rees Smith B, Kolouskova S. The dynamic changes of zinc transporter 8 autoantibodies in Czech children from the onset of Type 1 diabetes mellitus. *Diabet Med* 2014; **31**: 165-171 [PMID: 23952619 DOI: 10.1111/dme.12308]
131 **Skärstrand H**, Lernmark A, Vaziri-Sani F. Antigenicity and epitope specificity of ZnT8 autoantibodies in type 1 diabetes. *Scand J Immunol* 2013; **77**: 21-29 [PMID: 23126564 DOI: 10.1111/sji.12008]
132 **Törn C**, Mueller PW, Schlosser M, Bonifacio E, Bingley PJ. Diabetes Antibody Standardization Program: evaluation of assays for autoantibodies to glutamic acid decarboxylase and islet antigen-2. *Diabetologia* 2008; **51**: 846-852 [PMID: 18373080 DOI: 10.1007/s00125-008-0967-2]
133 **Vaziri-Sani F**, Oak S, Radtke J, Lernmark K, Lynch K, Agardh CD, Cilio CM, Lethagen AL, Ortqvist E, Landin-Olsson M, Törn C, Hampe CS. ZnT8 autoantibody titers in type 1 diabetes patients decline rapidly after clinical onset. *Autoimmunity* 2010; **43**: 598-606 [PMID: 20298127 DOI: 10.3109/08916930903555927]
134 **Zhang L**, Eisenbarth GS. Prediction and prevention of Type 1 diabetes mellitus. *J Diabetes* 2011; **3**: 48-57 [PMID: 21073664 DOI: 10.1111/j.1753-0407.2010.00102.x]
135 **Thomson G**, Robinson WP, Kuhner MK, Joe S, MacDonald MJ, Gottschall JL, Barbosa J, Rich SS, Bertrams J, Baur MP. Genetic heterogeneity, modes of inheritance, and risk estimates for a joint study of Caucasians with insulin-dependent diabetes mellitus. *Am J Hum Genet* 1988; **43**: 799-816 [PMID: 3057885]
136 **Cooper GS**, Bynum ML, Somers EC. Recent insights in the epidemiology of autoimmune diseases: improved prevalence estimates and understanding of clustering of diseases. *J Autoimmun* 2009; **33**: 197-207 [PMID: 19819109 DOI: 10.1016/j.jaut.2009.09.008]
137 **Unnikrishnan AG**, Kumaravel V, Nair V, Rao A, Jayakumar RV, Kumar H, Sanjeevi CB. TSH receptor antibodies in subjects with type 1 diabetes mellitus. *Ann N Y Acad Sci* 2006; **1079**: 220-225 [PMID: 17130558 DOI: 10.1196/annals.1375.034]
138 **Premawardhana LD**, Wijeyaratne CN, Chen S, Wijesuriya M, Illangasekera U, Brooking H, Amoroso M, Jeffreys J, Bolton J, Lazarus JH, Furmaniak J, Rees Smith B. Islet cell, thyroid, adrenal and celiac disease related autoantibodies in patients with Type 1 diabetes from Sri Lanka. *J Endocrinol Invest* 2006; **29**: 968-974 [PMID: 17259793]
139 **Orgiazzi J**. Anti-TSH receptor antibodies in clinical practice. *Endocrinol Metab Clin North Am* 2000; **29**: 339-355, vii [PMID: 10874533]
140 **Pinto AL**, Dantas JR, Araujo D, Barone B, de Souza Papi JÂ, de Oliveira JE, Zajdenverg L, Rodacki M. Anti-parietal cell antibodies and pernicious anemia in patients with type 1 diabetes mellitus and multiethnic background. *Diabetes Res Clin Pract* 2013; **102**: e41-e43 [PMID: 24083984 DOI: 10.1016/j.diabres.2013.08.008]
141 **Perros P**, Singh RK, Ludlam CA, Frier BM. Prevalence of pernicious anaemia in patients with Type 1 diabetes mellitus and autoimmune thyroid disease. *Diabet Med* 2000; **17**: 749-751 [PMID: 11110510]
142 **Caturegli P**, Newschaffer C, Olivi A, Pomper MG, Burger PC, Rose NR. Autoimmune hypophysitis. *Endocr Rev* 2005; **26**: 599-614 [PMID: 15634713 DOI: 10.1210/er.2004-0011]
143 **Løvås K**, Husebye ES. High prevalence and increasing incidence of Addison's disease in western Norway. *Clin Endocrinol* (Oxf) 2002; **56**: 787-791 [PMID: 12072049]
144 **Ban Y**, Davies TF, Greenberg DA, Concepcion ES, Tomer Y. The influence of human leucocyte antigen (HLA) genes on autoimmune thyroid disease (AITD): results of studies in HLA-DR3 positive AITD families. *Clin Endocrinol* (Oxf) 2002; **57**: 81-88 [PMID: 12100074]
145 **Brozzetti A**, Marzotti S, Tortoioli C, Bini V, Giordano R, Dotta F, Betterle C, De Bellis A, Arnaldi G, Toscano V, Arvat E, Bellastella A, Mantero F, Falorni A. Cytotoxic T lymphocyte antigen-4 Ala17 polymorphism is a genetic marker of autoimmune adrenal insufficiency: Italian association study and meta-analysis of European studies. *Eur J Endocrinol* 2010; **162**: 361-369 [PMID: 19884265 DOI: 10.1530/eje-09-0618]
146 **Criswell LA**, Pfeiffer KA, Lum RF, Gonzales B, Novitzke J, Kern M, Moser KL, Begovich AB, Carlton VE, Li W, Lee AT, Ortmann W, Behrens TW, Gregersen PK. Analysis of families in the multiple autoimmune disease genetics consortium (MADGC) collection: the PTPN22 620W allele associates with multiple autoimmune phenotypes. *Am J Hum Genet* 2005; **76**: 561-571 [PMID: 15719322 DOI: 10.1086/429096]
147 **Gambelunghe G**, Falorni A, Ghaderi M, Laureti S, Tortoioli C, Santeusanio F, Brunetti P, Sanjeevi CB. Microsatellite polymorphism of the MHC class I chain-related (MIC-A and MIC-B) genes marks the risk for autoimmune Addison's disease. *J Clin Endocrinol Metab* 1999; **84**: 3701-3707 [PMID: 10523017 DOI: 10.1210/jcem.84.10.6069]
148 **Heward JM**, Allahabadia A, Daykin J, Carr-Smith J, Daly A, Armitage M, Dodson PM, Sheppard MC, Barnett AH, Franklyn JA, Gough SC. Linkage disequilibrium between the human leukocyte antigen class II region of the major histocompatibility complex and Graves' disease: replication using a population case control and family-based study. *J Clin Endocrinol Metab* 1998; **83**: 3394-3397 [PMID: 9768636 DOI: 10.1210/jcem.83.10.5137]
149 **Ide M**, Dittmar M, Wurm M, Kanitz M, Kahaly GJ. [Polymorphisms of MICA microsatellites in thyroidal autoimmunity]. *Med Klin* (Munich) 2007; **102**: 11-15 [PMID: 17221346 DOI: 10.1007/s00063-007-1001-z]
150 **Kavvoura FK**, Ioannidis JP. CTLA-4 gene polymorphisms and susceptibility to type 1 diabetes mellitus: a HuGE Review and meta-analysis. *Am J Epidemiol* 2005; **162**: 3-16 [PMID: 15961581 DOI: 10.1093/aje/kwi165]
151 **Menconi F**, Monti MC, Greenberg DA, Oashi T, Osman R, Davies TF, Ban Y, Jacobson EM, Concepcion ES, Li CW, Tomer Y. Molecular amino acid signatures in the MHC class II peptide-binding pocket predispose to autoimmune thyroiditis in humans and in mice. *Proc Natl Acad Sci USA* 2008; **105**: 14034-14039 [PMID: 18779568 DOI: 10.1073/pnas.0806584105]
152 **Park Y**, Lee H, Sanjeevi CB, Eisenbarth GS. MICA polymorphism is associated with type 1 diabetes in the Korean population. *Diabetes Care* 2001; **24**: 33-38 [PMID: 11194237]
153 **Roycroft M**, Fichna M, McDonald D, Owen K, Zurawek M, Gryczyńska M, Januszkiewicz-Lewandowska D, Fichna P, Cordell H, Donaldson P, Nowak J, Pearce S. The tryptophan 620 allele of the lymphoid tyrosine phosphatase (PTPN22) gene predisposes to autoimmune Addison's disease. *Clin Endocrinol* (Oxf) 2009; **70**: 358-362 [PMID: 18710467 DOI: 10.1111/j.1365-2265.2008.03380.x]
154 **Saleh HM**, Rohowsky N, Leski M. The CTLA4 -819 C/T and +49 A/G dimorphisms are associated with Type 1 diabetes in Egyptian children. *Indian J Hum Genet* 2008; **14**: 92-98 [PMID:

20300303 DOI: 10.4103/0971-6866.45001]
155 **Sanjeevi CB**, Sedimbi SK, Landin-Olsson M, Kockum I, Lernmark A. Risk conferred by HLA-DR and DQ for type 1 diabetes in 0-35-year age group in Sweden. *Ann N Y Acad Sci* 2008; **1150**: 106-111 [PMID: 19120278 DOI: 10.1196/annals.1447.061]
156 **Stenszky V**, Kozma L, Balázs C, Rochlitz S, Bear JC, Farid NR. The genetics of Graves' disease: HLA and disease susceptibility. *J Clin Endocrinol Metab* 1985; **61**: 735-740 [PMID: 3861611 DOI: 10.1210/jcem-61-4-735]
157 **Thorsby E**. Invited anniversary review: HLA associated diseases. *Hum Immunol* 1997; **53**: 1-11 [PMID: 9127141 DOI: 10.1016/s0198-8859(97)00024-4]
158 **Ueda H**, Howson JM, Esposito L, Heward J, Snook H, Chamberlain G, Rainbow DB, Hunter KM, Smith AN, Di Genova G, Herr MH, Dahlman I, Payne F, Smyth D, Lowe C, Twells RC, Howlett S, Healy B, Nutland S, Rance HE, Everett V, Smink LJ, Lam AC, Cordell HJ, Walker NM, Bordin C, Hulme J, Motzo C, Cucca F, Hess JF, Metzker ML, Rogers J, Gregory S, Allahabadia A, Nithiyananthan R, Tuomilehto-Wolf E, Tuomilehto J, Bingley P, Gillespie KM, Undlien DE, Rønningen KS, Guja C, Ionescu-Tîrgovişte C, Savage DA, Maxwell AP, Carson DJ, Patterson CC, Franklyn JA, Clayton DG, Peterson LB, Wicker LS, Todd JA, Gough SC. Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. *Nature* 2003; **423**: 506-511 [PMID: 12724780 DOI: 10.1038/nature01621]
159 **Urcelay E**, Santiago JL, de la Calle H, Martínez A, Méndez J, Ibarra JM, Maluenda C, Fernández-Arquero M, de la Concha EG. Type 1 diabetes in the Spanish population: additional factors to class II HLA-DR3 and -DR4. *BMC Genomics* 2005; **6**: 56 [PMID: 15842729 DOI: 10.1186/1471-2164-6-56]
160 **Van Autreve JE**, Koeleman BP, Quartier E, Aminkeng F, Weets I, Gorus FK, Van der Auwera BJ. MICA is associated with type 1 diabetes in the Belgian population, independent of HLA-DQ. *Hum Immunol* 2006; **67**: 94-101 [PMID: 16698430 DOI: 10.1016/j.humimm.2006.02.032]
161 **Meager A**, Visvalingam K, Peterson P, Möll K, Murumägi A, Krohn K, Eskelin P, Perheentupa J, Husebye E, Kadota Y, Willcox N. Anti-interferon autoantibodies in autoimmune polyendocrinopathy syndrome type 1. *PLoS Med* 2006; **3**: e289 [PMID: 16784312 DOI: 10.1371/journal.pmed.0030289]
162 **Maurer A**, Schwarting A, Kahaly GJ. [Polyglandular autoimmune syndromes]. *Z Rheumatol* 2011; **70**: 752-754, 756-759 [PMID: 22033826 DOI: 10.1007/s00393-011-0786-6]
163 **Alimohammadi M**, Björklund P, Hallgren A, Pöntynen N, Szinnai G, Shikama N, Keller MP, Ekwall O, Kinkel SA, Husebye ES, Gustafsson J, Rorsman F, Peltonen L, Betterle C, Perheentupa J, Akerström G, Westin G, Scott HS, Holländer GA, Kämpe O. Autoimmune polyendocrine syndrome type 1 and NALP5, a parathyroid autoantigen. *N Engl J Med* 2008; **358**: 1018-1028 [PMID: 18322283 DOI: 10.1056/NEJMoa0706487]
164 **Cheng MH**, Anderson MS. Insights into type 1 diabetes from the autoimmune polyendocrine syndromes. *Curr Opin Endocrinol Diabetes Obes* 2013; **20**: 271-278 [PMID: 23770732 DOI: 10.1097/MED.0b013e32836313eb]
165 **Tsuda M**, Torgerson TR, Selmi C, Gambineri E, Carneiro-Sampaio M, Mannurita SC, Leung PS, Norman GL, Gershwin ME. The spectrum of autoantibodies in IPEX syndrome is broad and includes anti-mitochondrial autoantibodies. *J Autoimmun* 2010; **35**: 265-268 [PMID: 20650610 DOI: 10.1016/j.jaut.2010.06.017]
166 **Cheng MH**, Anderson MS. Monogenic autoimmunity. *Annu Rev Immunol* 2012; **30**: 393-427 [PMID: 22224765 DOI: 10.1146/annurev-immunol-020711-074953]

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REVIEW

Diabetes and cardiac autonomic neuropathy: Clinical manifestations, cardiovascular consequences, diagnosis and treatment

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Abstract

Cardiac autonomic neuropathy (CAN) is a frequent chronic complication of diabetes mellitus with potentially life-threatening outcomes. CAN is caused by the impairment of the autonomic nerve fibers regulating heart rate, cardiac output, myocardial contractility, cardiac electrophysiology and blood vessel constriction and dilatation. It causes a wide range of cardiac disorders, including resting tachycardia, arrhythmias, intraoperative cardiovascular instability, asymptomatic myocardial ischemia and infarction and increased rate of mortality after myocardial infarction. Etiological factors associated with autonomic neuropathy include insufficient glycemic control, a longer period since the onset of diabetes, increased age, female sex and greater body mass index. The most commonly used methods for the diagnosis of CAN are based upon the assessment of heart rate variability (the physiological variation in the time interval between heartbeats), as it is one of the first findings in both clinically asymptomatic and symptomatic patients. Clinical symptoms associated with CAN generally occur late in the disease process and include early fatigue and exhaustion during exercise, orthostatic hypotension, dizziness, presyncope and syncope. Treatment is based on early diagnosis, life style changes, optimization of glycemic control and management of cardiovascular risk factors. Medical therapies, including aldose reductase inhibitors, angiotensin-converting enzyme inhibitors, prostoglandin analogs and alpha-lipoic acid, have been found to be effective in randomized controlled trials. The following article includes the epidemiology, clinical findings and cardiovascular consequences, diagnosis, and approaches to prevention and treatment of CAN.

Key words: Diabetes mellitus; Autonomic neuropathy; Heart rate variability; Cardiac; Cardiovascular reflex tests

Core tip: Although very frequent, cardiac autonomic neuropathy (CAN) is one of the most commonly overlooked complication of diabetes. Higher incidence of cardiovascular events is encountered with CAN due to its relation with silent myocardial ischemia, arrhythmias, intraoperative cardiovascular instability,

orthostatic hypotension and cardiomyopathy. Diabetic patients should be screened for CAN due to the possibility of reversal of cardiovascular denervation in the early stages of the disease. Cardiovascular reflex tests and Holter-derived time- and frequency-domain measurements are frequently used for the diagnosis. Therapeutic approaches are promising and may hinder or reverse the progression of the disease when initiated during the early stages.

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INTRODUCTION

Cardiac autonomic neuropathy (CAN), a type of generalized symmetric polyneuropathy, is the most examined and clinically significant diabetic autonomic neuropathy[1]. The autonomic nervous system has 2 major components: the parasympathetic and the sympathetic nervous systems. These may operate independently of each other or interact cooperatively to control heart rate, cardiac output, myocardial contractility, cardiac electrophysiology, and the constriction and dilatation of blood vessels[2]. CAN is caused by damage to the autonomic nerve fibers that innervate the heart and blood vessels and leads to abnormalities in cardiovascular dynamics[2]. The earliest finding of CAN, even at the subclinical stage, is a decrease in heart rate variability (HRV)[3].

EPIDEMIOLOGY

Diabetes is estimated to affect approximately 350 million people globally[4]. Diabetic neuropathies, including CAN, are frequent chronic complications of type 1 and 2 diabetes that influence quality of life and have potentially fatal outcomes[2]. Prevalence rates between 1.6% to 90% have been reported, varying according to the diagnostic methods used, population studied and disease stage[1]. The Diabetes Control and Complications Trial (DCCT) showed abnormal HRV values of 1.65%, 6.2% and 12.2% in patients with diabetes for a duration of less than 5 years, 5 to 9 years and more than 9 years, respectively[5]. A study including 1171 patients with both type of diabetes mellitus reported impaired HRV tests in 25.3% of type 1 and 34.3% of type 2 patients[6]. The different methodology between various studies makes epidemiological comparison difficult. Risk factors of decreased HRV in patients with type 2 diabetes include age, duration of diabetes, obesity and smoking[2]. In type 1 diabetes, risk factors of CAN include higher levels of HbA1c, hypertension, distal symmetrical polyneuropathy, retinopathy and hyperglycemic exposure[7,8].

PATHOGENESIS

Diabetic CAN is eventually caused by complex interactions among a number of pathogenic pathways. Hyperglycemia is the leading cause of the initiation of this pathogenic process[9,10]. The pathogenesis of diabetic CAN is multifactorial, including increased mitochondrial production of free radicals due to hyperglycemia-induced oxidative stress. Neuronal activity, mitochondrial function, membrane permeability and endothelial function are impacted by advanced glycosylation end product formation, polyol aldose reductase signaling and poly(ADP ribose) polymerase activation and the alteration of the Na^+/K^+-ATPase pump function. Neuronal apoptotic processes are precipitated by endoplasmic reticulum stress induced by hyperglycemia, along with impaired nerve perfusion, dyslipidemia, alterations in redox status, low-grade inflammation and disturbance in calcium balance[11]. The literature has described these mechanisms and their interactions but is beyond the scope of this article[9-11].

CLINICAL MANIFESTATIONS

Diabetes can lead to dysfunction in the autonomic nervous system, causing various cardiovascular disorders, including resting tachycardia, postural hypotension, higher intra/perioperative cardiovascular instability, more frequent asymptomatic myocardial ischemia and infarction, and greater mortality after myocardial infarction[2]. Clinical symptoms associated with CAN generally occur at later stages and include postural hypotension, dizziness, lightheadedness, presyncope, syncope, and early fatigue and exhaustion during exercise[1]. However, subclinical autonomic dysfunction, revealed as deterioration in HRV, can occur in the 1st year following diagnosis in patients with type 2 diabetes and within 2 years following diagnosis in type 1[12]. Low *et al*[13] reported a higher rate of autonomic symptoms in type 1 than in type 2 diabetes. A greater number of autonomic symptoms have been associated with an increased risk of CAN as measured by HRV[14].

Because neuropathy first affects the longest nerve fibers, the first manifestation of diabetic CAN tends to be related with vagus nerve damage, which is responsible for nearly 75% of parasympathetic activity[9]. This damage causes resting tachycardia as the sympathetic tone becomes dominant[15]. Tachycardia eventually diminishes in a few years due to progressive sympathetic nerve fiber damage. However, increased heart rate persists in these patients[16]. The progressive damage of the autonomic balance is indicated by additional symptoms, including intolerance to exercise, orthostatic hypotension and a further HRV reduction[17]. Cardiac pain perception often deteriorates with the involvement of sensory nerve fibers, making patients prone to silent ischemia and myocardial infarction[18,19].

Etiological factors associated with autonomic neuropathy include poor glycemic control, longer diabetes duration, increased age, female sex and greater body

mass index[20]. Mortality rates of 25% to 50% within 5 to 10 years of diagnosis have been found in patients with symptomatic autonomic dysfunction[21,22]. Among diabetic patients, the 5-year mortality rate is 3 times higher in those with autonomic involvement than in those without[23].

CARDIOVASCULAR CONSEQUENCES

Impaired heart rate variability

HRV is a physiological variation in the interval between heartbeats and is regulated by the interaction of the sympathetic and parasympathetic tone[24]. The functional response to the instantaneous metabolic needs of the body is regulated by this beat-to-beat variation. As high variability reflects the cardiac ability to adapt and implies good health, damage or disturbances to this control system results in lower HRV values. Even in a normal heart rate, the first finding of CAN is a decrease in HRV, which is apparent at the subclinical stage and can be detected through deep respiration[25].

Resting tachycardia

Due to the dominant sympathetic tone, resting heart rates of 90 to 100 beats per minute with occasional increases to as many as 130 beats per minute are frequent findings in CAN with vagal impairment[16,26]. Highest resting heart rates have been shown in patients with lone parasympathetic impairment[16,26]. As the disease progresses and involves both parasympathetic and sympathetic nerves, the heart rate tends to return to the normal range but remains higher than in healthy individuals[16,26]. Tang *et al*[27] showed that resting heart rate is independently associated with CAN and has a high predictive value in predicting CAN in the general population[27]. A steady heart rate less responsive to exercise, stress or sleep is suggestive of almost total cardiac denervation, which indicates severe CAN[20].

Exercise intolerance

Exercise tolerance is worsened by CAN through the blunting of the increases in heart rate, blood pressure and cardiac output response to exertion[20]. The development of hypotension or hypertension following strenuous exercise is more likely in individuals with CAN, particularly in the onset of a new exercise program[1]. Therefore, patients with diabetes probably to have CAN should be checked for cardiac stress before beginning to exercise[1]. Due to poor thermoregulation, such patients should avoid exercising in environments that are too hot or cold and hydrate adequately[1].

Intraoperative and perioperative cardiovascular instability

The perioperative risk of cardiovascular morbidity and mortality are 2 to 3 times higher in diabetic individuals[28]. Since the normal autonomic response of vasoconstriction and the increase in heart rate cannot appropriately compensate for the vasodilatation and negative chronotropic effects of anesthesia, diabetic patients with CAN are subject to more pronounced decreases in blood pressure and heart rate during induction of anesthesia and, to a lesser extent, after intubation and extubation[20,28]. In addition, more severe intraoperative hypothermia in patients with CAN resulting in decelerated metabolism of anesthetic drugs may cause deepening of anesthesia and/or delayed recovery[29]. Accordingly, screening for CAN is recommended during the preoperative evaluation of diabetic patients for anesthetic management planning.

Non-dipping blood pressure profile and orthostatic hypotension

Blood pressure has diurnal variations. Decreases in nighttime and increases in daytime blood pressure show its circadian rhythm[30]. Declines in parasympathetic tone occur at night and nocturnal unopposed sympathetic activity leads to deterioration of the circadian rhythm of blood pressure and results in the lack of or a less than 10% reduction in nocturnal blood pressure in patients with CAN[31]. Such "non-dipper" CAN subjects experience more frequent left ventricular hypertrophy and are predisposed to cardiovascular events[32]. Another blood pressure regulation abnormality related to CAN is orthostatic hypotension, which is defined as a decrease in systolic blood pressure by a minimum of 20 mmHg (at least 30 mmHg in patients with hypertension) or diastolic blood pressure of 10 mmHg in response to postural shifts from the supine to the standing[2,20]. In diabetic individuals, orthostatic hypotension develops as a consequence of denervation of the efferent sympathetic vasomotor nerves, especially in the splanchnic vascular bed[33]. Additionally, pathogenesis of orthostatic hypotension includes lower cutaneous, splanchnic and total vascular resistance[10]. Impaired chronotropic and/or blood pressure response to exercise may accompany this condition[10]. Orthostatic hypotension can cause many symptoms that reduce quality of life or lead to serious injury due to falling, such as lightheadedness, dizziness, faintness, visual blurring, presyncope and syncope while standing[20]. However, an important number of patients are asymptomatic despite significant decreases in blood pressure[2]. Orthostatic hypotension can be provoked by several drugs, which may be used concurrently for treatment of diabetes or its complications, including, vasodilators, diuretics, phenothiazines, insulin (through endothelium-dependent vasodilation) and tricyclic antidepressants for symptomatic relief of pain associated with diabetic neuropathy[9].

Silent myocardial ischemia

Coronary artery disease has long been considered as a major complication of diabetes mellitus[34]. Numerous studies have reported more extensive atherosclerotic disease, particularly that of the coronary arteries, in diabetic individuals[35,36]. Silent myocardial ischemia is defined as the objective documentation of myocardial ischemia without angina or its equivalents[37]. Several

reports have shown the predictive role of silent ischemia during exercise testing[38] or ambulatory electrocardiography (ECG) monitoring[39] on poor clinical outcomes and survival. A threefold increase in cardiac deaths was witnessed over a 2-year follow-up in individuals with silent ischemia during ambulatory ECG monitoring[39]. Painless presentation of myocardial infarction in patients with CAN may include diaphoresis, dyspnea, fatigue, lightheadedness, palpitations, acute confusion, indigestion, nausea, and vomiting[20]. Several explanations are possible for the variety of symptom patterns in individuals with diabetes, such as various pain perception thresholds, sensorial denervation secondary to autonomic neuropathy and psychological disavowal[34]. Therefore, despite increasing ischemia, individuals with CAN and coronary artery disease may resume exercise as the longer threshold or subthreshold ischemia is not sufficient to induce pain, thus endangering the patient[40]. Likewise, the Framingham Heart Study reported higher incidences of painless myocardial infarction in patients with diabetes than without (39% *vs* 22%)[41,42]. Similarly, the National Registry of Myocardial Infarction 2 (NRMI-2) survey found that one-third of patients presented without angina[43]. In the NRMI-2, diabetes was present in 32% of subjects without chest pain and 25.4% with angina[43]. The Detection of Ischemia in Asymptomatic Diabetic Study, including 1123 patients with type 2 diabetes, reported that CAN is able to strongly predict silent ischemia and succeeding adverse cardiovascular events[44]. Vinik *et al*[10], in their meta-analysis of 12 studies, reported the association between CAN and the existence of silent ischemia detected by exercise stress tests with prevalence rate ratios of 0.85 to 15.53[10]. Therefore, patients with CAN require more rigorous evaluation of the presence of coronary artery disease. The presence and extent of macrovascular coronary artery disease in such patients can be noninvasively tested by resting and stress thallium myocardial scintigraphy[1]. Moreover, cardiovascular autonomic function testing should be included in the coronary artery risk assessment of all diabetic patients.

Myocardial infarction and increased risk of mortality

Myocardial infarction tends to be more extensive and severe in patients with diabetes[42,45]. The timely diagnosis of myocardial ischemia or infarction is often delayed due to diminished angina perception and therefore the period before first medical contact is prolonged[46]. Long-term survival rates following acute myocardial infarction are lower in patients with diabetes[20]. In diabetic patients, 5-year survival rates of 38% have been reported following the first major coronary event, lowering to just 25% for those with subsequent events, while the rates are 75% and 50%, respectively, in non-diabetic patients[45,47]. HRV has been demonstrated to be a good predictor of post-myocardial infarction mortality[48-50]. For this reason, cardiovascular autonomic function testing is advisable for all diabetic patients after a myocardial infarction to identify the candidates who have a high risk of death[51].

Sudden death

CAN is associated with a higher risk of malignant arrhythmias and sudden death[9]. Previous studies have found 5-year mortality rates between 16% and 50% in patients with both CAN and either type of diabetes, often attributed to sudden cardiac death[14,23]. Severe asymptomatic ischemia inducing fatal arrhythmias has been reported as the leading potential cause[22]. Additionally, life-threatening arrhythmias and sudden death may be predisposed by QT prolongation[52]. The European Diabetes Insulin-Dependent Diabetes Mellitus (EURODIAB IDDM) Complications Study established the association between impaired HRV and corrected QT prolongation[53]. In addition, unopposed increases in sympathetic activity and resultant norepinephrine signaling and metabolism[9], along with increased mitochondrial oxidative stress[54] and calcium-dependent apoptosis[55], is thought to contribute to myocardial injury[54,56] and clarify the higher risk of sudden cardiac events and deaths. The EURODIAB IDDM Prospective Cohort Study, including 2787 patients with type 1 diabetes, reported CAN to be the strongest predictor of mortality over the 7-year follow-up period, even greater than traditional cardiovascular risk factors[57]. A meta-analysis including 15 studies and 2900 diabetic patients showed CAN patients to have a pooled relative risk of mortality of 3.45 (95%CI: 2.66-4.47) and an increase in line with higher numbers of cardiovascular autonomic function abnormalities[58]. Similar results were confirmed in 2 other studies of patients with type 1 and type 2 diabetes, strengthening the role in predicting mortality of abnormalities in both HRV and the QT index independent of conventional risk factors[59,60]. The Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial[61] also confirmed the association between CAN and mortality in type 2 diabetic patients. In this trial, Pop-Busui *et al*[62] showed that mortality was between 1.55 and 2.14 times more likely in patients with baseline CAN than those without[62]. Three large studies (the ACCORD trial[61], the Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation trial[63], and the Veterans Affairs Diabetes Trial[64]) investigated the role of more intensive treatment and the frequency of cardiovascular events on individuals with long-term type 2 diabetes. In these trials, tight glycemic control was not shown to reduce cardiovascular adverse events[2] and the ACCORD trial was terminated early due to the increased mortality risk in the intensive therapy group[65]. Hypoglycemia is known to reduce the threshold for malignant ventricular arrhythmias that can result in sudden death[9,66]. In addition, hypoglycemic periods were reported to lead to impaired autonomic cardiovascular function even in healthy volunteers[67]. Hence, the cause of a lack of a decrease, or even an increase, in cardiovascular events in the intensive therapy arm may be linked to deterioration of cardiac autonomic function due to episodes of hypoglycemia.

Cardiomyopathy

Diabetic cardiomyopathy is defined as structural and

functional myocardial abnormalities without coronary artery disease, hypertension or valvular heart disease[68]. It is characterized by diastolic dysfunction[69]. The responsible mechanisms are left ventricular hypertrophy (increased left ventricular mass and concentric remodeling[70]), myocardial lipotoxicity, increased oxidative stress, cell death, interstitial and perivascular fibrosis, impaired contractile reserve, changes in myocardial substrate and energy metabolism, altered substrate utilization and mitochondrial dysfunction[69]. In patients with CAN, the initial augmentation in cardiac sympathetic activity stimulates the renin-angiotensin-aldosterone system and increases heart rate, stroke volume and peripheral vascular resistance[71]. In addition, the combination of sympathetic hyperactivity and regional myocardial sympathetic denervation cause reduced coronary blood flow and diastolic dysfunction, which may lead to impairment of systolic function[2].

Stroke

Previous studies have revealed the relationship between CAN and cerebrovascular events. Ko *et al*[72] reported that the presence of CAN, assessed by HRV testing, was significantly associated with the ischemic stroke in a study of 1458 patients with type 2 diabetes with a 7-year follow-up[72]. Another study of 133 subjects with type 2 diabetes showed that stroke could be predicted by parasympathetic and sympathetic autonomic function abnormalities[73].

DIAGNOSTIC METHODS OF CARDIAC AUTONOMIC NEUROPATHY

Variability in heart rate and blood pressure values can provide data regarding both parasympathetic and sympathetic autonomic function and is useful in clinical settings.

Heart rate variability

The most commonly used methods for the diagnosis of CAN are based on HRV assessment. HRV testing is noninvasive and objective in the evaluation of cardiac autonomic function and can be performed by recording electrocardiograms during deep breathing, standing, and Valsalva maneuvers[74]. HRV analysis enables the independent measurement of the sympathetic and parasympathetic components of the autonomic nervous system and can be assessed with a number of simple clinical tests[75] or easier digital 24-h electrocardiographic recordings[24]. In the 1970s, Ewing *et al*[75] discovered 5 simple tests of short-term R-R alterations to identify CAN in patients with diabetes: (1) heart rate response to respiration, which measures beat to beat sinus arrhythmia (R-R variation) during paced deep expiration and inspiration (E:I ratio); (2) heart rate response to standing, known as the 30:15 ratio, which is the ratio of the longest R-R interval (between beats 20 to 40) to the shortest R-R interval (between beats 5 to 25); (3) the Valsalva maneuver, measuring the heart rate response during and after a provoked increase in intra-thoracic and intra-abdominal pressures; (4) blood pressure response to orthostasis, which evaluates baroreflex mediated blood pressure fluctuations after changes in posture; and (5) blood pressure response to isometric exercise, as defined by the diastolic blood pressure increase due to continuous muscle contraction using a handgrip dynamometer[75]. Tests 1 and 2 reflect parasympathetic function, and 4 and 5, sympathetic function[76,77]. Although the Valsalva ratio primarily represents parasympathetic activity, the resultant autonomic changes are complicated and include both the sympathetic and parasympathetic components[78]. An American Diabetes Association statement describes these validated cardiac autonomic reflex tests (CART) in detail and recommends their use in the diagnosis of CAN (Table 1)[1]. HRV with deep breathing is the most commonly used autonomic function test and has a specificity of approximately 80%[79,80].

The assessment of HRV has become easier and more detailed due to new, digital, high frequency, 24-h multi-channel electrocardiographic recorders and the use of statistical indexes in the time and frequency domains.

Time domain methods

Time domain methods determine the heart rate at any point in time or the intervals between consecutive normal complexes[24]. Each QRS complex is detected and the normal-to-normal (NN) intervals (that is all intervals between adjacent QRS complexes resulting from sinus node depolarizations) or the instantaneous heart rate is determined through a continuous ECG record[24]. Simple time-domain variables that can be analyzed include mean NN interval, mean heart rate, the difference between the longest and the shortest NN interval and the variation between night and day heart rate[24]. More complex time-domain parameter calculations can be performed from a series of instantaneous heart rates or cycle intervals, particularly those recorded during 24-h periods[24]. The complex parameters can be divided into 2 classes: those derived from direct measurements of the NN intervals and those derived from the differences between NN intervals. The parameters calculated by direct measurements include: standard deviation of the NN interval (SDNN), reflecting all cyclic components responsible for variability in the recording period; standard deviation of the average NN interval (SDANN) calculated over periods (usually 5 min), estimating heart rate changes from cycles longer than 5 min; and the mean of the 5-min standard deviation of the NN interval (SDNN index) calculated over 24 h, measuring variability from cycles of less than 5 min. Secondly, the parameters calculated by the differences between NN intervals include the square root of the mean squared differences of successive NN intervals (RMSSD), the number of interval differences of successive NN intervals of more than 50 msec (NN50), and the division of NN50 by the total number of NN intervals (pNN50)[24]. Although influenced by several

Table 1 Cardiovascular autonomic reflex tests[1]

Test	Technique	Normal response and values
Beat-to-beat HRV	With the patient at rest and supine, heart rate is monitored by ECG while the patient breathes in and out at 6 breaths per minute, paced by a metronome or similar device	A difference in heart rate of > 15 beats per minute is normal and < 10 beats per minute is abnormal. The lowest normal value for the expiration-to inspiration ratio of the R-R interval decreases with age: age 20-24 yr, 1.17; 25-29, 1.15; 30-34, 1.13; 35-39, 1.12; 40-44, 1.10; 45-49, 1.08; 50-54, 1.07; 55-59, 1.06; 60-64, 1.04; 65-69, 1.03; and 70-75, 1.02
Heart rate response to standing	During continuous ECG monitoring, the R-R interval is measured at beats 15 and 30 after standing	Normally, a tachycardia is followed by reflex bradycardia. The 30:15 ratio should be > 1.03
Heart rate response to the Valsalva maneuver	The subject forcibly exhales into the mouthpiece of a manometer to 40 mmHg for 15 s during ECG monitoring	Healthy subjects develop tachycardia and peripheral vasoconstriction during strain and an overshoot bradycardia and rise in blood pressure with release. The normal ratio of longest R-R to shortest R-R is > 1.2
Systolic blood pressure response to standing	Systolic blood pressure is measured in the supine subject. The patient stands and the systolic blood pressure is measured after 2 min	Normal response is a fall of < 10 mmHg, borderline fall is a fall of 10-29 mmHg and abnormal fall is a decrease of > 30 mmHg with symptoms
Diastolic blood pressure response to isometric exercise	The subject squeezes a handgrip dynamometer to establish a maximum. Grip is then squeezed at 30% maximum for 5 min	The normal response for diastolic blood pressure is a rise of > 16 mmHg in the other arm

HRV: Heart rate variability; ECG: Electrocardiogram.

arrhythmias and requiring normal sinus rhythm and atrioventricular nodal function, these short-term variation measurements estimate high frequency variations in heart rate and are therefore highly correlated[24]. SDNN represents both the sympathetic and parasympathetic modulation of HRV, and RMSSD and pNN50 the parasympathetic system[24].

Frequency domain methods

CAN may also be evaluated using spectral analysis of HRV, which divides the R-R signal into sine and cosine waves to estimate the amount of variability as a function of frequency[24]. Three main spectral components are distinguished in a spectrum calculated from short-term recordings of 2 to 5 min: very-low-frequency (≤ 0.04 Hz) of fluctuations in vasomotor tone related to thermoregulation, low-frequency (0.04-0.15 Hz) associated with the baroreceptor reflex, and high-frequency (0.15-0.4 Hz) related to respiratory activity[24]. The sympathetic system is thought to modulate the 2 low-frequency components and the parasympathetic system the high-frequency component[20]. Accordingly, while decreases in very-low- and low-frequency peaks indicate sympathetic dysfunction, a decrease in the high-frequency peak is a sign of parasympathetic dysfunction[1]. A decrease in the ratio of low-frequency-to-high-frequency demonstrates sympathetic imbalance[1]. Various mathematical methods can be used to analyze the spectral components of HRV. Most common is the Fourier transform because of its simplicity and high processing speed[24]. A noise-free signal is necessary in order to correctly perform the spectral analysis. Because artefacts and extra beats must be removed, this correction leads to data loss and is associated with an underestimation in each case. Additionally, specific reference values must be obtained as each HRV-analysing device has different technical properties for spectral measurements.

It is not yet clear which of these 2 methods is preferable: time-domain methods including the standardized CART of Ewing or frequency-domain methods. However, many time- and frequency-domain variables obtained over the 24-h period are highly correlated with each other[24]. On the other hand, studies comparing Holter-based analysis and CART found a high (83%) correlation between the both techniques[81]. The advantages of Holter-based techniques include simpler, less stressful and faster implementation during daily routine use, independence of patient cooperation and greater sensitivity allowing for the identification of disorders in the early stages. However, CART can be applied quickly (less than 15 min) using stand-alone operator friendly devices during routine physical examination.

Heart rate turbulence

Another Holter-based technique for evaluating CAN is the heart rate turbulence (HRT)[82]. HRT refers to sinus rhythm cycle length fluctuations following isolated premature ventricular beats. After an initial acceleration, the sinus rate decelerates after a premature ventricular beat. There are 2 components of HRT; turbulence onset and turbulence slope. A transient vagal inhibition triggers the mentioned initial acceleration in heart rate as a response to the missed baroreflex afferent input due to hemodynamically ineffective ventricular contraction. The successive deceleration in heart rate is caused by a sympathetically mediated overshoot of arterial pressure through vagal recruitment[82]. HRT evaluation can be used in the risk assessment after acute myocardial infarction and in the monitoring of disease progression in heart failure and CAN[82]. We previously demonstrated that among HRV and HRT indexes, turbulence slope has the greatest correlation with CAN severity[83]. A turbulence slope of below 3.32 msec/R-R is 97% sensitive and 71% specific for the diagnosis of CAN as detected by the

CART in patients with type 2 diabetes[83].

Other diagnostic tools

Other methods currently used in research settings are scintigraphic evaluation of sympathetic innervation of the heart, which can reveal cardiac sympathetic nerve population changes and early anatomical regional deficits of sympathetic denervation[84-86]; microneurography, which records electrical activity released by peroneal, tibial or radial sympathetic nerves and identifies sympathetic dysfunction[87]; neurovascular flow, using noninvasive laser Doppler measures of peripheral sympathetic reactions to nociception[88]; and baroreflex sensitivity, which evaluates the capability to reflexively increase vagal activity in response to a sudden increase in blood pressure[89]. As many of these tests assess the influence on sympathetic component, they do not provide information about early stage CAN. In a recent study, it was shown that altered cardiac autonomic balance can be detected through exercise stress testing in diabetic subjects even with minimal evidence of CAN[90].

Criteria for diagnosis and staging

CART are the gold standard clinical tests for cardiovascular autonomic neuropathy[91]. Following the 8th international symposium on diabetic neuropathy in 2010, criteria for diagnosis and staging of CAN are defined in the CAN Subcommittee of the Toronto Consensus Panel statement[92]. Accordingly, only 1 abnormal CART result is sufficient to diagnose possible or early CAN; among the 7 autonomic function analysis (5 CART, time-domain and frequency-domain HRV tests), 2 or 3 abnormal tests indicate definite or confirmed CAN; and severe/advanced CAN can be indicated by concurrent orthostatic hypotension[91].

SCREENING FOR CAN

By the time clinical signs occur, CAN has often reached to a late stage, making management more difficult. Therefore, patients should be screened for CAN at the time of diagnosis of type 2 diabetes and within 5 years of diagnosis of type 1 diabetes (except presence of symptoms suggesting autonomic neuropathy earlier)[1]. In addition, screening may be of benefit before undergoing an operation or beginning a new intense exercise program[93,94]. Screening should include a clinical history and an evaluation for evidence of autonomic dysfunction. Main HRV tests (E:I ratio, heart rate response to Valsalva maneuver, and heart rate response to standing) should also be performed. As an alternative to CART, easier screening methods has been attempted to develop. For instance, sudomotor function tests that assess the cholinergic innervation of sweat glands have been found to be useful for early screening of CAN[95]. Ge *et al*[96] offered a new risk score system not requiring specific tests for screening CAN, using clinical parameters including age, body mass index, hypertension and resting heart rate. The risk score can be between 0 and 15, and a score of 6 can detect CAN in 72.87% of previously undiagnosed individuals[96]. Screening should be repeated annually in the presence of negative results[1].

THERAPEUTIC APPROACHES

Early determination of CAN is significant for the success of therapeutic strategies as cardiovascular denervation seems to be reversible at onset[97]. In less affected patients, lifestyle changes including graded supervised exercise associated with weight loss improve HRV[97].

Optimizing glycemic control

Blood glucose optimization is the essential treatment for CAN. The Framingham Heart Study showed the significant association between reduced HRV and increased fasting plasma glucose level[98]. This finding is present in diabetics as well as individuals with impaired glucose tolerance[99]. Additionally, the DCCT reported that intensive insulin therapy reduced the incidence of CAN in comparison to conventional insulin therapy after approximately 5 years (14% *vs* 7%; $P < 0.004$) in type 1 diabetics[100]. The Epidemiology of Diabetes Intervention and Complication Study (EDIC) is a longitudinal cohort follow-up study for the DCCT[101]. Pop-Busui *et al*[102] demonstrated that during EDIC follow-up, CAN progressed in both the conventional and intensive insulin therapy groups, while its incidence and prevalence remained lower in the intensive therapy group despite similar glycemic control[102]. Accordingly, the early initiation of intensive glucose control in type 1 diabetics can help to minimize the development of CAN[102]. On the other hand, the benefit of glycemic control in type 2 diabetics is less certain[1,2]. The Veterans Affairs Cooperative Study reported a similar prevalence of autonomic neuropathy in type 2 diabetics after 2 years of intense glucose control in comparison with conventional glycemic control[103]. Similarly, in the Anglo-Danish-Dutch Study of Intensive Treatment in People With Screen Detected Diabetes in Primary Care Danish arm, CAN was frequently found 6 years following diagnosis of type 2 diabetes and this prevalence was not significantly affected by intensive multifactorial treatment in comparison with routine care[104]. Conversely, in the Steno-2 Trial patients with type 2 diabetes were given intensive multifactorial treatment (*e.g.*, targeting hyperglycemia, hypertension and dyslipidemia, including acetylsalicylic acid for secondary prevention) and targeted strict glycemic control as well as other cardiovascular risk factor modification, which reduced the incidence of autonomic dysfunction by approximately 60%[105]. Briefly, the intensive blood glucose, HbA1c, blood pressure and lipid levels control using pharmacological therapy with lifestyle changes are recommended for all diabetic patients[1].

Other therapies

Functional disorders of the autonomic nervous system can be treated with a variety of medications. In a trial

including 73 type 2 diabetic subjects, a four-month period of treatment with alpha-lipoic acid, which reduces oxygen free radicals, improved HRV detected by standardized CART[1,106]. While the use of aldose reductase inhibitors (epalrestat, fidarestat and AS-3201), which reduce nerve sorbitol, had a positive influence on HRV in patients with mild abnormalities, they were ineffective in advanced CAN patients[1,107]. Total HRV has been shown to be increased and parasympathetic/sympathetic balance improved by angiotensin-converting enzyme (ACE) inhibition in patients with mild autonomic neuropathy through increases in nerve blood flow[1]. Prostoglandin analogs have been shown to be effective through the same mechanism[1]. Cardioselective beta-blockers are considered to have positive effects on autonomic dysfunction. For example, the addition of metoprolol to ramipril therapy in patients with type 1 diabetes resulted in recovery of HRV parameters[108]. Furthermore, bisoprolol improved HRV in heart failure[109]. In a study including individuals with long-term diabetes and diabetic neuropathy, the combination of ACE inhibition and angiotensin-receptor blockade improved autonomic neuropathy[110]. In addition, Ozdemir *et al*[111] showed that losartan therapy significantly improved HRV in patients with ischemic cardiomyopathy already receiving ACE inhibitors and beta-blockers. Similarly, sympathovagal imbalance in heart failure patients was improved following the administration of spironolactone along with enalapril, furosemide, and digoxin[112]. Such evidence reveals that combination therapies appear to provide better results than monotherapies.

Orthostatic hypotension

Because orthostatic hypotension is a relatively late complication of CAN, the treatment is challenging due to advanced disease. Nonpharmacological treatments include: increased water consumption; the use of lower-extremity stockings; avoidance of sudden postural changes to standing up; avoidance of medicines such as vasodilators, diuretics, phenothiazines and tricyclic antidepressants that provoke hypotension; eating frequent, small meals to prevent postprandial hypotension; and avoidance of exercises and maneuvers that increase intra-abdominal and intra-thoracic pressure resulting in venous return decrease[20]. Some physical preventive maneuvers, such as crossing of the legs and squatting may counter decreases in blood pressure[9]. While pharmacological treatments, such as midodrine, clonidine, octreotide, fludrocortisone acetate, erythropoietin, nonselective beta-blockers and prydostigmine bromide appear promising, all have mild to severe side effects, including hypertension[9].

CONCLUSION

Although very common and serious, CAN is a frequently overlooked complication of diabetes. Related with intraoperative and perioperative cardiovascular instability, abnormal blood pressure profile, orthostatic hypotension, silent myocardial ischemia, arrhythmias, diabetic cardiomyopathy, and stroke, CAN is associated with significant increases in morbidity and mortality. Patients may have subclinical CAN for several years before it becomes clinically apparent. Because the progression of cardiovascular denervation is partly reversible or can be slowed down in the early stages of the disease, recent guidelines strongly recommend screening for CAN in patients with diabetes. Assessment of CAN is possible through a variety of methods, such as CART, HRV and imaging modalities. Operator friendly devices and use of Holter-based analysis has simplified CAN testing. Treatment principles include early diagnosis, optimization of glycemic control, life style changes and management of cardiovascular risk factors. Medical therapy, including aldose reductase inhibitors, ACE inhibitors, prostoglandin analogs and alpha-lipoic acid, have been found to be effective in randomized control trials for the treatment of autonomic neuropathies. Orthostatic hypotension, which may lead to life-threatening injuries, is an undesired manifestation and indicates severe or advanced CAN.

REFERENCES

1 **Boulton AJ**, Vinik AI, Arezzo JC, Bril V, Feldman EL, Freeman R, Malik RA, Maser RE, Sosenko JM, Ziegler D. Diabetic neuropathies: a statement by the American Diabetes Association. *Diabetes Care* 2005; **28**: 956-962 [PMID: 15793206 DOI: 10.2337/diacare.28.4.956]

2 **Vinik AI**, Erbas T. Diabetic autonomic neuropathy. *Handb Clin Neurol* 2013; **117**: 279-294 [PMID: 24095132 DOI: 10.1016/B978-0-444-53491-0.00022-5]

3 **Ewing DJ**, Campbell IW, Clarke BF. The natural history of diabetic autonomic neuropathy. *Q J Med* 1980; **49**: 95-108 [PMID: 7433630]

4 **World Health Organization**. Diabetes Programme. Available from: URL: http: //www.who.int/diabetes/en/

5 **The Diabetes Control and Complications Trial Research Group.** The effect of intensive diabetes therapy on measures of autonomic nervous system function in the Diabetes Control and Complications Trial (DCCT). *Diabetologia* 1998; **41**: 416-423 [PMID: 9562345 DOI: 10.1007/s001250050924]

6 **Ziegler D**, Dannehl K, Mühlen H, Spüler M, Gries FA. Prevalence of cardiovascular autonomic dysfunction assessed by spectral analysis, vector analysis, and standard tests of heart rate variation and blood pressure responses at various stages of diabetic neuropathy. *Diabet Med* 1992; **9**: 806-814 [PMID: 1473320 DOI: 10.1111/j.1464-5491.1992.tb01898.x]

7 **Witte DR**, Tesfaye S, Chaturvedi N, Eaton SE, Kempler P, Fuller JH. Risk factors for cardiac autonomic neuropathy in type 1 diabetes mellitus. *Diabetologia* 2005; **48**: 164-171 [PMID: 15619072 DOI: 10.1007/s00125-004-1617-y]

8 **Ziegler D**, Zentai C, Perz S, Rathmann W, Haastert B, Meisinger C, Löwel H. Selective contribution of diabetes and other cardiovascular risk factors to cardiac autonomic dysfunction in the general population. *Exp Clin Endocrinol Diabetes* 2006; **114**: 153-159 [PMID: 16710813 DOI: 10.1055/s-2006-924083]

9 **Pop-Busui R**. Cardiac autonomic neuropathy in diabetes: a clinical perspective. *Diabetes Care* 2010; **33**: 434-441 [PMID: 20103559 DOI: 10.2337/dc09-1294]

10 **Vinik AI**, Maser RE, Mitchell BD, Freeman R. Diabetic autonomic neuropathy. *Diabetes Care* 2003; **26**: 1553-1579 [PMID: 12716821 DOI: 10.2337/diacare.26.5.1553]

11 **Albers JW**, Pop-Busui R. Diabetic neuropathy: mechanisms, emerging treatments, and subtypes. *Curr Neurol Neurosci Rep* 2014; **14**: 473 [PMID: 24954624 DOI: 10.1007/s11910-014-0473-5]
12 **Pfeifer MA**, Weinberg CR, Cook DL, Reenan A, Halter JB, Ensinck JW, Porte D. Autonomic neural dysfunction in recently diagnosed diabetic subjects. *Diabetes Care* 1984; **7**: 447-453 [PMID: 6499637 DOI: 10.2337/diacare.7.5.447]
13 **Low PA**, Benrud-Larson LM, Sletten DM, Opfer-Gehrking TL, Weigand SD, O'Brien PC, Suarez GA, Dyck PJ. Autonomic symptoms and diabetic neuropathy: a population-based study. *Diabetes Care* 2004; **27**: 2942-2947 [PMID: 15562211 DOI: 10.2337/diacare.27.12.2942]
14 **Navarro X**, Kennedy WR, Sutherland DE. Autonomic neuropathy and survival in diabetes mellitus: effects of pancreas transplantation. *Diabetologia* 1991; **34** Suppl 1: S108-S112 [PMID: 1936671 DOI: 10.1007/BF00587633]
15 **Schönauer M**, Thomas A, Morbach S, Niebauer J, Schönauer U, Thiele H. Cardiac autonomic diabetic neuropathy. *Diab Vasc Dis Res* 2008; **5**: 336-344 [PMID: 18958844 DOI: 10.3132/dvdr.2008.047]
16 **Ewing DJ**, Campbell IW, Clarke BF. Heart rate changes in diabetes mellitus. *Lancet* 1981; **1**: 183-186 [PMID: 6109858 DOI: 10.1016/S0140-6736(81)90061-1]
17 **Ziegler D**. Diabetic cardiovascular autonomic neuropathy: prognosis, diagnosis and treatment. *Diabetes Metab Rev* 1994; **10**: 339-383 [PMID: 7796704 DOI: 10.1002/dmr.5610100403]
18 **Airaksinen KE**, Koistinen MJ. Association between silent coronary artery disease, diabetes, and autonomic neuropathy. Fact of fallacy? *Diabetes Care* 1992; **15**: 288-292 [PMID: 1547688]
19 **Marchant B**, Umachandran V, Stevenson R, Kopelman PG, Timmis AD. Silent myocardial ischemia: role of subclinical neuropathy in patients with and without diabetes. *J Am Coll Cardiol* 1993; **22**: 1433-1437 [PMID: 8227802 DOI: 10.1016/0735-1097(93)90554-E]
20 **Vinik AI**, Ziegler D. Diabetic cardiovascular autonomic neuropathy. *Circulation* 2007; **115**: 387-397 [PMID: 17242296 DOI: 10.1161/CIRCULATIONAHA.106.634949]
21 **Ewing DJ**, Boland O, Neilson JM, Cho CG, Clarke BF. Autonomic neuropathy, QT interval lengthening, and unexpected deaths in male diabetic patients. *Diabetologia* 1991; **34**: 182-185 [PMID: 1884890 DOI: 10.1007/BF00418273]
22 **Rathmann W**, Ziegler D, Jahnke M, Haastert B, Gries FA. Mortality in diabetic patients with cardiovascular autonomic neuropathy. *Diabet Med* 1993; **10**: 820-824 [PMID: 8281726 DOI: 10.1111/j.1464-5491.1993.tb00173.x]
23 **O'Brien IA**, McFadden JP, Corrall RJ. The influence of autonomic neuropathy on mortality in insulin-dependent diabetes. *Q J Med* 1991; **79**: 495-502 [PMID: 1946930]
24 Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *Circulation* 1996; **93**: 1043-1065 [PMID: 8598068 DOI: 10.1161/01.CIR.93.5.1043]
25 **Metelka R**. Heart rate variability - current diagnosis of the cardiac autonomic neuropathy. A review. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* 2014; **158**: 327-338 [PMID: 25004914 DOI: 10.5507/bp.2014.025]
26 **Pop-Busui R**. What do we know and we do not know about cardiovascular autonomic neuropathy in diabetes. *J Cardiovasc Transl Res* 2012; **5**: 463-478 [PMID: 22644723 DOI: 10.1007/s12265-012-9367-6]
27 **Tang ZH**, Zeng F, Li Z, Zhou L. Association and predictive value analysis for resting heart rate and diabetes mellitus on cardiovascular autonomic neuropathy in general population. *J Diabetes Res* 2014; **2014**: 215473 [PMID: 24772443 DOI: 10.1155/2014/215473]
28 **Burgos LG**, Ebert TJ, Asiddao C, Turner LA, Pattison CZ, Wang-Cheng R, Kampine JP. Increased intraoperative cardiovascular morbidity in diabetics with autonomic neuropathy. *Anesthesiology* 1989; **70**: 591-597 [PMID: 2929996 DOI: 10.1097/00000542-198904000-00006]
29 **Kitamura A**, Hoshino T, Kon T, Ogawa R. Patients with diabetic neuropathy are at risk of a greater intraoperative reduction in core temperature. *Anesthesiology* 2000; **92**: 1311-1318 [PMID: 10781276 DOI: 10.1097/00000542-200005000-00019]
30 **Furlan R**, Guzzetti S, Crivellaro W, Dassi S, Tinelli M, Baselli G, Cerutti S, Lombardi F, Pagani M, Malliani A. Continuous 24-hour assessment of the neural regulation of systemic arterial pressure and RR variabilities in ambulant subjects. *Circulation* 1990; **81**: 537-547 [PMID: 2297860 DOI: 10.1161/01.CIR.81.2.537]
31 **Spallone V**, Bernardi L, Ricordi L, Soldà P, Maiello MR, Calciati A, Gambardella S, Fratino P, Menzinger G. Relationship between the circadian rhythms of blood pressure and sympathovagal balance in diabetic autonomic neuropathy. *Diabetes* 1993; **42**: 1745-1752 [PMID: 8243821 DOI: 10.2337/diab.42.12.1745]
32 **Schwartz PJ**, La Rovere MT, Vanoli E. Autonomic nervous system and sudden cardiac death. Experimental basis and clinical observations for post-myocardial infarction risk stratification. *Circulation* 1992; **85**: I77-I91 [PMID: 1728509]
33 **Low PA**, Walsh JC, Huang CY, McLeod JG. The sympathetic nervous system in diabetic neuropathy. A clinical and pathological study. *Brain* 1975; **98**: 341-356 [PMID: 810214 DOI: 10.1093/brain/98.3.341]
34 **Chiariello M**, Indolfi C. Silent myocardial ischemia in patients with diabetes mellitus. *Circulation* 1996; **93**: 2089-2091 [PMID: 8925575 DOI: 10.1161/01.CIR.93.12.2089]
35 **Dortimer AC**, Shenoy PN, Shiroff RA, Leaman DM, Babb JD, Liedtke AJ, Zelis R. Diffuse coronary artery disease in diabetic patients: fact or fiction? *Circulation* 1978; **57**: 133-136 [PMID: 618380 DOI: 10.1161/01.CIR.57.1.133]
36 **Garcia MJ**, McNamara PM, Gordon T, Kannel WB. Morbidity and mortality in diabetics in the Framingham population. Sixteen year follow-up study. *Diabetes* 1974; **23**: 105-111 [PMID: 4359625]
37 **Cohn PF**, Fox KM, Daly C. Silent myocardial ischemia. *Circulation* 2003; **108**: 1263-1277 [PMID: 12963683 DOI: 10.1161/01.CIR.0000088001.59265.EE]
38 **Weiner DA**, Ryan TJ, McCabe CH, Luk S, Chaitman BR, Sheffield LT, Tristani F, Fisher LD. Significance of silent myocardial ischemia during exercise testing in patients with coronary artery disease. *Am J Cardiol* 1987; **59**: 725-729 [PMID: 3825930 DOI: 10.1016/0002-9149(87)91081-2]
39 **Deedwania PC**, Carbajal EV. Silent ischemia during daily life is an independent predictor of mortality in stable angina. *Circulation* 1990; **81**: 748-756 [PMID: 2306826 DOI: 10.1161/01.CIR.81.3.748]
40 **Shakespeare CF**, Katritsis D, Crowther A, Cooper IC, Coltart JD, Webb-Peploe MW. Differences in autonomic nerve function in patients with silent and symptomatic myocardial ischaemia. *Br Heart J* 1994; **71**: 22-29 [PMID: 8297687 DOI: 10.1136/hrt.71.1.22]
41 **Kannel WB**, Abbott RD. Incidence and prognosis of unrecognized myocardial infarction. An update on the Framingham study. *N Engl J Med* 1984; **311**: 1144-1147 [PMID: 6482932 DOI: 10.1056/NEJM198411013111802]
42 **Margolis JR**, Kannel WS, Feinleib M, Dawber TR, McNamara PM. Clinical features of unrecognized myocardial infarction--silent and symptomatic. Eighteen year follow-up: the Framingham study. *Am J Cardiol* 1973; **32**: 1-7 [PMID: 4713110 DOI: 10.1016/S0002-9149(73)80079-7]
43 **Canto JG**, Shlipak MG, Rogers WJ, Malmgren JA, Frederick PD, Lambrew CT, Ornato JP, Barron HV, Kiefe CI. Prevalence, clinical characteristics, and mortality among patients with myocardial infarction presenting without chest pain. *JAMA* 2000; **283**: 3223-3229 [PMID: 10866870 DOI: 10.1001/jama.283.24.3223]

44 **Wackers FJ**, Young LH, Inzucchi SE, Chyun DA, Davey JA, Barrett EJ, Taillefer R, Wittlin SD, Heller GV, Filipchuk N, Engel S, Ratner RE, Iskandrian AE. Detection of silent myocardial ischemia in asymptomatic diabetic subjects: the DIAD study. *Diabetes Care* 2004; **27**: 1954-1961 [PMID: 15277423 DOI: 10.2337/diacare.27.8.1954]

45 **Weitzman S**, Wagner GS, Heiss G, Haney TL, Slome C. Myocardial infarction site and mortality in diabetes. *Diabetes Care* 1982; **5**: 31-35 [PMID: 7140497 DOI: 10.2337/diacare.5.1.31]

46 **Ambepityia G**, Kopelman PG, Ingram D, Swash M, Mills PG, Timmis AD. Exertional myocardial ischemia in diabetes: a quantitative analysis of anginal perceptual threshold and the influence of autonomic function. *J Am Coll Cardiol* 1990; **15**: 72-77 [PMID: 2295745 DOI: 10.1016/0735-1097(90)90178-R]

47 **Partamian JO**, Bradley RF. Acute myocardial infarction in 258 cases of diabetes. Immediate mortality and five-year survival. *N Engl J Med* 1965; **273**: 455-461 [PMID: 5826160 DOI: 10.1056/NEJM196508262730901]

48 **Bigger JT**, Fleiss JL, Steinman RC, Rolnitzky LM, Kleiger RE, Rottman JN. Frequency domain measures of heart period variability and mortality after myocardial infarction. *Circulation* 1992; **85**: 164-171 [PMID: 1728446 DOI: 10.1161/01.CIR.85.1.164]

49 **Kleiger RE**, Miller JP, Bigger JT, Moss AJ. Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. *Am J Cardiol* 1987; **59**: 256-262 [PMID: 3812275 DOI: 10.1016/0002-9149(87)90795-8]

50 **Miettinen H**, Lehto S, Salomaa V, Mähönen M, Niemelä M, Haffner SM, Pyörälä K, Tuomilehto J. Impact of diabetes on mortality after the first myocardial infarction. The FINMONICA Myocardial Infarction Register Study Group. *Diabetes Care* 1998; **21**: 69-75 [PMID: 9538972 DOI: 10.2337/diacare.21.1.69]

51 **Katz A**, Liberty IF, Porath A, Ovsyshcher I, Prystowsky EN. A simple bedside test of 1-minute heart rate variability during deep breathing as a prognostic index after myocardial infarction. *Am Heart J* 1999; **138**: 32-38 [PMID: 10385760 DOI: 10.1016/S0002-8703(99)70242-5]

52 **Sivieri R**, Veglio M, Chinaglia A, Scaglione P, Cavallo-Perin P. Prevalence of QT prolongation in a type 1 diabetic population and its association with autonomic neuropathy. The Neuropathy Study Group of the Italian Society for the Study of Diabetes. *Diabet Med* 1993; **10**: 920-924 [PMID: 8306587 DOI: 10.1111/j.1464-5491.1993.tb00007.x]

53 **Veglio M**, Borra M, Stevens LK, Fuller JH, Perin PC. The relation between QTc interval prolongation and diabetic complications. The EURODIAB IDDM Complication Study Group. *Diabetologia* 1999; **42**: 68-75 [PMID: 10027581 DOI: 10.1007/s001250051115]

54 **Givertz MM**, Sawyer DB, Colucci WS. Antioxidants and myocardial contractility: illuminating the "Dark Side" of beta-adrenergic receptor activation? *Circulation* 2001; **103**: 782-783 [PMID: 11171781 DOI: 10.1161/01.CIR.103.6.782]

55 **Iwai-Kanai E**, Hasegawa K, Araki M, Kakita T, Morimoto T, Sasayama S. alpha- and beta-adrenergic pathways differentially regulate cell type-specific apoptosis in rat cardiac myocytes. *Circulation* 1999; **100**: 305-311 [PMID: 10411857 DOI: 10.1161/01.CIR.100.3.305]

56 **Paulson DJ**, Light KE. Elevation of serum and ventricular norepinephrine content in the diabetic rat. *Res Commun Chem Pathol Pharmacol* 1981; **33**: 559-562 [PMID: 7330457]

57 **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]

58 **Maser RE**, Mitchell BD, Vinik AI, Freeman R. The association between cardiovascular autonomic neuropathy and mortality in individuals with diabetes: a meta-analysis. *Diabetes Care* 2003; **26**: 1895-1901 [PMID: 12766130 DOI: 10.2337/diacare.26.6.1895]

59 **Lykke JA**, Tarnow L, Parving HH, Hilsted J. A combined abnormality in heart rate variation and QT corrected interval is a strong predictor of cardiovascular death in type 1 diabetes. *Scand J Clin Lab Invest* 2008; **68**: 654-659 [PMID: 19378439 DOI: 10.1080/00365510802018330]

60 **Ziegler D**, Zentai CP, Perz S, Rathmann W, Haastert B, Döring A, Meisinger C. Prediction of mortality using measures of cardiac autonomic dysfunction in the diabetic and nondiabetic population: the MONICA/KORA Augsburg Cohort Study. *Diabetes Care* 2008; **31**: 556-561 [PMID: 18086873 DOI: 10.2337/dc07-1615]

61 **Buse JB**, Bigger JT, Byington RP, Cooper LS, Cushman WC, Friedewald WT, Genuth S, Gerstein HC, Ginsberg HN, Goff DC, Grimm RH, Margolis KL, Probstfield JL, Simons-Morton DG, Sullivan MD. Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial: design and methods. *Am J Cardiol* 2007; **99**: 21i-33i [PMID: 17599422 DOI: 10.1016/j.amjcard.2007.03.003]

62 **Pop-Busui R**, Evans GW, Gerstein HC, Fonseca V, Fleg JL, Hoogwerf BJ, Genuth S, Grimm RH, Corson MA, Prineas R. Effects of cardiac autonomic dysfunction on mortality risk in the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial. *Diabetes Care* 2010; **33**: 1578-1584 [PMID: 20215456 DOI: 10.2337/dc10-0125]

63 **Patel A**, MacMahon S, Chalmers J, Neal B, Billot L, Woodward M, Marre M, Cooper M, Glasziou P, Grobbee D, Hamet P, Harrap S, Heller S, Liu L, Mancia G, Mogensen CE, Pan C, Poulter N, Rodgers A, Williams B, Bompoint S, de Galan BE, Joshi R, Travert F. Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. *N Engl J Med* 2008; **358**: 2560-2572 [PMID: 18539916 DOI: 10.1056/NEJMoa0802987]

64 **Duckworth W**, Abraira C, Moritz T, Reda D, Emanuele N, Reaven PD, Zieve FJ, Marks J, Davis SN, Hayward R, Warren SR, Goldman S, McCarren M, Vitek ME, Henderson WG, Huang GD. Glucose control and vascular complications in veterans with type 2 diabetes. *N Engl J Med* 2009; **360**: 129-139 [PMID: 19092145 DOI: 10.1056/NEJMoa0808431]

65 **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]

66 **Cryer PE**. Hypoglycemia-associated autonomic failure in diabetes. *Handb Clin Neurol* 2013; **117**: 295-307 [PMID: 24095133 DOI: 10.1016/B978-0-444-53491-0.00023-7]

67 **Adler GK**, Bonyhay I, Failing H, Waring E, Dotson S, Freeman R. Antecedent hypoglycemia impairs autonomic cardiovascular function: implications for rigorous glycemic control. *Diabetes* 2009; **58**: 360-366 [PMID: 19056608 DOI: 10.2337/db08-1153]

68 **Bellmann B**, Tschöpe C. [Heart failure. Cardiovascular autonomic neuropathy in patients with diabetes mellitus]. *Herz* 2014; **39**: 306-311 [PMID: 24715197 DOI: 10.1007/s00059-014-4093-2]

69 **Boudina S**, Abel ED. Diabetic cardiomyopathy, causes and effects. *Rev Endocr Metab Disord* 2010; **11**: 31-39 [PMID: 20180026 DOI: 10.1007/s11154-010-9131-7]

70 **Pop-Busui R**, Cleary PA, Braffett BH, Martin CL, Herman WH, Low PA, Lima JA, Bluemke DA. Association between cardiovascular autonomic neuropathy and left ventricular dysfunction: DCCT/EDIC study (Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications). *J Am Coll Cardiol* 2013; **61**: 447-454 [PMID: 23265339 DOI: 10.1016/j.jacc.2012.10.028]

71 **Acar E**, Ural D, Bildirici U, Sahin T, Yılmaz I. Diabetic

cardiomyopathy. *Anadolu Kardiyol Derg* 2011; **11**: 732-737 [PMID: 22137942 DOI: 10.5152/akd.2011.196]
72 **Ko SH**, Song KH, Park SA, Kim SR, Cha BY, Son HY, Moon KW, Yoo KD, Park YM, Cho JH, Yoon KH, Ahn YB. Cardiovascular autonomic dysfunction predicts acute ischaemic stroke in patients with Type 2 diabetes mellitus: a 7-year follow-up study. *Diabet Med* 2008; **25**: 1171-1177 [PMID: 19046195 DOI: 10.1111/j.1464-5491.2008.02567.x]
73 **Töyry JP**, Niskanen LK, Mäntysaari MJ, Länsimies EA, Uusitupa MI. Occurrence, predictors, and clinical significance of autonomic neuropathy in NIDDM. Ten-year follow-up from the diagnosis. *Diabetes* 1996; **45**: 308-315 [PMID: 8593935 DOI: 10.2337/diab.45.3.308]
74 **Ewing DJ**, Campbell IW, Clarke BF. Assessment of cardiovascular effects in diabetic autonomic neuropathy and prognostic implications. *Ann Intern Med* 1980; **92**: 308-311 [PMID: 7356219 DOI: 10.7326/0003-4819-92-2-308]
75 **Ewing DJ**, Martyn CN, Young RJ, Clarke BF. The value of cardiovascular autonomic function tests: 10 years experience in diabetes. *Diabetes Care* 1985; **8**: 491-498 [PMID: 4053936 DOI: 10.2337/diacare.8.5.491]
76 **Ewing DJ**, Campbell IW, Murray A, Neilson JM, Clarke BF. Immediate heart-rate response to standing: simple test for autonomic neuropathy in diabetes. *Br Med J* 1978; **1**: 145-147 [PMID: 620228]
77 **Pfeifer MA**, Cook D, Brodsky J, Tice D, Reenan A, Swedine S, Halter JB, Porte D. Quantitative evaluation of cardiac parasympathetic activity in normal and diabetic man. *Diabetes* 1982; **31**: 339-345 [PMID: 7152130 DOI: 10.2337/diab.31.4.339]
78 **Sandroni P**, Benarroch EE, Low PA. Pharmacological dissection of components of the Valsalva maneuver in adrenergic failure. *J Appl Physiol* (1985) 1991; **71**: 1563-1567 [PMID: 1757382]
79 **England JD**, Gronseth GS, Franklin G, Carter GT, Kinsella LJ, Cohen JA, Asbury AK, Szigeti K, Lupski JR, Latov N, Lewis RA, Low PA, Fisher MA, Herrmann D, Howard JF, Lauria G, Miller RG, Polydefkis M, Sumner AJ. Evaluation of distal symmetric polyneuropathy: the role of autonomic testing, nerve biopsy, and skin biopsy (an evidence-based review). *Muscle Nerve* 2009; **39**: 106-115 [PMID: 19086069 DOI: 10.1002/mus.21227]
80 **Stranieri A**, Abawajy J, Kelarev A, Huda S, Chowdhury M, Jelinek HF. An approach for Ewing test selection to support the clinical assessment of cardiac autonomic neuropathy. *Artif Intell Med* 2013; **58**: 185-193 [PMID: 23768975 DOI: 10.1016/j.artmed.2013.04.007]
81 **Howorka K**, Pumprla J, Schabmann A. Optimal parameters of short-term heart rate spectrogram for routine evaluation of diabetic cardiovascular autonomic neuropathy. *J Auton Nerv Syst* 1998; **69**: 164-172 [PMID: 9696273 DOI: 10.1016/S0165-1838(98)00015-0]
82 **Bauer A**, Malik M, Schmidt G, Barthel P, Bonnemeier H, Cygankiewicz I, Guzik P, Lombardi F, Müller A, Oto A, Schneider R, Watanabe M, Wichterle D, Zareba W. Heart rate turbulence: standards of measurement, physiological interpretation, and clinical use: International Society for Holter and Noninvasive Electrophysiology Consensus. *J Am Coll Cardiol* 2008; **52**: 1353-1365 [PMID: 18940523 DOI: 10.1016/j.jacc.2008.07.041]
83 **Balcioğlu S**, Arslan U, Türkoğlu S, Ozdemir M, Cengel A. Heart rate variability and heart rate turbulence in patients with type 2 diabetes mellitus with versus without cardiac autonomic neuropathy. *Am J Cardiol* 2007; **100**: 890-893 [PMID: 17719340 DOI: 10.1016/j.amjcard.2007.03.106]
84 **Schnell O**, Muhr D, Weiss M, Dresel S, Haslbeck M, Standl E. Reduced myocardial 123I-metaiodobenzylguanidine uptake in newly diagnosed IDDM patients. *Diabetes* 1996; **45**: 801-805 [PMID: 8635656 DOI: 10.2337/diab.45.6.801]
85 **Stevens MJ**, Raffel DM, Allman KC, Dayanikli F, Ficaro E, Sandford T, Wieland DM, Pfeifer MA, Schwaiger M. Cardiac sympathetic dysinnervation in diabetes: implications for enhanced cardiovascular risk. *Circulation* 1998; **98**: 961-968 [PMID: 9737515 DOI: 10.1161/01.CIR.98.10.961]
86 **Stevens MJ**, Raffel DM, Allman KC, Schwaiger M, Wieland DM. Regression and progression of cardiac sympathetic dysinnervation complicating diabetes: an assessment by C-11 hydroxyephedrine and positron emission tomography. *Metabolism* 1999; **48**: 92-101 [PMID: 9920151 DOI: 10.1016/S0026-0495(99)90016-1]
87 **Hamner JW**, Taylor JA. Automated quantification of sympathetic beat-by-beat activity, independent of signal quality. *J Appl Physiol* (1985) 2001; **91**: 1199-1206 [PMID: 11509516]
88 **Vinik AI**, Erbas T. Recognizing and treating diabetic autonomic neuropathy. *Cleve Clin J Med* 2001; **68**: 928-930, 932, 934-944 [PMID: 11718432 DOI: 10.3949/ccjm.68.11.928]
89 **La Rovere MT**, Bigger JT, Marcus FI, Mortara A, Schwartz PJ. Baroreflex sensitivity and heart-rate variability in prediction of total cardiac mortality after myocardial infarction. ATRAMI (Autonomic Tone and Reflexes After Myocardial Infarction) Investigators. *Lancet* 1998; **351**: 478-484 [PMID: 9482439 DOI: 10.1016/S0140-6736(97)11144-8]
90 **Banthia S**, Bergner DW, Chicos AB, Ng J, Pelchovitz DJ, Subacius H, Kadish AH, Goldberger JJ. Detection of cardiovascular autonomic neuropathy using exercise testing in patients with type 2 diabetes mellitus. *J Diabetes Complications* 2013; **27**: 64-69 [PMID: 23083925 DOI: 10.1016/j.jdiacomp.2012.09.002]
91 **Spallone V**, Ziegler D, Freeman R, Bernardi L, Frontoni S, Pop-Busui R, Stevens M, Kempler P, Hilsted J, Tesfaye S, Low P, Valensi P. Cardiovascular autonomic neuropathy in diabetes: clinical impact, assessment, diagnosis, and management. *Diabetes Metab Res Rev* 2011; **27**: 639-653 [PMID: 21695768 DOI: 10.1002/dmrr.1239]
92 **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]
93 **Buse JB**, Ginsberg HN, Bakris GL, Clark NG, Costa F, Eckel R, Fonseca V, Gerstein HC, Grundy S, Nesto RW, Pignone MP, Plutzky J, Porte D, Redberg R, Stitzel KF, Stone NJ. Primary prevention of cardiovascular diseases in people with diabetes mellitus: a scientific statement from the American Heart Association and the American Diabetes Association. *Circulation* 2007; **115**: 114-126 [PMID: 17192512 DOI: 10.1161/CIRCULATIONAHA.106.179294]
94 **Kadoi Y**. Anesthetic considerations in diabetic patients. Part I: preoperative considerations of patients with diabetes mellitus. *J Anesth* 2010; **24**: 739-747 [PMID: 20640453 DOI: 10.1007/s00540-010-0987-1]
95 **Yajnik CS**, Kantikar V, Pande A, Deslypere JP, Dupin J, Calvet JH, Bauduceau B. Screening of cardiovascular autonomic neuropathy in patients with diabetes using non-invasive quick and simple assessment of sudomotor function. *Diabetes Metab* 2013; **39**: 126-131 [PMID: 23159130 DOI: 10.1016/j.diabet.2012.09.004]
96 **Ge X**, Pan SM, Zeng F, Tang ZH, Wang YW. A simple Chinese risk score model for screening cardiovascular autonomic neuropathy. *PLoS One* 2014; **9**: e89623 [PMID: 24621478 DOI: 10.1371/journal.pone.0089623]
97 **Howorka K**, Pumprla J, Haber P, Koller-Strametz J, Mondrzyk J, Schabmann A. Effects of physical training on heart rate variability in diabetic patients with various degrees of cardiovascular autonomic neuropathy. *Cardiovasc Res* 1997; **34**: 206-214 [PMID: 9217892 DOI: 10.1016/S0008-6363(97)00040-0]
98 **Singh JP**, Larson MG, O'Donnell CJ, Wilson PF, Tsuji H, Lloyd-Jones DM, Levy D. Association of hyperglycemia with reduced heart rate variability (The Framingham Heart Study). *Am J Cardiol* 2000; **86**: 309-312 [PMID: 10922439 DOI: 10.1016/S0002-9149(00)00920-6]

99 **The Diabetes Control and Complications Trial Research Group.** The absence of a glycemic threshold for the development of long-term complications: the perspective of the Diabetes Control and Complications Trial. *Diabetes* 1996; **45**: 1289-1298 [PMID: 8826962 DOI: 10.2337/diab.45.10.1289]

100 **The Diabetes Control and Complications Trial Research Group.** The effect of intensive diabetes therapy on the development and progression of neuropathy. The Diabetes Control and Complications Trial Research Group. *Ann Intern Med* 1995; **122**: 561-568 [PMID: 7887548 DOI: 10.7326/0003-4819-122-8-199504150-00001]

101 Writing Team for the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group. Sustained effect of intensive treatment of type 1 diabetes mellitus on development and progression of diabetic nephropathy: the Epidemiology of Diabetes Interventions and Complications (EDIC) study. *JAMA* 2003; **290**: 2159-2167 [PMID: 14570951 DOI: 10.1001/jama.290.16.2159]

102 **Pop-Busui R**, Low PA, Waberski BH, Martin CL, Albers JW, Feldman EL, Sommer C, Cleary PA, Lachin JM, Herman WH. Effects of prior intensive insulin therapy on cardiac autonomic nervous system function in type 1 diabetes mellitus: the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications study (DCCT/EDIC). *Circulation* 2009; **119**: 2886-2893 [PMID: 19470886 DOI: 10.1161/CIRCULATIONAHA.108.837369]

103 **Azad N**, Emanuele NV, Abraira C, Henderson WG, Colwell J, Levin SR, Nuttall FQ, Comstock JP, Sawin CT, Silbert C, Rubino FA. The effects of intensive glycemic control on neuropathy in the VA cooperative study on type II diabetes mellitus (VA CSDM). *J Diabetes Complications* 1999; **13**: 307-313 [PMID: 10765007 DOI: 10.1016/S1056-8727(99)00062-8]

104 **Charles M**, Fleischer J, Witte DR, Ejskjaer N, Borch-Johnsen K, Lauritzen T, Sandbaek A. Impact of early detection and treatment of diabetes on the 6-year prevalence of cardiac autonomic neuropathy in people with screen-detected diabetes: ADDITION-Denmark, a cluster-randomised study. *Diabetologia* 2013; **56**: 101-108 [PMID: 23064291 DOI: 10.1007/s00125-012-2744-5]

105 **Gaede P**, Vedel P, Parving HH, Pedersen O. Intensified multifactorial intervention in patients with type 2 diabetes mellitus and microalbuminuria: the Steno type 2 randomised study. *Lancet* 1999; **353**: 617-622 [PMID: 10030326 DOI: 10.1016/S0140-6736(98)07368-1]

106 **Ziegler D**, Schatz H, Conrad F, Gries FA, Ulrich H, Reichel G. Effects of treatment with the antioxidant alpha-lipoic acid on cardiac autonomic neuropathy in NIDDM patients. A 4-month randomized controlled multicenter trial (DEKAN Study). Deutsche Kardiale Autonome Neuropathie. *Diabetes Care* 1997; **20**: 369-373 [PMID: 9051389 DOI: 10.2337/diacare.20.3.369]

107 **Hu X**, Li S, Yang G, Liu H, Boden G, Li L. Efficacy and safety of aldose reductase inhibitor for the treatment of diabetic cardiovascular autonomic neuropathy: systematic review and meta-analysis. *PLoS One* 2014; **9**: e87096 [PMID: 24533052 DOI: 10.1371/journal.pone.0087096]

108 **Ebbehøj E**, Poulsen PL, Hansen KW, Knudsen ST, Mølgaard H, Mogensen CE. Effects on heart rate variability of metoprolol supplementary to ongoing ACE-inhibitor treatment in Type I diabetic patients with abnormal albuminuria. *Diabetologia* 2002; **45**: 965-975 [PMID: 12136395 DOI: 10.1007/s00125-002-0869-7]

109 **Pousset F**, Copie X, Lechat P, Jaillon P, Boissel JP, Hetzel M, Fillette F, Remme W, Guize L, Le Heuzey JY. Effects of bisoprolol on heart rate variability in heart failure. *Am J Cardiol* 1996; **77**: 612-617 [PMID: 8610612 DOI: 10.1016/S0002-9149(97)89316-2]

110 **Didangelos TP**, Arsos GA, Karamitsos DT, Athyros VG, Georga SD, Karatzas ND. Effect of quinapril or losartan alone and in combination on left ventricular systolic and diastolic functions in asymptomatic patients with diabetic autonomic neuropathy. *J Diabetes Complications* 2006; **20**: 1-7 [PMID: 16389160 DOI: 10.1016/j.jdiacomp.2005.05.002]

111 **Ozdemir M**, Arslan U, Türkoğlu S, Balcioğlu S, Cengel A. Losartan improves heart rate variability and heart rate turbulence in heart failure due to ischemic cardiomyopathy. *J Card Fail* 2007; **13**: 812-817 [PMID: 18068613 DOI: 10.1016/j.cardfail.2007.08.002]

112 **Korkmaz ME**, Müderrisoğlu H, Uluçam M, Ozin B. Effects of spironolactone on heart rate variability and left ventricular systolic function in severe ischemic heart failure. *Am J Cardiol* 2000; **86**: 649-653 [PMID: 10980217 DOI: 10.1016/S0002-9149(00)01046-8]

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REVIEW

Ocular complications of diabetes mellitus

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Abstract

Diabetes mellitus (DM) is a important health problem that induces ernestful complications and it causes significant morbidity owing to specific microvascular complications such as, retinopathy, nephropathy and neuropathy, and macrovascular complications such as, ischaemic heart disease, and peripheral vasculopathy. It can affect children, young people and adults and is becoming more common. Ocular complications associated with DM are progressive and rapidly becoming the world's most significant cause of morbidity and are preventable with early detection and timely treatment. This review provides an overview of five main ocular complications associated with DM, diabetic retinopathy and papillopathy, cataract, glaucoma, and ocular surface diseases.

Key words: Diabetes mellitus; Diabetic retinopathy; Ocular complication; Neovascular glaucoma; Cataract; Ocular diseases

Core tip: Ocular complications associated with diabetes mellitus (DM) are progressive and rapidly becoming the world's most significant cause of morbidity and are preventable with early detection and timely treatment. This review provides an overview of five main ocular complications associated with DM, diabetic retinopathy and papillopathy, cataract, glaucoma, and ocular surface diseases.

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INTRODUCTION

Complications of diabetes mellitus (DM) are progressive and almost resulting by chronic exposure to high blood levels of glucose caused by impairments in insulin metabolism and biological macromolecules such as carbohydrates, lipids, proteins and nucleic acids[1]. DM and its complications are rapidly becoming the world's most significant cause of morbidity and mortality[2,3]. The DM pandemic has expanded speedily in the developed

and developing countries. It is expected that DM will reach epidemic proportions within the near future[4]. DM affects more than 240 million people worldwide, and this number is expected to reach roughly 370 million by 2030[5,6]. DM can lead to several ocular complications such as diabetic retinopathy, diabetic papillopathy, glaucoma, cataract, and ocular surface diseases[7]. Diabetes related ocular complications are general public health problem, so we purpose of putting emphasis on the frequencies, pathogenesis, and management of these ocular complications.

DIABETIC RETINOPATHY

Diabetic retinopathy (DR), a microangiopathy affecting all of the small retinal vessels, such as arterioles, capillaries and venules, is characterized by increased vascular permeability, ocular haemorrhages, lipid exudate, by vascular closure mediated by the development of new vessels on the retina and the posterior vitreous surface[8]. DR, the most common microvascular complication of DM, is predicted to be the principal reason of new blindness among working population[9,10]. DR is the major reason of blindness in adults 20-74 years of age in the United States of America[11]. In patients with type 1 and type 2 diabetics with disease duration of over twenty years, the prevalences of DR are 95% and 60%, respectively[12]. Roughly 25% of type 1 diabetic patients have been reported to be influenced with DR, with the frequency increasing to about 80% after 15 years of anguish[13]. The type 2 DM is responsible for a higher percentage of patients with visual loss[13]. The incidence of DR is related primarily to duration and control of diabetes and is related to hyperglycemia, hypertension, hyperlipidemia, pregnancy, nephropathy, and anemia[14-16]. According to reports published by Wisconsin epidemiologic study of diabetic retinopathy (WESDR)[17], the general 10-year incidence of DR was 74%. Moreover in 64% of people with baseline DR developed more severe DR and 17% of those advanced to occur proliferative DR[18].

Pathogenesis

There is a very strong relationship between chronic hyperglycemia and the development of DR[19,20]. Hyperglycemia triggers a sequence of events causing vascular endothelial dysfunction. Many interdependent metabolic pathways have been put forward as important connections between hyperglycemia and DR. These implicated metabolic pathways include increased polyol[21] and protein kinase C (PKC) pathway[22] activity, upregulation of growth factors of which vascular endothelial growth factor (VEGF)[22], generation of advanced glycation endproducts (AGEs)[23,24], chronic oxidative damage[25], increased activation of the renin angiotensin system (RAS)[26], chronic inflammation and abnormal clumping of leukocytes (leukostasis)[26].

When excessive amounts of glucose increase the polyol way is activated to reduce glucose into sorbitol. The aldose reductase enzyme and nicotinamide adenine dinucleotide phosphate are involved in this biochemical reaction. Sorbitol is further metabolized to fructose by sorbitol dehydrogenase. Since sorbitol movement is severely restricted by cellular membrane, excessive accumulation of sorbitol in the cell occurs[27,28]. The increased sorbitol has potential osmotic damage in retinal cells[29] (Figure 1).

Chronic hyperglycemia increases quantity of diacylglycerol (DAG), which is leading to activate protein kinase C[30]. This activation leads to increase vascular permeability and upregulation of VEGF in the retinal structure. However, this abnormal pathway may lead to increase the activation of leukostasis[31-33] and significant changes in extracellular matrix (ECM) protein synthesis (Figure 2). Eventually, DAG and PKC pathway adversely affect inflammation, neovascularization, and retinal haemodynamics, which redounds to progression of DR[26].

VEGF is a crucial mediator in microvascular complications of DM. Normally, numerous retinal cells such as, retinal pigment epithelial (RPE) cells, Mueller cells, and pericytes, produce VEGF[31-33]. When a hypoxia occurs VEGF is secreted much more than normal production by hypoxic retinal tissues[31]. Clinical studies have reported that there is a strong correlation between DR and intraocular VEGF concentrations. Intravitreal and intracameral VEGF levels were prominently increased in patients with proliferative diabetic retinopathy (PDR)[34]. Additionally, VEGF has a crucial role in the pathogenesis of diabetic macular edema (DME) by increasing vascular permeability[35,36].

AGEs have been implicated in several diabetic complications, such as DR, and DME. Under chronic hyperglycemic circumstances, proteins are nonenzymatically glycated and the excessive amount of AGEs alter structures and functions of ECM, basement membranes, and vessel wall.

Oxidative stress is also a serious condition that may result in microvascular complications[37,38]. Severe production of reactive oxygen radicals may increase the oxidative stress and reduce antioxidant capacity[39].

RAAS is the endocrine system that takes an essential role to regulate vascular blood pressure, electrolyte, and fluid balance and shows an aberration in patients with DM[40], although the accurate process of RAAS leads to DR is not well clarified.

Inflammation is a prominent part of the pathogenesis of DR[41,42]. In response to hyperglycemic stress, AGE formation, and hypertension, a sequence of inflammatory mediators are increased in DM. Retinal subclinical inflammation contributes to elevated intraocular perfusion pressure by means of endothelial nitric oxide synthase (eNOS), the development of neovascularization (NV) due to hypoxia and VEGF. Although there are no strong association between systemic inflammation and development of DR[43,44], leukostasis is a likely to be a significant local factor in DR pathogenesis, causing capillary occlusion.

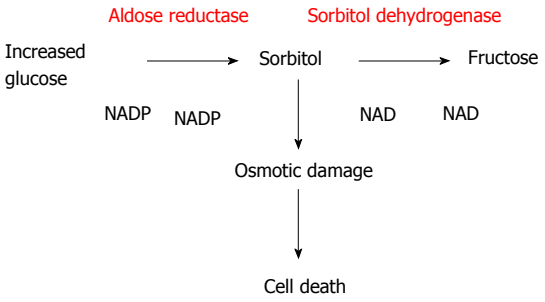

Figure 1 The polyol pathway.

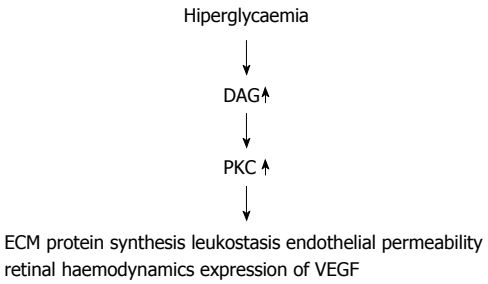

Figure 2 The protein kinase C pathway. DAG: Diacylglycerol; PKC; Protein kinase C; VEGF: Vascular endothelial growth factor; ECM: Extracellular matrix.

The classification of DR

Previously, DR was classified into three forms, such as, background, pre-proliferative, and proliferative DR. The current classification is based on the location, extent, and degree of various clinically significant features, such as microaneurysms, intraretinal hemorrhages, venous abnormaities such as beading, intraretinal microvascular abnormalities (IRMA), and NV. Recently, DR is classified as either nonproliferative or proliferative.

Nonproliferative diabetic retinopathy: (1) Mild non-proliferative diabetic retinopathy (NPDR): There are a few microaneurysms; (2) Moderate NPDR: In this form, there are less than 20 microaneurysms. Hard yellow exudates, cotton wool spots, and venous beading are present also in only one quadrant; (3) Severe NPDR: It is identified as any of following clinic features; Microaneurysms in all 4 quadrants; Venous beading in 2 or more quadrants; IRMA in 1 or more quadrant; and (4) Very severe NPDR: This form includes 2 or more of the criteria for severe NPDR.

PDR: As a response to ischemia, NV grows at the optic nerve (NVD) and elsewhere in the retina except the optic disc (NVE). In general, NV grows at the border zone of perfused and non-perfused retina. These new vessels are permeable, and the leakage of plasma contents probably causes a structural change in the adjacent vitreous. Also, NV may cause preretinal and subhyaloid vitreous hemorrhages and can become membrane formations on the posterior hyaloid surface.

Diabetic macular edema

Macular edema is defined as retinal thickening or the existence of hard exudates at 2 disk diameter of the macula. Diabetic macular edema (DME) is the most common cause of moderate or severe visual loss in diabetic patients. DME occurs apart from the stage of DR, so it should be evaluated independently. In diabetic eyes, central macular thickness does not correlate directly with visual acuity, but there is a vigorous link between the unity of the photoreceptor inner/outer segment junction and visual acuity[45].

Clinically significant macular edema

The Early Treatment Diabetic Retinopathy Study (ETDRS) described the clinically significant macular edema (CSME) as the following conditions: (1) Retinal thickening within 500 microns of the center of the fovea; (2) Hard yellow exudate within 500 microns of the center of the fovea with adjacent retinal thickening; and (3) Retinal thickening 1 disc area or larger, any part of which is within 1 disc diameter of the center of the fovea.

The ETDRS indicated that the presence of CMSE guide ophthalmologyst for the focal laser treatment.

DME classification based on optical coherence tomography

Optical coherence tomography (OCT) shows four different types of DME: Sponge like retinal swelling, cystoid macular edema (CME), macular edema with serous retinal detachment (SRD) and tractional macular edema (TDME)[46-48].

Sponge like retinal swelling: There is an increased diffuse retinal thickness with reduced intraretinal reflectivity. This type of retinal swelling has a better visual outcome than the CME, SRD and TRD types after laser treatment[49].

CME: In this type, there is diffuse or focal retinal thickening with intraretinal cystic spaces.

SRD: There is an accumulation of subretinal fluid below reflective elevation. It is possible to confirm the presence of SRD only by OCT.

TDME: TDME is identified by a hyperreflective membrane on OCT with loss of foveal depression and macular edema.

First examination and follow-up

The WESDR study represented that, for type 1 diabetic patients, the frequency of NPDR at less than 5 years was 17% and the frequency of PDR was nearly 0%[50]. These frequencies were nearly 99%, and 50% after 20 years later, respectively. So, the first eye exam should be performed almost 4 years after diagnosis with annual follow-up exams.

The same study indicated that, for type 2 diabetic patients, the frequency of NPDR at 5 years was nearly 30% and the frequency of PDR was nearly 2%[51]. These frequencies were nearly 80%, and 15% after 15

years later, respectively. So, the first eye exam should be examined at diagnosis with annual follow-up exams.

Mild NPDR can be followed with dilated fundus exams every 12 mo. If DME that is not CSME is present, follow-up every 3 mo is advised. If CSME is present, treatment is advised promptly. Severe NPDR should be followed up every 2 mo. If very severe NPDR is present, patients should be followed more closely. After treatment of PDR, they should be observed every 3 mo not to overlook complications, such as TRD and CSME.

Current therapy

The treatment of DR includes increased metabolic control, laser treatment, intravitreal medication, and surgery.

Metabolic control

Poor metabolic control is a good marker for development and progression of DR. So, related risk factor such as, hyperglycemia, hypertension, and hyperlipidemia should be controlled. It reduces the risk of retinopathy occurrence and progression[52].

Glysemic control

The trial research group[53] showed that, for type 1 diabetic patients, a 10% reduction in the hemoglobin A1c (HbA1c) was associated with a 43% and 45% diminution in improvement of DR in the rigorous and traditional treatment group, respectively[53]. The another trial group[54] found that, for type 2 diabetic patients, tighter blood glucose control had been found to correlate most closely with a lower rate of DR[54]. However, very strict control of blood glucose may lead to cause worsening of DR due to up regulation of insulin-like growth factor-1 (IGF-1)[52,55,56].

Control of blood pressure

Hypertension is more common in type 2 diabetic patients rather than patients with type 1 DM. Approximately 40%-60% of patients with hypertension are over the age range of 45 to 75[57]. Although the relationship between hypertension and progression of retinopathy is not certain, good blood pressure control pulls down the risk of DR. An another study[58] reported that strict control of blood pressure reduces the risk of diabetic ocular complications[58].

Control of serum lipids

There is a positive correlations between the severity of DR and plasma lipid levels, particularly LDL-HDL cholesterol ratio[59]. Hard yellow exudates, which are lipid rich, have been found to correlate with plasma protein levels. Dietary and medicine therapy may reduce hard exudates[60,61]. Systemic lipid-lowering drugs such as, fenofibrate reduced the need for focal laser treatment of CSME in type 2 diabetic patients[62].

Laser treatment

Laser treatment has been considered the evidence-based treatment for DME and PDR for a long time. Randomized studies have demonstrated the efficacy of laser photocoagulation to prevent vision loss from DME[63,64]. In eyes observed with CSME, prompt photocoagulation is highly recommended. Treatment is performed at areas of focal leaking microaneurysms by using focal laser photocoagulation or at areas of diffuse leakage by using grid laser photocoagulation. Laser spot size should not be greater than 100 μm for focal laser treatment. Grid laser treatment is characterized by mild RPE whitening spots as far as 2 optic disks diameters from the center of the fovea[65]. Combination treatment is applied in most patients, which involves focal and grid laser treatment.

Patients are reevaluated for retreatment at 3 mo intervals. For each retreatment, clinicians repeat the fluorescein angiogram to determine sites of persistent dye leakage. If patients have focal leakage with a circinate lipid ring, it may not be necessary to repeat angiogram before the treatment because the leaking focal lesions are in the lipid ring.

Panretinal laser photocoagulation (PRP) treatment became a standard of care for DR when the results of the Diabetic Retinopathy Study (DRS) were published[66,67]. DRS showed that PRP enormously reduced the risk of severe vision loss from 16% to 6.4% in patient with PDR. The goal of PRP is not to improve visual acuity. It is applied to regress of the NVD or NVE and to prevent the blinding complications of DRP. Generally, laser treatment should be performed over a period of 4-6 wk by applying 1.500-2.000 burns, with a size of 500 μm, spacing spots 0.5 burn widths from each other with a 0.1-0.2 s duration[65].

Intravitreal medication

The results of several investigations showed that these different intravitreal agents are effective not only in the prevention of visual loss, but also allowed a regain of visual acuity. The two main categories of intravitreal drugs recently used in the management of DME and PDR are steroids and anti-VEGF agents.

The use of intravitreal steroids are preferred to manage the DME. They have antiinflammatory and antiangiogenic effects that stabilize of the inner blood-retina barrier. Intraocular steroid injections have beneficial effects in PDR, by inhibiting production of the VEGF[68,69]. Many various studies reported the benefits of injections of triamcinolone acetonide (IVTA) to reduce DME and increase visual acuity[70-74].

The effects of intravitreal steroids are temporary and last for about 3 mo. In this cases, intravitreal steroids may be repeated. But complications such as elevated intraocular pressure and infection may occur. However, IVTA is more likely to be associated with cataract progression. Combination of IVTA and laser treatment has more beneficial effects in pseudophakic eyes than laser alone[74].

Recently, a novel, biodegradable, slow-release dexamethasone implant (DEX implant, Ozurdex) was

developed to gradually release 0.7 mg of preservative-free dexamethasone in the vitreous cavity after a small incision[75]. DEX implant have the advantage of a lower incidence of cataract and glaucoma than IVTA[76]. The maximum effects of the DEX implant occur at 3 mo and gradually diminish from month 4 to 6[77].

Anti-VEGF agents (pegaptanib, bavacizumab, ranibizumab, aflibercept) have been investigated as a treatment for DME and for PDR. Also, anti-VEGF injections might be useful adjuncts to facilitate effective fibrovascular membrane dissection in eyes with active vascularity components[78]. TRD occur or progress within 1-4 wk of anti-VEGF injection, so, in general, these cases should be scheduled in a timely manner after the injection[79].

Nowadays, clinicians have the option of four anti VEGF agents: Pegaptanib (Macugen), Bevacizumab (Avastin), Ranibizumab (Lucentis), Aflibercept (Eylea).

Pegaptanib is a selective VEGF antagonist that binds to the VEGF165 isoform. Intravitreal pegaptanib is currently an approved treatment in neovascular choroidal membrane, but several trials addressed the efficacy and safety of intravitreal pegaptanib injections in the treatment of PDR and DME[80-82].

Bevacizumab[83] is a full-size humanized antibody that binds to all VEGF-A isoform. Intravitreal bevacizumab is currently used beneficially in the off-label treatment of DR. There have been many studies with intravitreal bevacizumab injections and DME. The results of these retrospective or prospective trials showed an improvement in visual acuity and OCT outcomes. However, bevacizumab injections were also associated with short-term efficacy and a high recurrence rate[83-88].

Ranibizumab is a high affinity anti-VEGF Fab specifically designed for ophthalmic use. It binds to all isoforms of VEGF-A and related degradation products and neutralizes their biological activity. Several studies confirmed its efficacy in treating DME[89-94].

Aflibercept[95] is an intravitreally administered fusion protein that is designed to bind both the VEGF-A and the placental growth factor with higher affinity in comparison to other anti- VEGF agents[95]. Aflibercept has a longer duration of action in the eye after intraocular injection. This new agent has been recently investigated in the treatment of DME[96,97].

Surgery

Pars plana vitrectomy (PPV) is considered an option for patients not responding to combined anti-VEGF- laser and/or steroid-laser theraphy in DME[98]. PPV, including posterior hyaloid, internal limiting membrane (ILM) and epiretinal membrane (ERM) removal, might achieve DME resolution. However, the removal of the vitreous gel might improve inner retina oxygenation and thus promote the resolution of DME[98-101].

PPV was introduced in the early 1970 as a promising treatment for the severe late complications of PDR, including vitreous hemorrhage, TRD, and fibrovascular proliferation[102]. The proper timing for PPV in PDR was under discussion for a long time. The Diabetic Retinopathy Vitrectomy Study (DRVS) considered the early PPV effects compared to deferral PPV in patients with severe vitreous hemorrhage (VH)[103]. The DRVS showed that at 2-year follow up, early PPV for nonclearing VH primarily increased the chance for retaining vision $\geqslant$ 20/40. Today, PPV can be performed as early as it is needed by the patients. The aim of PPV in PDR includes removal of opacity from the vitreous space, and the removal of tractional membrane from the retinal surface. Anti-VEGF injections might be useful adjuncts to ease effective fibrovascular membrane dissection in eyes with active vascularity components[78].

Finally, enzymatic vitrectomy performed by the intravitreal injection of autologous plasmin enzyme might be effective and could be considered as an alternative for diabetic patients before performing other treatments, such as intravitreal injections of anti-VEGF or steroids, surgical vitrectomy or laser. Several investigations on enzymatic vitreolysis, such as microplasmin, showed that many agents might achieve vitreous dissolution, PVD, or VH clearance[104,105].

Indications for PPV in PDR: Severe nonclearing vitreous hemorrhage; Nonclearing vitreous hemorrhage; Premacular subhyaloid hemorrhage; TRD involving the fovea; Tractional and rhegmatogenous retinal detach-ment; Macular edema due to vitreomacular traction; Nontractional macular edema that is refractory to pharmacotherapy and laser therapy.

DIABETIC PAPILLOPATHY

Definition and incidence

Diabetic papillopathy (DP) is an uncommon ocular manifestation of DM identified by unilateral or bilateral disk swelling associated with minimal or no optic nerve dysfunction[106-108]. DP, which is self-limited disease, was repoted in 1971 in T1DM patients for the first time[109]. So, it is very difficult to predict the exact incidence of DP. The prevalence of DP in both types of DM is about 0.5%, regardless of glycemic control and seriousness of DRP[106-108]. The percentage of patients with DP presenting a NPDRP is higher than in the PDRP.

Pathogenesis

The pathophysiology is not fully understood and several theories have been suggested. There are no links between DP and either DRP or metabolic control. Some researchers suggest that DP is a subtype of non-arteritic anterior ischemic optic neuropathy (NAION), but there are some differential features between NAION and DP, for insance, DP is an asymptomatic optic disc edema, whereas NAION is an acute optic disc infarction[110,111]. However, the most plausible mechanism responsible for DP is a limited impairment to the peripapillary vascular network, and superficial capillary network endothelial

cells[111,112].

Clinical evaluation

The other causes of disk swelling, and PDRP with NV on the disc have been ruled out to verify the diagnosis of DP[113]. DP, which occurs generally in patients with uncontrolled diabetes, has following features: painless visual loss, macular edema, disk hyperfluorescence on fluorescein angiography, and significant visual improvement after the treatment[106].

However, several diseases can imitate DP, such as infection, inflammation, metastatic infiltration, hypertension, and papilledema[106,108,114]. Pseudopapilloedema, that is seen in patients with disc drusen[113], can be confused with DP.

In order to reach differential diagnosis, investigations are required, such as fluorescein angiography, orbital magnetic resonance imaging, blood tests including serum angiotensin-converting enzyme, anti nuclear antibody, vitamin B12, folate, erythrocyte sedimentation rate, C reactive protein, and fluorescent treponemal antibody test.

Current therapy

So far, definitive treatment has not been found to change its native progression, as in most cases the disc edema resolves within a few months with no visual impairment. Intravitreal anti-VEGF injection increased visual acuity and decreased disk edema in patients with DP[114-117]. At the same time, it is unknown that how anti-VEGF agents affect to the patients with DP. Another study showed that periocular corticosteroids stabilize the blood-ocular barrier at the disc and the macula and causes resolution of the disc and macular edema[118]. Some degree of optic atrophy is seldom present after treatment. Tight control of blood pressure optimises the visual outcome.

GLAUCOMA

Association of DM and glaucoma has been investigated much in the literature. DM is the major etiologic factor for neovascular glaucoma (NVG)[119]. However, the association of DM with other types of glaucoma such as open angle glaucoma (OAG) and angle closure glaucoma (ACG) is controversial. Since glaucoma is a type of optic neuropathy and DM alone could cause optic neuropathy, a complex relation may occur between DM and glaucomatous optic neuropathy. On the other hand, central corneal thickness (CCT) is found to be thicker in patients with DM that could cause higher intraocular pressure (IOP) readings[120]. Since the mechanisms of glaucoma subtypes are different from each other; it would be more logical to investigate the association of glaucoma subtypes individually with DM.

OAG and DM

OAG is one of the most common causes of vision loss worldwide. In several studies, DM was reported as a risk factor for OAG, along with other risk factors such as elevated IOP, older age, family history of glaucoma and black race[121-123]. It was found that as the duration of type 2 DM increases, risk of having OAG also increases[123]. On the other hand, an association of having a history of DM and risk of OAG was not found in several studies[124,125]. It is possible that diabetic patients are more likely to have an ocular examination than the general population and are thus more likely to be diagnosed with OAG[122]. Small vascular abnormalities including optic nerve vessels and oxidative damage are some of the possible mechanisms by which DM might increase risk of OAG[122]. In the aspect of treatment, OAG patients with DM undergoing trabeculectomy do not have the same long-term IOP control and surgical survival rate when compared with patients without DM[126]. Medical treatment, laser trabeculoplasty, and surgery (filtering surgery, aqueous drainage devices, *etc*.) are the treatment options.

ACG and DM

The association between DM and ACG is not very clear. But several studies showed that DM might be considered as a risk factor for ACG[127,128]. Saw and colleagues[127] reported that diabetic patients have shallower anterior chambers than individuals without DM, irrespective of age, gender, and socioeconomic factors. Senthil *et al*[128] found that DM is associated with ACG, possibly because of the thicker lenses of diabetic patients. Weinreb *et al*[129] reported that pseudophakic pupillary block with ACG might occur in patients with DM. Also, treatment of DR with argon laser panretinal photocoagulation could cause ACG soon after the laser[130]. Medical treatment (topical, oral, and intravenous agents) and laser iridotomy are the treatment options.

NVG and DM

NVG is a severe and intractable glaucoma type. DR is one of the most common etiologic factors for NVG. NVG might occur in cases with no retinal or optic disc neovascularization, but it is more likely seen in PDR[131]. The association of iris and angle NV with DM mostly increase with the duration of the disease and blood sugar control[132]. Although iris and angle NVs are common in DM, they do not always progress to NVG; but NVs always develop prior to IOP increase[132]. This is due to a fibrovascular membrane that occurs on the anterior surface of the iris and iridocorneal angle. This membrane then causes anterior synechiae, angle closure, and rise of IOP[131,132].

NVG may develop in diabetic patients after cataract surgery, laser posterior capsulotomy and pars plana vitrectomy[132]. NVG following these operations probably results from a combination of surgical inflammation and disruption of a barrier preventing diffusion of angiogenesis factors to the anterior segment[132]. Prompt diagnosis and treatment are very important to prevent blindness due to NVG. Panretinal photocoagulation is the

key treatment method for prevention of NVG in DRP[131]. Panretinal photocoagulation laser therapy in the early stages may be efficacious in inhibiting and even reversing new vessel proliferation in the anterior segment of the eye. Medical treatment, cyclophotocoagulation, cryotherapy, and surgery (trabeculectomy with antimetabolites and valve implantation) are the other therapeutic options.

Other glaucoma types and DM

Pseudoexfoliation (Psx) has been supposed to be a generalized or systemic disorder of the extracellular matrix[133]. Psx increases the risk of glaucoma development[133]. It was reported that there is not a significant relationship between DM and psx[134]. Also, HbA1c levels do not vary among patients with DM based on psx status[134]. Ellis *et al*[135] found that DM is not associated with ocular hypertension. On the other hand, it was revealed that DM is significantly associated with bilateral eye involvement in normotension glaucoma, maybe due to several impaired neurovascular autoregulation processes related to DM[136].

Glaucomatous optic neuropathy and DM

Retinal ganglion cell death is the major cause of blindness in glaucoma. DM may increase susceptibility of retinal ganglion cells to apoptosis when there is a co-morbidity with elevated IOP in glaucoma[137]. DM disrupts vascular tissues, compromises neuro-glial functions, and thus may take a role in the pathogenesis of optic neuropathy related with glaucoma[138]. In the literature, it was shown that DM may accelerate apoptosis of retinal inner neurons, alter metabolism of astrocytes and Müller cells, and impair microglial function[138]. All of these factors contribute to visual acuity, contrast sensitivity and color vision loss in comorbidity of DM and glaucoma[138].

Miscellaneous issues related to glaucoma and DM

DM is associated with increased corneal stiffness, and corneal hysteresis which have been shown to have an effect on glaucoma risk[125,139]. IOP may increase in patients with DM due to aqueous outflow resistance in trabecular meshwork, because of glycation and crosslinking of meshwork glycoproteins[140].

Since DM is frequently found with other systemic disorders, such as hypertension, this comorbid condition may also affect glaucoma risk. Shoshani *et al*[141] reported that DM may interfere with normal vascular regulation and contribute to glaucoma progression. Moïse *et al*[142] suggested that blindness due to glaucoma may be prevented by using a regular Mediterranean diet and maintaining regular intake of vegetables in patients with DM.

CATARACT

Definition and incidence

Cataract, the commonest cause of curable blindness worldwide, is the opacification of the crystalline lens[143,144]. Diabetic cataract is considered a complication of DM, which can affect individuals at younger ages[145]. Cataract formation in diabetics seems to be related to the hyperglicemia or to hastened senile lens opacity. A snowflake like cataract is occured commonly in patients with insulin-dependent diabetes and more prones to progress than others.

Diabetic patients are 2-5 times more at risk for cataract formation and and are more likely to get it at an earlier age[146,147]. Although cataract frequency varies based on ethnic populations and geographic locations (ranges from 35% to 48%), it is higher in diabetics when compared to non-diabetics[148-152]. In a study by Raman *et al*[153], it has been indicated that the mixed cataract was more common than mono type cataract (42% *vs* 19%, respectively). A combination of cortical, nuclear, and posterior subcapsular cataract was the most common form of the mixed types (20%), followed by the combined posterior subcapsular cataract and cortical (16%). Among the monotype cataracts, rate of cortical cataract was the highest (15%), followed by nuclear cataract (5%) and posterior subcapsular cataract (1%)[153]. On the other hand, cataract frequency varies from 1% to 27% in patients with type 1 diabetes[154].

Pathogenesis

Several different pathogenetic mechanisms that may precipitate formation of diabetic cataracts have been proposed: increased osmotic stress caused by activation of the polyol pathway[155], non-enzymatic glycation of lens proteins[156-159], and increased oxidative stress[160-164].

The polyol pathway

In cases of high blood glucose levels in diabetic patients, the crystalline lens is exposed to a hyperosmotic aqueous humour and its glucose concentration progressively increases. During hyperglycemic conditions excess glucose to sorbitol. Sorbitol is further metabolized to fructose. In diabetic patients, the excessive accumulation of sorbitol in the crystalline lens produces a high osmotic gradient that leads to a fluid infusion to equilibrate the osmotic gradient. The accumulation of sorbitol in lens cell causes a collapse and liquefaction of lens fibers, which eventually results in the cataract formation[165,166]. Moreover, increased osmotic stress in the crystalline lens produced by excess accumulation of sorbitol initiates apoptotic process in epithelial cells which contributes to the cataractogenesis[155,167,168].

Non-enzymatic glycation

Advanced glycation occurs during normal aging but to a greater degree in diabetic patients in which it contributes the formation of lens opacity[156]. Advanced glycation produced by a nonenzymatically reaction between the piece of the excess glucose and proteins, which may leads to production of superoxide radicals and AGE formation[169]. Excessive accumulation of AGEs in the crystalline lens of diabetic patients plays an essential role in cataractogenesis[157-161].

Increased oxidative stress

It is well known that chronic hyperglycemia may increase the oxidant load[162] and facilitate the onset of senile cataract[163]. In diabetic eyes, antioxidant capacity is reduced free radical load is increased, which increases the susceptibility of crystalline lens to oxidative damage. The decrease in antioxidant capacity is facilitated by advanced glycation and defects of antioxidant enzyme activity [164].

Clinical evaluation

DM can cause anterior segment changes as well as posterior segment; therefore, a comprehensive ophthalmologic examination including visual acuity measurement, evaluation of relative afferent pupil defect, slit-lamb biomicroscopy, gonioscopy, intraocular pressure measurement, and dilated fundus examination are mandatory. In selected cases, ancillary tests such as fundus angiography and OCT may also be useful.

The level of cataract should correspond to patient's visual complaints including decreased visual acuity, decreased contrast sensitivity, and glare. If the biomicroscopic examination shows mild cataract but the patient reports severe visual dysfunction, other ocular diabetic complications such as DR should be investigated. Recently, there has been a shift in emphasis towards early cataract removal in diabetics to enable adequate identification for examination of posterior segment, and facilitate panretinal photocoagulation and treatment of underlying macular edema[170]. Pre-existing PDR and macular edema may exacerbate after cataract surgery[171] which contributes to the poor visual outcomes[172]. Therefore if posterior segment is visualized, diabetic patients with pre-existing retinopathy should be preoperatively treated.

Current therapy

First of all, good blood glucose control is main goal to prevention of diabetic cataract. It has however been suggested that cataractogenesis can be prevented through nutrition and supplementation, including high content of nutritional antioxidants[173], lower dietary carbohydrate[174] and linolenic acid intake[175], and aldose reductase inhibitors[144,176].

Currently, the main treatment for the diabetic cataract is surgery. Phacoemulsification results in better visual results, less intraocular inflammation and less capsular opacification as compared to extracapsular surgery[177]. Femtosecond assisted cataract surgery may be a better option for diabetics; however, there has been no comparative study comparing the results of femtosecond assisted to conventional cataract surgery in diabetics. It is advisable to perform a large capsulorrhexis with a large diameter IOLs, thus allowing better visualization of the posterior segment for examination and further treatment of DR.

After cataract surgery, using topical anti-inflammatory drugs such as steroids and nonsteroidal anti-inflammatory drops may be useful to control inflammation and macular edema. Despite an uneventfully performed cataract surgery, DR and macular edema can become exacerbated after surgery, hence patients should be followed closely with fundus examinations and ancillary tests.

OCULAR SURFACE DISEASES

Ocular surface diseases, such as dry eye is frequently present in diabetic patients. Ocular surface diseases related with DM are developed in many mechanisms including abnormal ocular surface sensitivity[178,179], decreased tear production[179-181], and delayed corneal re-epithelialization[181].

DRY EYE SYNDROME

Definition and incidence

Dry eye is a condition which is a complex disease of tear film and anterior surface of the cornea. The resulting changes in the ocular surface may lead to ocular discomfort, and visual disturbance. Tear osmolarity, and ocular surface inflammation[182] are also increased in diabetic patients causing dry eye disease. Burning, foreign body sensation, photophobia, blurred vision[183], and blurred vision are present in patients with dry eye. Both dry eye disease and DM increase the risk of corneal infections and scarring, in advanced disease, corneal perforation and irreversible tissue damages[184] may occur. Patients with dry eye have serious corneal complications such as, superficial punctuate keratitis, neurotrophic keratopathy, and persistent epithelial defect[185]. Dry eye syndrome (DES) is more like to occur in the industrial country. Studies showed that approximately 1.68 million men and 3.2 million women[186] aged 50 and older are affected with DES in the United States[187]. DES, one of the most common diagnosis for diabetic patients[188], is a condition in which abnormal tear film and an changed anterior surface of the cornea is present. Studies show at least 50% of DM patients have either symptomatic or asymptomatic DES. 92 patients with diabetes types I and II have been evaluated by Seifart[189]. The patients were aged from 7 to 69 years old as well as normal healthy controls comparable in number, age and sex. The study demonstrated that 52.8 of all diabetic patients complained about eye dry symptoms, whereas 9.3% of the healthy controls complained about dry eye symptoms.

Pathogenesis

DM can lead to DES through a variety of mechanisms[190-192], but the association between DM and DES is unclear[193]. The most possible mechanism responsible for dry eye in DM is extensive hyperglycemia bring about corneal neuropathy. Corneal neuropathy leads to tear film instability and lower tear break up time (TBUT) values due to conjunctival goblet cell loss. Mucin, which covers the villus surface of the corneal epithelium and reduce evaporative tear loss[181] is produced by conjunctival goblet cells.

The other suggested mechanisms for disruption of

corneal integrity include AGE accumulation[194,195] and polyol pathway[196,197] bi-product accumulation within the corneal layers. It is believed that DM affects tear production and quality by compromising the functional integrity of the lacrimal gland. Corneal sensitivity is also reduced in DM, which affects the stimulation of basal tear production. Both lacrimal gland integrity[180] and corneal sensitivity are shown to be affected by diabetic neuropathy[180,198]. These proposed mechanisms imply that DM affects both tear production and corneal integrity, suggesting disruption to one or both may cause and lead to the exacerbation of DES.

Clinical evaluation

During routine eye examination clinicians should be aware of dry eye in diabetic patients[199]. Dry eye index scores can be used for uncovering the presence of dry eye and for evaluating the response to therapeutic treatment. Several questionnaires are available, with the most common being the Ocular Surface Disease Index (OSDI)[200]. However, there is still no standardized dry eye disease questionnaire that is universally accepted.

The most common test for determining tear film quality in use today is the TBUT which shows the tear film stability. The TBUT value is the time from the last complete blink to the appearance of dry spot. The Schirmer test is used for measuring the aqueous tear manufacture. Normally, the Schirmer filter paper gets wet 10 mm for 5 min. A result yielding less than 5 mm shows aqueous tear deficiency. Fluorescein is useful in assessing dry eye where its application can detect the epithelial defects due to dry eye disease.

Risk factors for DES include duration of DM and higher HbA1c levels[188,201]. So, strict blood glucose control and close follow-up reduce the risk of DES[188].

Current therapy

DES may cause loss of vision, scarring, perforation, and corneal infection. If patients with dry eye are treated in time, there will be no complications of DES[185]. The patients should be treated with tear supplements called "artificial tears" which contains surfactans, different viscosity agents, and electrolytes[202].

Dry eye disease is the outcome of many factors resulting in inflammation of the cornea and conjunctiva. Artificial tears can reduce blurred vision, and the symptoms of dry eye, temporarily. These agents do not contain the cytokines and growth factors which are comprised in normal tears and do not have direct anti-inflammatory effect[203,204]. Anti-inflammatory drugs are widely used for the treatment of DES. The most widely used anti-inflammatory agents are topical corticosteroids, NSAID, and cyclosporine A[203-205].

Corticosteroids can reduce the symptoms and signs of dry eye[206] to control inflammatory process. On the other hand, after long-term use, steroids produce severe side effects such as bacterial, viral, and fungal infection, elevated IOP, and cataract formation. NSAIDs are increasingly used as dry eye treatment instead of steroids because of their non-severe side effects. Topical cyclosporine A are used to increase tear production[207] and the number of goblet cells decreased by chronic inflammation due to dry eye disease[207].

DIABETIC KERATOPATHY

Definition and incidence

DM can trigger acceleration of ocular surface abnormalities which have been termed diabetic keratopathy[208]. In contrast to healthy persons, patients with diabetes have corneal epithelial erosions that may recur and be associated with unresponsiveness to conventional treatment regimens[209-211]. This clinical condition is known as diabetic keratopathy[212-214]. Diabetic keratopathy includes various symptomatic corneal conditions, such as, punctate keratopathy and persistent corneal epithelial defect[208].

Diabetic keratopathy is a common complication of patients with evidence of DR. A study reported that several symptomatic corneal epithelial lesions have been occured in diabetic patients at the rate of 47% to 64%[208]. In another study, authors showed that the incidence of diabetic keratopathy in diabetic patients with DR was 2 times greater than that of patients without DR[215]. Several studies reported that the incidence of diabetic keratopathy increased following pars plana vitrectomy[216,217], penetrating keratoplasty[218], laser iridectomy[219], and refractive surgery[220] in diabetic patients.

Pathogenesis

Several pathophysiological abnormalities have been shown in diabetic keratopathy, including, an abnormally thickened and discontinuous basement membrane, abnormal adhesion between the stroma and basement membrane[219-223], increased epithelial fragility[206], decreased epithelial healing rates, increased sorbitol concentrations[224], decreased oxygen consumption and uptake[225], increase in the polyol metabolism[196], decreased or alter epithelial hemidesmosomes, and increased glycosyltransferas activity[214,226].

Recently, studies have demonstrated[194,195,227] that there is a relationship between AGE and development of diabetic keratopathy. Increased AGE in the laminin of the corneal epithelial basement membrane causes abnormal weak attachment between the basal cells and basement membrane of the cornea in diabetics[194]. Also, the loss of the corneal sensation and neural stimulus have been regarded as the reason of the development of diabetic keratopathy[228]. Axonal degeneration of corneal unmyelinated nerves occurs under chronic hyperglycemic conditions.

Clinical evaluation

Diabetic keratopathy is a condition that can result in blindness and should be closely monitored. Early diagnosis and treatment of diabetic keratopathy, particularly, before corneal complications occur, is very crucial. If

the diagnosis is late, patients will become resistance to the routine treatment of corneal defects. Nonhealing corneal epithelial erosion may also occur after pars plana vitrectomy for advanced PDR[208,211]. If corneal epithelium is removed manually for clarity by surgeons, this conditions may accelerate dramatically. So, when diabetic patients are examined after vitrectomy their corneas should be examined carefully.

Current therapy

Keratopathy is generally treated with artificial tears, and antibiotics. Additionally, bandage contact lens, and tarsorrhaphy can be used for re-epithelialization. In selected cases new treatments modalities will be used such as, topical administration of naltrexone, nicergoline[229], aldose reductase inhibitor[194,214,230], and some growth hormones[231] to accelerate re-epithelialization. All of these drugs were associated with a high corneal epithelial wound healing rate.

Recently, new topical drugs such as substance P and IGF-1 were tested on diabetic animals to accelerate re-epithelialization. Successful outcomes were obtained with these new drugs[231]. Corneal epithelial barrier function was improved by topical aldose reductase inhibitors, but superficial punctate keratopathy could not be prevented by these topical drugs. Aminoguanidine had beneficial effects in corneal epithelial defects, by improving attachment between the epithelial cells and basement membrane of the cornea[185,194]. The *in vivo* beneficial effect of aminoguanidine were unknown[194]. In additional to these new drugs, amniotic membrane transplantation is used to treat persistent corneal epithelial defects[232].

CONCLUSION

DM and its ocular complications remain a major cause of blindness despite increased understanding of these ocular conditions and identification of successful treatments. All of diabetic ocular complications can be prevented by early diagnosis and theraphy. Therefore, periodic eye examinations are required for the reduction of diabetes-related vision loss. Good blood glucose control and other systemic risk factors such as hypertension, and hyperlipidemia are main goal to prevention of ocular complications of DM.

REFERENCES

1 **Kowluru RA**, Chan PS. Oxidative stress and diabetic retinopathy. *Exp Diabetes Res* 2007; **2007**: 43603 [PMID: 17641741 DOI: 10.1155/2007/43603]
2 **Forbes JM**, Soldatos G, Thomas MC. Below the radar: advanced glycation end products that detour "around the side". Is HbA1c not an accurate enough predictor of long term progression and glycaemic control in diabetes? *Clin Biochem Rev* 2005; **26**: 123-134 [PMID: 16648883]
3 **Jang C**, Lim JH, Park CW, Cho YJ. Regulator of Calcineurin 1 Isoform 4 (RCAN1.4) Is Overexpressed in the Glomeruli of Diabetic Mice. *Korean J Physiol Pharmacol* 2011; **15**: 299-305 [PMID: 22128263 DOI: 10.4196/kjpp.2011.15.5.299]
4 **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]
5 **International Diabetes Federation**. The Diabetes Atlas 2006. 3rd ed. [accessed 2013 May 17]. Available from: URL: http://www.idf.org/sites/default/files/Diabetes-Atlas-3rd-edition.pdf
6 **International Diabetes Federation**. The Diabetes Atlas 2011. 5th ed. [accessed on 2013 May 17]. Available from: URL: http://www.drsharma.ca/world-diabetes-atlas-5th-edition.html
7 **Threatt J**, Williamson JF, Huynh K, Davis RM. Ocular disease, knowledge and technology applications in patients with diabetes. *Am J Med Sci* 2013; **345**: 266-270 [PMID: 23531956 DOI: 10.1097/MAJ.0b013e31828aa6fb]
8 **Singh PP**, Mahadi F, Roy A, Sharma P. Reactive oxygen species, reactive nitrogen species and antioxidants in etiopathogenesis of diabetes mellitus type-2. *Indian J Clin Biochem* 2009; **24**: 324-342 [PMID: 23105858 DOI: 10.1007/s12291-009-0062-6]
9 **Moss SE**, Klein R, Klein BE. The 14-year incidence of visual loss in a diabetic population. *Ophthalmology* 1998; **105**: 998-1003 [PMID: 9627648 DOI: 10.1016/S0161-6420(98)96025-0]
10 **Aiello LM**. Perspectives on diabetic retinopathy. *Am J Ophthalmol* 2003; **136**: 122-135 [PMID: 12834680 DOI: 10.1016/S0002-9394(03)00219-8]
11 **Klein R**, Klein B. National Diabetes Data Group. Diabetes in America. 2. National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases. Vision disorders in diabetes. USA: Bethesda, MD, 1995: 293-337
12 **Garg S,** Davis RM. Diabetic Retinopathy Screening Update. *Clinical Diabetes Fall* 2009; **4**: 140-145 [DOI: 10.2337/diaclin.27.4.140]
13 **Kumari S**, Panda S, Mangaraj M, Mandal MK, Mahapatra PC. Plasma MDA and antioxidant vitamins in diabetic retinopathy. *Indian J Clin Biochem* 2008; **23**: 158-162 [PMID: 23105743 DOI: 10.1007/s12291-008-0035-1]
14 Retinopathy and nephropathy in patients with type 1 diabetes four years after a trial of intensive therapy. The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group. *N Engl J Med* 2000; **342**: 381-389 [PMID: 10666428 DOI: 10.1056/NEJM200002103420603]
15 **Stratton IM**, Kohner EM, Aldington SJ, Turner RC, Holman RR, Manley SE, Matthews DR. UKPDS 50: risk factors for incidence and progression of retinopathy in Type II diabetes over 6 years from diagnosis. *Diabetologia* 2001; **44**: 156-163 [PMID: 11270671 DOI: 10.1007/s001250051594]
16 **Kaštelan S**, Tomić M, Pavan J, Orešković S. Maternal immune system adaptation to pregnancy--a potential influence on the course of diabetic retinopathy. *Reprod Biol Endocrinol* 2010; **8**: 124 [PMID: 20964838 DOI: 10.1186/1477-7827-8-124]
17 **Varma R**. From a population to patients: the Wisconsin epidemiologic study of diabetic retinopathy. *Ophthalmology* 2008; **115**: 1857-1858 [PMID: 19068373 DOI: 10.1016/j.ophtha.2008.09.023]
18 **Klein R**. Epidemiology of Diabetic Retinopathy. In: Duh E, ed. Diabetic Retinopathy. Totowa: Humana Press, 2008
19 **Matthews DR**, Stratton IM, Aldington SJ, Holman RR, Kohner EM. Risks of progression of retinopathy and vision loss related to tight blood pressure control in type 2 diabetes mellitus: UKPDS 69. *Arch Ophthalmol* 2004; **122**: 1631-1640 [PMID: 15534123 DOI: 10.1001/archopht]
20 **White NH**, Cleary PA, Dahms W, Goldstein D, Malone J, Tamborlane WV. Beneficial effects of intensive therapy of diabetes during adolescence: outcomes after the conclusion of the Diabetes Control and Complications Trial (DCCT). *J Pediatr* 2001; **139**: 804-812 [PMID: 11743505 DOI: 10.1067/mpd.2001.118887]
21 **Naruse K**, Nakamura J, Hamada Y, Nakayama M, Chaya S, Komori T, Kato K, Kasuya Y, Miwa K, Hotta N. Aldose reductase inhibition prevents glucose-induced apoptosis

in cultured bovine retinal microvascular pericytes. *Exp Eye Res* 2000; **71**: 309-315 [PMID: 10973739 DOI: 10.1006/exer.2000.0882]
22 **Kowluru RA**. Diabetic retinopathy: mitochondrial dysfunction and retinal capillary cell death. *Antioxid Redox Signal* 2005; **7**: 1581-1587 [PMID: 16356121]
23 **Stitt AW**. The role of advanced glycation in the pathogenesis of diabetic retinopathy. *Exp Mol Pathol* 2003; **75**: 95-108 [PMID: 12834631 DOI: 10.1016/S0014-4800(03)00035-2]
24 **Chu J**, Ali Y. Diabetic Retinopathy: A Review. *Drug Dev Res* 2008; **69**: 1-14 [DOI: 10.1002/ddr.20222]
25 **Kowluru RA**, Tang J, Kern TS. Abnormalities of retinal metabolism in diabetes and experimental galactosemia. VII. Effect of long-term administration of antioxidants on the development of retinopathy. *Diabetes* 2001; **50**: 1938-1942 [PMID: 11473058 DOI: 10.2337/diabetes.50.8.1938]
26 **Tarr JM**, Kaul K, Chopra M, Kohner EM, Chibber R. Pathophysiology of diabetic retinopathy. *ISRN Ophthalmol* 2013; **2013**: 343560 [PMID: 24563789 DOI: 10.1155/2013/343560]
27 **Gabbay KH**. Hyperglycemia, polyol metabolism, and complications of diabetes mellitus. *Annu Rev Med* 1975; **26**: 521-536 [PMID: 238458]
28 **Kinoshita JH**. A thirty year journey in the polyol pathway. *Exp Eye Res* 1990; **50**: 567-573 [PMID: 2115448]
29 **Gabbay KH**. The sorbitol pathway and the complications of diabetes. *N Engl J Med* 1973; **288**: 831-836 [PMID: 4266466 DOI: 10.1056/NEJM197304192881609]
30 **Wang QJ**. PKD at the crossroads of DAG and PKC signaling. *Trends Pharmacol Sci* 2006; **27**: 317-323 [PMID: 16678913 DOI: 10.1016/j.tips.2006.04.003]
31 **Aiello LP**, Northrup JM, Keyt BA, Takagi H, Iwamoto MA. Hypoxic regulation of vascular endothelial growth factor in retinal cells. *Arch Ophthalmol* 1995; **113**: 1538-1544 [PMID: 7487623 DOI: 10.1001/archopht.1995.01100120068012]
32 **Simorre-Pinatel V**, Guerrin M, Chollet P, Penary M, Clamens S, Malecaze F, Plouet J. Vasculotropin-VEGF stimulates retinal capillary endothelial cells through an autocrine pathway. *Invest Ophthalmol Vis Sci* 1994; **35**: 3393-3400 [PMID: 8056513]
33 **Adamis AP**, Shima DT, Yeo KT, Yeo TK, Brown LF, Berse B, D'Amore PA, Folkman J. Synthesis and secretion of vascular permeability factor/vascular endothelial growth factor by human retinal pigment epithelial cells. *Biochem Biophys Res Commun* 1993; **193**: 631-638 [PMID: 8512562 DOI: 10.1006/bbrc.1993.1671]
34 **Aiello LP**, Avery RL, Arrigg PG, Keyt BA, Jampel HD, Shah ST, Pasquale LR, Thieme H, Iwamoto MA, Park JE. Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. *N Engl J Med* 1994; **331**: 1480-1487 [PMID: 7526212 DOI: 10.1056/NEJM199412013312203]
35 **Aiello LP**, Bursell SE, Clermont A, Duh E, Ishii H, Takagi C, Mori F, Ciulla TA, Ways K, Jirousek M, Smith LE, King GL. Vascular endothelial growth factor-induced retinal permeability is mediated by protein kinase C in vivo and suppressed by an orally effective beta-isoform-selective inhibitor. *Diabetes* 1997; **46**: 1473-1480 [PMID: 9287049 DOI: 10.2337/diab.46.9.1473]
36 **Murata T**, Ishibashi T, Khalil A, Hata Y, Yoshikawa H, Inomata H. Vascular endothelial growth factor plays a role in hyperpermeability of diabetic retinal vessels. *Ophthalmic Res* 1995; **27**: 48-52 [PMID: 7596559 DOI: 10.1159/000267567]
37 **Zong H**, Ward M, Stitt AW. AGEs, RAGE, and diabetic retinopathy. *Curr Diab Rep* 2011; **11**: 244-252 [PMID: 21590515 DOI: 10.1007/s11892-011-0198-7]
38 **Cui Y**, Xu X, Bi H, Zhu Q, Wu J, Xia X, Qiushi Ren PC. Expression modification of uncoupling proteins and MnSOD in retinal endothelial cells and pericytes induced by high glucose: the role of reactive oxygen species in diabetic retinopathy. *Exp Eye Res* 2006; **83**: 807-816 [PMID: 16750827 DOI: 10.1016/j.Exer.2006.0]
39 **Baynes JW**. Role of oxidative stress in development of complications in diabetes. *Diabetes* 1991; **40**: 405-412 [PMID: 2010041 DOI: 10.2337/diab.40.4.405]
40 **Wilkinson-Berka JL**. Angiotensin and diabetic retinopathy. *Int J Biochem Cell Biol* 2006; **38**: 752-765 [PMID: 16165393 DOI: 10.1016/j.biocel.2005.08.002]
41 **Antonetti DA**, Barber AJ, Bronson SK, Freeman WM, Gardner TW, Jefferson LS, Kester M, Kimball SR, Krady JK, LaNoue KF, Norbury CC, Quinn PG, Sandirasegarane L, Simpson IA. Diabetic retinopathy: seeing beyond glucose-induced microvascular disease. *Diabetes* 2006; **55**: 2401-2411 [PMID: 16936187 DOI: 10.2337/db05-1635]
42 **Xu H**, Chen M, Forrester JV. Para-inflammation in the aging retina. *Prog Retin Eye Res* 2009; **28**: 348-368 [PMID: 19560552 DOI: 10.1016/j.preteyeres.2009.06.001]
43 **Klein BE**, Knudtson MD, Tsai MY, Klein R. The relation of markers of inflammation and endothelial dysfunction to the prevalence and progression of diabetic retinopathy: Wisconsin epidemiologic study of diabetic retinopathy. *Arch Ophthalmol* 2009; **127**: 1175-1182 [PMID: 19752427]
44 **Nguyen TT**, Alibrahim E, Islam FM, Klein R, Klein BE, Cotch MF, Shea S, Wong TY. Inflammatory, hemostatic, and other novel biomarkers for diabetic retinopathy: the multi-ethnic study of atherosclerosis. *Diabetes Care* 2009; **32**: 1704-1709 [PMID: 19549733 DOI: 10.2337/dc09-0102]
45 **Maheshwary AS**, Oster SF, Yuson RM, Cheng L, Mojana F, Freeman WR. The association between percent disruption of the photoreceptor inner segment-outer segment junction and visual acuity in diabetic macular edema. *Am J Ophthalmol* 2010; **150**: 63-67.e1 [PMID: 20451897 DOI: 10.1016/j.ajo.2010.01.039]
46 **Otani T**, Kishi S, Maruyama Y. Patterns of diabetic macular edema with optical coherence tomography. *Am J Ophthalmol* 1999; **127**: 688-693 [PMID: 10372879 DOI: 10.1016/S0002-9394(99)00033-1]
47 **Kim NR**, Kim YJ, Chin HS, Moon YS. Optical coherence tomographic patterns in diabetic macular oedema: prediction of visual outcome after focal laser photocoagulation. *Br J Ophthalmol* 2009; **93**: 901-905 [PMID: 19254904 DOI: 10.1136/bjo.2008.152553]
48 **Yamamoto S**, Yamamoto T, Hayashi M, Takeuchi S. Morphological and functional analyses of diabetic macular edema by optical coherence tomography and multifocal electroretinograms. *Graefes Arch Clin Exp Ophthalmol* 2001; **239**: 96-101 [PMID: 11372551 DOI: 10.1007/s004170000238]
49 **Otani T**, Kishi S. Tomographic assessment of vitreous surgery for diabetic macular edema. *Am J Ophthalmol* 2000; **129**: 487-494 [PMID: 10764858 DOI: 10.1016/S0002-9394(99)00409-2]
50 **Klein R**, Klein BE, Moss SE, Davis MD, DeMets DL. The Wisconsin epidemiologic study of diabetic retinopathy. II. Prevalence and risk of diabetic retinopathy when age at diagnosis is less than 30 years. *Arch Ophthalmol* 1984; **102**: 520-526 [PMID: 6367724 DOI: 10.1001/archopht.1984.01040030398010]
51 **Klein R**, Klein BE, Moss SE, Davis MD, DeMets DL. The Wisconsin epidemiologic study of diabetic retinopathy. III. Prevalence and risk of diabetic retinopathy when age at diagnosis is 30 or more years. *Arch Ophthalmol* 1984; **102**: 527-532 [PMID: 6367725 DOI: 10.1001/archopht.1984.01040030405011]
52 Early worsening of diabetic retinopathy in the Diabetes Control and Complications Trial. *Arch Ophthalmol* 1998; **116**: 874-886 [PMID: 9682700 DOI: 10.1001/archopht.116.7.874]
53 The relationship of glycemic exposure (HbA1c) to the risk of development and progression of retinopathy in the diabetes control and complications trial. *Diabetes* 1995; **44**: 968-983 [PMID: 7622004 DOI: 10.2337/diab.44.8.968]
54 Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. *Lancet* 1998; **352**: 837-853 [PMID: 9742976 DOI: 10.1016/

S0140-6736(98)07019-6]
55 **Chantelau E**. Evidence that upregulation of serum IGF-1 concentration can trigger acceleration of diabetic retinopathy. *Br J Ophthalmol* 1998; **82**: 725-730 [PMID: 9924360 DOI: 10.1136/bjo.82.7.725]
56 **Chantelau E**, Meyer-Schwickerath R. Reversion of 'early worsening' of diabetic retinopathy by deliberate restoration of poor metabolic control. *Ophthalmologica* 2003; **217**: 373-377 [PMID: 12913330 DOI: 10.1159/000071355]
57 **RR Associates.** Blood pressure and diabetes: everyone's concern. London: British Diabetic Association, 1994
58 **Prospective Diabetes Study Group.** Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 38. UK Prospective Diabetes Study Group. *BMJ* 1998; **317**: 703-713 [PMID: 9732337 DOI: 10.1136/bmj.317.7160.703]
59 **Kissebah AH**, Kohner EM, Lewis B, Siddiq YK, Lowy C, Fraser TR. Plasma-lipids and glucose/insulin relationship in non-insulin-requiring diabetics with and without retinopathy. *Lancet* 1975; **1**: 1104-1108 [PMID: 49469 DOI: 10.1016/S0140-6736(75)92497-6]
60 **Duncan LJ**, Cullen JF, Ireland JT, Nolan J, Clarke BF, Oliver MF. A three-year trial of atromid therapy in exudative diabetic retinopathy. *Diabetes* 1968; **17**: 458-467 [PMID: 4875170 DOI: 10.2337/diab.17.7.458]
61 **Houtsmuller AJ**, Zahn KJ, Henkes HE. Unsaturated fats and progression of diabetic retinopathy. *Doc Ophthalmol* 1980; **48**: 363-371 [PMID: 6995054]
62 **Keech AC**, Mitchell P, Summanen PA, O'Day J, Davis TM, Moffitt MS, Taskinen MR, Simes RJ, Tse D, Williamson E, Merrifield A, Laatikainen LT, d'Emden MC, Crimet DC, O'Connell RL, Colman PG. Effect of fenofibrate on the need for laser treatment for diabetic retinopathy (FIELD study): a randomised controlled trial. *Lancet* 2007; **370**: 1687-1697 [PMID: 17988728 DOI: 10.1016/S0140-6736(07)61607-9]
63 Photocoagulation for diabetic macular edema. Early Treatment Diabetic Retinopathy Study report number 1. Early Treatment Diabetic Retinopathy Study research group. *Arch Ophthalmol* 1985; **103**: 1796-1806 [PMID: 2866759 DOI: 10.1001/archopht.1985.01050120030015]
64 **Olk RJ**. Modified grid argon (blue-green) laser photocoagulation for diffuse diabetic macular edema. *Ophthalmology* 1986; **93**: 938-950 [PMID: 3763140]
65 Treatment techniques and clinical guidelines for photocoagulation of diabetic macular edema. Early Treatment Diabetic Retinopathy Study Report Number 2. Early Treatment Diabetic Retinopathy Study Research Group. *Ophthalmology* 1987; **94**: 761-774 [PMID: 3658348]
66 Preliminary report on effects of photocoagulation therapy. The Diabetic Retinopathy Study Research Group. *Am J Ophthalmol* 1976; **81**: 383-396 [PMID: 944535]
67 Photocoagulation treatment of proliferative diabetic retinopathy: the second report of diabetic retinopathy study findings. *Ophthalmology* 1978; **85**: 82-106 [PMID: 345173]
68 **Edelman JL**, Lutz D, Castro MR. Corticosteroids inhibit VEGF-induced vascular leakage in a rabbit model of blood-retinal and blood-aqueous barrier breakdown. *Exp Eye Res* 2005; **80**: 249-258 [PMID: 15670803 DOI: 10.1016/j.exer.2004.09.013]
69 **Brooks HL**, Caballero S, Newell CK, Steinmetz RL, Watson D, Segal MS, Harrison JK, Scott EW, Grant MB. Vitreous levels of vascular endothelial growth factor and stromal-derived factor 1 in patients with diabetic retinopathy and cystoid macular edema before and after intraocular injection of triamcinolone. *Arch Ophthalmol* 2004; **122**: 1801-1807 [PMID: 15596583 DOI: 10.1001/archopht.122.12.1801]
70 **Audren F**, Erginay A, Haouchine B, Benosman R, Conrath J, Bergmann JF, Gaudric A, Massin P. Intravitreal triamcinolone acetonide for diffuse diabetic macular oedema: 6-month results of a prospective controlled trial. *Acta Ophthalmol Scand* 2006; **84**: 624-630 [PMID: 16965492 DOI: 10.1111/j.1600-0420.2006.00700.x]
71 **Gillies MC**, Sutter FK, Simpson JM, Larsson J, Ali H, Zhu M. Intravitreal triamcinolone for refractory diabetic macular edema: two-year results of a double-masked, placebo-controlled, randomized clinical trial. *Ophthalmology* 2006; **113**: 1533-1538 [PMID: 16828501 DOI: 10.1016/j.ophtha.2006.02.065]
72 **Massin P**, Audren F, Haouchine B, Erginay A, Bergmann JF, Benosman R, Caulin C, Gaudric A. Intravitreal triamcinolone acetonide for diabetic diffuse macular edema: preliminary results of a prospective controlled trial. *Ophthalmology* 2004; **111**: 218-224; discussion 224-225 [PMID: 15019365 DOI: 10.1016/j.ophtha.2003.05.037]
73 **Martidis A**, Duker JS, Greenberg PB, Rogers AH, Puliafito CA, Reichel E, Baumal C. Intravitreal triamcinolone for refractory diabetic macular edema. *Ophthalmology* 2002; **109**: 920-927 [PMID: 11986098 DOI: 10.1016/S0161-6420(02)00975-2]
74 **Elman MJ**, Aiello LP, Beck RW, Bressler NM, Bressler SB, Edwards AR, Ferris FL, Friedman SM, Glassman AR, Miller KM, Scott IU, Stockdale CR, Sun JK. Randomized trial evaluating ranibizumab plus prompt or deferred laser or triamcinolone plus prompt laser for diabetic macular edema. *Ophthalmology* 2010; **117**: 1064-1077.e35 [PMID: 20427088 DOI: 10.1016/j.Ophtha.2010.02.031]
75 **Haller JA**, Dugel P, Weinberg DV, Chou C, Whitcup SM. Evaluation of the safety and performance of an applicator for a novel intravitreal dexamethasone drug delivery system for the treatment of macular edema. *Retina* 2009; **29**: 46-51 [PMID: 18827732 DOI: 10.1097/IAE.0b013e318188c814]
76 **Haller JA**, Kuppermann BD, Blumenkranz MS, Williams GA, Weinberg DV, Chou C, Whitcup SM. Randomized controlled trial of an intravitreous dexamethasone drug delivery system in patients with diabetic macular edema. *Arch Ophthalmol* 2010; **128**: 289-296 [PMID: 20212197 DOI: 10.1001/archophthalmol.2010.21]
77 **Zucchiatti I**, Lattanzio R, Querques G, Querques L, Del Turco C, Cascavilla ML, Bandello F. Intravitreal dexamethasone implant in patients with persistent diabetic macular edema. *Ophthalmologica* 2012; **228**: 117-122 [PMID: 22310491 DOI: 10.1159/000336225]
78 **Avery RL**, Pearlman J, Pieramici DJ, Rabena MD, Castellarin AA, Nasir MA, Giust MJ, Wendel R, Patel A. Intravitreal bevacizumab (Avastin) in the treatment of proliferative diabetic retinopathy. *Ophthalmology* 2006; **113**: 1695.e1-1695.15 [PMID: 17011951 DOI: 10.1016/j.Ophtha.2006.05.064]
79 **Arevalo JF**, Maia M, Flynn HW, Saravia M, Avery RL, Wu L, Eid Farah M, Pieramici DJ, Berrocal MH, Sanchez JG. Tractional retinal detachment following intravitreal bevacizumab (Avastin) in patients with severe proliferative diabetic retinopathy. *Br J Ophthalmol* 2008; **92**: 213-216 [PMID: 17965108]
80 **Gragoudas ES**, Adamis AP, Cunningham ET, Feinsod M, Guyer DR. Pegaptanib for neovascular age-related macular degeneration. *N Engl J Med* 2004; **351**: 2805-2816 [PMID: 15625332 DOI: 10.1056/NEJMoa042760]
81 **Cunningham ET**, Adamis AP, Altaweel M, Aiello LP, Bressler NM, D'Amico DJ, Goldbaum M, Guyer DR, Katz B, Patel M, Schwartz SD. A phase II randomized double-masked trial of pegaptanib, an anti-vascular endothelial growth factor aptamer, for diabetic macular edema. *Ophthalmology* 2005; **112**: 1747-1757 [PMID: 16154196]
82 **Loftus JV**, Sultan MB, Pleil AM. Changes in vision- and health-related quality of life in patients with diabetic macular edema treated with pegaptanib sodium or sham. *Invest Ophthalmol Vis Sci* 2011; **52**: 7498-7505 [PMID: 21896838 DOI: 10.1167/iovs.11-7613]
83 **Scott IU**, Edwards AR, Beck RW, Bressler NM, Chan CK, Elman MJ, Friedman SM, Greven CM, Maturi RK, Pieramici DJ, Shami M, Singerman LJ, Stockdale CR. A phase II randomized clinical trial of intravitreal bevacizumab for diabetic macular edema. *Ophthalmology* 2007; **114**: 1860-1867 [PMID: 17698196 DOI: 10.1016/j.ophtha.2007.05.062]

84 **Lam DS**, Lai TY, Lee VY, Chan CK, Liu DT, Mohamed S, Li CL. Efficacy of 1.25 MG versus 2.5 MG intravitreal bevacizumab for diabetic macular edema: six-month results of a randomized controlled trial. *Retina* 2009; **29**: 292-299 [PMID: 19287286 DOI: 10.1097/IAE.0b013e31819a2d61]

85 **Arevalo JF**, Sanchez JG, Wu L, Maia M, Alezzandrini AA, Brito M, Bonafonte S, Lujan S, Diaz-Llopis M, Restrepo N, Rodríguez FJ, Udaondo-Mirete P. Primary intravitreal bevacizumab for diffuse diabetic macular edema: the Pan-American Collaborative Retina Study Group at 24 months. *Ophthalmology* 2009; **116**: 1488-1497, 1497.e1 [PMID: 19545900 DOI: 10.1016/j.ophtha.2009.03.016]

86 **Kook D**, Wolf A, Kreutzer T, Neubauer A, Strauss R, Ulbig M, Kampik A, Haritoglou C. Long-term effect of intravitreal bevacizumab (avastin) in patients with chronic diffuse diabetic macular edema. *Retina* 2008; **28**: 1053-1060 [PMID: 18779710 DOI: 10.1097/IAE.0b013e318176de48]

87 **Michaelides M**, Kaines A, Hamilton RD, Fraser-Bell S, Rajendram R, Quhill F, Boos CJ, Xing W, Egan C, Peto T, Bunce C, Leslie RD, Hykin PG. A prospective randomized trial of intravitreal bevacizumab or laser therapy in the management of diabetic macular edema (BOLT study) 12-month data: report 2. *Ophthalmology* 2010; **117**: 1078-1086. e2 [PMID: 20416952 DOI: 10.1016/j.ophtha.2010.03.045]

88 **Rajendram R**, Fraser-Bell S, Kaines A, Michaelides M, Hamilton RD, Esposti SD, Peto T, Egan C, Bunce C, Leslie RD, Hykin PG. A 2-year prospective randomized controlled trial of intravitreal bevacizumab or laser therapy (BOLT) in the management of diabetic macular edema: 24-month data: report 3. *Arch Ophthalmol* 2012; **130**: 972-979 [PMID: 22491395 DOI: 10.1001/archophthalmol.2012.393]

89 **Massin P**, Bandello F, Garweg JG, Hansen LL, Harding SP, Larsen M, Mitchell P, Sharp D, Wolf-Schnurrbusch UE, Gekkieva M, Weichselberger A, Wolf S. Safety and efficacy of ranibizumab in diabetic macular edema (RESOLVE Study): a 12-month, randomized, controlled, double-masked, multicenter phase II study. *Diabetes Care* 2010; **33**: 2399-2405 [PMID: 20980427 DOI: 10.2337/dc10-0493]

90 **Nguyen QD**, Brown DM, Marcus DM, Boyer DS, Patel S, Feiner L, Gibson A, Sy J, Rundle AC, Hopkins JJ, Rubio RG, Ehrlich JS. Ranibizumab for diabetic macular edema: results from 2 phase III randomized trials: RISE and RIDE. *Ophthalmology* 2012; **119**: 789-801 [PMID: 22330964 DOI: 10.1016/j.ophtha.2011.12.039]

91 **Nguyen QD**, Shah SM, Heier JS, Do DV, Lim J, Boyer D, Abraham P, Campochiaro PA. Primary End Point (Six Months) Results of the Ranibizumab for Edema of the mAcula in diabetes (READ-2) study. *Ophthalmology* 2009; **116**: 2175-2181. e1 [PMID: 19700194 DOI: 10.1016/j.ophtha.2009.04.023]

92 **Nguyen QD**, Shah SM, Khwaja AA, Channa R, Hatef E, Do DV, Boyer D, Heier JS, Abraham P, Thach AB, Lit ES, Foster BS, Kruger E, Dugel P, Chang T, Das A, Ciulla TA, Pollack JS, Lim JI, Eliott D, Campochiaro PA. Two-year outcomes of the ranibizumab for edema of the mAcula in diabetes (READ-2) study. *Ophthalmology* 2010; **117**: 2146-2151 [PMID: 20855114 DOI: 10.1016/j.ophtha.2010.08.016]

93 **Mitchell P**, Bandello F, Schmidt-Erfurth U, Lang GE, Massin P, Schlingemann RO, Sutter F, Simader C, Burian G, Gerstner O, Weichselberger A. The RESTORE study: ranibizumab monotherapy or combined with laser versus laser monotherapy for diabetic macular edema. *Ophthalmology* 2011; **118**: 615-625 [PMID: 21459215 DOI: 10.1016/j.ophtha.2011.01.031]

94 **Filho JA**, Messias A, Almeida FP, Ribeiro JA, Costa RA, Scott IU, Jorge R. Panretinal photocoagulation (PRP) versus PRP plus intravitreal ranibizumab for high-risk proliferative diabetic retinopathy. *Acta Ophthalmol* 2011; **89**: e567-e572 [PMID: 21726427 DOI: 10.1111/j.1755-3768.2011.02184.x]

95 **Economides AN**, Carpenter LR, Rudge JS, Wong V, Koehler-Stec EM, Hartnett C, Pyles EA, Xu X, Daly TJ, Young MR, Fandl JP, Lee F, Carver S, McNay J, Bailey K, Ramakanth S, Hutabarat R, Huang TT, Radziejewski C, Yancopoulos GD, Stahl N. Cytokine traps: multi-component, high-affinity blockers of cytokine action. *Nat Med* 2003; **9**: 47-52 [PMID: 12483208 DOI: 10.1038/nm811]

96 **Do DV**, Schmidt-Erfurth U, Gonzalez VH, Gordon CM, Tolentino M, Berliner AJ, Vitti R, Rückert R, Sandbrink R, Stein D, Yang K, Beckmann K, Heier JS. The DA VINCI Study: phase 2 primary results of VEGF Trap-Eye in patients with diabetic macular edema. *Ophthalmology* 2011; **118**: 1819-1826 [PMID: 21546089 DOI: 10.1016/j.ophtha.2011.02.018]

97 **Do DV**, Nguyen QD, Boyer D, Schmidt-Erfurth U, Brown DM, Vitti R, Berliner AJ, Gao B, Zeitz O, Ruckert R, Schmelter T, Sandbrink R, Heier JS. One-year outcomes of the da Vinci Study of VEGF Trap-Eye in eyes with diabetic macular edema. *Ophthalmology* 2012; **119**: 1658-1665 [PMID: 22537617 DOI: 10.1016/j.ophtha.2012.02.010]

98 **Stefánsson E**. Ocular oxygenation and the treatment of diabetic retinopathy. *Surv Ophthalmol* 2006; **51**: 364-380 [PMID: 16818083 DOI: 10.1016/j.survophthal.2006.04.005]

99 **Stefánsson E**, Hatchell DL, Fisher BL, Sutherland FS, Machemer R. Panretinal photocoagulation and retinal oxygenation in normal and diabetic cats. *Am J Ophthalmol* 1986; **101**: 657-664 [PMID: 3717248]

100 **Stefansson E**, Landers MB, Wolbarsht ML. Increased retinal oxygen supply following pan-retinal photocoagulation and vitrectomy and lensectomy. *Trans Am Ophthalmol Soc* 1981; **79**: 307-334 [PMID: 7200671]

101 **Yamamoto T**, Akabane N, Takeuchi S. Vitrectomy for diabetic macular edema: the role of posterior vitreous detachment and epimacular membrane. *Am J Ophthalmol* 2001; **132**: 369-377 [PMID: 11530050 DOI: 10.1016/S0002-9394(01)01050-9]

102 **Machemer R**, Buettner H, Norton EW, Parel JM. Vitrectomy: a pars plana approach. *Trans Am Acad Ophthalmol Otolaryngol* 1971; **75**: 813-820 [PMID: 5566980]

103 **Diabetic Retinopathy Vitrectomy Study Group.** Early vitrectomy for severe vitreous hemorrhage in diabetic retinopathy. Four-year results of a randomized trial: Diabetic Retinopathy Vitrectomy Study Report 5. *Arch Ophthalmol* 1990; **108**: 958-964 [PMID: 2196036]

104 **Lopez-Lopez F**, Rodriguez-Blanco M, Gómez-Ulla F, Marticorena J. Enzymatic vitreolysis. *Curr Diabetes Rev* 2009; **5**: 57-62 [PMID: 19199900]

105 **Benz MS**, Packo KH, Gonzalez V, Pakola S, Bezner D, Haller JA, Schwartz SD. A placebo-controlled trial of microplasmin intravitreous injection to facilitate posterior vitreous detachment before vitrectomy. *Ophthalmology* 2010; **117**: 791-797 [PMID: 20138368 DOI: 10.1016/j.ophtha.2009.11.005]

106 **Regillo CD**, Brown GC, Savino PJ, Byrnes GA, Benson WE, Tasman WS, Sergott RC. Diabetic papillopathy. Patient characteristics and fundus findings. *Arch Ophthalmol* 1995; **113**: 889-895 [PMID: 7605280]

107 **Friedrich Y**, Feiner M, Gawi H, Friedman Z. Diabetic papillopathy with macular star mimicking clinically significant diabetic macular edema. *Retina* 2001; **21**: 80-82 [PMID: 11217941]

108 **Bayraktar Z**, Alacali N, Bayraktar S. Diabetic papillopathy in type II diabetic patients. *Retina* 2002; **22**: 752-758 [PMID: 12476102]

109 **Lubow M**, Makley TA. Pseudopapilledema of juvenile diabetes mellitus. *Arch Ophthalmol* 1971; **85**: 417-422 [PMID: 5554869 DOI: 10.1001/archopht.1971.009900]

110 **Hayreh SS**, Zimmerman MB. Nonarteritic anterior ischemic optic neuropathy: clinical characteristics in diabetic patients versus nondiabetic patients. *Ophthalmology* 2008; **115**: 1818-1825 [PMID: 18502511]

111 **Slagle WS**, Musick AN, Eckermann DR. Diabetic papillopathy and its relation to optic nerve ischemia. *Optom Vis Sci* 2009; **86**: e395-e403 [PMID: 19225435 DOI: 10.1097/OPX.0b013e318198927c]

112 **Wise GN**, Dollery CT, Henkind P. The Retinal Circulation. New York: Harper & Row, 1971

113 **Zachariah S**, Sharfi O, Burton B, Nussey SS, Bano G. Diabetic papillopathy diagnosed on retinal screening in an asymptomatic patient. *BJDVD* 2007; **7**: 140 [DOI: 10.1177/14746514070070030701]

114 **Kim M**, Lee JH, Lee SJ. Diabetic papillopathy with macular edema treated with intravitreal ranibizumab. *Clin Ophthalmol* 2013; **7**: 2257-2260 [PMID: 24348012 DOI: 10.2147/OPTH.S55076]

115 **Al-Hinai AS**, Al-Abri MS, Al-Hajri RH. Diabetic papillopathy with macular edema treated with intravitreal bevacizumab. *Oman J Ophthalmol* 2011; **4**: 135-138 [PMID: 22279402 DOI: 10.4103/0974-620X.91270]

116 **Al-Dhibi H**, Khan AO. Response of diabetic papillopathy to intravitreal bevacizumab. *Middle East Afr J Ophthalmol* 2011; **18**: 243-245 [PMID: 21887082 DOI: 10.4103/0974-9233.84056]

117 **Willerslev A**, Munch IC, Larsen M. Resolution of diabetic papillopathy after a single intravitreal injection of ranibizumab. *Acta Ophthalmol* 2012; **90**: e407-e409 [PMID: 22268957 DOI: 10.1111/j.1755-3768.2011.02282.x]

118 **Mansour AM**, El-Dairi MA, Shehab MA, Shahin HK, Shaaban JA, Antonios SR. Periocular corticosteroids in diabetic papillopathy. *Eye* (Lond) 2005; **19**: 45-51 [PMID: 15094720 DOI: 10.1038/sj.eye.6701418]

119 **Al-Shamsi HN**, Dueker DK, Nowilaty SR, Al-Shahwan SA. Neovascular glaucoma at king khaled eye specialist hospital - etiologic considerations. *Middle East Afr J Ophthalmol* 2009; **16**: 15-19 [PMID: 20142954 DOI: 10.4103/0974-9233.48860]

120 **Ozdamar Y**, Cankaya B, Ozalp S, Acaroglu G, Karakaya J, Ozkan SS. Is there a correlation between diabetes mellitus and central corneal thickness? *J Glaucoma* 2010; **19**: 613-616 [PMID: 20051882 DOI: 10.1097/IJG.0b013e3181ca7c62]

121 **Bonovas S**, Peponis V, Filioussi K. Diabetes mellitus as a risk factor for primary open-angle glaucoma: a meta-analysis. *Diabet Med* 2004; **21**: 609-614 [PMID: 15154948 DOI: 10.1111/j.1464-5491.2004.01173.x]

122 **Newman-Casey PA**, Talwar N, Nan B, Musch DC, Stein JD. The relationship between components of metabolic syndrome and open-angle glaucoma. *Ophthalmology* 2011; **118**: 1318-1326 [PMID: 21481477 DOI: 10.1016j.Ophhtha]

123 **Chopra V**, Varma R, Francis BA, Wu J, Torres M, Azen SP. Type 2 diabetes mellitus and the risk of open-angle glaucoma the Los Angeles Latino Eye Study. *Ophthalmology* 2008; **115**: 227-232.e1 [PMID: 17716734]

124 **de Voogd S**, Ikram MK, Wolfs RC, Jansonius NM, Witteman JC, Hofman A, de Jong PT. Is diabetes mellitus a risk factor for open-angle glaucoma? The Rotterdam Study. *Ophthalmology* 2006; **113**: 1827-1831 [PMID: 16884777]

125 **Tan GS**, Wong TY, Fong CW, Aung T. Diabetes, metabolic abnormalities, and glaucoma. *Arch Ophthalmol* 2009; **127**: 1354-1361 [PMID: 19822853 DOI: 10.1001/archophthalmol.2009.268]

126 **Law SK**, Hosseini H, Saidi E, Nassiri N, Neelakanta G, Giaconi JA, Caprioli J. Long-term outcomes of primary trabeculectomy in diabetic patients with primary open angle glaucoma. *Br J Ophthalmol* 2013; **97**: 561-566 [PMID: 23355527 DOI: 10.1136/bjophthalmol-2012-302227]

127 **Saw SM**, Wong TY, Ting S, Foong AW, Foster PJ. The relationship between anterior chamber depth and the presence of diabetes in the Tanjong Pagar Survey. *Am J Ophthalmol* 2007; **144**: 325-326 [PMID: 17659975 DOI: 10.1016/j.ajo.2007.03.038]

128 **Senthil S**, Garudadri C, Khanna RC, Sannapaneni K. Angle closure in the Andhra Pradesh Eye Disease Study. *Ophthalmology* 2010; **117**: 1729-1735 [PMID: 20466426 DOI: 10.1016/j.ophtha.2010.01.021]

129 **Weinreb RN**, Wasserstrom JP, Forman JS, Ritch R. Pseudophakic pupillary block with angle-closure glaucoma in diabetic patients. *Am J Ophthalmol* 1986; **102**: 325-328 [PMID: 3752197 DOI: 10.1016/0002-9394(86)90006-1]

130 **Blondeau P**, Pavan PR, Phelps CD. Acute pressure elevation following panretinal photocoagulation. *Arch Ophthalmol* 1981; **99**: 1239-1241 [PMID: 7196215 DOI: 10.1001/archopht.1981.03930020113011]

131 **Hayreh SS**. Neovascular glaucoma. *Prog Retin Eye Res* 2007; **26**: 470-485 [PMID: 17690002 DOI: 10.1016/j.preteyeres.2007.06.001]

132 **Morrison JC**, Pollack IP. Neovascular glaucoma (Chapter 21). Glaucoma Science and Practice, 1st ed. New York: Thieme Medical Publishers, 2003: 226-236

133 **Miyazaki M**, Kubota T, Kubo M, Kiyohara Y, Iida M, Nose Y, Ishibashi T. The prevalence of pseudoexfoliation syndrome in a Japanese population: the Hisayama study. *J Glaucoma* 2005; **14**: 482-484 [PMID: 16276281 DOI: 10.109701.ijg.0000185436.1]

134 **Wood SD**, Asefzadeh B, Fisch B, Jiwani A, Lee RK, Conlin PR, Pasquale LR. The relationship between diabetes mellitus and exfoliation syndrome in a United States Veterans Affairs population: a case-control study. *J Glaucoma* 2011; **20**: 278-281 [PMID: 20577098 DOI: 10.1097/IJG.0b013e3181e3d483]

135 **Ellis JD**, Evans JM, Ruta DA, Baines PS, Leese G, MacDonald TM, Morris AD. Glaucoma incidence in an unselected cohort of diabetic patients: is diabetes mellitus a risk factor for glaucoma? DARTS/MEMO collaboration. Diabetes Audit and Research in Tayside Study. Medicines Monitoring Unit. *Br J Ophthalmol* 2000; **84**: 1218-1224 [PMID: 11049943 DOI: 10.1136/bjo.84.11.1218]

136 **Kim C**, Kim TW. Comparison of risk factors for bilateral and unilateral eye involvement in normal-tension glaucoma. *Invest Ophthalmol Vis Sci* 2009; **50**: 1215-1220 [PMID: 18836170 DOI: 10.1167/iovs.08-1886]

137 **Kanamori A**, Nakamura M, Mukuno H, Maeda H, Negi A. Diabetes has an additive effect on neural apoptosis in rat retina with chronically elevated intraocular pressure. *Curr Eye Res* 2004; **28**: 47-54 [PMID: 14704913 DOI: 10.1076/ceyr.28.1.47.23487]

138 **Nakamura M**, Kanamori A, Negi A. Diabetes mellitus as a risk factor for glaucomatous optic neuropathy. *Ophthalmologica* 2005; **219**: 1-10 [PMID: 15627820 DOI: 10.1159/000081775]

139 **Congdon NG**, Broman AT, Bandeen-Roche K, Grover D, Quigley HA. Central corneal thickness and corneal hysteresis associated with glaucoma damage. *Am J Ophthalmol* 2006; **141**: 868-875 [PMID: 16527231 DOI: 10.1016/j.ajo.2005.12.007]

140 **Chihara E**. Myopia and diabetes mellitus as modificatory factors of glaucomatous optic neuropathy. *Jpn J Ophthalmol* 2014; **58**: 16-25 [PMID: 23942995 DOI: 10.1007/s10384-013-0267-3]

141 **Shoshani Y**, Harris A, Shoja MM, Arieli Y, Ehrlich R, Primus S, Ciulla T, Cantor A, Wirostko B, Siesky BA. Impaired ocular blood flow regulation in patients with open-angle glaucoma and diabetes. *Clin Experiment Ophthalmol* 2012; **40**: 697-705 [PMID: 22394354 DOI: 10.1111/j.1442-9071.2012.02778.x]

142 **Moïse MM**, Benjamin LM, Doris TM, Dalida KN, Augustin NO. Role of Mediterranean diet, tropical vegetables rich in antioxidants, and sunlight exposure in blindness, cataract and glaucoma among African type 2 diabetics. *Int J Ophthalmol* 2012; **5**: 231-237 [PMID: 22762057 DOI: 10.3980/j.issn.2222-3959.2012.02.23]

143 **Kothadia AD,** Shenoy AM, Shabaraya AR, Rajan MS, Viradia UM, Patel NH. Evaluation of cataract preventive action of phycocyanin. *Int J Pharm Sci Drug Res* 2011; **3**: 42-44 [DOI: 10.2337/diaclin.27.4.140]

144 **Kato A**, Yasuko H, Goto H, Hollinshead J, Nash RJ, Adachi I. Inhibitory effect of rhetsinine isolated from Evodia rutaecarpa on aldose reductase activity. *Phytomedicine* 2009; **16**: 258-261 [PMID: 17498942 DOI: 10.1016/j.phymed.2007.04.008]

145 **Falck A**, Laatikainen L. Diabetic cataract in children. *Acta Ophthalmol Scand* 1998; **76**: 238-240 [PMID: 9591961 DOI: 10.1034/j.1600-0420.1998.760223.x]

146 **Klein BE**, Klein R, Moss SE. Incidence of cataract surgery in the Wisconsin Epidemiologic Study of Diabetic Retinopathy. *Am J Ophthalmol* 1995; **119**: 295-300 [PMID: 7872389]

147 **Klein BE**, Klein R, Wang Q, Moss SE. Older-onset diabetes and lens opacities. The Beaver Dam Eye Study. *Ophthalmic*

Epidemiol 1995; **2**: 49-55 [PMID: 7585233]
148 **Foster PJ**, Wong TY, Machin D, Johnson GJ, Seah SK. Risk factors for nuclear, cortical and posterior subcapsular cataracts in the Chinese population of Singapore: the Tanjong Pagar Survey. *Br J Ophthalmol* 2003; **87**: 1112-1120 [PMID: 12928278 DOI: 10.1136/bjo.87.9.1112]
149 **Nirmalan PK**, Robin AL, Katz J, Tielsch JM, Thulasiraj RD, Krishnadas R, Ramakrishnan R. Risk factors for age related cataract in a rural population of southern India: the Aravind Comprehensive Eye Study. *Br J Ophthalmol* 2004; **88**: 989-994 [PMID: 15258010 DOI: 10.1136/bjo.2003.038380]
150 **Husain R**, Tong L, Fong A, Cheng JF, How A, Chua WH, Lee L, Gazzard G, Tan DT, Koh D, Saw SM. Prevalence of cataract in rural Indonesia. *Ophthalmology* 2005; **112**: 1255-1262 [PMID: 15993241 DOI: 10.1016/j.ophtha.2005.02.015]
151 **Dandona L**, Dandona R, Naduvilath TJ, McCarty CA, Mandal P, Srinivas M, Nanda A, Rao GN. Population-based assessment of the outcome of cataract surgery in an urban population in southern India. *Am J Ophthalmol* 1999; **127**: 650-658 [PMID: 10372874 DOI: 10.1016/S0002-9394(99)00044-6]
152 **Chen SJ**, Liu JH, Shih HC, Chou P, Tsai CY, Tung TH. Prevalence and associated factors of lens opacities among Chinese type 2 diabetics in Kinmen, Taiwan. *Acta Diabetol* 2008; **45**: 7-13 [PMID: 17828461]
153 **Raman R**, Pal SS, Adams JS, Rani PK, Vaitheeswaran K, Sharma T. Prevalence and risk factors for cataract in diabetes: Sankara Nethralaya Diabetic Retinopathy Epidemiology and Molecular Genetics Study, report no. 17. *Invest Ophthalmol Vis Sci* 2010; **51**: 6253-6261 [PMID: 20610838 DOI: 10.1167/iovs.10-5414]
154 **Bron AJ**, Cheng H. Cataract and retinopathy: screening for treatable retinopathy. *Clin Endocrinol Metab* 1986; **15**: 971-999 [PMID: 3096617]
155 **Srivastava SK**, Ramana KV, Bhatnagar A. Role of aldose reductase and oxidative damage in diabetes and the consequent potential for therapeutic options. *Endocr Rev* 2005; **26**: 380-392 [PMID: 15814847 DOI: 10.1210/er.2004-0028]
156 **Ahmed N**. Advanced glycation endproducts--role in pathology of diabetic complications. *Diabetes Res Clin Pract* 2005; **67**: 3-21 [PMID: 15620429 DOI: 10.1016/j.diabres.2004.09.004]
157 **Araki N**, Ueno N, Chakrabarti B, Morino Y, Horiuchi S. Immunochemical evidence for the presence of advanced glycation end products in human lens proteins and its positive correlation with aging. *J Biol Chem* 1992; **267**: 10211-10214 [PMID: 1587810]
158 **Duhaiman AS**. Glycation of human lens proteins from diabetic and (nondiabetic) senile cataract patients. *Glycoconj J* 1995; **12**: 618-621 [PMID: 8595250]
159 **Lyons TJ**, Silvestri G, Dunn JA, Dyer DG, Baynes JW. Role of glycation in modification of lens crystallins in diabetic and nondiabetic senile cataracts. *Diabetes* 1991; **40**: 1010-1015 [PMID: 1907246 DOI: 10.2337/diab.40.8.1010]
160 **Nagaraj RH**, Sell DR, Prabhakaram M, Ortwerth BJ, Monnier VM. High correlation between pentosidine protein crosslinks and pigmentation implicates ascorbate oxidation in human lens senescence and cataractogenesis. *Proc Natl Acad Sci USA* 1991; **88**: 10257-10261 [PMID: 1946446]
161 **Shamsi FA**, Sharkey E, Creighton D, Nagaraj RH. Maillard reactions in lens proteins: methylglyoxal-mediated modifications in the rat lens. *Exp Eye Res* 2000; **70**: 369-380 [PMID: 10712823 DOI: 10.1006/exer.1999.0800]
162 **Agte VV**, Tarwadi KV. Combination of diabetes and cataract worsens the oxidative stress and micronutrient status in Indians. *Nutrition* 2008; **24**: 617-624 [PMID: 18472398 DOI: 10.1016/j.nut.2008.03.005]
163 **Jeganathan VS**, Wang JJ, Wong TY. Ocular associations of diabetes other than diabetic retinopathy. *Diabetes Care* 2008; **31**: 1905-1912 [PMID: 18753669 DOI: 10.2337/dc08-0342]
164 **Ookawara T**, Kawamura N, Kitagawa Y, Taniguchi N. Site-specific and random fragmentation of Cu,Zn-superoxide dismutase by glycation reaction. Implication of reactive oxygen species. *J Biol Chem* 1992; **267**: 18505-18510 [PMID: 1326527]
165 **Kinoshita JH**. Mechanisms initiating cataract formation. Proctor Lecture. *Invest Ophthalmol* 1974; **13**: 713-724 [PMID: 4278188]
166 **Kinoshita JH**. Cataracts in galactosemia. The Jonas S. Friedenwald Memorial Lecture. *Invest Ophthalmol* 1965; **4**: 786-799 [PMID: 5831988]
167 **Takamura Y**, Sugimoto Y, Kubo E, Takahashi Y, Akagi Y. Immunohistochemical study of apoptosis of lens epithelial cells in human and diabetic rat cataracts. *Jpn J Ophthalmol* 2001; **45**: 559-563 [PMID: 11754895 DOI: 10.1016/S0021-5155(01)00418-X]
168 **Li WC**, Kuszak JR, Dunn K, Wang RR, Ma W, Wang GM, Spector A, Leib M, Cotliar AM, Weiss M. Lens epithelial cell apoptosis appears to be a common cellular basis for non-congenital cataract development in humans and animals. *J Cell Biol* 1995; **130**: 169-181 [PMID: 7790371]
169 **Stitt AW**. The maillard reaction in eye diseases. *Ann N Y Acad Sci* 2005; **1043**: 582-597 [PMID: 16037281 DOI: 10.1196/annals.1338.066]
170 **Chew EY**, Benson WE, Remaley NA, Lindley AA, Burton TC, Csaky K, Williams GA, Ferris FL. Results after lens extraction in patients with diabetic retinopathy: early treatment diabetic retinopathy study report number 25. *Arch Ophthalmol* 1999; **117**: 1600-1606 [PMID: 10604663 DOI: 10.1001/archopht.117.12.1600]
171 **Pollack A**, Dotan S, Oliver M. Course of diabetic retinopathy following cataract surgery. *Br J Ophthalmol* 1991; **75**: 2-8 [PMID: 1991081]
172 **Squirrell D**, Bhola R, Bush J, Winder S, Talbot JF. A prospective, case controlled study of the natural history of diabetic retinopathy and maculopathy after uncomplicated phacoemulsification cataract surgery in patients with type 2 diabetes. *Br J Ophthalmol* 2002; **86**: 565-571 [PMID: 11973256 DOI: 10.1136/bjo.86.5.565]
173 **Pollreisz A**, Schmidt-Erfurth U. Diabetic cataract-pathogenesis, epidemiology and treatment. *J Ophthalmol* 2010; **2010**: 608751 [PMID: 20634936 DOI: 10.1155/2010/608751]
174 **Chiu CJ**, Morris MS, Rogers G, Jacques PF, Chylack LT, Tung W, Hankinson SE, Willett WC, Taylor A. Carbohydrate intake and glycemic index in relation to the odds of early cortical and nuclear lens opacities. *Am J Clin Nutr* 2005; **81**: 1411-1416 [PMID: 15941895]
175 **Lu M**, Taylor A, Chylack LT, Rogers G, Hankinson SE, Willett WC, Jacques PF. Dietary linolenic acid intake is positively associated with five-year change in eye lens nuclear density. *J Am Coll Nutr* 2007; **26**: 133-140 [PMID: 17536124]
176 **Drel VR**, Pacher P, Ali TK, Shin J, Julius U, El-Remessy AB, Obrosova IG. Aldose reductase inhibitor fidarestat counteracts diabetes-associated cataract formation, retinal oxidative-nitrosative stress, glial activation, and apoptosis. *Int J Mol Med* 2008; **21**: 667-676 [PMID: 18506358 DOI: 10.3892/ijmm.21.6.667]
177 **Dowler JG**, Hykin PG, Hamilton AM. Phacoemulsification versus extracapsular cataract extraction in patients with diabetes. *Ophthalmology* 2000; **107**: 457-462 [PMID: 10711881 DOI: 10.1016/S0161-6420(99)00136-0]
178 **Arthur SN**, Peng Q, Apple DJ, Escobar-Gomez M, Bianchi R, Pandey SK, Werner L. Effect of heparin surface modification in reducing silicone oil adherence to various intraocular lenses. *J Cataract Refract Surg* 2001; **27**: 1662-1669 [PMID: 11687368 DOI: 10.1016/S0886-3350(01)00891-4]
179 **Rosenberg ME**, Tervo TM, Immonen IJ, Müller LJ, Grönhagen-Riska C, Vesaluoma MH. Corneal structure and sensitivity in type 1 diabetes mellitus. *Invest Ophthalmol Vis Sci* 2000; **41**: 2915-2921 [PMID: 10967045]
180 **Cousen P**, Cackett P, Bennett H, Swa K, Dhillon B. Tear production and corneal sensitivity in diabetes. *J Diabetes*

Complications 2007; **21**: 371-373 [PMID: 17967709 DOI: 10.1016/j.jdiacomp.2006.05.008]
181 **Dogru M**, Katakami C, Inoue M. Tear function and ocular surface changes in noninsulin-dependent diabetes mellitus. *Ophthalmology* 2001; **108**: 586-592 [PMID: 11237914 DOI: 10.1016/S0161-6420(00)00599-6]
182 The definition and classification of dry eye disease: report of the Definition and Classification Subcommittee of the International Dry Eye WorkShop (2007). *Ocul Surf* 2007; **5**: 75-92 [PMID: 17508116]
183 **Lemp MA**. Report of the National Eye Institute/Industry workshop on Clinical Trials in Dry Eyes. *CLAO J* 1995; **21**: 221-232 [PMID: 8565190]
184 **Lubniewski AJ**, Houchin KW, Holland EJ, Weeks DA, Wessels IF, McNeill JI, Cameron JD. Posterior infectious crystalline keratopathy with Staphylococcus epidermidis. *Ophthalmology* 1990; **97**: 1454-1459 [PMID: 2255518]
185 **Riordan-Eva**, Asbury T, Whitcher JP. Vaughan and Asbury' s General Ophthalmology. USA: McGraw-Hill Medical, 2003: 308-310
186 **Schaumberg DA**, Sullivan DA, Buring JE, Dana MR. Prevalence of dry eye syndrome among US women. *Am J Ophthalmol* 2003; **136**: 318-326 [PMID: 12888056 DOI: 10.1016/S0002-9394(03)00218-6]
187 **Schaumberg DA**, Dana R, Buring JE, Sullivan DA. Prevalence of dry eye disease among US men: estimates from the Physicians' Health Studies. *Arch Ophthalmol* 2009; **127**: 763-768 [PMID: 19506195 DOI: 10.1001/archophthalmol.2009.103]
188 **Seifart U**, Strempel I. [The dry eye and diabetes mellitus]. *Ophthalmologe* 1994; **91**: 235-239 [PMID: 8012143]
189 **Kaiserman I**, Kaiserman N, Nakar S, Vinker S. Dry eye in diabetic patients. *Am J Ophthalmol* 2005; **139**: 498-503 [PMID: 15767060 DOI: 10.1016/j.ajo.2004.10.022]
190 **Alves Mde C**, Carvalheira JB, Módulo CM, Rocha EM. Tear film and ocular surface changes in diabetes mellitus. *Arq Bras Oftalmol* 2005; **71**: 96-103 [PMID: 19274419]
191 **Goebbels M**. Tear secretion and tear film function in insulin dependent diabetics. *Br J Ophthalmol* 2000; **84**: 19-21 [PMID: 10611093 DOI: 10.1136/bjo.84.1.19]
192 **Figueroa-Ortiz LC**, Jiménez Rodríguez E, García-Ben A, García-Campos J. [Study of tear function and the conjunctival surface in diabetic patients]. *Arch Soc Esp Oftalmol* 2011; **86**: 107-112 [PMID: 21569919 DOI: 10.1016/j.oftal.2010.12.010]
193 **Hom M**, De Land P. Self-reported dry eyes and diabetic history. *Optometry* 2006; **77**: 554-558 [PMID: 17145567 DOI: 10.1016/j.optm.2006.08.002]
194 **Kaji Y**, Usui T, Oshika T, Matsubara M, Yamashita H, Araie M, Murata T, Ishibashi T, Nagai R, Horiuchi S, Amano S. Advanced glycation end products in diabetic corneas. *Invest Ophthalmol Vis Sci* 2000; **41**: 362-368 [PMID: 10670463]
195 **Sato E**, Mori F, Igarashi S, Abiko T, Takeda M, Ishiko S, Yoshida A. Corneal advanced glycation end products increase in patients with proliferative diabetic retinopathy. *Diabetes Care* 2001; **24**: 479-482 [PMID: 11289471 DOI: 10.2337/diacare.24.3.479]
196 **Kinoshita JH**, Fukushi S, Kador P, Merola LO. Aldose reductase in diabetic complications of the eye. *Metabolism* 1979; **28**: 462-469 [PMID: 45423]
197 **Kador PF**, Kinoshita JH. Role of aldose reductase in the development of diabetes-associated complications. *Am J Med* 1985; **79**: 8-12 [PMID: 3934965]
198 **Inoue K**, Kato S, Ohara C, Numaga J, Amano S, Oshika T. Ocular and systemic factors relevant to diabetic keratoepitheliopathy. *Cornea* 2001; **20**: 798-801 [PMID: 11685054]
199 **Jin J**, Chen LH, Liu XL, Jin GS, Lou SX, Fang FN. [Tear film function in non-insulin dependent diabetics]. *Zhonghua YanKe ZaZhi* 2003; **39**: 10-13 [PMID: 12760806]
200 **Schiffman RM**, Christianson MD, Jacobsen G, Hirsch JD, Reis BL. Reliability and validity of the Ocular Surface Disease Index. *Arch Ophthalmol* 2000; **118**: 615-621 [PMID: 10815152 DOI: 10.1001/archopht.118.5.615]
201 **Akinci A**, Cetinkaya E, Aycan Z. Dry eye syndrome in diabetic children. *Eur J Ophthalmol* 2007; **17**: 873-878 [PMID: 18050110]
202 **Albietz JM**, Bruce AS. The conjunctival epithelium in dry eye subtypes: effect of preserved and non-preserved topical treatments. *Curr Eye Res* 2001; **22**: 8-18 [PMID: 11402374 DOI: 10.1076/ceyr.22.1.8.6977]
203 Management and therapy of dry eye disease: report of the Management and Therapy Subcommittee of the International Dry Eye WorkShop (2007). *Ocul Surf* 2007; **5**: 163-178 [PMID: 17508120]
204 **Jackson WB**. Management of dysfunctional tear syndrome: a Canadian consensus. *Can J Ophthalmol* 2009; **44**: 385-394 [PMID: 19606158 DOI: 10.3129/i09-015]
205 **Pflugfelder SC**. Anti-inflammatory therapy of dry eye. *Ocul Surf* 2003; **1**: 31-36 [PMID: 17075627 DOI: 10.1016/S1542-0124(12)70005-8]
206 **Yang CQ**, Sun W, Gu YS. A clinical study of the efficacy of topical corticosteroids on dry eye. *J Zhejiang Univ Sci B* 2006; **7**: 675-678 [PMID: 16845723 DOI: 10.1631/jzus.2006.B0675]
207 **Zhou XQ**, Wei RL. Topical cyclosporine A in the treatment of dry eye: a systematic review and meta-analysis. *Cornea* 2014; **33**: 760-767 [PMID: 24815112]
208 **Schultz RO**, Van Horn DL, Peters MA, Klewin KM, Schutten WH. Diabetic keratopathy. *Trans Am Ophthalmol Soc* 1981; **79**: 180-199 [PMID: 7342400]
209 **Friend J**, Thoft RA. The diabetic cornea. *Int Ophthalmol Clin* 1984; **24**: 111-123 [PMID: 6500867]
210 **Datiles MB**, Kador PF, Fukui HN, Hu TS, Kinoshita JH. Corneal re-epithelialization in galactosemic rats. *Invest Ophthalmol Vis Sci* 1983; **24**: 563-569 [PMID: 6841002]
211 **Perry HD**, Foulks GN, Thoft RA, Tolentino FI. Corneal complications after closed vitrectomy through the pars plana. *Arch Ophthalmol* 1978; **96**: 1401-1403 [PMID: 678179 DOI: 10.1001/archopht.1978.03910060155011]
212 **Cisarik-Fredenburg P**. Discoveries in research on diabetic keratopathy. *Optometry* 2001; **72**: 691-704 [PMID: 12363257]
213 **Kaji Y**. Prevention of diabetic keratopathy. *Br J Ophthalmol* 2005; **89**: 254-255 [PMID: 15722297 DOI: 10.1136/bjo.2004.055541]
214 **Sánchez-Thorin JC**. The cornea in diabetes mellitus. *Int Ophthalmol Clin* 1998; **38**: 19-36 [PMID: 9604736]
215 **Saini JS**, Khandalavla B. Corneal epithelial fragility in diabetes mellitus. *Can J Ophthalmol* 1995; **30**: 142-146 [PMID: 7627899]
216 **Chung H**, Tolentino FI, Cajita VN, Acosta J, Refojo MF. Reevaluation of corneal complications after closed vitrectomy. *Arch Ophthalmol* 1988; **106**: 916-919 [PMID: 3390054 DOI: 10.1001/archopht.1988.01060140062025]
217 **Foulks GN**, Thoft RA, Perry HD, Tolentino FI. Factors related to corneal epithelial complications after closed vitrectomy in diabetics. *Arch Ophthalmol* 1979; **97**: 1076-1078 [PMID: 444136 DOI: 10.1001/archopht.1979.01020010530002]
218 **Chou L**, Cohen EJ, Laibson PR, Rapuano CJ. Factors associated with epithelial defects after penetrating keratoplasty. *Ophthalmic Surg* 1994; **25**: 700-703 [PMID: 7898864]
219 **Jeng S**, Lee JS, Huang SC. Corneal decompensation after argon laser iridectomy--a delayed complication. *Ophthalmic Surg* 1991; **22**: 565-569 [PMID: 1961612]
220 **Simpson RG**, Moshirfar M, Edmonds JN, Christiansen SM. Laser in-situ keratomileusis in patients with diabetes mellitus: a review of the literature. *Clin Ophthalmol* 2012; **6**: 1665-1674 [PMID: 23109803 DOI: 10.2147/OPTH.S36382/OPTH]
221 **Gipson IK**, Grill SM, Spurr SJ, Brennan SJ. Hemidesmosome formation in vitro. *J Cell Biol* 1983; **97**: 849-857 [PMID: 6885921]
222 **Gipson IK**, Spurr-Michaud SJ, Tisdale AS. Anchoring fibrils form a complex network in human and rabbit cornea. *Invest Ophthalmol Vis Sci* 1987; **28**: 212-220 [PMID: 8591898]
223 **McDermott AM**, Xiao TL, Kern TS, Murphy CJ. Non-

enzymatic glycation in corneas from normal and diabetic donors and its effects on epithelial cell attachment in vitro. *Optometry* 2003; **74**: 443-452 [PMID: 12877277]

224 **Yue DK**, Hanwell MA, Satchell PM, Turtle JR. The effect of aldose reductase inhibition on motor nerve conduction velocity in diabetic rats. *Diabetes* 1982; **31**: 789-794 [PMID: 6819173 DOI: 10.2337/diab.31.9.789]

225 **Graham CR**, Richard RD, Varma SD. Oxygen consumption by normal and diabetic rat and human corneas. *Ophthalmol Res* 1981; **13**: 65–71 [DOI: 10.1159/000265134]

226 **Akimoto Y**, Kawakami H, Yamamoto K, Munetomo E, Hida T, Hirano H. Elevated expression of O-GlcNAc-modified proteins and O-GlcNAc transferase in corneas of diabetic Goto-Kakizaki rats. *Invest Ophthalmol Vis Sci* 2003; **44**: 3802-3809 [PMID: 12939295 DOI: 10.1167/iovs.03-0227]

227 **Kaji Y**, Amano S, Usui T, Oshika T, Yamashiro K, Ishida S, Suzuki K, Tanaka S, Adamis AP, Nagai R, Horiuchi S. Expression and function of receptors for advanced glycation end products in bovine corneal endothelial cells. *Invest Ophthalmol Vis Sci* 2003; **44**: 521-528 [PMID: 12556378 DOI: 10.1167/iovs.02-0268]

228 **Saito J**, Enoki M, Hara M, Morishige N, Chikama T, Nishida T. Correlation of corneal sensation, but not of basal or reflex tear secretion, with the stage of diabetic retinopathy. *Cornea* 2003; **22**: 15-18 [PMID: 12502941]

229 **Awata T**, Sogo S, Yamagami Y, Yamamoto Y. Effect of an aldose reductase inhibitor, CT-112, on healing of the corneal epithelium in galactose-fed rats. *J Ocul Pharmacol* 1988; **4**: 195-201 [PMID: 3143793]

230 **Nakamura M**, Kawahara M, Morishige N, Chikama T, Nakata K, Nishida T. Promotion of corneal epithelial wound healing in diabetic rats by the combination of a substance P-derived peptide (FGLM-NH2) and insulin-like growth factor-1. *Diabetologia* 2003; **46**: 839-842 [PMID: 12764579]

231 **Abdelkader H**, Patel DV, McGhee CNj, Alany RG. New therapeutic approaches in the treatment of diabetic keratopathy: a review. *Clin Experiment Ophthalmol* 2011; **39**: 259-270 [PMID: 20973888 DOI: 10.1111/j.1442-9071.2010.02435.x]

232 **Kheirkhah A**, Casas V, Raju VK, Tseng SC. Sutureless amniotic membrane transplantation for partial limbal stem cell deficiency. *Am J Ophthalmol* 2008; **145**: 787-794 [PMID: 18329626 DOI: 10.1016/j.ajo.2008.01.009]

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REVIEW

β-cell dysfunction: Its critical role in prevention and management of type 2 diabetes

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Abstract

Type 2 diabetes (T2DM) is characterized by insulin resistance and β-cell dysfunction. Although, in contrast to type 1 diabetes, insulin resistance is assumed to be a major pathophysiological feature of T2DM, T2DM never develops unless β-cells fail to compensate insulin resistance. Recent studies have revealed that a deficit of β-cell functional mass is an essential component of the pathophysiology of T2DM, implying that β-cell deficit is a common feature of both type 1 and type 2 diabetes. β-cell dysfunction is present at the diagnosis of T2DM and progressively worsens with disease duration. β-cell dysfunction is associated with worsening of glycemic control and treatment failure; thus, it is important to preserve or recover β-cell functional mass in the management of T2DM. Since β-cell regenerative capacity appears somewhat limited in humans, reducing β-cell workload appears to be the most effective way to preserve β-cell functional mass to date, underpinning the importance of lifestyle modification and weight loss for the treatment and prevention of T2DM. This review summarizes the current knowledge on β-cell functional mass in T2DM and discusses the treatment strategy for T2DM.

Key words: β-cell; Insulin secretion; Type 2 diabetes; Prevention; Treatment

Core tip: Recent studies have revealed that a deficit of β-cell functional mass is an essential component of the pathophysiology of type 2 diabetes (T2DM). β-cell dysfunction is present at the diagnosis of T2DM and progressively worsens with disease duration. β-cell dysfunction is associated with worsening of glycemic control and treatment failure; thus, it is important to preserve or recover β-cell functional mass in the management of T2DM. This review summarizes the current knowledge on β-cell functional mass in T2DM and discusses the treatment strategy for T2DM.

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INTRODUCTION

The number of patients with diabetes is countinuously increasing all over the world. Worldwide, there were

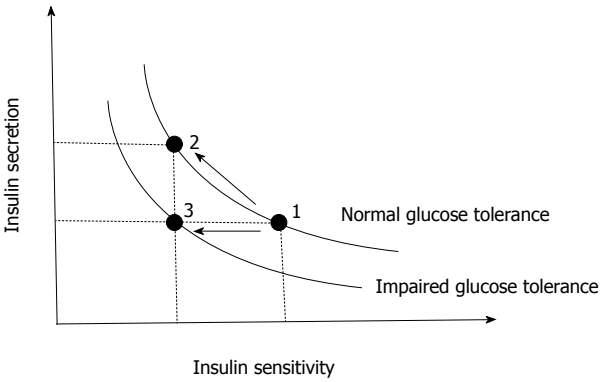

Figure 1 Insulin secretion-insulin sensitivity relationship. In a physiological condition, when insulin sensitivity decreases, insulin secretion increases to maintain normoglycemia (1→2), showing a hyperbolic curve. When insulin secretion fails to compensate, the hyperbolic curve shifts to the left and abnormal glucose tolerance develops (1→3).

382 million patients with diabetes in 2013, which will rise to 592 million in 2035[1]. Diabetes is associated not only with diabetic microangiopathy such as retinopathy, nephropathy and neuropathy, but also with a 2- to 4-fold increase in risk of cardiovascular disease[2,3]. Among the people with diabetes, more than 90% have type 2 diabetes (T2DM). Therefore, optimal treatment and prevention strategies for T2DM are urgently needed.

T2DM is characterized by insulin resistance and β-cell dysfunction. Recent evidence suggests an important role of β-cell function in the development and management of T2DM. In this review, the current knowledge regarding β-cell dysfunction in T2DM is summarized and its critical role in the prevention and treatment of T2DM is discussed.

DEFICITS OF β-CELL FUNCTION AND β-CELL MASS IN T2DM

Disposition index: A true assessment of β-cell function

T2DM is characterized by insulin resistance and β-cell dysfunction[4,5]. However, since the development of an insulin radioimmunoassay, it was found that in people with T2DM, plasma insulin concentration is rather higher than that in those with normal glucose tolerance (NGT), indicating that insulin resistance rather than insulin deficiency is central in the pathogenesis of T2DM. Therefore, in contrast to type 1 diabetes, obesity, hyperinsulinemia and insulin resistance are often emphasized as characteristics of T2DM, and β-cell function in T2DM is often less emphasized or even ignored.

However, the higher plasma insulin concentration in patients with T2DM is often confounded by a higher plasma glucose level, which itself stimulates insulin secretion. Moreover, insulin sensitivity also affects insulin secretion. In normal physiological conditions, normoglycemia is maintained under a balance between insulin sensitivity and insulin secretion, and when insulin sensitivity decreases, insulin secretion increases to maintain normoglycemia. Thus, insulin secretion should always be assessed in relation to insulin sensitivity. Bergman and Cobelli have found that this relationship between insulin secretion and insulin sensitivity is expressed as a hyperbolic curve, and as a result the product of insulin sensitivity and insulin secretion is constant as long as normoglycemia is maintained[6,7] (Figure 1). The product of insulin sensitivity and insulin secretion, called the disposition index, refers to insulin secretion adjusted by insulin sensitivity and reflects true β-cell function *in vivo*.

Once insulin secretion is not able to sufficiently increase to compensate the decrease in insulin sensitivity, the insulin sensitivity-insulin secretion relationship is shifted to the left and abnormal glucose tolerance develops (Figure 1). In this case, the disposition index is decreased, indicating that abnormal glucose tolerance develops only when β-cells are no longer able to compensate decreased insulin sensitivity.

β-cell function in T2DM

When β-cell function is assessed using the disposition index, a number of studies have consistently shown that β-cell function is diminished in people with T2DM[4,8,9]. Using the disposition index, DeFronzo *et al*[9] have shown that β-cell function is decreased by -80% in patients with impaired glucose tolerance (IGT) and is even less in patients with T2DM (Figure 2). Importantly, β-cell function starts to decline with higher plasma glucose levels, even within the range of normal plasma glucose levels[9], which suggests that β-cell function is already impaired prior to the development of IGT.

β-cell mass in T2DM

If β-cell function is impaired in patients with T2DM, what about the β-cell mass? β-cells are located in the islets of Langerhans, which are scattered within the exocrine pancreas. Each islet contains -1000 β-cells together with other endocrine cells such as alpha cells, delta cells, pancreatic polypeptide (PP) cells and epsilon cells, and a total of -1 million islets exist in the pancreas. β-cell mass refers to the total mass of β-cells and is approximately 1 g in humans.

Due to the anatomical characteristics of β-cells scattered throughout the whole pancreas, it is difficult to visualize β-cells *in vivo*, and direct measurement of β-cell mass *in vivo* in humans remains to be established[10,11]. Thus, to date, the measurement of β-cell mass inevitably relies on histological analysis of the pancreas obtained surgically or at autopsy.

Since insulin resistance and hyperinsulinemia are often emphasized in people with T2DM, β-cell mass in people with T2DM is also often assumed to be increased or at least not decreased. However, based on histological analysis, Butler *et al*[12] have reported that β-cell mass is decreased by -40% and -65% in lean and obese people with T2DM, respectively, compared with non-diabetic controls matched for age and BMI. Other groups have

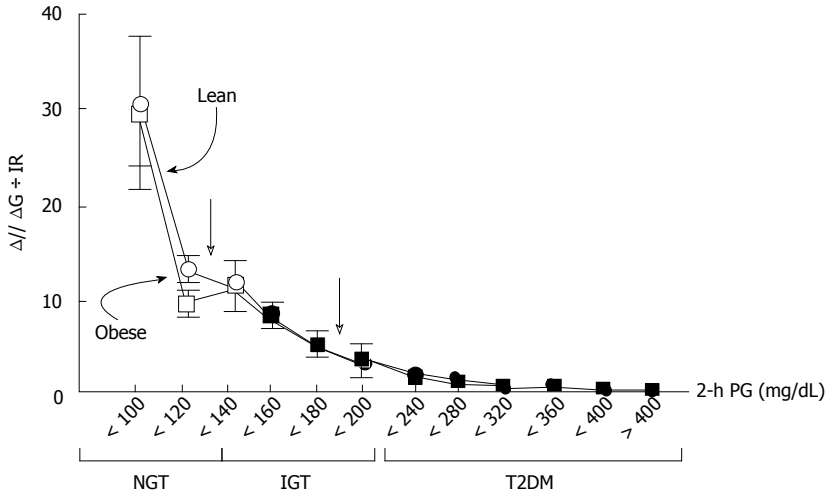

Figure 2 Insulin secretion/insulin resistance (disposition) index (I/G ÷ IR) during 75g-oral glucose tolerance test in individuals with normal glucose tolerance, impaired glucose tolerance, and type 2 diabetes as a function of the 2 h plasma glucose concentration in lean and obese subjects. I/G: Insulinogenic index (Insulin 0-30 min/Glucose 0-30 min); IR: Homeostasis model assessment of insulin resistance [HOMA-IR; fasting insulin (mU/L) x glucose (mmol/L)/22.5]. Adapted from ref.[9]. NGT: Normal glucose tolerance; IGT: Impaired glucose tolerance; T2DM: Type 2 diabetes; PG: Plasma glucose.

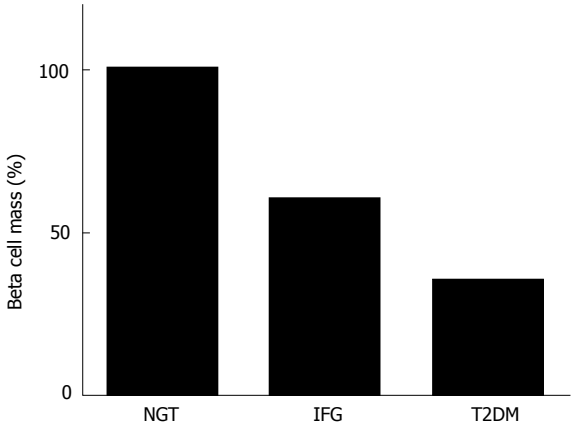

Figure 3 β-cell mass in patients with normal glucose tolerance, impaired fasting glycemia and type 2 diabetes. Adapted and modified from the study by Butler *et al*[12]. NGT: Normal glucose tolerance; IFG: Impaired fasting glycemia; T2DM: Type 2 diabetes.

also reported a significant (-30%-40%) decrease in β-cell mass in patients with T2DM[13-15]. These findings suggest that deficit of β-cell mass is a common pathophysiological feature of type 1 and T2DM (Figure 3), while the cause and degree of the deficit are different between type 1 and T2DM.

Mechanisms of β-cell deficit in T2DM

β-cell mass is regulated by the balance of newly formed β-cells and β-cell loss[16-18]. Butler *et al*[12] have shown that β-cell apoptosis is increased in patients with T2DM, whereas neither β-cell replication nor neogenesis is decreased, suggesting that increased β-cell loss is the main cause of reduced β-cell mass in T2DM. Various mechanisms that induce β-cell apoptosis have been proposed such as hyperglycemia (glucotoxicity)[19], fatty acids (lipotoxicity)[20], amyloid or islet amyloid polypeptide (IAPP, also called amylin)[21-24], oxidative stress[25], inflammatory cytokines[26], mitochondrial dysfunction[27], endoplasmic reticulum (ER) stress[28,29] and dysfunction of autophagy[30]. A recent study suggested that several mechanisms are simultaneously associated with β-cell failure in humans with T2DM[31].

Recently, transdifferentiation of β-cells to alpha cells has been suggested as a mechanism of β-cell loss in a rodent model of diabetes[32]. This mechanism could explain β-cell loss and the reciprocal increase in alpha cell mass observed in humans with T2DM[15,31], although whether an increase in alpha cell mass occurs in humans with T2DM remains controversial[33,34].

Association between β-cell mass and glucose metabolism

The deficit of β-cell mass in patients with T2DM raises the next question of whether the change in β-cell mass is associated with the severity of glucose intolerance. It has been reported that there is a reciprocal relationship between β-cell mass and fasting plasma glucose level[35], suggesting that glucose intolerance develops when the β-cell mass decreases by -50% of the normal level. A similar relationship has been observed in rodents[36], pigs[37] and monkeys[38]. An increased risk of the development of IGT or diabetes after hemipancreatectomy has been reported in dogs and humans[39-43]. It has also been reported that β-cell mass was decreased by -20%-40% in patients with IGT and impaired fasting glycemia (IFG)[12,44]. We have also reported that there was a significant negative correlation between β-cell mass and glycated hemoglobin (HbA1c) level in non-diabetic individuals[45], suggesting that β-cell mass is related to glucose intolerance even prior to the development of T2DM. A significant correlation between β-cell mass and HbA1c was also observed in patients with T2DM[31].

Association between β-cell mass and β-cell function

The relationship between β-cell mass and β-cell function is more complicated. Whether β-cell dysfunction in T2DM is mainly due to a functional defect of each β-cell or due to a defect of β-cell mass has been extensively argued[46]. A close correlation between β-cell function assessed by maximum acute insulin response (AIRmax) induced by arginine infusion under a hyperglycemic state and β-cell mass of transplanted islets has been reported[47]. On the other hand, β-cell dysfunction was markedly improved after an overnight β-cell rest by somatostatin

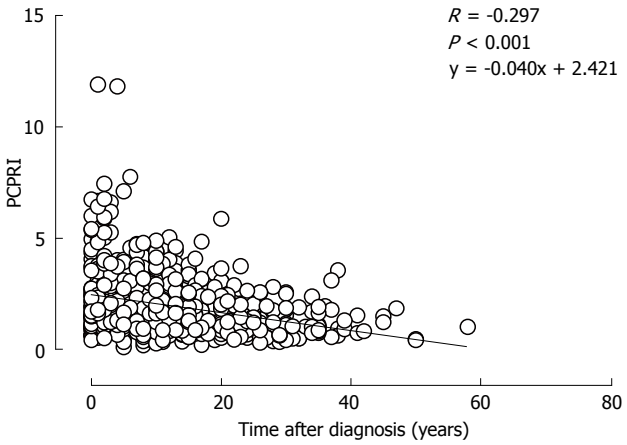

Figure 4 Relationship between postprandial C-peptide to glucose ratio (postprandial C-peptide index) and time after diagnosis in patients with Type 2 diabetes. Reproduced with permission from ref.[51]. PCPRI: Postprandial C-peptide index.

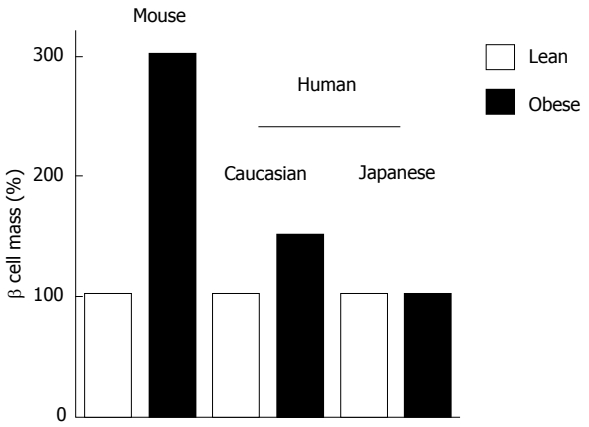

Figure 5 Change in β-cell mass with obesity. In mice, β-cell mass increases 3-fold with obesity. In humans, a 50% increase in β-cell mass has been reported in Caucasians, while no increase was reported in Japanese. Adopted and modified from ref.[54,59,85].

infusion[48]. Thus, it remains uncertain whether β-cell function *in vivo* sufficiently reflects β-cell mass in patients with T2DM.

Meier *et al*[49] assessed the relationship between β-cell mass and β-cell function in patients who had undergone pancreatic surgery and found that there was a significant positive correlation between β-cell mass and β-cell function, especially postprandial C-peptide level, suggesting that C-peptide measurement in clinical settings reflects β-cell mass.

Taken together, these results indicate that β-cell function and β-cell mass seem to be correlated with each other, although on some occasions they can be dissociated, and both β-cell function and mass seem to decrease during the development of glucose intolerance. Since β-cell function and mass are difficult to separate, currently they are referred to as "β-cell functional mass", and it is now certain that β-cell functional mass decreases during the development of T2DM.

Progressive decline in β-cell functional mass in T2DM

A deficit of β-cell functional mass is not only present in patients with T2DM, but it also progressively declines with disease duration. In the UK Prospective Diabetes Study (UKPDS), β-cell function assessed by homeostasis model assessment (HOMA) in patients with T2DM was already decreased by -50% at the time of diagnosis and progressively declined by -5% annually[50]. This also indicates that in patients with T2DM, β-cell function starts to decline -10 years prior to the onset of the disease. A gradual but significant decline in β-cell function assessed by C-peptide level has been confirmed in cross-sectional cohort studies of Japanese patients with T2DM[51,52] (Figure 4). Intriguingly, a significant negative correlation between β-cell mass and duration of T2DM has also been reported[13].

Limited β-cell regenerative capacity in humans

Since deficits of β-cell function and mass are now recognized as hallmarks of T2DM as well as type 1 diabetes, β-cell regeneration is considered to be an important therapeutic strategy for both types of diabetes.

Rodent studies show an adaptive change in β-cell mass in response to obesity or pregnancy[53-58], suggesting the presence of endogenous β-cell regenerative capacity in the postnatal period. However, recent observation of the human pancreas suggests that endogenous β-cell regenerative capacity is limited in humans.

We have reported that β-cell mass in obese non-diabetic individuals is -1.2 g compared with -0.8 g in lean non-diabetic individuals, an -50% increase[59], whereas β-cell mass increases 3- to 10-fold in response to obesity or insulin resistance in rodents[53,54] (Figure 5). This striking difference in β-cell regenerative capacity between humans and rodents suggests that the results of rodent studies are not necessarily applicable to humans[60,61].

In humans, β-cell mass increases from -37 mg to -1 g in the first five years of life, and during this period replicating β-cells are often observed[62,63]. However, after that, replicating β-cells are rarely seen and β-cell mass reaches a plateau. The β-cell mass then remains constant during adulthood[13,59,64], suggesting that β-cell turnover is limited in humans after the first five years of life. Estimation of β-cell life using either ^{14}C measurement or cellular lipofuscin body content also suggests very slow turnover of β-cells in adult humans[65,66]. Recent studies have suggested that there is an increase in β-cell neogenesis in humans with obesity, pregnancy and IGT[67-70]; however, the extent of its contribution to β-cell mass remains unclear. Limited β-cell regenerative capacity has also been observed in monkeys[71,72]. Even in rodents, β-cell regenerative capacity significantly decreases with aging[54,73,74].

A hypothetical schema of the change in β-cell functional mass during the development of T2DM is shown in Figure 6. The magnitude of the increased demand for insulin due to insulin resistance caused by excess caloric intake and physical inactivity exceeds the magnitude of β-cell mass expansion, resulting in an increase in β-cell

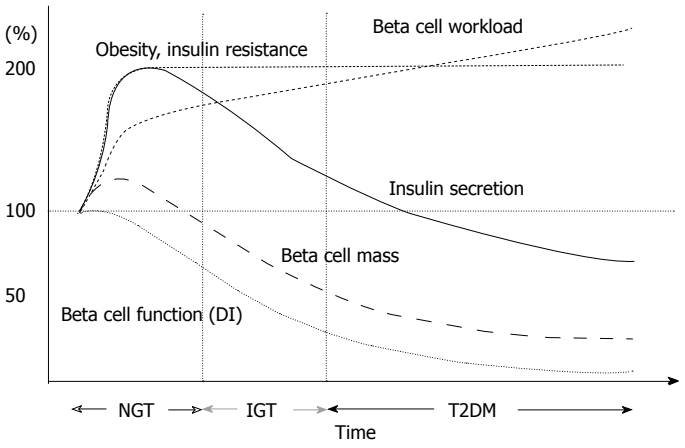

Figure 6 Hypothesis for change in β-cell function and mass during development of abnormal glucose tolerance. The magnitude of the increased demand for insulin due to insulin resistance caused by excess caloric intake and physical inactivity exceeds the magnitude of β-cell mass expansion, resulting in an increase in β-cell workload. In individuals who are susceptible to type 2 diabetes (T2DM), increased β-cell workload may lead to β-cell failure and the development of T2DM. Adopted and modified from ref.[109].

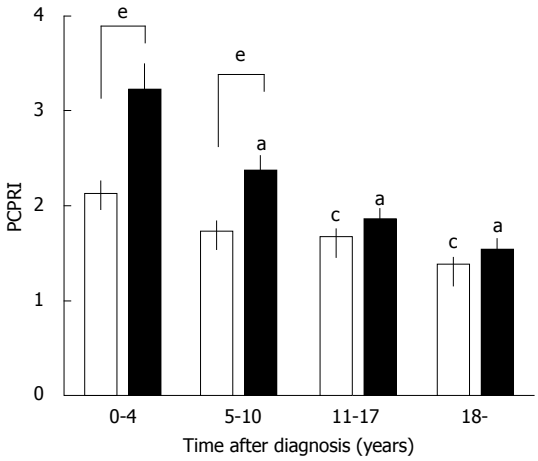

Figure 7 Postprandial C-peptide to glucose ratio (postprandial C-peptide index; PCPRI) in subjects according to obesity and time after diagnosis (0-4, 5-10, 11-17 and ⩾ 18 years). There were significant differences in PCPRI between lean (open bars) and obese subjects (solid bars) in the first and second quartiles of time after diagnosis, but no significant difference was observed in the third and fourth quartiles. $^{a}P < 0.05$ *vs* obese subjects ⩽ 4 yr after diagnosis, $^{c}P < 0.05$ *vs* lean subjects ⩽ 4 yr after diagnosis, $^{e}P < 0.05$ *vs* lean subjects. Reproduced with permission from ref.[51].

workload. In individuals who are susceptible to T2DM, increased β-cell workload may lead to β-cell failure and the development of T2DM. In addition, once hyperglycemia develops, it also causes β-cell dysfunction and apoptosis, which further exacerbate β-cell failure. Importantly, because insulin resistance persists, the β-cell workload continues to increase, with a reduction in β-cell mass. As a result, glucose metabolism progressively deteriorates in patients with T2DM. In our retrospective cohort, the progressive decline in β-cell function seemed to be exaggerated in the presence of obesity in Japanese patients with T2DM[51] (Figure 7). Another Japanese cohort showed a decreasing trend in fasting insulin level despite an increasing trend in BMI at the first clinic/hospital visit of patients with T2DM during the past ten years[75]. Recent studies have shown that even metabolically healthy obese individuals are at increased risk of future development of diabetes, cardiovascular events and all-cause mortality[76-78]. Thus, weight loss itself may be important to preserve β-cell function and improve clinical outcomes.

β-cell functional mass in Asian population

T2DM is characterized by obesity, but the degree of obesity differs between ethnic groups[79,80]. In Caucasians, most patients with T2DM are obese, and the mean BMI of patients with T2DM is ~30 kg/m^2. In contrast, the mean BMI of Asian patients with T2DM is ~23 kg/m^2, suggesting that about half of patients with T2DM are not even overweight (*i.e.*, BMI ⩾ 25 kg/m^2, the definition of obesity in Asian countries).

The difference in adiposity between Caucasians and Asians has been postulated to explain this ethnic difference. Visceral adiposity is more apparent in Asians compared to Caucasians with the same BMI[81,82], indicating that Asians have a lower capacity for subcutaneous fat deposition and are more vulnerable to visceral fat accumulation compared with Caucasians. Nonetheless, a meta-analysis of studies examining the insulin sensitivity-insulin secretion relationship in individuals with NGT clearly showed that Asians have less insulin secretion with higher insulin sensitivity compared with Caucasians[83]. Direct comparison of insulin sensitivity and insulin secretion between Japanese and Caucasians showed that most of the difference in insulin secretion between the two ethnicities can be explained by the difference in BMI between the two[84]. Since the incidence of T2DM is comparable between the two ethnicities despite the different degree of obesity[1], it is plausible that the lower degree of obesity in Asians could be attributable to the lower β-cell functional capacity in this population.

We have recently examined the change in β-cell mass in Japanese obese nondiabetic individuals (mean BMI 20.4 kg/m^2) compared to age- and sex-matched lean individuals (mean BMI 28.5 kg/m^2)[85]. As a result, in contrast to the studies in Caucasians showing a significant increase in β-cell mass with obesity[13,59], there was no significant increase in β-cell mass in Japanese obese individuals (Figure 5). Another Japanese study also confirmed our findings[86]. These studies suggest that Asians have less β-cell regenerative capacity compared with Caucasians, which is probably derived from both genetic and environmental factors, and the lower β-cell

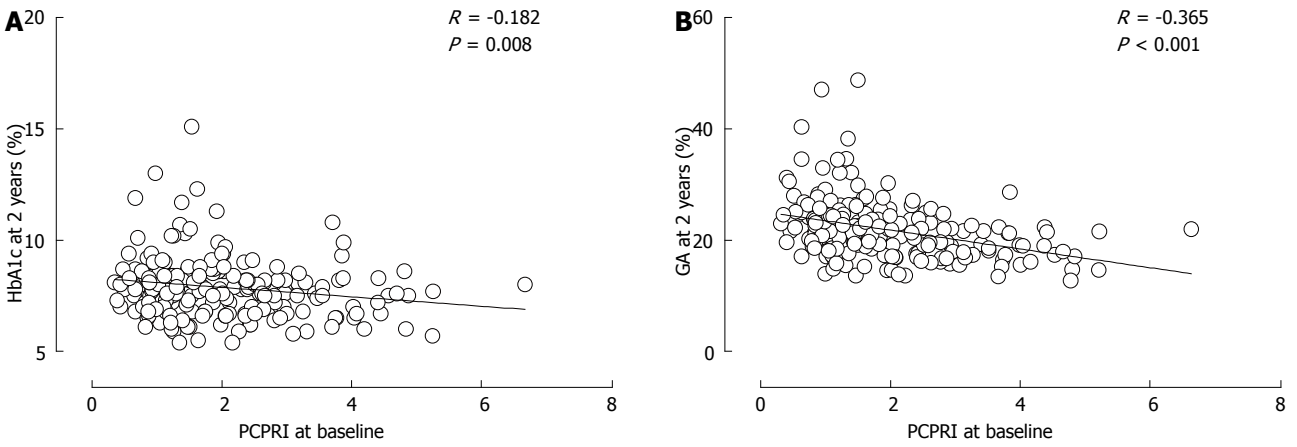

Figure 8 Correlation between baseline postprandial C-peptide index and HbA1c (A) and glycated albumin (B) after 2 years. Reproduced with permission from ref.[93]. PCPRI: Postprandial C-peptide index; GA: Glycated albumin.

functional capacity in Asians may contribute the different phenotype of T2DM between the two ethnicities. Because of the limited capacity of β-cell regeneration in Asians, excess β-cell workload could be induced in individuals with less obesity compared with Caucasians, which may lead to β-cell failure and the development of T2DM.

IMPLICATIONS FOR TREATMENT AND PREVENTION OF T2DM

β-cell function and glycemic control

If a deficit of β-cell functional mass is a hallmark of T2DM, what is the clinical consequence? In UKPDS and A Diabetes Outcome Progression Trial (ADOPT), treatment failure was associated with a progressive decline in β-cell function[50,87,88]. In the Treatment Options for T2DM Adolescents and Youth (TODAY) study, similar results were observed in adolescents with T2DM, and in this study baseline β-cell function was associated with treatment efficacy[89]. In our retrospective cohort analysis, we found that a lower baseline C-peptide level was associated with poorer glycemic control and the need for insulin therapy thereafter[90-93] (Figure 8). In these studies, postprandial C-peptide index [*i.e.,* postprandial serum C-peptide (ng/mL)/plasma glucose (mg/dL) × 100] was the best predictor of future insulin therapy among other C-peptide indices such as fasting C-peptide index and urinary C-peptide level. Since it was also significantly correlated with β-cell mass[49], postprandial C-peptide index may be a useful marker of β-cell function in clinical settings.

Thus, poorer β-cell function is associated with poorer glycemic control and treatment failure, indicating the important role of β-cell function in the treatment of T2DM.

β-cell function and glycemic variability

Furthermore, β-cell function is associated with glycemic variability. In patients with T1DM, it has been reported that lower β-cell functional capacity is associated with greater glycemic variability[94-96].

We and others have reported that serum and urinary C-peptide levels are negatively correlated with glycated albumin (GA) to HbA1c ratio in patients with T2DM[97-99] (Figure 9). Since albumin is more susceptable to glycation than is hemoglobin[100,101], GA more sensitively reflects glycemic variability than does HbA1c[97,102,103]. Thus, the inverse association between C-peptide level and GA to HbA1c ratio in patients with T2DM indicates that β-cell dysfunction is associated with greater glycemic variability in not only patients with type 1 diabetes but also those with T2DM.

Notably, we found that the relationship between postprandial C-peptide index and GA to HbA1c ratio in patients with T2DM was comparable to that in those with type 1 diabetes[97] (Figure 9B). This suggests that the impact of β-cell dysfunction on glycemic variability is irrespective of the type of diabetes, again indicating the central role of β-cell function in the pathogenesis of diabetes.

Recently, it has been reported that greater glycemic variability as well as poorer glycemic control is associated with the development of micro- and macroangiopathy[104-107]. Thus, it should be stressed that greater glycemic variability and poorer glycemic control due to β-cell dysfunction may result in increased risk of diabetic complications.

Treatment strategy for T2DM

Since β-cell dysfunction is associated with poor glycemic control in patients with T2DM, preservation and recovery of β-cell functional mass is an important therapeutic strategy for T2DM. Moreover, the current issues in the treatment of T2DM summarized in Table 1 are, to put it simply, all associated with either excess or insufficiency of insulin supplementation[108]. Thus, the recovery of physiological insulin secretion in patients with T2DM is also a key to resolving these issues.

To preserve or recover β-cell function, a reduction in excess β-cell workload appears to be the most effective strategy to date. In ADOPT, better glycemic control was

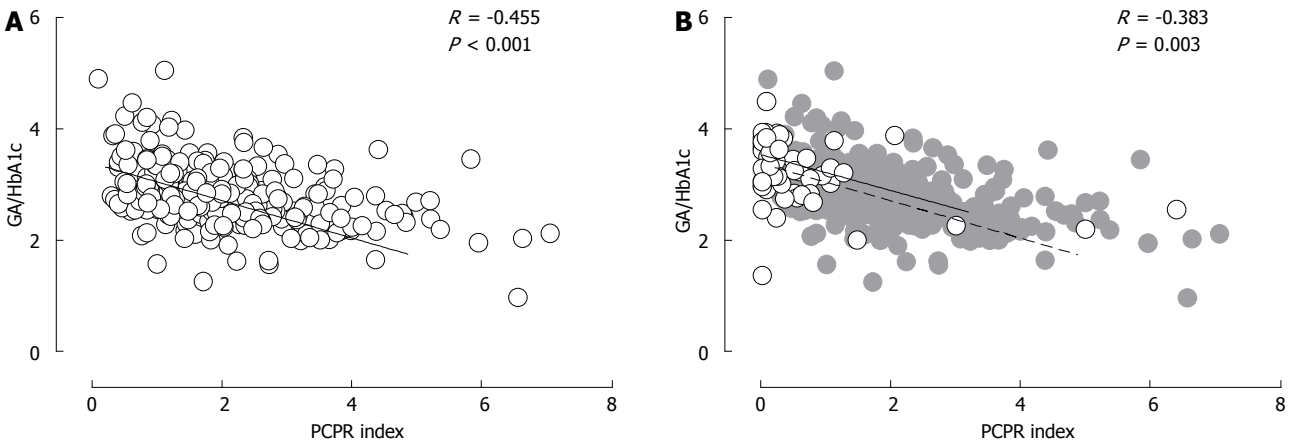

Figure 9 Correlation between postprandial C-peptide index and glycated albumin to HbA1c ratio in patients with type 2 diabetes (A) and type 1 diabetes (B). In Figure 9B, the data of patients with type 1 diabetes are superimposed on the data of those with type 2 diabetes (gray circles and dotted line). Reproduced with permission from ref.[97]. PCPRI: Postprandial C-peptide index; GA: Glycated albumin.

Table 1 Current issues in treatment of type 2 diabetes

Issue	Cause
Hypoglycemia	Excess insulin
Weight gain	Excess insulin
Concern of increased risk of malignancy and/or atherosclerosis	Excess insulin, especially peripheral hyperinsulinemia
Postprandial hyperglycemia	Insufficient insulin in postprandial state, especially in portal vein

obtained with metfomin or rosiglitazone monotherapy compared with glyburide in patients with T2DM[87]. Thus, therapy should be focused on improving insulin sensitivity to reduce β-cell workload.

A proposed treatment strategy for T2DM is shown in Figure 10, as also described previously[108,109]. It is emphasized that, to reduce β-cell workload, lifestyle modification and weight reduction remain the most important therapy at any stage of T2DM. Although lifestyle modification failed to reduce the incidence of cardiovascular disease in the Action for Health in Diabetes (Look AHEAD) trial[110], it has been reported that lifestyle modification improved cardiovascular risk factors, reduced the need for and cost of medication, reduced the rate of sleep apnea and urinary incontinence, improved well-being and depression symptoms, and increased the rate of diabetes remission[111-116]. In a cohort analysis of the ADDITION-Cambridge study, it has been reported that healthy behavioral changes after the diagnosis of T2DM were associated with a significant reduction in risk of cardiovascular events[117], suggesting that early lifestyle intervention may be important to improve cardiovascular outcome.

Metformin is currently positioned as first-line therapy in most guidelines for the treatment of T2DM[118,119]. Since metformin is effective in lean patients as well as obese patients with T2DM[120,121], it should be used in both lean and obese individuals unless contraindicated. Its efficacy in reducing HbA1c (by -1.5%), low risk of hypoglycemia, favorable effect on body weight and low cost also support metformin as a first-line drug.

Thiazolidinediones (TZDs) have also been shown to reduce β-cell workload and maintain glycemic control in the long term[87,88]. Rosiglitazone has been shown to increase low-density lipoprotein (LDL) cholesterol and the risk of coronary heart disease in patients with T2DM[122], and its use has been suspended or strictly restricted in Europe and United States[123,124], although recently the US Food and Drug Administration has lifted most of its restrictions[125]. On the other hand, pioglitazone has been shown to suppress the progression of atherosclerosis and reduce the risk of cardiovascular disease[126-129]. However, TZDs often induce weight gain and edema due to fluid retention, and are contraindicated in patients with heart failure[118]. Recent studies have also shown an increase in risk of bone fracture in women[130] and risk of bladder cancer[131-133] in patients treated with pioglitazone. The risk of bladder cancer may be dose dependent. In addition, since low-dose pioglitazone also reduces the risk of weight gain and edema, it may be preferable to use pioglitazone at lower doses, especially in women. Pioglitazone should also be used with caution in postmenopausal women with osteoporosis because of the increased fracture risk.

α-glucosidase inhibitors (AGIs) delay the absorption of carbohydrate from the small intestine, and thereby reduce postprandial hyperglycemia, resulting in reduced β-cell workload in a postprandial state. AGIs have also been reported to reduce the progression to T2DM in patients with IGT[134,135]. Improving postprandial hyperglycemia by AGIs may also improve the cardiovascular outcome[136-138]. Therefore, although the reduction in HbA1c by AGIs is relatively small (-0.5%), their use is also considered in patients with T2DM, especially those with postprandial hyperglycemia. The major side effect of AGIs is gastrointestinal disturbance such as flatulence, diarrhea and abdominal pain. In Japan, AGIs are the only medication indicated for patients with IGT.

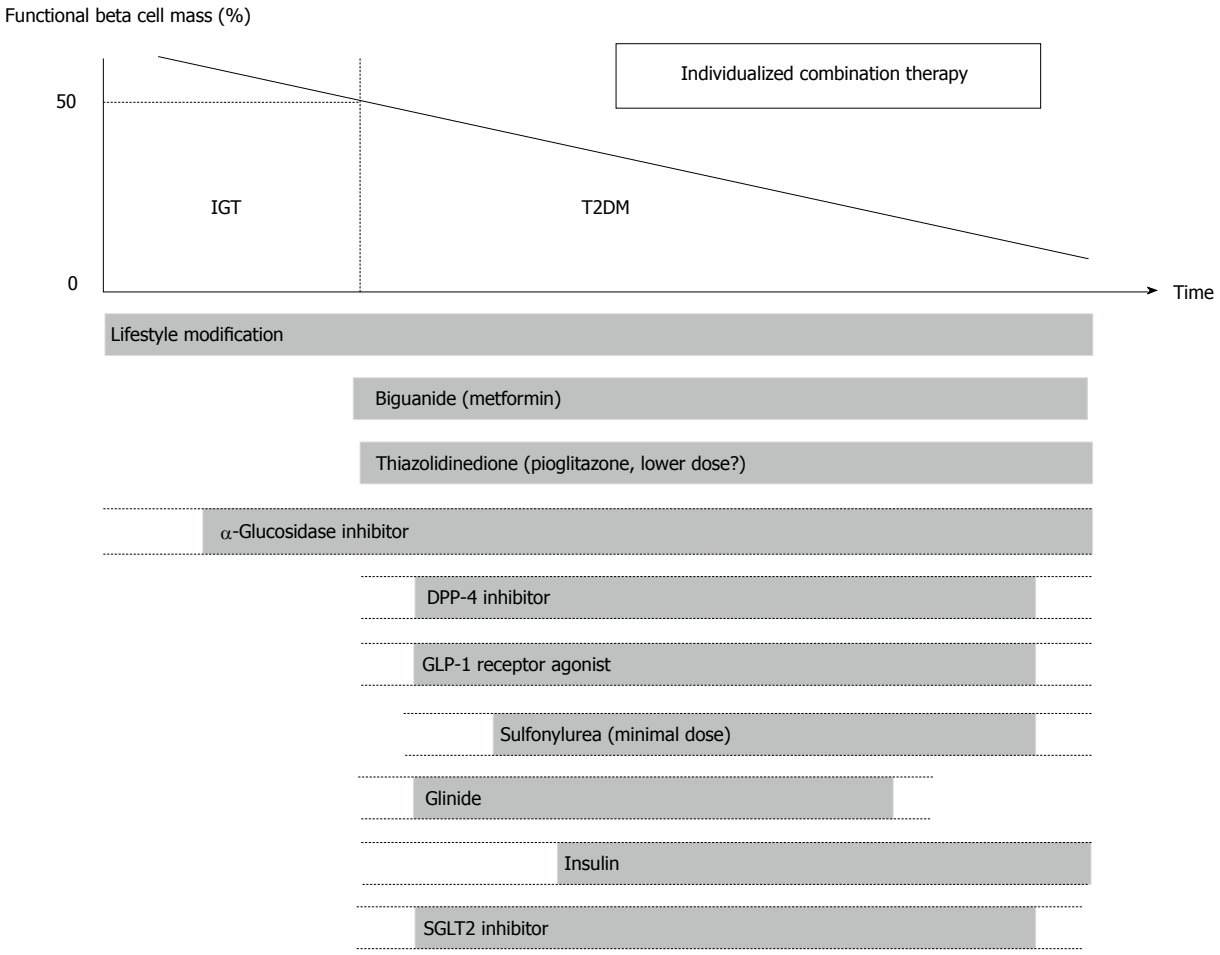

Figure 10 Proposed concept of treatment strategy for type 2 diabetes in relation to functional β-cell mass. α-glucosidase inhibitor is partly approved for use in patients with impaired glucose tolerance in Japan. Medications not approved in Japan are not included in the figure. Since currently no single therapy or agent can cure and even manage type 2 diabetes (T2DM), an effective combination of current medications in addition to lifestyle modification aiming at reduction in β-cell workload is important to preserve or recover β-cell function. Adopted and modified from ref.[108,109]. IGT: Impaired glucose tolerance.

Thus, AGIs are also considered for the treatment of T2DM at the early stage of the disease, if tolerated.

On the other hand, the use of insulin secretagogues, which increase β-cell workload, may be somewhat limited. Sulfonylureas (SUs), while remaining among the most highly prescribed drugs for the treatment of T2DM, increase the risk of hypoglycemia and weight gain, resulting in a high rate of treatment failure[87]. These issues of SUs may be derived from their non-physiological augmentation of insulin secretion from β-cells.

Incretin drugs include dipeptidyl peptidase-4 (DPP-4) inhibitors and glucagon-like peptide-1 receptor agonists (GLP-1RAs). Both drug types reduce HbA1c mainly through an increase in insulin secretion, but also through suppression of glucagon secretion[139]. GLP-1RAs also slow gastric emptying and reduce appetite, resulting in weight loss. The most important characteristic of incretin drugs is probably that the enhancement of insulin secretion occurs in a glucose-dependent manner. Thus, the action of incretin drugs as insulin secretagogues is more physiological than that of SUs, thereby resulting in a low risk of hypoglycemia and weight gain with incretin therapy[140-142]. Whether this physiological enhancement of insulin secretion results in long-term maintenance of glycemic control remains to be elucidated. Although an increase in β-cell mass with incretin therapy has been reported in rodent studies[143,144], this effect has not been confirmed in humans[145-147]. Since incretin therapy is usually well tolerated without serious adverse effects, the use of incretin drugs is rapidly increasing[148].

Glinides, short-acting insulin secretagogues, enhance early-phase insulin secretion, thereby reducing post-prandial hyperglycemia[149]. Since a defect in early-phase insulin secretion is a hallmark of glucose intolerance[150], the enhancement of early-phase insulin secretion without prolonged hyperinsulinemia by glinides is more physiological, unlike the action of SUs, and is assumed to increase β-cell workload as well as the risk of hypoglycemia to a lesser degree compared with SUs.

Thus, the use of insulin secretagogues may be limited because of an increase in β-cell workload as well as increased risk of hypoglycemia. Since incretin enhances insulin secretion in a more physiological manner and is also expected to improve β-cell function and/or mass, incretin drugs could be used at any stage of T2DM.

On the other hand, SUs may be used rather to enhance incretin action at only a minimal dose. To recover physiological insulin secretion, a combination of an incretin drug and a glinide may also be useful.

Insulin has been shown to improve β-cell function in patients with IGT and T2DM[151-153]. Since initial intensive insulin therapy has been shown to preserve β-cell function thereafter[152], insulin therapy should be considered as early as possible in patients with T2DM. Insulin therapy is also the most effective medication to reduce HbA1c[118]. However, the increased risk of hypoglycemia, weight gain and non-physiological insulin delivery (*i.e.,* systemic *vs* portal), in addition to the fear of injections, limit its use. Insulin therapy to overpower insulin resistance without eliminating excess calories may worsen ectopic lipid overload[154].

A sodium-glucose cotransporter 2 (SGLT2) inhibitor has recently been approved in several countries including United States, EU and Japan. SGLT2 inhibitors suppress reabsorption of glucose by SGLT2 in the proximal renal tubule and increase glucose excretion in urine (-60-80 g glucose/d)[155]. As a result, SGLT2 inhibitors not only decrease HbA1c, but also reduce body weight and blood pressure and improve the lipid profile. The action of SGLT2 inhibitors is independent of insulin. Thus, the efficacy of SGLT2 inhibitors seems to be regardless of β-cell function. SGLT2 inhibitors show a low risk of hypoglycemia but increase the incidence of bacterial urinary tract infections and fungal genital infections especially in women. A higher risk of hypotension has also been reported[156]. SGLT2 inhibitors may be suitable for obese patients with T2DM and metabolic syndrome; however, their longer term safety including cardiovascular and cancer risk and efficacy remain unknown[156,157].

Nonetheless, since currently no single therapy or agent can cure or even manage T2DM, an effective combination of current medications in addition to lifestyle modification aiming at reduction of β-cell workload is important to preserve or recover β-cell function.

Finally, marked weight reduction by bariatric surgery such as gastric bypass or sleeve gastrectomy has been reported to markedly improve glycemic control and even achieve remission of T2DM in severely obese T2DM patients[158,159]. This also suggests the importance of reducing β-cell workload, although change in incretin secretion has also been proposed as another mechanism by which glucose metabolism is improved after gastric bypass. On the other hand, it has been reported that gastric bypass markedly improved incretin's effect on insulin secretion, but not insulin secretion induced by intravenous glucose infusion[160], suggesting limited recovery of β-cell function even with marked weight loss. Also, the remission of T2DM after bariatric surgery is associated with residual β-cell function[161,162], indicating the importance of residual β-cell function to manage and/or cure T2DM.

Implications for prevention

The progressive decline in β-cell functional capacity during the development of glucose intolerance also implies the important role of preservation or recovery of β-cell function to prevent T2DM.

Similarly to the treatment of T2DM, prevention strategies should focus on reducing β-cell workload or inducing β-cell rest. These include lifestyle modification and/or weight reduction, and use of metformin or TZD. Lifestyle modification, *i.e.,* nutritional therapy and increase in physical activity, and weight reduction improve insulin sensitivity and thereby reduce β-cell workload. A number of studies have shown the efficacy of lifestyle intervention to prevent the development of T2DM in patients with IGT[163-166]. In the Diabetes Prevention Program (DPP), intensive lifestyle modification with more than 7% weight loss suppressed the progression to T2DM by -58% in patients with IGT[165]. In the same study, metformin therapy also reduced the progression to T2DM by -31%[165]. TZDs have also been shown to effectively suppress the progression from IGT to T2DM[167-169]. A significant reduction in the development of diabetes was also observed in patients with IGT treated with AGIs[134,135]. In the Outcome Reduction with Initial Glargine Intervention (ORIGIN) trial, adding basal insulin was also shown to suppress progression from IGT to T2DM[151], probably through inducing β-cell rest. On the other hand, in the Nateglinide And Valsartan in Impaired Glucose Tolerance Outcomes Research (NAVIGATOR) trial, nateglinide, a short-acting insulin secretagogue, failed to show a reduction in progression to T2DM in patients with IGT[170], suggesting that therapeutic strategies to increase β-cell workload may not be effective to prevent deterioration of glucose metabolism.

Importance of empowerment of patients

Although several anti-diabetic agents have been shown to effectively prevent the onset of T2DM, the importance of lifestyle modification remains unchanged, since the rapid increase in incidence of T2DM is certainly associated with the change in diet (*i.e.,* westernization) and physical inactivity, resulting in increased incidence of obesity. In the NAVIGATOR trial, it has been reported that both baseline level and change in daily ambulatory activity were associated with a reduced risk of cardiovascular events in patients with IGT[171]. A six-year lifestyle intervention program for Chinese people with IGT showed a significant reduction in the incidence of cardiovascular and all-cause mortality as well as diabetes during 23 years of follow-up[172]. A combination of diet and exercise appears more beneficial than either alone in obese older adults[173]. Lifestyle modification may improve cardiovascular outcomes even after the onset of T2DM[117].

Nonetheless, it is difficult to continue lifestyle modification in most patients. Patients' motivation is one of the most important factors in successful patient-centered management of T2DM[118]. Therefore, it is important to motivate and encourage them to improve their adherence to daily lifestyle modification. In this context,

understanding the natural history of the development of T2DM and the importance of reducing β-cell workload to prevent or manage the disease may help to motivate or encourage patients to adhere to daily lifestyle changes.

Furthermore, as a whole society, not only patients with IGT or T2DM, but the healthy, general population should also be educated to motivate or encourage them to pursue a healthy lifestyle to prevent diseases associated with obesity and physical inactivity, resulting in improvement of quality of life (QOL). Changing our understanding of T2DM and a "modern" lifestyle may be needed to overcome this pandemic burden of T2DM all over the world.

CONCLUSION

This review summarizes the current knowledge of β-cell function and β-cell mass in T2DM. Recent evidence has emerged that a deficit of β-cell function along with β-cell mass is a hallmark of T2DM. Therefore, it is now acknowledged that a deficit of β-cell functional mass is a common characteristic of both type 1 and type 2 diabetes, indicating a core pathogenesis of diabetes. Genome-wide association studies have currently detected over 60 genetic loci associated with T2DM, most of which are assumed to relate to the β-cell, also indicating the imporance of β-cells in the pathogenesis of T2DM[174-178]. It is important to stress that diabetes never develops unless β-cells fail to compensate insulin resistance. In addition, β-cell function is related to treatment failure and glycemic control, suggesting its critical role in the management of T2DM. These findings suggest that recovery of β-cell functional mass is an important therapeutic strategy to manage or even cure T2DM. Although, unfortunately, currently no treatment strategy or medication to recover β-cell functional mass has been established, current evidence suggests that reducing β-cell workload is most effective to preserve β-cell functional mass. Thus, therapy or prevention of T2DM should focus on this point, and, therefore, lifestyle modification and weight loss remain the most important therapeutic strategy. Use of medication without lifestyle modification may even result in adverse outcomes. From the point of view of prevention, we need to tackle this pandemic burden of T2DM as a whole society, and correct understanding of the pathogenesis of T2DM may help motivate people to maintain a healthy lifestyle.

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I apologize to the many authors of original research whose publications I could not cite owing to space restrictions.

REFERENCES

1 **Aguiree F,** Brown A, Cho NH, Dahlquist G, Dodd S, Dunning T, Hirst M, Hwang C, Magliano D, Patterson C, Scott C, Shaw J, Soltesz G, Usher-Smith J, Whiting D. IDF Diabetes Atlas. IDF diabetes Atlas. 6th ed. Brussels, Belgium: International Diabetes Federation, 2013

2 **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]

3 **Seshasai SR**, Kaptoge S, Thompson A, Di Angelantonio E, Gao P, Sarwar N, Whincup PH, Mukamal KJ, Gillum RF, Holme I, Njølstad I, Fletcher A, Nilsson P, Lewington S, Collins R, Gudnason V, Thompson SG, Sattar N, Selvin E, Hu FB, Danesh J. Diabetes mellitus, fasting glucose, and risk of cause-specific death. *N Engl J Med* 2011; **364**: 829-841 [PMID: 21366474 DOI: 10.1056/NEJMoa1008862]

4 **Defronzo RA**. Banting Lecture. From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus. *Diabetes* 2009; **58**: 773-795 [PMID: 19336687]

5 **Kahn SE**, Cooper ME, Del Prato S. Pathophysiology and treatment of type 2 diabetes: perspectives on the past, present, and future. *Lancet* 2014; **383**: 1068-1083 [PMID: 24315620 DOI: 10.1016/S0140-6736(13)62154-6]

6 **Bergman RN**, Phillips LS, Cobelli C. Physiologic evaluation of factors controlling glucose tolerance in man: measurement of insulin sensitivity and beta-cell glucose sensitivity from the response to intravenous glucose. *J Clin Invest* 1981; **68**: 1456-1467 [PMID: 7033284 DOI: 10.1172/JCI110398]

7 **Bergman RN**, Ader M, Huecking K, Van Citters G. Accurate assessment of beta-cell function: the hyperbolic correction. *Diabetes* 2002; **51** Suppl 1: S212-S220 [PMID: 11815482 DOI: 10.2337/diabetes.51.2007.S212]

8 **Jensen CC**, Cnop M, Hull RL, Fujimoto WY, Kahn SE. Beta-cell function is a major contributor to oral glucose tolerance in high-risk relatives of four ethnic groups in the U.S. *Diabetes* 2002; **51**: 2170-2178 [PMID: 12086947]

9 **DeFronzo RA**, Eldor R, Abdul-Ghani M. Pathophysiologic approach to therapy in patients with newly diagnosed type 2 diabetes. *Diabetes Care* 2013; **36** Suppl 2: S127-S138 [PMID: 23882037 DOI: 10.2337/dcS13-2011]

10 **Ichise M**, Harris PE. Imaging of beta-cell mass and function. *J Nucl Med* 2010; **51**: 1001-1004 [PMID: 20554742 DOI: 10.2967/jnumed.109.068999]

11 **Tiedge M**. Inside the pancreas: progress and challenges of human beta cell mass quantification. *Diabetologia* 2014; **57**: 856-859 [PMID: 24599112 DOI: 10.1007/s00125-014-3206-z]

12 **Butler AE**, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC. Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. *Diabetes* 2003; **52**: 102-110 [PMID: 12502499 DOI: 10.2337/diabetes.52.1.102]

13 **Rahier J**, Guiot Y, Goebbels RM, Sempoux C, Henquin JC. Pancreatic beta-cell mass in European subjects with type 2 diabetes. *Diabetes Obes Metab* 2008; **10** Suppl 4: 32-42 [PMID: 18834431 DOI: 10.1111/j.1463-1326.2008.00969.x]

14 **Sakuraba H**, Mizukami H, Yagihashi N, Wada R, Hanyu C, Yagihashi S. Reduced beta-cell mass and expression of oxidative stress-related DNA damage in the islet of Japanese Type II diabetic patients. *Diabetologia* 2002; **45**: 85-96 [PMID: 11845227 DOI: 10.1007/s001250200009]

15 **Yoon KH**, Ko SH, Cho JH, Lee JM, Ahn YB, Song KH, Yoo SJ, Kang MI, Cha BY, Lee KW, Son HY, Kang SK, Kim HS, Lee IK, Bonner-Weir S. Selective beta-cell loss and alpha-cell expansion in patients with type 2 diabetes mellitus in Korea. *J Clin Endocrinol Metab* 2003; **88**: 2300-2308 [PMID: 12727989 DOI: 10.1210/jc.2002-020735]

16 **Butler PC**, Meier JJ, Butler AE, Bhushan A. The replication of beta cells in normal physiology, in disease and for therapy. *Nat Clin Pract Endocrinol Metab* 2007; **3**: 758-768 [PMID: 17955017 DOI: 10.1038/ncpendmet0647]

17 **Manesso E**, Toffolo GM, Saisho Y, Butler AE, Matveyenko AV, Cobelli C, Butler PC. Dynamics of beta-cell turnover: evidence for beta-cell turnover and regeneration from sources of beta-cells other than beta-cell replication in the HIP rat.

Am J Physiol Endocrinol Metab 2009; **297**: E323-E330 [PMID: 19470833]
18 **Weir GC**, Bonner-Weir S. Islet β cell mass in diabetes and how it relates to function, birth, and death. *Ann N Y Acad Sci* 2013; **1281**: 92-105 [PMID: 23363033 DOI: 10.1111/nyas.12031]
19 **Poitout V**, Robertson RP. Glucolipotoxicity: fuel excess and beta-cell dysfunction. *Endocr Rev* 2008; **29**: 351-366 [PMID: 18048763 DOI: 10.1210/er.2007-0023]
20 **Kusminski CM**, Shetty S, Orci L, Unger RH, Scherer PE. Diabetes and apoptosis: lipotoxicity. *Apoptosis* 2009; **14**: 1484-1495 [PMID: 19421860 DOI: 10.1007/s10495-009-0352-8]
21 **Haataja L**, Gurlo T, Huang CJ, Butler PC. Islet amyloid in type 2 diabetes, and the toxic oligomer hypothesis. *Endocr Rev* 2008; **29**: 303-316 [PMID: 18314421 DOI: 10.1210/er.2007-0037]
22 **Costes S,** Langen R, Gurlo T, Matveyenko AV, Butler PC. β-Cell failure in type 2 diabetes: a case of asking too much of too few? *Diabetes* 2013; **62**: 327-335 [PMID: 23349537 DOI: 10.2337/db12-1326]
23 **Hull RL**, Westermark GT, Westermark P, Kahn SE. Islet amyloid: a critical entity in the pathogenesis of type 2 diabetes. *J Clin Endocrinol Metab* 2004; **89**: 3629-3643 [PMID: 15292279 DOI: 10.1210/jc.2004-0405]
24 **Jurgens CA**, Toukatly MN, Fligner CL, Udayasankar J, Subramanian SL, Zraika S, Aston-Mourney K, Carr DB, Westermark P, Westermark GT, Kahn SE, Hull RL. β-cell loss and β-cell apoptosis in human type 2 diabetes are related to islet amyloid deposition. *Am J Pathol* 2011; **178**: 2632-2640 [PMID: 21641386 DOI: 10.1016/j.ajpath.2011.02.036]
25 **Robertson RP**. Antioxidant drugs for treating beta-cell oxidative stress in type 2 diabetes: glucose-centric versus insulin-centric therapy. *Discov Med* 2010; **9**: 132-137 [PMID: 20193639]
26 **Dinarello CA**, Donath MY, Mandrup-Poulsen T. Role of IL-1beta in type 2 diabetes. *Curr Opin Endocrinol Diabetes Obes* 2010; **17**: 314-321 [PMID: 20588114 DOI: 10.1097/MED.0b013e32833bf6dc]
27 **Supale S**, Li N, Brun T, Maechler P. Mitochondrial dysfunction in pancreatic β cells. *Trends Endocrinol Metab* 2012; **23**: 477-487 [PMID: 22766318 DOI: 10.1016/j.tem.2012.06.002]
28 **Scheuner D**, Kaufman RJ. The unfolded protein response: a pathway that links insulin demand with beta-cell failure and diabetes. *Endocr Rev* 2008; **29**: 317-333 [PMID: 18436705 DOI: 10.1210/er.2007-0039]
29 **Eizirik DL**, Cardozo AK, Cnop M. The role for endoplasmic reticulum stress in diabetes mellitus. *Endocr Rev* 2008; **29**: 42-61 [PMID: 18048764 DOI: 10.1210/er.2007-0015]
30 **Masini M**, Bugliani M, Lupi R, del Guerra S, Boggi U, Filipponi F, Marselli L, Masiello P, Marchetti P. Autophagy in human type 2 diabetes pancreatic beta cells. *Diabetologia* 2009; **52**: 1083-1086 [PMID: 19367387 DOI: 10.1007/s00125-009-1347-2]
31 **Mizukami H**, Takahashi K, Inaba W, Tsuboi K, Osonoi S, Yoshida T, Yagihashi S. Involvement of oxidative stress-induced DNA damage, endoplasmic reticulum stress, and autophagy deficits in the decline of β-cell mass in Japanese type 2 diabetic patients. *Diabetes Care* 2014; **37**: 1966-1974 [PMID: 24705612 DOI: 10.2337/dc13-2018]
32 **Talchai C**, Xuan S, Lin HV, Sussel L, Accili D. Pancreatic β cell dedifferentiation as a mechanism of diabetic β cell failure. *Cell* 2012; **150**: 1223-1234 [PMID: 22980982 DOI: 10.1016/j.cell.2012.07.029]
33 **Henquin JC**, Rahier J. Pancreatic alpha cell mass in European subjects with type 2 diabetes. *Diabetologia* 2011; **54**: 1720-1725 [PMID: 21465328 DOI: 10.1007/s00125-011-2118-4]
34 **Weir GC**, Aguayo-Mazzucato C, Bonner-Weir S. β-cell dedifferentiation in diabetes is important, but what is it? *Islets* 2013; **5**: 233-237 [PMID: 24356710 DOI: 10.4161/isl.27494]
35 **Ritzel RA**, Butler AE, Rizza RA, Veldhuis JD, Butler PC. Relationship between beta-cell mass and fasting blood glucose concentration in humans. *Diabetes Care* 2006; **29**: 717-718 [PMID: 16505537 DOI: 10.2337/diacare.29.03.06.dc05-1538]
36 **Matveyenko AV**, Butler PC. Beta-cell deficit due to increased apoptosis in the human islet amyloid polypeptide transgenic (HIP) rat recapitulates the metabolic defects present in type 2 diabetes. *Diabetes* 2006; **55**: 2106-2114 [PMID: 16804082 DOI: 10.2337/db05-1672]
37 **Kjems LL**, Kirby BM, Welsh EM, Veldhuis JD, Straume M, McIntyre SS, Yang D, Lefèbvre P, Butler PC. Decrease in beta-cell mass leads to impaired pulsatile insulin secretion, reduced postprandial hepatic insulin clearance, and relative hyperglucagonemia in the minipig. *Diabetes* 2001; **50**: 2001-2012 [PMID: 11522665 DOI: 10.2337/diabetes.50.9.2001]
38 **Saisho Y**, Butler AE, Manesso E, Galasso R, Zhang L, Gurlo T, Toffolo GM, Cobelli C, Kavanagh K, Wagner JD, Butler PC. Relationship between fractional pancreatic beta cell area and fasting plasma glucose concentration in monkeys. *Diabetologia* 2010; **53**: 111-114 [PMID: 19847395 DOI: 10.1007/s00125-009-1552-z]
39 **Matveyenko AV**, Veldhuis JD, Butler PC. Mechanisms of impaired fasting glucose and glucose intolerance induced by an approximate 50% pancreatectomy. *Diabetes* 2006; **55**: 2347-2356 [PMID: 16873700 DOI: 10.2337/db06-0345]
40 **Kendall DM**, Sutherland DE, Najarian JS, Goetz FC, Robertson RP. Effects of hemipancreatectomy on insulin secretion and glucose tolerance in healthy humans. *N Engl J Med* 1990; **322**: 898-903 [PMID: 2179721 DOI: 10.1056/NEJM199003293221305]
41 **Robertson RP**, Lanz KJ, Sutherland DE, Seaquist ER. Relationship between diabetes and obesity 9 to 18 years after hemipancreatectomy and transplantation in donors and recipients. *Transplantation* 2002; **73**: 736-741 [PMID: 11907419 DOI: 10.1097/00007890-200203150-00013]
42 **Kumar AF**, Gruessner RW, Seaquist ER. Risk of glucose intolerance and diabetes in hemipancreatectomized donors selected for normal preoperative glucose metabolism. *Diabetes Care* 2008; **31**: 1639-1643 [PMID: 18469205]
43 **Jin SM**, Oh SH, Kim SK, Jung HS, Choi SH, Jang KT, Lee KT, Kim JH, Lee MS, Lee MK, Kim KW. Diabetes-free survival in patients who underwent islet autotransplantation after 50% to 60% distal partial pancreatectomy for benign pancreatic tumors. *Transplantation* 2013; **95**: 1396-1403 [PMID: 23558506 DOI: 10.1097/TP.0b013e31828c0c29]
44 **Meier JJ**, Breuer TG, Bonadonna RC, Tannapfel A, Uhl W, Schmidt WE, Schrader H, Menge BA. Pancreatic diabetes manifests when beta cell area declines by approximately 65% in humans. *Diabetologia* 2012; **55**: 1346-1354 [PMID: 22286529 DOI: 10.1007/s00125-012-2466-8]
45 **Kou K**, Saisho Y, Sato S, Yamada T, Itoh H. Islet number rather than islet size is a major determinant of β- and α-cell mass in humans. *J Clin Endocrinol Metab* 2014; **99**: 1733-1740 [PMID: 24517149 DOI: 10.1210/jc.2013-3731]
46 **Meier JJ**, Bonadonna RC. Role of reduced β-cell mass versus impaired β-cell function in the pathogenesis of type 2 diabetes. *Diabetes Care* 2013; **36** Suppl 2: S113-S119 [PMID: 23882035 DOI: 10.2337/dcS13-2008]
47 **Robertson RP**. Estimation of beta-cell mass by metabolic tests: necessary, but how sufficient? *Diabetes* 2007; **56**: 2420-2424 [PMID: 17606873]
48 **Laedtke T**, Kjems L, Pørksen N, Schmitz O, Veldhuis J, Kao PC, Butler PC. Overnight inhibition of insulin secretion restores pulsatility and proinsulin/insulin ratio in type 2 diabetes. *Am J Physiol Endocrinol Metab* 2000; **279**: E520-E528 [PMID: 10950818]
49 **Meier JJ**, Menge BA, Breuer TG, Müller CA, Tannapfel A, Uhl W, Schmidt WE, Schrader H. Functional assessment of pancreatic beta-cell area in humans. *Diabetes* 2009; **58**: 1595-1603 [PMID: 19509022 DOI: 10.2337/db08-1611]
50 U.K. prospective diabetes study 16. Overview of 6 years' therapy of type II diabetes: a progressive disease. U.K. Prospective Diabetes Study Group. *Diabetes* 1995; **44**:

1249-1258 [PMID: 7589820]
51 **Saisho Y**, Tanaka K, Abe T, Shimada A, Kawai T, Itoh H. Effect of obesity on declining beta cell function after diagnosis of type 2 diabetes: a possible link suggested by cross-sectional analysis. *Endocr J* 2012; **59**: 187-195 [PMID: 22156325]
52 **Funakoshi S**, Fujimoto S, Hamasaki A, Fujiwara H, Fujita Y, Ikeda K, Hamamoto Y, Hosokawa M, Seino Y, Inagaki N. Analysis of factors influencing pancreatic beta-cell function in Japanese patients with type 2 diabetes: association with body mass index and duration of diabetic exposure. *Diabetes Res Clin Pract* 2008; **82**: 353-358 [PMID: 18950889 DOI: 10.1016/j.diabres.2008.09.010]
53 **Brüning JC**, Winnay J, Bonner-Weir S, Taylor SI, Accili D, Kahn CR. Development of a novel polygenic model of NIDDM in mice heterozygous for IR and IRS-1 null alleles. *Cell* 1997; **88**: 561-572 [PMID: 9038347]
54 **Tschen SI**, Dhawan S, Gurlo T, Bhushan A. Age-dependent decline in beta-cell proliferation restricts the capacity of beta-cell regeneration in mice. *Diabetes* 2009; **58**: 1312-1320 [PMID: 19228811 DOI: 10.2337/db08-1651]
55 **Parsons JA**, Brelje TC, Sorenson RL. Adaptation of islets of Langerhans to pregnancy: increased islet cell proliferation and insulin secretion correlates with the onset of placental lactogen secretion. *Endocrinology* 1992; **130**: 1459-1466 [PMID: 1537300 DOI: 10.1210/endo.130.3.1537300]
56 **Scaglia L**, Smith FE, Bonner-Weir S. Apoptosis contributes to the involution of beta cell mass in the post partum rat pancreas. *Endocrinology* 1995; **136**: 5461-5468 [PMID: 7588296 DOI: 10.1210/endo.136.12.7588296]
57 **Karnik SK**, Chen H, McLean GW, Heit JJ, Gu X, Zhang AY, Fontaine M, Yen MH, Kim SK. Menin controls growth of pancreatic beta-cells in pregnant mice and promotes gestational diabetes mellitus. *Science* 2007; **318**: 806-809 [PMID: 17975067 DOI: 10.1126/science.1146812]
58 **Kim H**, Toyofuku Y, Lynn FC, Chak E, Uchida T, Mizukami H, Fujitani Y, Kawamori R, Miyatsuka T, Kosaka Y, Yang K, Honig G, van der Hart M, Kishimoto N, Wang J, Yagihashi S, Tecott LH, Watada H, German MS. Serotonin regulates pancreatic beta cell mass during pregnancy. *Nat Med* 2010; **16**: 804-808 [PMID: 20581837 DOI: 10.1038/nm.2173]
59 **Saisho Y**, Butler AE, Manesso E, Elashoff D, Rizza RA, Butler PC. β-cell mass and turnover in humans: effects of obesity and aging. *Diabetes Care* 2013; **36**: 111-117 [PMID: 22875233 DOI: 10.2337/dc12-0421]
60 **Jiao Y**, Le Lay J, Yu M, Naji A, Kaestner KH. Elevated mouse hepatic betatrophin expression does not increase human β-cell replication in the transplant setting. *Diabetes* 2014; **63**: 1283-1288 [PMID: 24353178 DOI: 10.2337/db13-1435]
61 **Stewart AF**. Betatrophin versus bitter-trophin and the elephant in the room: time for a new normal in β-cell regeneration research. *Diabetes* 2014; **63**: 1198-1199 [PMID: 24651805 DOI: 10.2337/db14-0009]
62 **Meier JJ**, Butler AE, Saisho Y, Monchamp T, Galasso R, Bhushan A, Rizza RA, Butler PC. Beta-cell replication is the primary mechanism subserving the postnatal expansion of beta-cell mass in humans. *Diabetes* 2008; **57**: 1584-1594 [PMID: 18334605]
63 **Gregg BE**, Moore PC, Demozay D, Hall BA, Li M, Husain A, Wright AJ, Atkinson MA, Rhodes CJ. Formation of a human β-cell population within pancreatic islets is set early in life. *J Clin Endocrinol Metab* 2012; **97**: 3197-3206 [PMID: 22745242 DOI: 10.1210/jc.2012-1206]
64 **Menge BA**, Tannapfel A, Belyaev O, Drescher R, Müller C, Uhl W, Schmidt WE, Meier JJ. Partial pancreatectomy in adult humans does not provoke beta-cell regeneration. *Diabetes* 2008; **57**: 142-149 [PMID: 17959931]
65 **Perl S**, Kushner JA, Buchholz BA, Meeker AK, Stein GM, Hsieh M, Kirby M, Pechhold S, Liu EH, Harlan DM, Tisdale JF. Significant human beta-cell turnover is limited to the first three decades of life as determined by in vivo thymidine analog incorporation and radiocarbon dating. *J Clin Endocrinol Metab* 2010; **95**: E234-E239 [PMID: 20660050 DOI: 10.1210/jc.2010-0932]
66 **Cnop M**, Hughes SJ, Igoillo-Esteve M, Hoppa MB, Sayyed F, van de Laar L, Gunter JH, de Koning EJ, Walls GV, Gray DW, Johnson PR, Hansen BC, Morris JF, Pipeleers-Marichal M, Cnop I, Clark A. The long lifespan and low turnover of human islet beta cells estimated by mathematical modelling of lipofuscin accumulation. *Diabetologia* 2010; **53**: 321-330 [PMID: 19855953 DOI: 10.1007/s00125-009-1562-x]
67 **Mezza T**, Muscogiuri G, Sorice GP, Clemente G, Hu J, Pontecorvi A, Holst JJ, Giaccari A, Kulkarni RN. Insulin resistance alters islet morphology in nondiabetic humans. *Diabetes* 2014; **63**: 994-1007 [PMID: 24215793 DOI: 10.2337/db13-1013]
68 **Butler AE**, Cao-Minh L, Galasso R, Rizza RA, Corradin A, Cobelli C, Butler PC. Adaptive changes in pancreatic beta cell fractional area and beta cell turnover in human pregnancy. *Diabetologia* 2010; **53**: 2167-2176 [PMID: 20523966 DOI: 10.1007/s00125-010-1809-6]
69 **Yoneda S**, Uno S, Iwahashi H, Fujita Y, Yoshikawa A, Kozawa J, Okita K, Takiuchi D, Eguchi H, Nagano H, Imagawa A, Shimomura I. Predominance of β-cell neogenesis rather than replication in humans with an impaired glucose tolerance and newly diagnosed diabetes. *J Clin Endocrinol Metab* 2013; **98**: 2053-2061 [PMID: 23539729 DOI: 10.1210/jc.2012-3832]
70 **Gargani S**, Thévenet J, Yuan JE, Lefebvre B, Delalleau N, Gmyr V, Hubert T, Duhamel A, Pattou F, Kerr-Conte J. Adaptive changes of human islets to an obesogenic environment in the mouse. *Diabetologia* 2013; **56**: 350-358 [PMID: 23192693 DOI: 10.1007/s00125-012-2775-y]
71 **Saisho Y**, Manesso E, Butler AE, Galasso R, Kavanagh K, Flynn M, Zhang L, Clark P, Gurlo T, Toffolo GM, Cobelli C, Wagner JD, Butler PC. Ongoing beta-cell turnover in adult nonhuman primates is not adaptively increased in streptozotocin-induced diabetes. *Diabetes* 2011; **60**: 848-856 [PMID: 21270238 DOI: 10.2337/db09-1368]
72 **Guardado-Mendoza R**, Jimenez-Ceja L, Majluf-Cruz A, Kamath S, Fiorentino TV, Casiraghi F, Velazquez AO, DeFronzo RA, Dick E, Davalli A, Folli F. Impact of obesity severity and duration on pancreatic β- and α-cell dynamics in normoglycemic non-human primates. *Int J Obes* (Lond) 2013; **37**: 1071-1078 [PMID: 23229736 DOI: 10.1038/ijo.2012.205]
73 **Teta M**, Long SY, Wartschow LM, Rankin MM, Kushner JA. Very slow turnover of beta-cells in aged adult mice. *Diabetes* 2005; **54**: 2557-2567 [PMID: 16123343]
74 **Rankin MM**, Kushner JA. Adaptive beta-cell proliferation is severely restricted with advanced age. *Diabetes* 2009; **58**: 1365-1372 [PMID: 19265026 DOI: 10.2337/db08-1198]
75 **Matsuba I**, Saito K, Takai M, Hirao K, Sone H. Fasting insulin levels and metabolic risk factors in type 2 diabetic patients at the first visit in Japan: a 10-year, nationwide, observational study (JDDM 28). *Diabetes Care* 2012; **35**: 1853-1857 [PMID: 22665215 DOI: 10.2337/dc12-0156]
76 **Bell JA**, Kivimaki M, Hamer M. Metabolically healthy obesity and risk of incident type 2 diabetes: a meta-analysis of prospective cohort studies. *Obes Rev* 2014; **15**: 504-515 [PMID: 24661566 DOI: 10.1111/obr.12157]
77 **Heianza Y**, Arase Y, Tsuji H, Fujihara K, Saito K, Hsieh SD, Tanaka S, Kodama S, Hara S, Sone H. Metabolically healthy obesity, presence or absence of fatty liver, and risk of type 2 diabetes in Japanese individuals: Toranomon Hospital Health Management Center Study 20 (TOPICS 20). *J Clin Endocrinol Metab* 2014; **99**: 2952-2960 [PMID: 24823457 DOI: 10.1210/jc.2013-4427]
78 **Kramer CK**, Zinman B, Retnakaran R. Are metabolically healthy overweight and obesity benign conditions?: A systematic review and meta-analysis. *Ann Intern Med* 2013; **159**: 758-769 [PMID: 24297192 DOI: 10.7326/0003-4819-159-11-201312030-00008]

79 **Sone H**, Ito H, Ohashi Y, Akanuma Y, Yamada N. Obesity and type 2 diabetes in Japanese patients. *Lancet* 2003; **361**: 85 [PMID: 12517507 DOI: 10.1016/S0140-6736(03)12151-4]

80 **Hsu WC**, Boyko EJ, Fujimoto WY, Kanaya A, Karmally W, Karter A, King GL, Look M, Maskarinec G, Misra R, Tavake-Pasi F, Arakaki R. Pathophysiologic differences among Asians, native Hawaiians, and other Pacific Islanders and treatment implications. *Diabetes Care* 2012; **35**: 1189-1198 [PMID: 22517940 DOI: 10.2337/dc12-0212]

81 **Gill TP**. Cardiovascular risk in the Asia-Pacific region from a nutrition and metabolic point of view: abdominal obesity. *Asia Pac J Clin Nutr* 2001; **10**: 85-89 [PMID: 11710363]

82 **Kadowaki T**, Sekikawa A, Murata K, Maegawa H, Takamiya T, Okamura T, El-Saed A, Miyamatsu N, Edmundowicz D, Kita Y, Sutton-Tyrrell K, Kuller LH, Ueshima H. Japanese men have larger areas of visceral adipose tissue than Caucasian men in the same levels of waist circumference in a population-based study. *Int J Obes* (Lond) 2006; **30**: 1163-1165 [PMID: 16446744 DOI: 10.1038/sj.ijo.0803248]

83 **Kodama K**, Tojjar D, Yamada S, Toda K, Patel CJ, Butte AJ. Ethnic differences in the relationship between insulin sensitivity and insulin response: a systematic review and meta-analysis. *Diabetes Care* 2013; **36**: 1789-1796 [PMID: 23704681 DOI: 10.2337/dc12-1235]

84 **Møller JB**, Pedersen M, Tanaka H, Ohsugi M, Overgaard RV, Lynge J, Almind K, Vasconcelos NM, Poulsen P, Keller C, Ueki K, Ingwersen SH, Pedersen BK, Kadowaki T. Body composition is the main determinant for the difference in type 2 diabetes pathophysiology between Japanese and Caucasians. *Diabetes Care* 2014; **37**: 796-804 [PMID: 24130359 DOI: 10.2337/dc13-0598]

85 **Kou K**, Saisho Y, Satoh S, Yamada T, Itoh H. Change in β-cell mass in Japanese nondiabetic obese individuals. *J Clin Endocrinol Metab* 2013; **98**: 3724-3730 [PMID: 23766518 DOI: 10.1210/jc.2013-1373]

86 **Mizukami H,** Takahashi K, Inaba W, Osonoi S, Kamata K, Tsuboi K, Yagihashi S. Age-associated changes of islet endocrine cells and the effects of body mass index in Japanese. *J Diabetes Investig* 2014; **5**: 38-47 [PMID: 24843735 DOI: 10.1111/jdi.12118]

87 **Kahn SE**, Haffner SM, Heise MA, Herman WH, Holman RR, Jones NP, Kravitz BG, Lachin JM, O'Neill MC, Zinman B, Viberti G. Glycemic durability of rosiglitazone, metformin, or glyburide monotherapy. *N Engl J Med* 2006; **355**: 2427-2443 [PMID: 17145742 DOI: 10.1056/NEJMoa066224]

88 **Kahn SE**, Lachin JM, Zinman B, Haffner SM, Aftring RP, Paul G, Kravitz BG, Herman WH, Viberti G, Holman RR. Effects of rosiglitazone, glyburide, and metformin on β-cell function and insulin sensitivity in ADOPT. *Diabetes* 2011; **60**: 1552-1560 [PMID: 21415383 DOI: 10.2337/db10-1392]

89 **TODAY Study Group.** Effects of metformin, metformin plus rosiglitazone, and metformin plus lifestyle on insulin sensitivity and β-cell function in TODAY. *Diabetes Care* 2013; **36**: 1749-1757 [PMID: 23704674 DOI: 10.2337/dc12-2393]

90 **Saisho Y**, Kou K, Tanaka K, Abe T, Kurosawa H, Shimada A, Meguro S, Kawai T, Itoh H. Postprandial serum C-peptide to plasma glucose ratio as a predictor of subsequent insulin treatment in patients with type 2 diabetes. *Endocr J* 2011; **58**: 315-322 [PMID: 21415555]

91 **Saisho Y**, Kou K, Tanaka K, Abe T, Shimada A, Kawai T, Itoh H. Postprandial serum C-peptide to plasma glucose ratio predicts future insulin therapy in Japanese patients with type 2 diabetes. *Acta Diabetol* 2013; **50**: 987-988 [PMID: 23212668 DOI: 10.1007/s00592-012-0441-y]

92 **Saisho Y**, Kou K, Tanaka K, Abe T, Shimada A, Kawai T, Itoh H. Association between beta cell function and future glycemic control in patients with type 2 diabetes. *Endocr J* 2013; **60**: 517-523 [PMID: 23268927]

93 **Saisho Y**, Tanaka K, Abe T, Kawai T, Itoh H. Lower beta cell function relates to sustained higher glycated albumin to glycated hemoglobin ratio in Japanese patients with type 2 diabetes. *Endocr J* 2014; **61**: 149-157 [PMID: 24212881]

94 **Fukuda M**, Tanaka A, Tahara Y, Ikegami H, Yamamoto Y, Kumahara Y, Shima K. Correlation between minimal secretory capacity of pancreatic beta-cells and stability of diabetic control. *Diabetes* 1988; **37**: 81-88 [PMID: 3275557]

95 **Nakanishi K**, Kobayashi T, Inoko H, Tsuji K, Murase T, Kosaka K. Residual beta-cell function and HLA-A24 in IDDM. Markers of glycemic control and subsequent development of diabetic retinopathy. *Diabetes* 1995; **44**: 1334-1339 [PMID: 7589833]

96 **Sassa M**, Yamada Y, Hosokawa M, Fukuda K, Fujimoto S, Toyoda K, Tsukiyama K, Seino Y, Inagaki N. Glycemic instability in type 1 diabetic patients: Possible role of ketosis or ketoacidosis at onset of diabetes. *Diabetes Res Clin Pract* 2008; **81**: 190-195 [PMID: 18514964 DOI: 10.1016/j.diabres.2008.04.009]

97 **Saisho Y,** Tanaka K, Abe T, Shimada A, Kawai T, Itoh H. Glycated albumin to glycated hemoglobin ratio reflects postprandial glucose excursion and relates to β-cell function in both type 1 and type 2 diabetes. *Diabetol Int* 2011; **2**: 146-153

98 **Tanaka C,** Saisho Y, Tanaka K, Kou K, Tanaka M, Meguro S, Irie J, Jo R, Kawai T, Itoh H. Factors associated with glycemic variability in Japanese patients with diabetes. *Diabetol Int* 2014; **5**: 36-42 [DOI: 10.1007/s13340-013-0129-8]

99 **Koga M**, Murai J, Saito H, Kasayama S. Glycated albumin and glycated hemoglobin are influenced differently by endogenous insulin secretion in patients with type 2 diabetes. *Diabetes Care* 2010; **33**: 270-272 [PMID: 19846794 DOI: 10.2337/dc09-1002]

100 **Day JF**, Ingebretsen CG, Ingebretsen WR, Baynes JW, Thorpe SR. Nonenzymatic glucosylation of serum proteins and hemoglobin: response to changes in blood glucose levels in diabetic rats. *Diabetes* 1980; **29**: 524-527 [PMID: 6991338]

101 **Tahara Y**, Shima K. Kinetics of HbA1c, glycated albumin, and fructosamine and analysis of their weight functions against preceding plasma glucose level. *Diabetes Care* 1995; **18**: 440-447 [PMID: 7497851]

102 **Yoshiuchi K**, Matsuhisa M, Katakami N, Nakatani Y, Sakamoto K, Matsuoka T, Umayahara Y, Kosugi K, Kaneto H, Yamasaki Y, Hori M. Glycated albumin is a better indicator for glucose excursion than glycated hemoglobin in type 1 and type 2 diabetes. *Endocr J* 2008; **55**: 503-507 [PMID: 18445997]

103 **Imai T**, Oikawa Y, Shimada A. Improved Monitoring of the Hyperglycemic State in Type 1 Diabetes Patients by Use of the Glycoalbumin/HbA1c Ratio. *Rev Diabet Stud* 2007; **4**: 44-48 [PMID: 17565415 DOI: 10.1900/RDS.2007.4.44]

104 **Takao T**, Ide T, Yanagisawa H, Kikuchi M, Kawazu S, Matsuyama Y. The effect of fasting plasma glucose variability on the risk of retinopathy in type 2 diabetic patients: retrospective long-term follow-up. *Diabetes Res Clin Pract* 2010; **89**: 296-302 [PMID: 20416966 DOI: 10.1016/j.diabres.2010.03.027]

105 **Hsu CC**, Chang HY, Huang MC, Hwang SJ, Yang YC, Lee YS, Shin SJ, Tai TY. HbA1c variability is associated with microalbuminuria development in type 2 diabetes: a 7-year prospective cohort study. *Diabetologia* 2012; **55**: 3163-3172 [PMID: 22923064 DOI: 10.1007/s00125-012-2700-4]

106 **Sugawara A**, Kawai K, Motohashi S, Saito K, Kodama S, Yachi Y, Hirasawa R, Shimano H, Yamazaki K, Sone H. HbA(1c) variability and the development of microalbuminuria in type 2 diabetes: Tsukuba Kawai Diabetes Registry 2. *Diabetologia* 2012; **55**: 2128-2131 [PMID: 22580991 DOI: 10.1007/s00125-012-2572-7]

107 **Hirakawa Y**, Arima H, Zoungas S, Ninomiya T, Cooper M, Hamet P, Mancia G, Poulter N, Harrap S, Woodward M, Chalmers J. Impact of visit-to-visit glycemic variability on the risks of macrovascular and microvascular events and all-cause mortality in type 2 diabetes: the ADVANCE trial. *Diabetes Care* 2014; **37**: 2359-2365 [PMID: 24812434 DOI:

10.2337/dc14-0199]
108 **Saisho Y.** Importance of B-cell Function for the Treatment of Type 2 Diabetes. *J Clin Med* 2014; **3**: 923-943 [DOI: 10.3390/jcm3030923]
109 **Saisho Y.** Obesity, type 2 diabetes and β-cell failure: An Asian perspective. *J Mol Genet Med* 2014; **S1**: 8 [DOI: 10.4172/1747-0862.S1-008]
110 **Wing RR**, Bolin P, Brancati FL, Bray GA, Clark JM, Coday M, Crow RS, Curtis JM, Egan CM, Espeland MA, Evans M, Foreyt JP, Ghazarian S, Gregg EW, Harrison B, Hazuda HP, Hill JO, Horton ES, Hubbard VS, Jakicic JM, Jeffery RW, Johnson KC, Kahn SE, Kitabchi AE, Knowler WC, Lewis CE, Maschak-Carey BJ, Montez MG, Murillo A, Nathan DM, Patricio J, Peters A, Pi-Sunyer X, Pownall H, Reboussin D, Regensteiner JG, Rickman AD, Ryan DH, Safford M, Wadden TA, Wagenknecht LE, West DS, Williamson DF, Yanovski SZ. Cardiovascular effects of intensive lifestyle intervention in type 2 diabetes. *N Engl J Med* 2013; **369**: 145-154 [PMID: 23796131 DOI: 10.1056/NEJMoa1212914]
111 **Williamson DA**, Rejeski J, Lang W, Van Dorsten B, Fabricatore AN, Toledo K. Impact of a weight management program on health-related quality of life in overweight adults with type 2 diabetes. *Arch Intern Med* 2009; **169**: 163-171 [PMID: 19171813 DOI: 10.1001/archinternmed.2008.544]
112 **Wing RR**, Lang W, Wadden TA, Safford M, Knowler WC, Bertoni AG, Hill JO, Brancati FL, Peters A, Wagenknecht L. Benefits of modest weight loss in improving cardiovascular risk factors in overweight and obese individuals with type 2 diabetes. *Diabetes Care* 2011; **34**: 1481-1486 [PMID: 21593294 DOI: 10.2337/dc10-2415]
113 **Gregg EW**, Chen H, Wagenknecht LE, Clark JM, Delahanty LM, Bantle J, Pownall HJ, Johnson KC, Safford MM, Kitabchi AE, Pi-Sunyer FX, Wing RR, Bertoni AG. Association of an intensive lifestyle intervention with remission of type 2 diabetes. *JAMA* 2012; **308**: 2489-2496 [PMID: 23288372 DOI: 10.1001/jama.2012.67929]
114 **Breyer BN,** Phelan S, Hogan PE, Rosen RC, Kitabchi AE, Wing RR, Brown JS; Look AHEAD Research Group. Intensive Lifestyle Intervention Reduces Urinary Incontinence in Overweight/Obese Men with Type 2 Diabetes: Results from the Look AHEAD Trial. *J Urol* 2014 Jun 1; Epub ahead of print [PMID: 24533998 DOI: 10.1016/j.juro.2014.02.036]
115 **Gerstein HC**. Do lifestyle changes reduce serious outcomes in diabetes? *N Engl J Med* 2013; **369**: 189-190 [PMID: 23796132 DOI: 10.1056/NEJMe1306987]
116 **Wadden TA;** Look AHEAD Research Group. Impact of Intensive Lifestyle Intervention on Depression and Health-Related Quality of Life in Type 2 Diabetes: The Look AHEAD Trial. *Diabetes Care* 2014; **37**: 1544-1553 [DOI: 10.2337/dc13-1928]
117 **Long GH,** Cooper AJM, Wareham NJ, Griffin SJ, Simmons RK. Healthy Behavior Change and Cardiovascular Outcomes in Newly Diagnosed Type 2 Diabetic Patients: A Cohort Analysis of the ADDITION-Cambridge Study. *Diabetes Care* 2014; **37**: 1712-1720 [DOI: 10.2337/dc13-1731]
118 **Inzucchi SE**, Bergenstal RM, Buse JB, Diamant M, Ferrannini E, Nauck M, Peters AL, Tsapas A, Wender R, Matthews DR. Management of hyperglycemia in type 2 diabetes: a patient-centered approach: position statement of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetes Care* 2012; **35**: 1364-1379 [PMID: 22517736 DOI: 10.2337/dc12-0413]
119 **Garber AJ**, Abrahamson MJ, Barzilay JI, Blonde L, Bloomgarden ZT, Bush MA, Dagogo-Jack S, Davidson MB, Einhorn D, Garvey WT, Grunberger G, Handelsman Y, Hirsch IB, Jellinger PS, McGill JB, Mechanick JI, Rosenblit PD, Umpierrez G, Davidson MH. AACE comprehensive diabetes management algorithm 2013. *Endocr Pract* 2013; **19**: 327-336 [PMID: 23598536]
120 **DeFronzo RA**, Barzilai N, Simonson DC. Mechanism of metformin action in obese and lean noninsulin-dependent diabetic subjects. *J Clin Endocrinol Metab* 1991; **73**: 1294-1301 [PMID: 1955512 DOI: 10.1210/jcem-73-6-1294]
121 **Kim CH**, Han KA, Oh HJ, Tan KE, Sothiratnam R, Tjokroprawiro A, Klein M. Safety, tolerability, and efficacy of metformin extended-release oral antidiabetic therapy in patients with type 2 diabetes: an observational trial in Asia. *J Diabetes* 2012; **4**: 395-406 [PMID: 22742083 DOI: 10.1111/j.1753-0407.2012.00220.x]
122 **Nissen SE**, Wolski K. Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. *N Engl J Med* 2007; **356**: 2457-2471 [PMID: 17517853 DOI: 10.1056/NEJMoa072761]
123 FDA places greater restrictions on access to rosiglitazone. *BMJ* 2010; **341**: c5287 [PMID: 20870699 DOI: 10.1136/bmj.c5287]
124 **Blind E**, Dunder K, de Graeff PA, Abadie E. Rosiglitazone: a European regulatory perspective. *Diabetologia* 2011; **54**: 213-218 [PMID: 21153629 DOI: 10.1007/s00125-010-1992-5]
125 **McCarthy M**. US regulators relax restrictions on rosiglitazone. *BMJ* 2013; **347**: f7144 [PMID: 24286989]
126 **Dormandy JA**, Charbonnel B, Eckland DJ, Erdmann E, Massi-Benedetti M, Moules IK, Skene AM, Tan MH, Lefèbvre PJ, Murray GD, Standl E, Wilcox RG, Wilhelmsen L, Betteridge J, Birkeland K, Golay A, Heine RJ, Korányi L, Laakso M, Mokán M, Norkus A, Pirags V, Podar T, Scheen A, Scherbaum W, Schernthaner G, Schmitz O, Skrha J, Smith U, Taton J. Secondary prevention of macrovascular events in patients with type 2 diabetes in the PROactive Study (PROspective pioglitAzone Clinical Trial In macroVascular Events): a randomised controlled trial. *Lancet* 2005; **366**: 1279-1289 [PMID: 16214598 DOI: 10.1016/S0140-6736(05)67528-9]
127 **Tzoulaki I**, Molokhia M, Curcin V, Little MP, Millett CJ, Ng A, Hughes RI, Khunti K, Wilkins MR, Majeed A, Elliott P. Risk of cardiovascular disease and all cause mortality among patients with type 2 diabetes prescribed oral antidiabetes drugs: retrospective cohort study using UK general practice research database. *BMJ* 2009; **339**: b4731 [PMID: 19959591]
128 **Loke YK**, Kwok CS, Singh S. Comparative cardiovascular effects of thiazolidinediones: systematic review and meta-analysis of observational studies. *BMJ* 2011; **342**: d1309 [PMID: 21415101]
129 **Nissen SE**, Nicholls SJ, Wolski K, Nesto R, Kupfer S, Perez A, Jure H, De Larochellière R, Staniloae CS, Mavromatis K, Saw J, Hu B, Lincoff AM, Tuzcu EM. Comparison of pioglitazone vs glimepiride on progression of coronary atherosclerosis in patients with type 2 diabetes: the PERISCOPE randomized controlled trial. *JAMA* 2008; **299**: 1561-1573 [PMID: 18378631 DOI: 10.1001/jama.299.13.1561]
130 **Meier C**, Kraenzlin ME, Bodmer M, Jick SS, Jick H, Meier CR. Use of thiazolidinediones and fracture risk. *Arch Intern Med* 2008; **168**: 820-825 [PMID: 18443256 DOI: 10.1001/archinte.168.8.820]
131 **Lewis JD**, Ferrara A, Peng T, Hedderson M, Bilker WB, Quesenberry CP, Vaughn DJ, Nessel L, Selby J, Strom BL. Risk of bladder cancer among diabetic patients treated with pioglitazone: interim report of a longitudinal cohort study. *Diabetes Care* 2011; **34**: 916-922 [PMID: 21447663 DOI: 10.2337/dc10-1068]
132 **Neumann A**, Weill A, Ricordeau P, Fagot JP, Alla F, Allemand H. Pioglitazone and risk of bladder cancer among diabetic patients in France: a population-based cohort study. *Diabetologia* 2012; **55**: 1953-1962 [PMID: 22460763 DOI: 10.1007/s00125-012-2538-9]
133 **Colmers IN**, Bowker SL, Majumdar SR, Johnson JA. Use of thiazolidinediones and the risk of bladder cancer among people with type 2 diabetes: a meta-analysis. *CMAJ* 2012; **184**: E675-E683 [PMID: 22761478 DOI: 10.1503/cmaj.112102]
134 **Chiasson JL**, Josse RG, Gomis R, Hanefeld M, Karasik A, Laakso M. Acarbose for prevention of type 2 diabetes

mellitus: the STOP-NIDDM randomised trial. *Lancet* 2002; **359**: 2072-2077 [PMID: 12086760 DOI: 10.1016/S0140-6736(02)08905-5]
135 **Kawamori R**, Tajima N, Iwamoto Y, Kashiwagi A, Shimamoto K, Kaku K. Voglibose for prevention of type 2 diabetes mellitus: a randomised, double-blind trial in Japanese individuals with impaired glucose tolerance. *Lancet* 2009; **373**: 1607-1614 [PMID: 19395079 DOI: 10.1016/S0140-6736(09)60222-1]
136 **Chiasson JL**, Josse RG, Gomis R, Hanefeld M, Karasik A, Laakso M. Acarbose treatment and the risk of cardiovascular disease and hypertension in patients with impaired glucose tolerance: the STOP-NIDDM trial. *JAMA* 2003; **290**: 486-494 [PMID: 12876091 DOI: 10.1001/jama.290.4.486]
137 **Hanefeld M**, Chiasson JL, Koehler C, Henkel E, Schaper F, Temelkova-Kurktschiev T. Acarbose slows progression of intima-media thickness of the carotid arteries in subjects with impaired glucose tolerance. *Stroke* 2004; **35**: 1073-1078 [PMID: 15073402 DOI: 10.1161/01.STR.0000125864.01546.f2]
138 **Hanefeld M**, Cagatay M, Petrowitsch T, Neuser D, Petzinna D, Rupp M. Acarbose reduces the risk for myocardial infarction in type 2 diabetic patients: meta-analysis of seven long-term studies. *Eur Heart J* 2004; **25**: 10-16 [PMID: 14683737 DOI: 10.1016/S0195-668X(03)00468-8]
139 **Drucker DJ**, Nauck MA. The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. *Lancet* 2006; **368**: 1696-1705 [PMID: 17098089 DOI: 10.1016/S0140-6736(06)69705-5]
140 **Kendall DM**, Cuddihy RM, Bergenstal RM. Clinical application of incretin-based therapy: therapeutic potential, patient selection and clinical use. *Am J Med* 2009; **122**: S37-S50 [PMID: 19464427 DOI: 10.1016/j.amjmed.2009.03.015]
141 **Karagiannis T**, Paschos P, Paletas K, Matthews DR, Tsapas A. Dipeptidyl peptidase-4 inhibitors for treatment of type 2 diabetes mellitus in the clinical setting: systematic review and meta-analysis. *BMJ* 2012; **344**: e1369 [PMID: 22411919 DOI: 10.1136/bmj.e1369]
142 **Aroda VR**, Henry RR, Han J, Huang W, DeYoung MB, Darsow T, Hoogwerf BJ. Efficacy of GLP-1 receptor agonists and DPP-4 inhibitors: meta-analysis and systematic review. *Clin Ther* 2012; **34**: 1247-1258.e22 [PMID: 22608780 DOI: 1016/j.clinthera.2012.04.013]
143 **Rolin B**, Larsen MO, Gotfredsen CF, Deacon CF, Carr RD, Wilken M, Knudsen LB. The long-acting GLP-1 derivative NN2211 ameliorates glycemia and increases beta-cell mass in diabetic mice. *Am J Physiol Endocrinol Metab* 2002; **283**: E745-E752 [PMID: 12217892 DOI: 10.1152/ajpendo.00030.2002]
144 **Lamont BJ**, Li Y, Kwan E, Brown TJ, Gaisano H, Drucker DJ. Pancreatic GLP-1 receptor activation is sufficient for incretin control of glucose metabolism in mice. *J Clin Invest* 2012; **122**: 388-402 [PMID: 22182839]
145 **Farilla L**, Bulotta A, Hirshberg B, Li Calzi S, Khoury N, Noushmehr H, Bertolotto C, Di Mario U, Harlan DM, Perfetti R. Glucagon-like peptide 1 inhibits cell apoptosis and improves glucose responsiveness of freshly isolated human islets. *Endocrinology* 2003; **144**: 5149-5158 [PMID: 12960095 DOI: 10.1210/en.2003-0323]
146 **Bunck MC**, Cornér A, Eliasson B, Heine RJ, Shaginian RM, Taskinen MR, Smith U, Yki-Järvinen H, Diamant M. Effects of exenatide on measures of β-cell function after 3 years in metformin-treated patients with type 2 diabetes. *Diabetes Care* 2011; **34**: 2041-2047 [PMID: 21868779]
147 **Foley JE**, Bunck MC, Möller-Goede DL, Poelma M, Nijpels G, Eekhoff EM, Schweizer A, Heine RJ, Diamant M. Beta cell function following 1 year vildagliptin or placebo treatment and after 12 week washout in drug-naive patients with type 2 diabetes and mild hyperglycaemia: a randomised controlled trial. *Diabetologia* 2011; **54**: 1985-1991 [PMID: 21547496 DOI: 10.1007/s00125-011-2167-8]
148 **Kohro T**, Yamazaki T, Sato H, Harada K, Ohe K, Komuro I, Nagai R. Trends in antidiabetic prescription patterns in Japan from 2005 to 2011. *Int Heart J* 2013; **54**: 93-97 [PMID: 23676369]
149 **Kodani N**, Saisho Y, Tanaka K, Kawai T, Itoh H. Effects of mitiglinide, a short-acting insulin secretagogue, on daily glycemic variability and oxidative stress markers in Japanese patients with type 2 diabetes mellitus. *Clin Drug Investig* 2013; **33**: 563-570 [PMID: 23797928 DOI: 10.1007/s40261-013-0098-5]
150 **Del Prato S**, Tiengo A. The importance of first-phase insulin secretion: implications for the therapy of type 2 diabetes mellitus. *Diabetes Metab Res Rev* 2001; **17**: 164-174 [PMID: 11424229 DOI: 10.1002/dmrr.198]
151 **Gerstein HC**, Bosch J, Dagenais GR, Díaz R, Jung H, Maggioni AP, Pogue J, Probstfield J, Ramachandran A, Riddle MC, Rydén LE, Yusuf S. Basal insulin and cardiovascular and other outcomes in dysglycemia. *N Engl J Med* 2012; **367**: 319-328 [PMID: 22686416 DOI: 10.1056/NEJMoa1203858]
152 **Weng J**, Li Y, Xu W, Shi L, Zhang Q, Zhu D, Hu Y, Zhou Z, Yan X, Tian H, Ran X, Luo Z, Xian J, Yan L, Li F, Zeng L, Chen Y, Yang L, Yan S, Liu J, Li M, Fu Z, Cheng H. Effect of intensive insulin therapy on beta-cell function and glycaemic control in patients with newly diagnosed type 2 diabetes: a multicentre randomised parallel-group trial. *Lancet* 2008; **371**: 1753-1760 [PMID: 18502299 DOI: 10.1016/S0140-6736(08)60762-X]
153 **Pennartz C**, Schenker N, Menge BA, Schmidt WE, Nauck MA, Meier JJ. Chronic reduction of fasting glycemia with insulin glargine improves first- and second-phase insulin secretion in patients with type 2 diabetes. *Diabetes Care* 2011; **34**: 2048-2053 [PMID: 21775756 DOI: 10.2337/dc11-0471]
154 **Unger RH**. Reinventing type 2 diabetes: pathogenesis, treatment, and prevention. *JAMA* 2008; **299**: 1185-1187 [PMID: 18334695 DOI: 10.1001/jama.299.10.1185]
155 **Tahrani AA**, Barnett AH, Bailey CJ. SGLT inhibitors in management of diabetes. *Lancet Diabetes Endocrinol* 2013; **1**: 140-151 [PMID: 24622320 DOI: 10.1016/S2213-8587(13)70050-0]
156 **Vasilakou D**, Karagiannis T, Athanasiadou E, Mainou M, Liakos A, Bekiari E, Sarigianni M, Matthews DR, Tsapas A. Sodium-glucose cotransporter 2 inhibitors for type 2 diabetes: a systematic review and meta-analysis. *Ann Intern Med* 2013; **159**: 262-274 [PMID: 24026259 DOI: 10.7326/0003-4819-159-4-201308200-00007]
157 **Riser Taylor S**, Harris KB. The clinical efficacy and safety of sodium glucose cotransporter-2 inhibitors in adults with type 2 diabetes mellitus. *Pharmacotherapy* 2013; **33**: 984-999 [PMID: 23744749 DOI: 10.1002/phar.1303]
158 **Gloy VL**, Briel M, Bhatt DL, Kashyap SR, Schauer PR, Mingrone G, Bucher HC, Nordmann AJ. Bariatric surgery versus non-surgical treatment for obesity: a systematic review and meta-analysis of randomised controlled trials. *BMJ* 2013; **347**: f5934 [PMID: 24149519 DOI: 10.1136/bmj.f5934]
159 **Schauer PR**, Bhatt DL, Kirwan JP, Wolski K, Brethauer SA, Navaneethan SD, Aminian A, Pothier CE, Kim ES, Nissen SE, Kashyap SR. Bariatric surgery versus intensive medical therapy for diabetes--3-year outcomes. *N Engl J Med* 2014; **370**: 2002-2013 [PMID: 24679060 DOI: 10.1056/NEJMoa1401329]
160 **Dutia R**, Brakoniecki K, Bunker P, Paultre F, Homel P, Carpentier AC, McGinty J, Laferrère B. Limited recovery of β-cell function after gastric bypass despite clinical diabetes remission. *Diabetes* 2014; **63**: 1214-1223 [PMID: 24296713 DOI: 10.2337/db13-1176]
161 **Dixon JB**, Chuang LM, Chong K, Chen SC, Lambert GW, Straznicky NE, Lambert EA, Lee WJ. Predicting the glycemic response to gastric bypass surgery in patients with type 2 diabetes. *Diabetes Care* 2013; **36**: 20-26 [PMID: 23033249 DOI: 10.2337/dc12-0779]
162 **Lee YC**, Lee WJ, Liew PL. Predictors of remission of type 2 diabetes mellitus in obese patients after gastrointestinal surgery. *Obes Res Clin Pract* 2013; **7**: e494-e500 [PMID: 24308892 DOI: 10.1016/j.orcp.2012.08.190]
163 **Pan XR**, Li GW, Hu YH, Wang JX, Yang WY, An ZX, Hu ZX,

Lin J, Xiao JZ, Cao HB, Liu PA, Jiang XG, Jiang YY, Wang JP, Zheng H, Zhang H, Bennett PH, Howard BV. Effects of diet and exercise in preventing NIDDM in people with impaired glucose tolerance. The Da Qing IGT and Diabetes Study. *Diabetes Care* 1997; **20**: 537-544 [PMID: 9096977 DOI: 10.2337/diacare.20.4.537]

164 **Tuomilehto J**, Lindström J, Eriksson JG, Valle TT, Hämäläinen H, Ilanne-Parikka P, Keinänen-Kiukaanniemi S, Laakso M, Louheranta A, Rastas M, Salminen V, Uusitupa M. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *N Engl J Med* 2001; **344**: 1343-1350 [PMID: 11333990 DOI: 10.1056/NEJM200105033441801]

165 **Knowler WC**, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, Nathan DM. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med* 2002; **346**: 393-403 [PMID: 11832527 DOI: 10.1056/NEJMoa012512]

166 **Yoon U**, Kwok LL, Magkidis A. Efficacy of lifestyle interventions in reducing diabetes incidence in patients with impaired glucose tolerance: a systematic review of randomized controlled trials. *Metabolism* 2013; **62**: 303-314 [PMID: 22959500 DOI: 10.1016/j.metabol.2012.07.009]

167 **DeFronzo RA**, Tripathy D, Schwenke DC, Banerji M, Bray GA, Buchanan TA, Clement SC, Henry RR, Hodis HN, Kitabchi AE, Mack WJ, Mudaliar S, Ratner RE, Williams K, Stentz FB, Musi N, Reaven PD. Pioglitazone for diabetes prevention in impaired glucose tolerance. *N Engl J Med* 2011; **364**: 1104-1115 [PMID: 21428766 DOI: 10.1056/NEJMoa1010949]

168 **Gerstein HC**, Yusuf S, Bosch J, Pogue J, Sheridan P, Dinccag N, Hanefeld M, Hoogwerf B, Laakso M, Mohan V, Shaw J, Zinman B, Holman RR. Effect of rosiglitazone on the frequency of diabetes in patients with impaired glucose tolerance or impaired fasting glucose: a randomised controlled trial. *Lancet* 2006; **368**: 1096-1105 [PMID: 16997664 DOI: 10.1016/S0140-6736(06)69420-8]

169 **Knowler WC**, Hamman RF, Edelstein SL, Barrett-Connor E, Ehrmann DA, Walker EA, Fowler SE, Nathan DM, Kahn SE. Prevention of type 2 diabetes with troglitazone in the Diabetes Prevention Program. *Diabetes* 2005; **54**: 1150-1156 [PMID: 15793255]

170 **Holman RR**, Haffner SM, McMurray JJ, Bethel MA, Holzhauer B, Hua TA, Belenkov Y, Boolell M, Buse JB, Buckley BM, Chacra AR, Chiang FT, Charbonnel B, Chow CC, Davies MJ, Deedwania P, Diem P, Einhorn D, Fonseca V, Fulcher GR, Gaciong Z, Gaztambide S, Giles T, Horton E, Ilkova H, Jenssen T, Kahn SE, Krum H, Laakso M, Leiter LA, Levitt NS, Mareev V, Martinez F, Masson C, Mazzone T, Meaney E, Nesto R, Pan C, Prager R, Raptis SA, Rutten GE, Sandstroem H, Schaper F, Scheen A, Schmitz O, Sinay I, Soska V, Stender S, Tamás G, Tognoni G, Tuomilehto J, Villamil AS, Vozár J, Califf RM. Effect of nateglinide on the incidence of diabetes and cardiovascular events. *N Engl J Med* 2010; **362**: 1463-1476 [PMID: 20228402 DOI: 10.1056/NEJMoa1001122]

171 **Yates T**, Haffner SM, Schulte PJ, Thomas L, Huffman KM, Bales CW, Califf RM, Holman RR, McMurray JJ, Bethel MA, Tuomilehto J, Davies MJ, Kraus WE. Association between change in daily ambulatory activity and cardiovascular events in people with impaired glucose tolerance (NAVIGATOR trial): a cohort analysis. *Lancet* 2014; **383**: 1059-1066 [PMID: 24361242 DOI: 10.1016/S0140-6736(13)62061-9]

172 **Li G**, Zhang P, Wang J, An Y, Gong Q, Gregg EW, Yang W, Zhang B, Shuai Y, Hong J, Engelgau MM, Li H, Roglic G, Hu Y, Bennett PH. Cardiovascular mortality, all-cause mortality, and diabetes incidence after lifestyle intervention for people with impaired glucose tolerance in the Da Qing Diabetes Prevention Study: a 23-year follow-up study. *Lancet Diabetes Endocrinol* 2014; **2**: 474-480 [PMID: 24731674 DOI: 10.1016/s2213-8587(14)70057-9]

173 **Villareal DT**, Chode S, Parimi N, Sinacore DR, Hilton T, Armamento-Villareal R, Napoli N, Qualls C, Shah K. Weight loss, exercise, or both and physical function in obese older adults. *N Engl J Med* 2011; **364**: 1218-1229 [PMID: 21449785 DOI: 10.1056/NEJMoa1008234]

174 **Pal A**, McCarthy MI. The genetics of type 2 diabetes and its clinical relevance. *Clin Genet* 2013; **83**: 297-306 [PMID: 23167659 DOI: 10.1111/cge.12055]

175 **Jonsson A**, Ladenvall C, Ahluwalia TS, Kravic J, Krus U, Taneera J, Isomaa B, Tuomi T, Renström E, Groop L, Lyssenko V. Effects of common genetic variants associated with type 2 diabetes and glycemic traits on α- and β-cell function and insulin action in humans. *Diabetes* 2013; **62**: 2978-2983 [PMID: 23557703 DOI: 10.2337/db12-1627]

176 **Andersson EA**, Allin KH, Sandholt CH, Borglykke A, Lau CJ, Ribel-Madsen R, Sparsø T, Justesen JM, Harder MN, Jørgensen ME, Jørgensen T, Hansen T, Pedersen O. Genetic risk score of 46 type 2 diabetes risk variants associates with changes in plasma glucose and estimates of pancreatic β-cell function over 5 years of follow-up. *Diabetes* 2013; **62**: 3610-3617 [PMID: 23835328 DOI: 10.2337/db13-0362]

177 **Iwata M**, Maeda S, Kamura Y, Takano A, Kato H, Murakami S, Higuchi K, Takahashi A, Fujita H, Hara K, Kadowaki T, Tobe K. Genetic risk score constructed using 14 susceptibility alleles for type 2 diabetes is associated with the early onset of diabetes and may predict the future requirement of insulin injections among Japanese individuals. *Diabetes Care* 2012; **35**: 1763-1770 [PMID: 22688542 DOI: 10.2337/dc11-2006]

178 **Pasquali L**, Gaulton KJ, Rodríguez-Seguí SA, Mularoni L, Miguel-Escalada I, Akerman I, Tena JJ, Morán I, Gómez-Marín C, van de Bunt M, Ponsa-Cobas J, Castro N, Nammo T, Cebola I, García-Hurtado J, Maestro MA, Pattou F, Piemonti L, Berney T, Gloyn AL, Ravassard P, Gómez-Skarmeta JL, Müller F, McCarthy MI, Ferrer J. Pancreatic islet enhancer clusters enriched in type 2 diabetes risk-associated variants. *Nat Genet* 2014; **46**: 136-143 [PMID: 24413736 DOI: 10.1038/ng.2870]

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REVIEW

Roles of interstitial fluid pH in diabetes mellitus: Glycolysis and mitochondrial function

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Abstract

The pH of body fluids is one the most important key factors regulating various cell function such as enzyme activity and protein-protein interaction *via* modification of its binding affinity. Therefore, to keep cell function normal, the pH of body fluids is maintained constant by various systems. Insulin resistance is one of the most important, serious factors making the body condition worse in diabetes mellitus. I have recently found that the pH of body (interstitial) fluids is lower in diabetes mellitus than that in non-diabetic control, and that the lowered pH is one of the causes producing insulin resistance. In this review article, I introduce importance of body (interstitial) fluid pH in regulation of body function, evidence on abnormal regulation of body fluid pH in diabetes mellitus, and relationship between the body fluid pH and insulin resistance. Further, this review proposes perspective therapies on the basis of regulation of body fluid pH including propolis (honeybee product) diet.

Key words: pH; Interstitial fluid; Insulin; Binding affinity to receptors; Propolis

Core tip: This review article provides new findings on changes of body (interstitial) fluid pH in type 2 diabetes mellitus, the role of body (interstitial) fluid pH in occurrence of insulin resistance, and future possibility of treatment for type 2 diabetes mellitus from a viewpoint of improvement of body (interstitial) fluid pH.

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INTRODUCTION

Metabolic syndrome increases the risk developing type

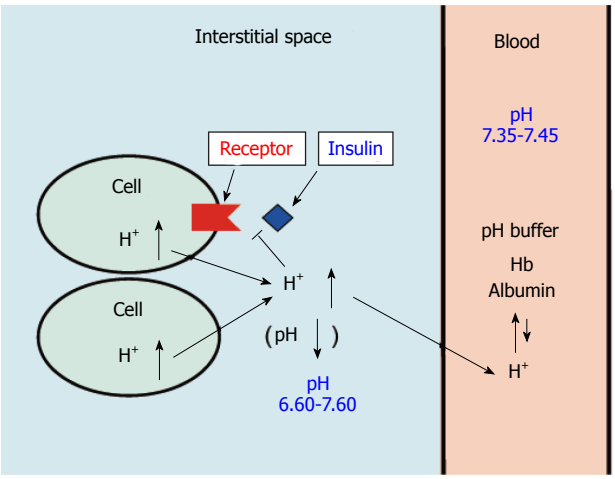

Figure 1 pH of interstitial fluid and blood, and binding affinity of insulin to its receptor. Interstitial fluids have little pH-buffering molecules, while blood has very strong, powerful pH buffering molecules such as hemoglobin and albumin. Thus, even under mild but not severe metabolic disorder conditions, blood pH is kept constant within a normal range (7.35-7.45), but interstitial fluid pH would be lower than a normal level.

2 diabetes mellitus, cardiovascular disease and cancer, *etc.*, meaning that it would be a pre-stage for diseases. Therefore, to prevent progression of metabolic syndrome is one of the most effective strategies for prevention of occurrence of type 2 diabetes mellitus. Insulin resistance is one of the most important, serious factors developing symptoms of type 2 diabetes mellitus. Patients with insulin resistance show hypertension, one of typical clinical symptoms for diagnosis of cardiovascular disorders[1-4]. Further, patients with hyperinsulinemia, which is generally caused by insulin resistance, show hypertension mediated through various types of disorders such as renal failure, vascular dysfunction, and hyper-activation of sympathetic nerve[5-10]. These findings suggest that preventing development of insulin resistance would be one of the most important key subjects keeping the healthy body function.

Pathogenesis of insulin resistance means insufficiency of insulin action on glucose uptake in skeletal muscle[11], sustaining blood glucose at high levels after meals; this is known as one of typical symptoms of type 2 diabetes mellitus. The number of patients suffering from type 2 diabetes mellitus still continuously increases, and this becomes one of the most serious worldwide social problems[12]. Thus, clarification of mechanisms causing insulin resistance is one of the most important key subjects on prevention and treatment for diabetes mellitus. Unfortunately, the mechanisms of occurrence of insulin resistance have not yet been fully clarified.

As described above, one of the most major symptoms of type 2 diabetes mellitus is insulin resistance causing hyperglycemia, which leads to progression of pancreatic β-cell dysfunction due to hyper-secretion of insulin. Continuous hyperglycemia due to poor uptake of glucose into cells such as skeletal muscles, adipocytes, hepatocytes, *etc.*, in general, irreversibly leads to macro- and micro-vascular complications, resulting in myocardial infarction, stroke, blindness, renal dysfunction, and peripheral neuropathy. International Diabetes Federation (IDF) reports that in 2012, 370 million people are recognized as diabetes mellitus in the worldwide, and the number of people with diabetes mellitus is considered to increase up to 550 million by 2030[12]. Various types of drugs have been developed for treatment of type 2 diabetes mellitus, however a tremendous number of people still suffer from type 2 diabetes mellitus. This means that although some newly developed drugs for treatment of type 2 diabetes mellitus are very efficient, the drugs are sill not effective to fully treat patients suffering from type 2 diabetes mellitus.

Interstitial fluids provide circumstances where extracellular signaling molecules such as hormones and neurotransmitters regulate cell function. This means that alteration of interstitial fluid composition affects efficiency of signal transduction of extracellular signaling molecules in intracellular signal transduction. Specially, it is notable that pH of interstitial fluids is very variable, since interstitial fluids contain little pH buffering molecules (Figures 1 and 2). On the other hand, blood has powerful pH buffering such as hemoglobin, albumin, *etc.,* (Figures 1 and 2), keeping strictly pH of blood at a range between 7.35-7.45. These facts mean that even if pH of blood stays at normal levels, 7.35-7.45, pH of interstitial fluids would deviate from the normal range under metabolically pathophysiological conditions. As described above, hormones and neurotransmitters act on their receptors in the interstitial (extracellular) fluids (spaces) but not inside blood vessels (Figures 1 and 2). Further, it is notable that pH regulates activity of various types of enzymes and binding affinity of hormones and neurotransmitters to their receptors (Figure 1). This means that pH of interstitial fluids plays one of the most important key roles in regulation of cell function keeping homeostasis of the body function and condition, nevertheless unfortunately little information on pH of interstitial fluids is available.

As mentioned above, activity of most enzymes and binding affinity of hormones and neurotransmitters to their receptors directly depend on pH of interstitial fluids. Therefore, keeping normal body/cell function requires maintenance of interstitial fluid pH within a normal range. Energy has to be also supplied to keep normal cell/body function. This process produces organic acids *via* glycolysis and CO_2 *via* TCA cycle (Figure 2). Under physiological conditions, these acids including CO_2 (H^+ produced from CO_2 and H_2O) and organic acids are extruded *via* the lung and the kidney to keep pH of interstitial fluids within a normal range. However, under metabolically pathophysiological conditions such as diabetes mellitus, pH of interstitial fluids would become lower; *i.e.*, interstitial fluids become acidic. For example, severe diabetes mellitus causes ketoacidosis detected as lowered pH (< 7.35) of "arterial" blood even containing strong pH buffers such as hemoglobin and albumin. This suggests that the interstitial fluid pH with little pH buffer in severe diabetes mellitus would be much lower than that in normal persons. The lowered pH of interstitial

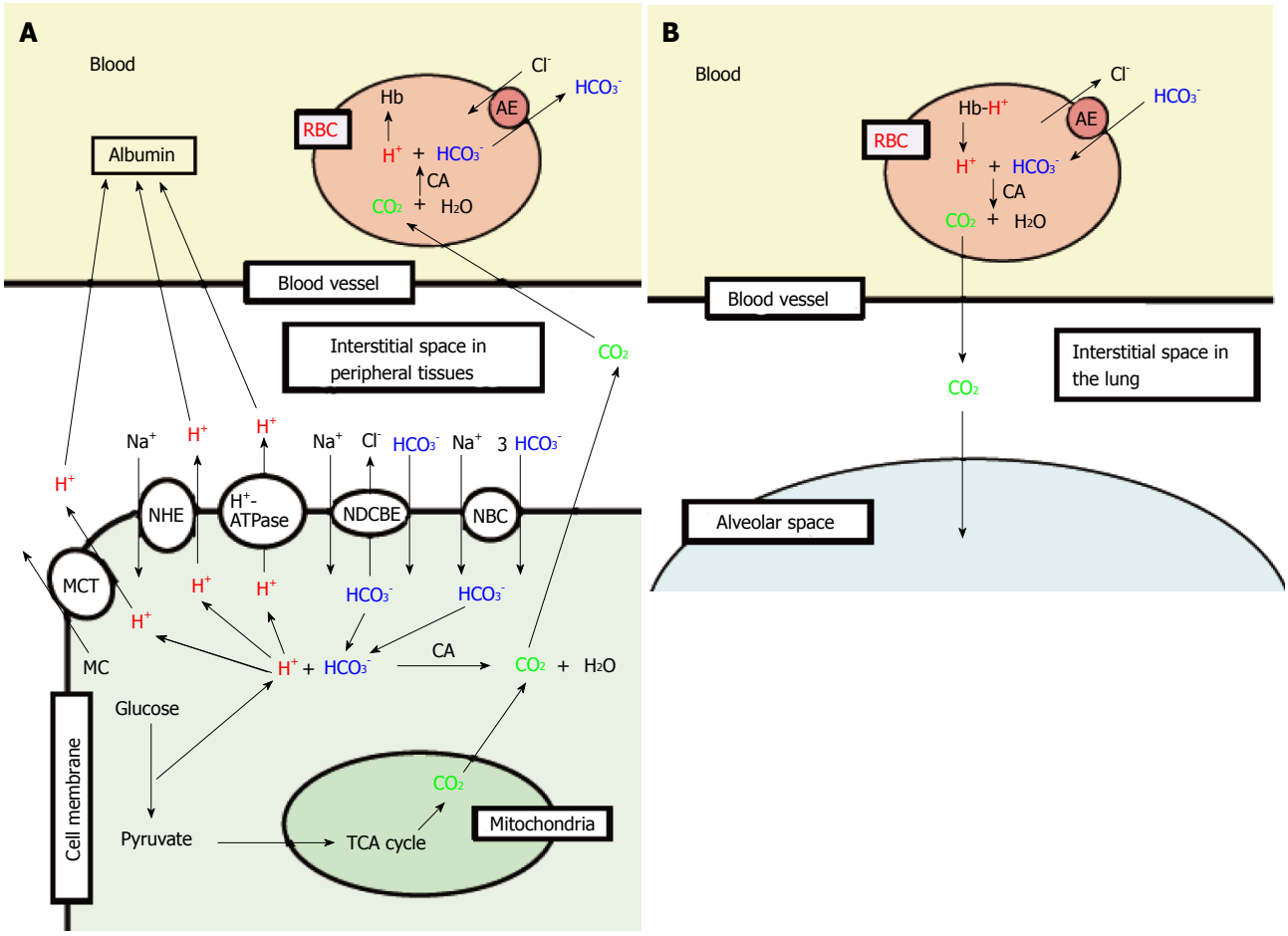

Figure 2 Production of H^+, and H^+ transporting systems in peripheral tissues (A) and the lung (B). A: In cells of peripheral tissues, H^+ is produced from organic acids generated as metabolites *via* glycolysis such as lactic acid. H^+ is directly extruded *via* Na^+/H^+ exchanger (NHE), H^+-ATPase and H^+-coupled monocarboxylate (MC) transporter (MCT) from intracellular to extracellular (interstitial) spaces, and moves into blood, and binds to albumin. Further, a part of H^+ produced from metabolites is converted to CO_2 and H_2O consuming HCO_3^- *via* carbonic anhydrase (CA)-mediated facilitation process. To supply HCO_3^- consumed for conversion of H^+ to CO_2 and H_2O in cells, Na^+-driven Cl^-/HCO_3^- exchanger (NDCBE) and Na^+-HCO_3^- cotransporter (NBC) participate in uptake of HCO_3^- into intracellular from extracellular (interstitial) spaces. CO_2 moves into red blood cell (RBC, erythrocyte) in blood *via* permeation across the plasma membrane of RBC due to high CO_2 permeability of the plasma membrane, and is converted to H^+ and HCO_3^- consuming H_2O *via* CA-mediated facilitation process. H^+ produced from CO_2 and H_2O *via* CA-mediated facilitation process in RBC binds to hemoglobin. HCO_3^- produced from CO_2 and H_2O *via* CA-mediated facilitation process in RBC is extruded from intracellular to extracellular (interstitial) spaces *via* exchange of Cl^- existing in the extracellular space by anion exchanger (AE): this exchanging step of HCO_3^- extrusion and Cl^- uptake is so called as Cl^- shift; B: In the lung, the reversible process occurs due to low CO_2 circumstances.

fluids under metabolically pathophysiological conditions leads patients to being further worse conditions of the disease[13,14]. Interestingly, even in pre-disease stages pH is drastically lowered in interstitial fluids around various tissues including the brain[15-17], developing diseases.

In this review article, I provide a new concept regarding insulin resistance and its improvement; particularly I discuss the role of interstitial fluid pH in cell function in diabetes mellitus.

INTERSTITIAL FLUIDS

Interstitial fluid pH kept within a normal range plays a role as one of the most important key factors in keeping normal cell/body functions and adaptation of body condition as mentioned above. However, unfortunately interstitial fluids have little pH buffers unlike blood. The reason why interstitial fluids have little pH buffers unlike blood containing hemoglobin and albumin, very strong pH buffers, is as follows. If interstitial fluids have pH-buffering proteins such as albumin, colloid osmotic pressure of interstitial fluids becomes larger than that without pH buffering proteins such as albumin. High colloid osmotic pressure of interstitial fluids leads to disturbance of transport and circulation of nutrition and metabolites between blood and interstitial fluids across walls of blood vessels. Namely, metabolites produced in peripheral tissues are collected into capillaries near veins by the larger colloid osmotic pressure in the capillary than that in the interstitial fluid. This driving force of metabolite collection into capillary becomes low if the colloid osmotic pressure becomes large. Therefore, the fact that interstitial fluids have little pH buffering proteins is a weak point for keeping activity of hormones and enzymes at normal levels, but is essentially required for collection of metabolites produced in peripheral tissues

into capillaries. In general, the pH-buffering capacity in blood is large enough to keep the interstitial fluid pH constant under physiological conditions. Unfortunately, under metabolically pathophysiological conditions, the pH-buffering capacity in blood is not large enough to maintain activity of hormones and enzymes in interstitial fluids, resulting in disorders such as insulin resistance.

REGULATION AND ABNORMALITIES IN pH OF INTERSTITIAL FLUIDS

The pH of mammalian "arterial" blood is accurately maintained at 7.40 ± 0.05 under normal physiological conditions. Severe metabolic disorders cause deviation of "arterial" blood pH more than 0.05 unit from a normal range of 7.35-7.45; *i.e.*, pH < 7.35 is defined as acidosis or pH > 7.45 is defined as alkalosis. Alkalosis, pH > 7.45, is caused by vomiting of gastric juices or diarrhea (metabolic alkalosis), or hyperventilation (respiratory alkalosis), *etc.* However, long-term alkalosis rarely occurs. On the other hand, acidosis (specially long-term acidosis) occurs in various metabolic disorders including diabetes mellitus. Acidosis and alkalosis are well recognized as severe disorders of body conditions, however little information is available on the interstitial fluid pH. Even under the condition with normal pH (7.35-7.45) of "arterial" blood, pH of interstitial fluids would deviate from the normal range. Our previous reports indicate that the pH of interstitial fluids is deviated form the normal range[15-17] even under conditions maintained at normal "arterial" blood pH. The pH of interstitial fluids is determined by the content of H^+ (proton) provided from organic acids as metabolites produced at ATP synthesis in living cells.

One of typical H^+ sources is lactate, CH_3-CH(OH)-COOH [CH_3-CH(OH)-COO^- + H^+], which is converted from pyruvate, CH_3-CO-COOH (CH_3-CO-COO^- + H^+), a product from glycolysis. In tissues requiring much energy (ATP) such as skeletal muscles, the anaerobically glycolytic metabolism mediates the conversion of glucose and glycogen into lactic acid *via* production of pyruvate. Under an aerobic condition, pyruvate is used for TCA cycle conducted in mitochondria. Therefore, under physiological conditions little amounts of lactate are generated, and most of the final product of glycolysis followed by TCA cycle is CO_2, which is facilitated to be converted into H^+ and HCO_3^- by carbonic anhydrase. Of course, CO_2 is one of major sources for H^+. However, to obtain a fixed amount of ATP, the amount of H^+ generated by organic acids and CO_2 produced in the process for generation of ATP mediated *via* both glycolysis and TCA cycle is much smaller than that produced only by glycolysis. Namely, under conditions with ATP synthesis predominantly mediated *via* glycolysis but not followed by function of TCA cycle, the total amount of produced H^+ is much larger than that under conditions with ATP synthesis *via* glycolysis associated with functional TCA cycle. Patients with diabetes mellitus are suggested to have reduced mitochondria function[11,18-20]. Based on this suggestion[11,18-20], the total amount of H^+ produced in patients with diabetes mellitus is much larger than that in healthy persons with normal mitochondrial function. Even in cases that blood pH in patients with diabetes mellitus except severe cases is within a normal range (7.35-7.45), pH of interstitial fluids would be less than 7.35.

Other sources of H^+ are ketone bodies; *i.e.*, metabolism of fatty acids in liver generates beta-hydroxybutyrate, which provides H^+ *via* dissociation into beta-hydroxybutyrate anion and H^+ (beta-hydroxybutyrate$^-$ + H^+)[21]. Beta-hydroxybutyrate is the major ketone body (approximately 70% of total ketone bodies) produced by TCA cycle in liver mitochondria *via* oxidation of free fatty acids released from adipocytes[22]. Another major ketone body is acetoacetate, which is converted to beta-hydroxybutyrate. The synthesis of these ketone bodies in liver mitochondria is, in general, occurs in response to an unavailability of blood glucose, contributing to overall energy metabolism. The ketone bodies produced in liver mitochondria are transported *via* blood to extra-hepatic tissues such as skeletal muscles and heart muscles. Under conditions with an unavailability or low availability of blood glucose, the transported ketone bodies such as beta-hydroxybutyrate and acetoacetate to muscles are used as sources of acetyl CoA, which is utilized in TCA cycle in mitochondria of muscles for generation of ATP[23]. Further, fatty acids can be converted to acetyl CoA without formation of ketone bodies, meaning that fatty acids are sources of ATP in mitochondria. However, when amounts of fatty acids is very large, abundant ketone bodies are produced in liver mitochondria and the amount of ketone bodies are exceeds the metabolizing capacity in mitochondria of muscles[23]. In this case, the body produces a large amount of ketone bodies, leading to elevation of H^+ concentration (lowered pH) as mentioned above. This means that when low utilization of glucose or low mitochondria function occurs, high levels of ketone bodies appear in periphery tissues, providing a large amount of H^+ (low pH).

METABOLITES INFLUENCING pH OF INTERSTITIAL FLUIDS AND pH-BUFFERING SYSTEMS

Lactate is dissociated into CH_3-CH(OH)-COO^- + H^+ under physiological conditions of pH (approximately 7.40) much higher than pKa of lactate (3.86), leading to production of H^+. In addition to lactate, as mentioned above H^+ is also provided from materials such as ketone bodies; for example, metabolism of fatty acids in liver generates beta-hydroxybutyrate, one of typical ketone bodies, provides H^+ *via* dissociation into beta-hydroxybutyrate anion and H^+ (beta-hydroxybutyrate$^-$ + H^+). The H^+ produced in cells *via* metabolism is extruded as a form of H^+ itself or CO_2 *via* various types of H^+ transporter such as Na^+/H^+ exchanger (NHE), H^+-ATPase, H^+-coupled monocarboxylate transporter (MCT),

Na^+-driven Cl^-/HCO_3^- exchanger (NDCBE), and Na^+-HCO_3^- cotransporter (NBC), *etc.,* (Figure 2A)[24-29]. Namely, extrusion of H^+ as a form of H^+ itself is mediated by NHE, H^+-ATPase and/or MCT, *etc.*, that directly transport H^+ to the extracellular (interstitial) space. On the other hand, NDCBE or NBC doesn't directly transport H^+, but uptake HCO_3^- into the intracellular space, resulting in production of $CO_2 + H_2O$ from H^+ and HCO_3^- *via* carbonic anhydrase (CA). CO_2 produced by H^+ and HCO_3^- *via* a CA-facilitated process easily moves to the extracellular space by permeating the plasma membrane, since the plasma membrane of cells has high permeability to CO_2 (Figure 2A). Thus, H^+ produced in the intracellular space is extruded to the extracellular (interstitial) space as a form of CO_2 *via* consumption of HCO_3^- transported from the extracellular (interstitial) space. Because, HCO_3^- originally generated from CO_2 produced *via* TCA cycle in cells associated with H^+ could not be a net source for conversion to CO_2 due to its origin, CO_2. Thus, H^+ produced from lactate, *etc.*, in the intracellular space is extruded to the extracellular (interstitial) space *via* directly NHE/H^+-ATPase/MCT, and indirectly NDCBE/NBC. Anyway, H^+ produced from metabolites such as lactate, *etc.*, consumes HCO_3^-, reducing the intracellular concentration of HCO_3^- associated with a compensatory increase in HCO_3^- uptake *via* NDCBE and NBC. The source of HCO_3^- under anaerobic conditions associated with dysfunction of mitochondria in cells such as muscles is HCO_3^- in blood, which is produced from $CO_2 + H_2O$ *via* facilitated conversion by carbonic anhydrase in other tissues. H^+ is finally extruded from the body *via* the kidney into urine. Therefore, severe overproduction of H^+ spends HCO_3^-, being converted into CO_2 an H_2O (Figure 2). This CO_2 produced from H^+ and HCO_3^- moves into red blood cells (RBC, erythrocytes), and is again converted into H^+ and HCO_3^- *via* a CA-facilitated process (Figure 2A). In red blood cells, H^+ binds to hemoglobin, and HCO_3^- is extruded to the extracellular space in blood *via* anion exchanger (AE) (Figure 2A). Namely, even though HCO_3^- is consumed in cells, HCO_3^- is again produced in red blood cells, suggesting that HCO_3^- is not consumed in peripheral tissues. However, HCO_3^- is transported into red blood cells *via* AE in blood at the lung due to low CO_2 pressure compared with peripheral tissues, and HCO_3^- is converted to CO_2 with H^+ released from hemoglobin (Figure 2B). CO_2 produced in this process is extruded to atmosphere (Figure 2B). Thus, *via* this overall process the concentration of HCO_3^- in blood is reduced under these metabolically pathophysiological conditions. Severe overproduction of H^+ causes metabolic acidosis consuming HCO_3^-, pH of "arterial" blood being less than 7.35. This acidosis has been previously recognized to occur as results from general metabolic disorders, however diabetes mellitus is recently indicated to be associated with mitochondrial dysfunction[11,18-20]. This mitochondrial dysfunction is one of main causes leading to acidosis. Further, as mentioned above, lowered pH is also caused by H^+ dissociated from ketone bodies generated in liver provide, which are mainly produced *via* oxidation of free fatty acids released from adipocytes[22]; representative ketone bodies are beta-hydroxybutyrate, and acetoacetate. As described above, the synthesis of these ketone bodies in the liver mitochondria are generally produced in response to an unavailability of blood glucose in muscles. Therefore, these ketone bodies are utilized as sources of acetyl CoA for generation of ATP *via* TCA cycle in mitochondria of muscles[23]. Under conditions with mitochondrial dysfunction in muscles, these ketone bodies generated in the liver are not utilized as sources of acetyl CoA for generation of ATP *via* TCA cycle in mitochondria of muscles[23]. Thus, under these conditions, the ketone bodies provide a lot of H^+, leading to much lower pH (acidosis) than that under conditions with normal mitochondrial function.

Lactate in intracellular spaces is a useful energy source *via* oxidation as a respiratory fuel[30,31]. Thus, the cytosolic lactate produced in fast muscles contracting relatively fast at heavy exercise under physiological conditions is extruded to the extracellular (interstitial) space *via* MCTs[32,33], being delivered to oxidative tissues *via* extracellular lactate shuttle through the blood[34]. Specially, in diabetes mellitus patients, lactate is generated due to mitochondrial dysfunction even in cases of regular exercise without heavy contraction of fast muscles[20,35]. In most mammalian cells, MCTs participate in the transport of lactate and other monocarboxylic acids such pyruvate, beta-hydroxybutyrate and acetoacetate across the cellular membrane[36-38]. It has been indicated that patients suffering from diabetes mellitus show alteration of MCTs expression[39,40]. Since MCTs carry monocarboxylate with H^+, MCTs function as H^+ extrusion coupled with monocarboxylate extrusion (Figure 2A), meaning that MCTs plays important, essential roles in pH and energy balance in patients suffering from diabetes mellitus.

ABNORMAL INTERSTITIAL FLUID pH AND PROGRESSION OF DIABETES MELLITUSS

As well known, insulin decreases blood glucose levels by stimulating glucose uptake into skeletal muscle cells *via* glucose transporter 4 (GLUT4), maintaining whole-body glucose homeostasis[41]. GLUT4 translocation to the plasma membrane from intracellular store sites is the main mechanism of insulin showing its stimulatory action on glucose uptake into skeletal muscle cells and adipocytes. Insulin regulates a dynamic process of GLUT4 trafficking between the plasma membrane and its intracellular store sites[42]. Insulin binds to its receptor located on the plasma membrane, immediately auto-phosphorylating tyrosine residues of the receptor. This auto-phosphorylation of insulin receptor subsequently induces phosphorylation of tyrosine residues of insulin receptor substrate-1 (IRS-1) phosphorylating (activating) PI3K, which catalyzes 3' phosphorylation of phosphatidylinositol 4,5-diphosphate,

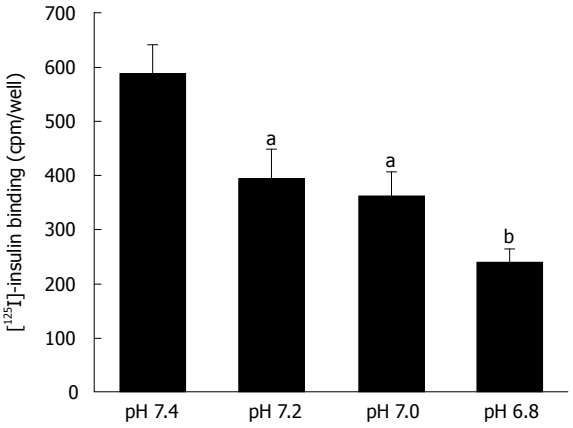

Figure 3 Insulin binding to insulin receptor under various pH conditions. After serum starved for 4 h, L6 myotubes were treated with 100 nmol/L insulin for 15 min in the HEPES buffer with different values of pH. Proteins expressed on the plasma membrane were biotinylated and precipitated. Differentiated L6 myotubes were treated with [^{125}I]-labeled insulin for 15 min in the indicated pH buffers, and the radioactivities were measured after cells were washed and suspended. The values of radioactivity from at least 6 experiments are shown. The values are shown as the mean ± SEM. $^{a}P < 0.05$, $^{b}P < 0.01$ *vs* pH 7.4. Modified from ref.[46] with allowance of non-profit use of figures.

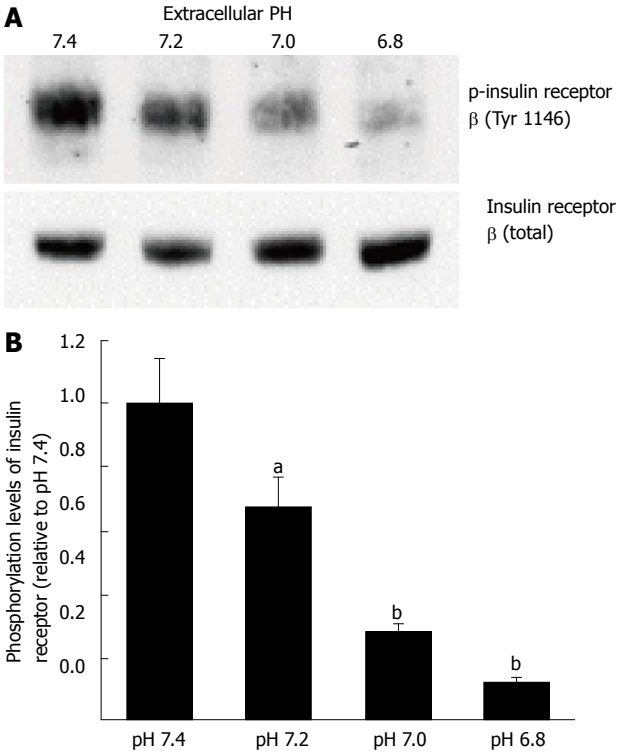

Figure 4 Phosphorylation levels of insulin receptor. After serum starvation for 4 h, L6 myotubes were treated with 100 nmol/L insulin for 15 min in the buffer with different pH. Total cell lysates were isolated and analyzed by Western blotting with indicated antibodies. A: Representative blots are shown; B: The quantative values of expression of insulin receptor using densitometry from 6 independent experiments using anti-phosphorylated-insulin receptor-β (Tyr 1146) antibody normalized to the level of total insulin receptor compared with that in pH 7.4 buffer. The values are shown as the mean ± SEM ($n = 6$). $^{a}P < 0.05$, $^{b}P < 0.01$ *vs* pH 7.4. Modified from ref.[46] with allowance of non-profit use of figures.

leading to activation of Akt. This PI3K/Akt-mediated signaling pathway in the insulin-induced down-stream pathway stimulates the intracellular translocation of GLUT4 to the plasma membrane, elevating glucose uptake in skeletal muscles. Dysfunction of this insulin signal transduction leads to reduced levels of insulin-stimulated glucose uptake into skeletal muscles in type 2 diabetic patients, and this dysfunction is so-called insulin resistance[43]. Our recent study has shown that pH of interstitial fluids is lower in Otsuka Long-Evans Tokushima Fatty (OLETF) rats, a model in type 2 diabetes mellitus, than normal one[15]. Many epidemiological studies have recently reported the relationship between metabolic acidosis and insulin resistance[44]. Organic acids-induced acidosis would contribute to early stages in the development of insulin resistance[15,44-46]. The relationship between production of organic acids and development of insulin sensitivity is an important subject in patients suffering from type 2 diabetes mellitus[47-49]. Insulin sensitivity and urine pH have negative correlation with body weight and waist size[34]. Persons with metabolic syndrome are reported to have a significantly lower value of 24-h urine pH than that in normal persons without metabolic syndrome[48]. Persons with higher amounts of anion gap in metabolic acidosis associated with lower serum HCO_3^- show lower insulin sensitivity[50]. Our recently studies[15,17] indicate that the interstitial fluid pH in ascites, brain hippocampus and metabolic tissues in Otsuka Long-Evans Tokushima Fatty (OLETF) rats in early developing stages of diabetic mellitus is lower than the normal pH (7.40). Although our studies have not yet clarified the molecular mechanism causing lowered pH of interstitial fluids, these phenomena would be due to dysfunction or hypo-function of mitochondria in diabetes mellitus[11,19,20]. As mentioned above, the pH-buffering capacity in the interstitial fluid is much lower than that in the cytosol and in the blood, meaning that pH of interstitial fluids in metabolic tissues has valuable values depending on metabolic conditions. Therefore, we have studied if the lowered extracellular (interstitial fluid) pH reduces insulin action on its signaling pathways in rat skeletal model cells[46,51]. As mentioned above, insulin shows its stimulatory action on glucose uptake in skeletal muscle in a phosphatidylinositol 3-kinase (PI3K)-mediated pathway after binding to its receptor located on the plasma membrane *via* phosphorylation of its receptor. Therefore, we first studied if the insulin binding to its receptor is affected by lowering extracellular (interstitial fluid) pH. We have found that lowered extracellular (interstitial) fluid pH diminishes binding affinity of insulin to its receptor (Figure 3) associated with diminution of insulin receptor phosphorylation (activation) (Figure 4) without any change in expression of insulin receptor on the plasma membrane of skeletal muscle (Figure 5)[46]. Further, levels of phosphorylated (activated) Akt, a down-stream molecule of insulin signaling pathway, are decreased under conditions with lowered extracellular (interstitial fluid) pH (Figure 6)[46]. Glucose uptake is also diminished under the condition[46]. These observations indicate the importance

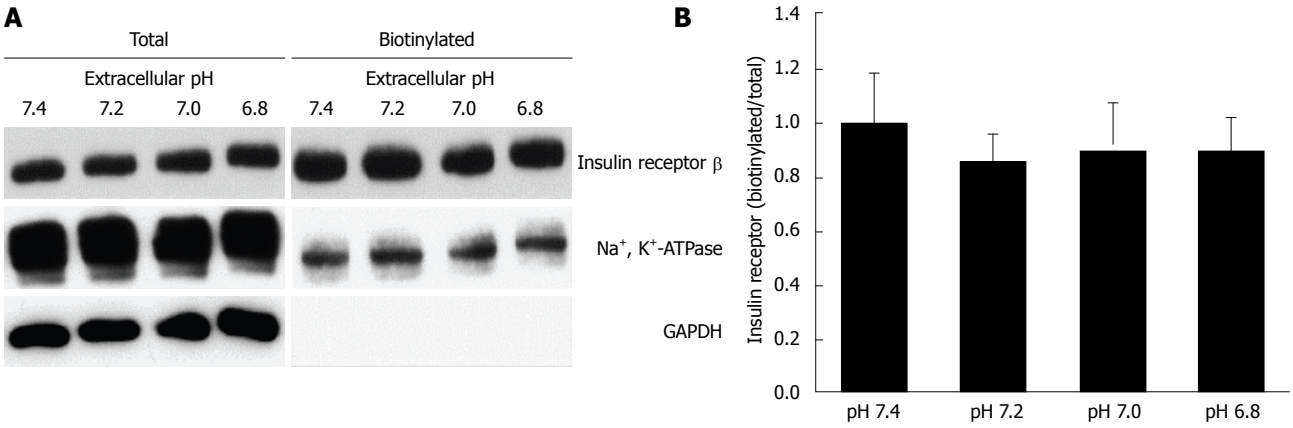

Figure 5 Effects of extracellular pH on the expression of insulin receptor on the plasma membrane. After serum starved for 4 h, L6 myotubes were treated with 100 nmol/L insulin for 15 min in the HEPES buffer with different pH. Proteins expressed on the plasma membrane were biotinylated and precipitated. A: Representative blots of total expression of insulin receptor on the plasma membrane, the Na^+, K^+-ATPase, and GAPDH; B: Quantative data of expression of insulin receptor on the plasma membrane at different pH normalized to that at pH 7.4. The results are presented as the mean ± SEM (n = 8). pH had no significant effects on the expression of insulin receptor on the plasma membrane. Modified from ref.[46] with allowance of non-profit use of figures.

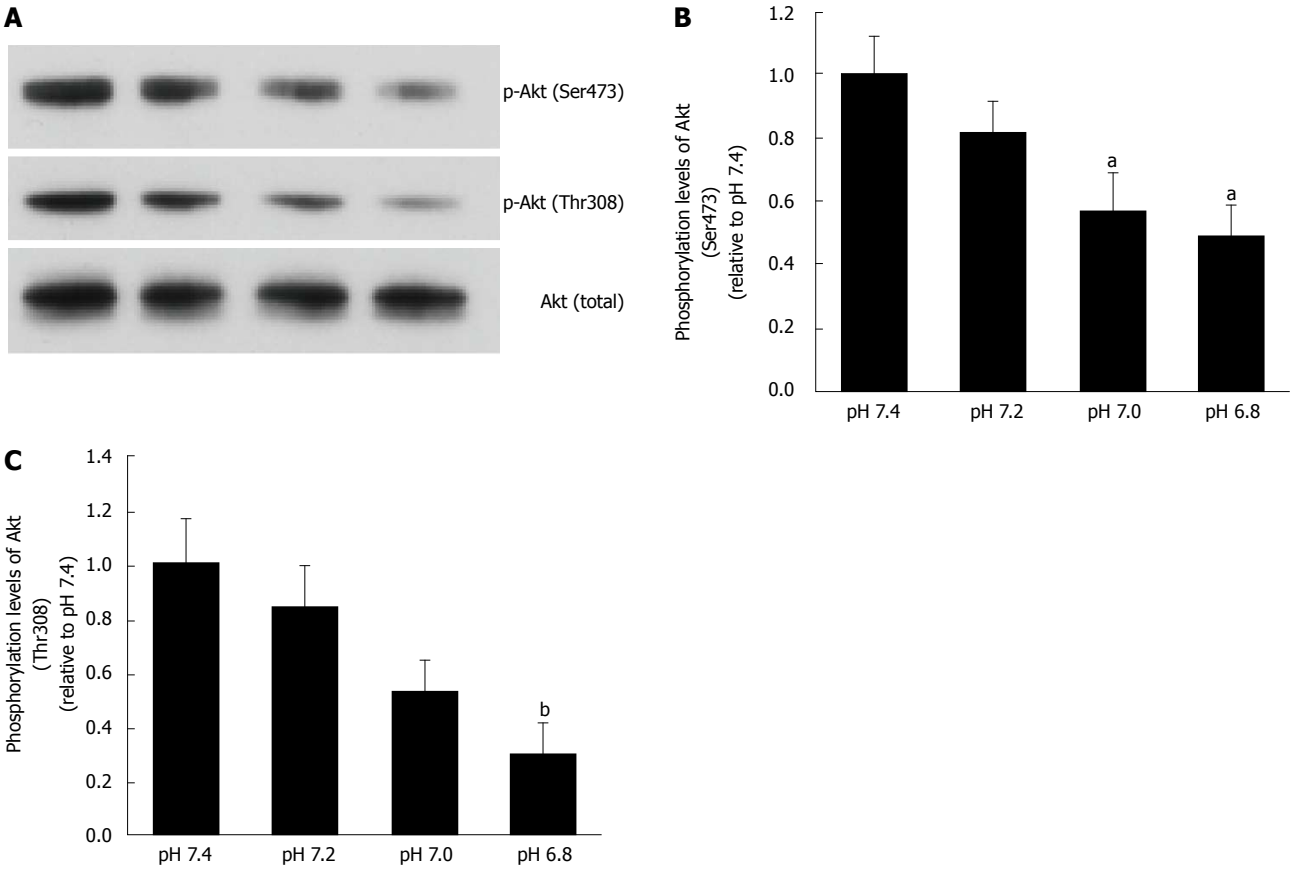

Figure 6 Phosphorylation levels of Akt. L6 myotubes were treated with 100 nmol/L insulin for 15 min in the buffer with different pH after serum starvation for 4 h. Total cell lysates were isolated, and were analyzed by Western blot with the indicated antibodies. A: Representative blots are shown using anti-phosphorylated (Ser473)-Akt, anti-phosphorylated (Thr308)-Akt, and anti-Akt antibodies. Phosphorylation levels of Ser473 (B) and Thr308 (C) are expressed as normalized values to the level of total Akt compared with those in pH 7.4. The values are shown as the mean ± SEM (n = 6). $^aP < 0.05$, $^bP < 0.01$ *vs* pH 7.4. Modified from ref.[46] with allowance of non-profit use of figures.

of interstitial fluid pH in occurrence of insulin resistance.

Our report also indicates an interesting observation regarding effects of propolis on various factors in type 2 diabetes mellitus model rats[15,16]. Propolis, a natural compound derived from plant resins collected by honeybees, contains various factors such as amino acids, steroids, phenolic aldehydes, polyphenols, sequiterpene quinines, and coumarins, *etc.*[52]. Propolis has been shown to possess anti-oxidant, anti-inflammation, and anti-tumor activities[53-56]. In addition to these actions, we

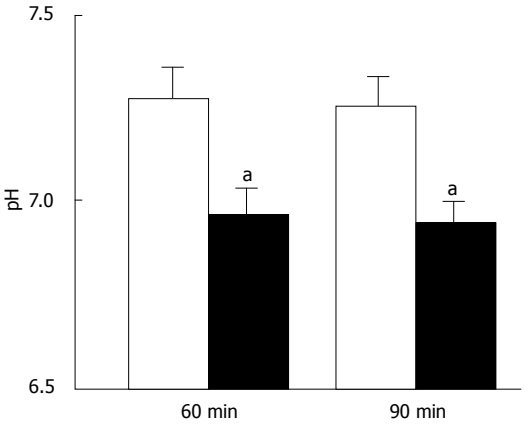

Figure 7 pH of interstitial (extracellular) fluid around the hippocampus of Otsuka Long-Evans Tokushima Fatty and normal (Wistar) rats. The pH value is shown as the mean ± SEM (n = 4). The pH values shown in Figure 7 were measured at 60 and 90 min after antimony pH electrodes reached interstitial (extracellular) fluids around the hippocampus of the Otsuka Long-Evans Tokushima Fatty rats (closed columns) and normal (Wistar) rats (open columns). $^{a}P < 0.05$ compared with that in normal (Wistar) rats at each measured time. Modified from ref.[17] with allowance of free use of figures.

have found that propolis improves insulin sensitivity[15,16]. Propolis improves (elevates) pH of interstitial fluids that is lower in type 2 diabetes mellitus than normal one[15,16]. Lowered pH of interstitial fluids diminishes binding affinity of insulin to its receptor (Figure 3), causing insulin resistance[46]. Although we have no information on molecular mechanisms how propolis improves lowered pH of interstitial fluids in type 2 diabetes mellitus at the present stage, these observations indicate us a very interesting point that propolis improves insulin sensitivity by elevating pH of interstitial fluids *via* recovery from diminished insulin binding affinity to insulin receptor in type 2 diabetes mellitus.

FLUIDS SECRETED INTO GASTROINTESTINAL LUMINAL SPACE AND FROM SEWEAT GLAND IN DIABETES MELLITUS

As mentioned above, the interstitial fluid has lower pH in type 2 diabetes mellitus than non-diabetic control. In addition to the interstitial fluid, I discuss about pH of fluids secreted from glands and gastrointestinal fluids. Before discussing about pH of those fluids, I should mention that these fluids are not secreted for maintenance of intracellular ionic conditions unlike the interstitial fluids. As mentioned above, pH of the interstitial fluids is lowered as a result from high production of H^+ due to mitochondrial dysfunction and/or disability of glucose in muscles, neurons and, *etc.* On the other hands, pH of fluids secreted from glands and gastrointestinal fluids is, in general, not directly influenced by high production of H^+ due to mitochondrial dysfunction and/or disability of glucose in muscles, neurons and *etc.* When mitochondrial dysfunction and/or disability of glucose occur in gland

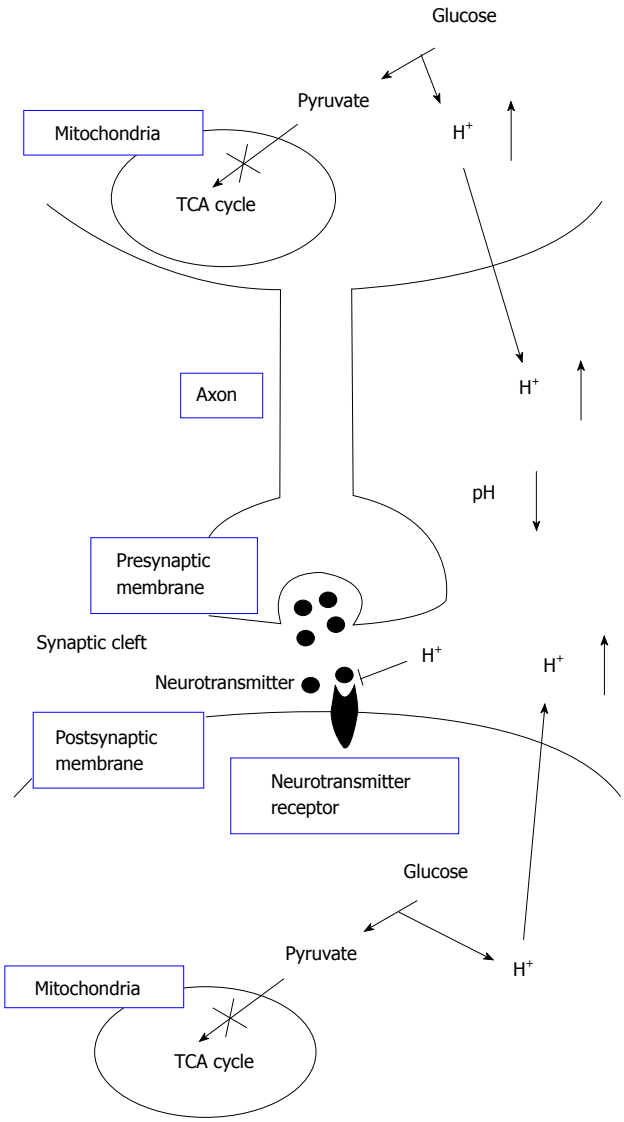

Figure 8 The pH-dependent mechanism of neural cell function in diabetes mellitus with dysfunction of mitochondria. Neural cells with dysfunction of mitochondria synthesize ATP required for maintenance of neural cell function only or mainly *via* glycolysis. Thus, neural cells with dysfunction of mitochondria produces much larger amounts of H^+ than neural cells with normal function of mitochondria. H^+ produced by glycolysis in neural cells with dysfunction of mitochondria[11,18-20] is released to the extracellular space, lowering pH of interstitial fluids[15-17] including the fluids in synaptic clefts. Lowered pH of synaptic cleft fluid diminishes the binding affinity of neurotransmitters to their receptors[67]. Thus, activity of neural cells is diminished at lowered pH of synaptic cleft fluid. Namely, the amount of neurotransmitters released into the synaptic cleft is large enough for generation of action potential under conditions with normal function of mitochondria. However, the amount of neurotransmitters released into the synaptic cleft is insufficient for generation of action potential under conditions with dysfunction of mitochondria, since lowered pH of synaptic cleft diminishes the binding affinity of neurotransmitters to receptors. Modified from ref.[17] with allowance of free use of figures.

or gastrointestinal cells, H^+ produced in these cells is, in general, extruded to the extracellular space across the basolateral membrane (so called interstitial fluid) but not to the luminal space across the apical membrane by H^+ transporter such as NHE, H^+-ATPase and *etc.* However, a study[57] indicates that the amounts of acid secreted from gastric gland under the basal condition and in response

to cholecystokinin are larger in diabetes than that in non-diabetic control. This suggests that the intra-gastric pH in diabetes is lower than that in control. On the other hand, HCO_3^- secretion from pancreas in response to secretin shows no difference between diabetes and non-diabetic control[57]. Further, observations on sweating in diabetes are reported[58,59]. Most studies on sweating in diabetes are focused on blood flow around sweat glands and blood-flow-dependent amounts of sweat secretion without studies on ionic composition[58,59], although a study indicates that Cl^- concentration is not changed in diabetes[60].

ALZHEIMER'S DISEASE IN DIABETES MELLITUS AND pH OF INTERSTITIAL FLUID

Patients with type 2 diabetes mellitus have been suggested to have a high risk of developing dementia and Alzheimer's disease with defective memory functions[61]. Insulin is suggested to be necessary for neuronal survival within the central nervous system[62,63]. Fluctuating levels of blood glucose resulting from dysfunction of insulin (insulin resistance) leads neurons including central nervous system to apoptosis, formation of neuritic plaques, neurofibrillary tangles, energy starvation, and altered acetylcholine levels in the hippocampus, which are observed in Alzheimer's disease[64,65]. Hippocampus is an important region participating in memory function[66]. We have found that pH of interstitial fluid around hippocampus is lower in type 2 diabetes mellitus model OLETF rats than that in normal ones (Figure 7), suggesting diminution of neuronal activity around hippocampus (Figure 8)[17,67].

Therefore, we suggest that maintenance of the interstitial fluid pH at the normal level or the recovery of the "interstitial" pH to normal from lowered levels would be a key factor in developing molecular and cellular therapies for metabolic brain disorders including Alzheimer's disease.

CONCLUSION

Interstitial fluids have little pH buffering capacity. Therefore, over production of acid metabolites lower pH of interstitial fluids even when the intracellular and "arterial" blood pH remains normal (Figures 1 and 2). The lowered pH of interstitial fluids causes insulin resistance *via* reduced binding affinity of insulin to its receptor (Figures 1 and 3). Acidic environments due to dysfunction of mitochondria occurring in type 2 diabetes mellitus lead to insulin resistance. Further, the acidic environment occurring in the brain would be related to diminution of neuronal function and onset of Alzheimer's disease (Figure 8).

REFERENCES

1 **Cederholm J**, Wibell L. Glucose intolerance in middle-aged subjects--a cause of hypertension? *Acta Med Scand* 1985; **217**: 363-371 [PMID: 4013827]
2 **Eriksson KF**, Lindgärde F. Contribution of estimated insulin resistance and glucose intolerance to essential hypertension. *J Intern Med Suppl* 1991; **735**: 75-83 [PMID: 2043225]
3 **Bao W**, Srinivasan SR, Berenson GS. Persistent elevation of plasma insulin levels is associated with increased cardiovascular risk in children and young adults. The Bogalusa Heart Study. *Circulation* 1996; **93**: 54-59 [PMID: 8616941]
4 **Haffner SM**, Ferrannini E, Hazuda HP, Stern MP. Clustering of cardiovascular risk factors in confirmed prehypertensive individuals. *Hypertension* 1992; **20**: 38-45 [PMID: 1618551]
5 **Marunaka Y**, Hagiwara N, Tohda H. Insulin activates single amiloride-blockable Na channels in a distal nephron cell line (A6). *Am J Physiol* 1992; **263**: F392-F400 [PMID: 1329533]
6 **Mills E**, Kuhn CM, Feinglos MN, Surwit R. Hypertension in CB57BL/6J mouse model of non-insulin-dependent diabetes mellitus. *Am J Physiol* 1993; **264**: R73-R78 [PMID: 8430889]
7 **Edwards JG**, Tipton CM. Influences of exogenous insulin on arterial blood pressure measurements of the rat. *J Appl Physiol* (1985) 1989; **67**: 2335-2342 [PMID: 2691488]
8 **Meehan WP**, Buchanan TA, Hsueh W. Chronic insulin administration elevates blood pressure in rats. *Hypertension* 1994; **23**: 1012-1017 [PMID: 8206584]
9 **Marunaka Y**, Niisato N, Taruno A, Ohta M, Miyazaki H, Hosogi S, Nakajima K, Kusuzaki K, Ashihara E, Nishio K, Iwasaki Y, Nakahari T, Kubota T. Regulation of epithelial sodium transport via epithelial Na+ channel. *J Biomed Biotechnol* 2011; **2011**: 978196 [PMID: 22028593]
10 **Marunaka Y**. Characteristics and pharmacological regulation of epithelial Na+ channel (ENaC) and epithelial Na+ transport. *J Pharmacol Sci* 2014; **126**: 21-36 [PMID: 25242083]
11 **Abdul-Ghani MA**, DeFronzo RA. Pathogenesis of insulin resistance in skeletal muscle. *J Biomed Biotechnol* 2010; **2010**: 476279 [PMID: 20445742 DOI: 10.1155/2010/476279]
12 **International Diabetes Federation.** Global Diabetes Plan 2011-2021. Available from: URL: http: //www.idf.org/sites/default/files/Global_Diabetes_Plan_Final.pdf
13 **Felig P**. Diabetic ketoacidosis. *N Engl J Med* 1974; **290**: 1360-1363 [PMID: 4208038]
14 **Reaven GM**, Olefsky JM. The role of insulin resistance in the pathogenesis of diabetes mellitus. *Adv Metab Disord* 1978; **9**: 313-331 [PMID: 417568]
15 **Aoi W**, Hosogi S, Niisato N, Yokoyama N, Hayata H, Miyazaki H, Kusuzaki K, Fukuda T, Fukui M, Nakamura N, Marunaka Y. Improvement of insulin resistance, blood pressure and interstitial pH in early developmental stage of insulin resistance in OLETF rats by intake of propolis extracts. *Biochem Biophys Res Commun* 2013; **432**: 650-653 [PMID: 23416075 DOI: 10.1016/j.bbrc.2013.02.029]
16 **Marunaka Y,** Aoi W, Hosogi S, Niisato N, Yokoyama N, Hayata H, Miyazaki H, Kusuzaki K, Taruno A, Nomura T. What is the role of interstitial pH in diabetes mellitus? Improving action of propolis on type diabetes mellitus via pH regulation. *Int J Mol Med* 2013; **32**: S50
17 **Marunaka Y,** Yoshimoto K, Aoi W, Hosogi S, Ikegaya H. Low pH of interstitial fluid around hippocampus of the brain in diabetic OLETF rats. *Mol Cell Therapies* 2014; **2**: 6 [DOI: 10.1186/2052-8426-2-6]
18 **Li R**, Guan MX. Human mitochondrial leucyl-tRNA synthetase corrects mitochondrial dysfunctions due to the tRNALeu(UUR) A3243G mutation, associated with mitochondrial encephalomyopathy, lactic acidosis, and stroke-like symptoms and diabetes. *Mol Cell Biol* 2010; **30**: 2147-2154 [PMID: 20194621 DOI: 10.1128/mcb.01614-09]
19 **El-Hattab AW**, Emrick LT, Hsu JW, Chanprasert S, Jahoor F, Scaglia F, Craigen WJ. Glucose metabolism derangements in adults with the MELAS m.3243A& gt; G mutation. *Mitochondrion* 2014; **18**: 63-69 [PMID: 25086207 DOI: 10.1016/j.mito.2014.07.008]
20 **Adeva-Andany M**, López-Ojén M, Funcasta-Calderón R,

Ameneiros-Rodríguez E, Donapetry-García C, Vila-Altesor M, Rodríguez-Seijas J. Comprehensive review on lactate metabolism in human health. *Mitochondrion* 2014; **17**: 76-100 [PMID: 24929216 DOI: 10.1016/j.mito.2014.05.007]
21 **Gosmanov AR,** Gosmanova EO, Dillard-Cannon E. Management of adult diabetic ketoacidosis. *Diabetes Metab Syndr Obes* 2014; **7**: 255-264 [PMID: 25061324 DOI: 10.2147/dmso.s50516]
22 **Persson B**. Determination of plasma acetoacetate and D-beta-hydroxybutyrate in new-born infants by an enzymatic fluorometric micro-method. *Scand J Clin Lab Invest* 1970; **25**: 9-18 [PMID: 5444963]
23 **Dedkova EN**, Blatter LA. Role of β-hydroxybutyrate, its polymer poly-β-hydroxybutyrate and inorganic polyphosphate in mammalian health and disease. *Front Physiol* 2014; **5**: 260 [PMID: 25101001 DOI: 10.3389/fphys.2014.00260]
24 **Ward CA**, Moffat MP. Modulation of sodium-hydrogen exchange activity in cardiac myocytes during acidosis and realkalinisation: effects on calcium, pHi, and cell shortening. *Cardiovasc Res* 1995; **29**: 247-253 [PMID: 7736502]
25 **Park CO**, Xiao XH, Allen DG. Changes in intracellular Na+ and pH in rat heart during ischemia: role of Na+/H+ exchanger. *Am J Physiol* 1999; **276**: H1581-H1590 [PMID: 10330242]
26 **Loh SH**, Chen WH, Chiang CH, Tsai CS, Lee GC, Jin JS, Cheng TH, Chen JJ. Intracellular pH regulatory mechanism in human atrial myocardium: functional evidence for Na(+)/H(+) exchanger and Na(+)/HCO(3)(-) symporter. *J Biomed Sci* 2002; **9**: 198-205 [PMID: 12065894]
27 **Hosogi S**, Miyazaki H, Nakajima K, Ashihara E, Niisato N, Kusuzaki K, Marunaka Y. An inhibitor of Na(+)/H(+) exchanger (NHE), ethyl-isopropyl amiloride (EIPA), diminishes proliferation of MKN28 human gastric cancer cells by decreasing the cytosolic Cl(-) concentration via DIDS-sensitive pathways. *Cell Physiol Biochem* 2012; **30**: 1241-1253 [PMID: 23075671]
28 **Hosogi S**, Kusuzaki K, Inui T, Wang X, Marunaka Y. Cytosolic chloride ion is a key factor in lysosomal acidification and function of autophagy in human gastric cancer cell. *J Cell Mol Med* 2014; **18**: 1124-1133 [PMID: 24725767 DOI: 10.1111/jcmm.12257]
29 **Marunaka Y.** Roles of ionic environments in growth of human cancer cell and potentials of ion transporter blockers in cancer therapies. *J Gastrointest Digest Syst* 2014; **3**: 163
30 **Liu L**, Duke BJ, Malik B, Yue Q, Eaton DC. Biphasic regulation of ENaC by TGF-{alpha} and EGF in renal epithelial cells. *Am J Physiol Renal Physiol* 2009; **296**: F1417-F1427 [PMID: 19297452]
31 **Aoi W,** Marunaka Y. The importance of regulation of body fluid pH in the development and progression of metabolic diseases. In: Berhardt LV, editor Advances in Medicine and Biology. Hauppauge, USA: Nova Publishers, 2014: 177-189
32 **Bonen A**. Lactate transporters (MCT proteins) in heart and skeletal muscles. *Med Sci Sports Exerc* 2000; **32**: 778-789 [PMID: 10776897]
33 **Juel C**. Lactate-proton cotransport in skeletal muscle. *Physiol Rev* 1997; **77**: 321-358 [PMID: 9114817]
34 **Han B**, Bai XH, Lodyga M, Xu J, Yang BB, Keshavjee S, Post M, Liu M. Conversion of mechanical force into biochemical signaling. *J Biol Chem* 2004; **279**: 54793-54801 [PMID: 15485829]
35 **Kourtoglou GI**. Insulin therapy and exercise. *Diabetes Res Clin Pract* 2011; **93** Suppl 1: S73-S77 [PMID: 21864755 DOI: 10.1016/s0168-8227(11)70017-1]
36 **Garcia CK**, Goldstein JL, Pathak RK, Anderson RG, Brown MS. Molecular characterization of a membrane transporter for lactate, pyruvate, and other monocarboxylates: implications for the Cori cycle. *Cell* 1994; **76**: 865-873 [PMID: 8124722]
37 **Xu AS**, Kuchel PW. Characterisation of erythrocyte transmembrane exchange of trifluoroacetate using 19F-NMR: evidence for transport via the monocarboxylate transporter. *Biochim Biophys Acta* 1993; **1150**: 35-44 [PMID: 8334136]
38 **Poole RC**, Halestrap AP. Transport of lactate and other monocarboxylates across mammalian plasma membranes. *Am J Physiol* 1993; **264**: C761-C782 [PMID: 8476015]
39 **Juel C**, Holten MK, Dela F. Effects of strength training on muscle lactate release and MCT1 and MCT4 content in healthy and type 2 diabetic humans. *J Physiol* 2004; **556**: 297-304 [PMID: 14724187 DOI: 10.1113/jphysiol.2003.058222]
40 **Opitz D**, Lenzen E, Schiffer T, Hermann R, Hellmich M, Bloch W, Brixius K, Brinkmann C. Endurance training alters skeletal muscle MCT contents in T2DM men. *Int J Sports Med* 2014; **35**: 1065-1071 [PMID: 25009968 DOI: 10.1055/s-0034-1371838]
41 **Huang S**, Czech MP. The GLUT4 glucose transporter. *Cell Metab* 2007; **5**: 237-252 [PMID: 17403369 DOI: 10.1016/j.cmet.2007.03.006]
42 **Stöckli J**, Fazakerley DJ, James DE. GLUT4 exocytosis. *J Cell Sci* 2011; **124**: 4147-4159 [PMID: 22247191 DOI: 10.1242/jcs.097063]
43 **Morgan BJ**, Chai SY, Albiston AL. GLUT4 associated proteins as therapeutic targets for diabetes. *Recent Pat Endocr Metab Immune Drug Discov* 2011; **5**: 25-32 [PMID: 22074575]
44 **Souto G**, Donapetry C, Calviño J, Adeva MM. Metabolic acidosis-induced insulin resistance and cardiovascular risk. *Metab Syndr Relat Disord* 2011; **9**: 247-253 [PMID: 21352078 DOI: 10.1089/met.2010.0108]
45 **Mandel EI**, Curhan GC, Hu FB, Taylor EN. Plasma bicarbonate and risk of type 2 diabetes mellitus. *CMAJ* 2012; **184**: E719-E725 [PMID: 22825995 DOI: 10.1503/cmaj.120438]
46 **Hayata H**, Miyazaki H, Niisato N, Yokoyama N, Marunaka Y. Lowered extracellular pH is involved in the pathogenesis of skeletal muscle insulin resistance. *Biochem Biophys Res Commun* 2014; **445**: 170-174 [PMID: 24502946 DOI: 10.1016/j.bbrc.2014.01.162]
47 **Otsuki M**, Kitamura T, Goya K, Saito H, Mukai M, Kasayama S, Shimomura I, Koga M. Association of urine acidification with visceral obesity and the metabolic syndrome. *Endocr J* 2011; **58**: 363-367 [PMID: 21441701]
48 **Maalouf NM**, Cameron MA, Moe OW, Adams-Huet B, Sakhaee K. Low urine pH: a novel feature of the metabolic syndrome. *Clin J Am Soc Nephrol* 2007; **2**: 883-888 [PMID: 17702734 DOI: 10.2215/CJN.00670207]
49 **Maalouf NM**, Cameron MA, Moe OW, Sakhaee K. Metabolic basis for low urine pH in type 2 diabetes. *Clin J Am Soc Nephrol* 2010; **5**: 1277-1281 [PMID: 20413437 DOI: 10.2215/cjn.08331109]
50 **Farwell WR**, Taylor EN. Serum bicarbonate, anion gap and insulin resistance in the National Health and Nutrition Examination Survey. *Diabet Med* 2008; **25**: 798-804 [PMID: 18644066 DOI: 10.1111/j.1464-5491.2008.02471.x]
51 **Hayata H,** Miyazaki H, Niisato N, Yokoyama N, Marunaka Y. Involvement of the extracellular pH in skeletal muscle insulin resistance. *J Physiol Sci* 2013; **63**: S199
52 **Khalil ML**. Biological activity of bee propolis in health and disease. *Asian Pac J Cancer Prev* 2006; **7**: 22-31 [PMID: 16629510]
53 **Krol W**, Czuba Z, Scheller S, Gabrys J, Grabiec S, Shani J. Anti-oxidant property of ethanolic extract of propolis (EEP) as evaluated by inhibiting the chemiluminescence oxidation of luminol. *Biochem Int* 1990; **21**: 593-597 [PMID: 2241984]
54 **Song YS**, Park EH, Jung KJ, Jin C. Inhibition of angiogenesis by propolis. *Arch Pharm Res* 2002; **25**: 500-504 [PMID: 12214863]
55 **Kimoto T**, Arai S, Kohguchi M, Aga M, Nomura Y, Micallef MJ, Kurimoto M, Mito K. Apoptosis and suppression of tumor growth by artepillin C extracted from Brazilian propolis. *Cancer Detect Prev* 1998; **22**: 506-515 [PMID: 9824373]
56 **Kimoto T**, Koya-Miyata S, Hino K, Micallef MJ, Hanaya T, Arai S, Ikeda M, Kurimoto M. Pulmonary carcinogenesis induced by ferric nitrilotriacetate in mice and protection

from it by Brazilian propolis and artepillin C. *Virchows Arch* 2001; **438**: 259-270 [PMID: 11315623]
57 **Tachibana I**, Akiyama T, Kanagawa K, Shiohara H, Furumi K, Watanabe N, Otsuki M. Defect in pancreatic exocrine and endocrine response to CCK in genetically diabetic OLETF rats. *Am J Physiol* 1996; **270**: G730-G737 [PMID: 8928805]
58 **Vinik AI**, Ziegler D. Diabetic cardiovascular autonomic neuropathy. *Circulation* 2007; **115**: 387-397 [PMID: 17242296 DOI: 10.1161/circulationaha.106.634949]
59 **Vinik AI**, Nevoret M, Casellini C, Parson H. Neurovascular function and sudorimetry in health and disease. *Curr Diab Rep* 2013; **13**: 517-532 [PMID: 23681491 DOI: 10.1007/s11892-013-0392-x]
60 **Doherty-Fuller E**, Copeland KC. Sweat tests in patients with diabetes insipidus. *Clin Pediatr* (Phila) 1988; **27**: 330-332 [PMID: 3390991]
61 **Mirza Z**, Kamal MA, Buzenadah AM, Al-Qahtani MH, Karim S. Establishing genomic/transcriptomic links between Alzheimer's disease and type 2 diabetes mellitus by meta-analysis approach. *CNS Neurol Disord Drug Targets* 2014; **13**: 501-516 [PMID: 24059308]
62 **Dudek H**, Datta SR, Franke TF, Birnbaum MJ, Yao R, Cooper GM, Segal RA, Kaplan DR, Greenberg ME. Regulation of neuronal survival by the serine-threonine protein kinase Akt. *Science* 1997; **275**: 661-665 [PMID: 9005851]
63 **Recio-Pinto E**, Rechler MM, Ishii DN. Effects of insulin, insulin-like growth factor-II, and nerve growth factor on neurite formation and survival in cultured sympathetic and sensory neurons. *J Neurosci* 1986; **6**: 1211-1219 [PMID: 3519887]
64 **Rasgon N**, Jarvik L. Insulin resistance, affective disorders, and Alzheimer's disease: review and hypothesis. *J Gerontol A Biol Sci Med Sci* 2004; **59**: 178-183; discussion 184-192 [PMID: 14999034]
65 **Steen E**, Terry BM, Rivera EJ, Cannon JL, Neely TR, Tavares R, Xu XJ, Wands JR, de la Monte SM. Impaired insulin and insulin-like growth factor expression and signaling mechanisms in Alzheimer's disease--is this type 3 diabetes? *J Alzheimers Dis* 2005; **7**: 63-80 [PMID: 15750215]
66 **Packard MG**, Goodman J. Factors that influence the relative use of multiple memory systems. *Hippocampus* 2013; **23**: 1044-1052 [PMID: 23929809 DOI: 10.1002/hipo.22178]
67 **Modest VE**, Butterworth JF. Effect of pH and lidocaine on beta-adrenergic receptor binding. Interaction during resuscitation? *Chest* 1995; **108**: 1373-1379 [PMID: 7587445]

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REVIEW

Ipragliflozin: A novel sodium-glucose cotransporter 2 inhibitor developed in Japan

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Abstract

Sodium-glucose cotransporter 2 (SGLT2) inhibition induces glucosuria and decreases blood glucose levels in diabetic patients and lowers hypoglycemic risk. SGLT1 is expressed in the kidney and intestine; SGLT1 inhibition causes abdominal symptoms such as diarrhea and reduces incretin secretion. Therefore, SGLT2 selectivity is important. Ipragliflozin is highly selective for SGLT2. In type 2 diabetes mellitus (T2DM), urinary glucose excretion increased to 90 g/24 h after 28 d of treatment with ipragliflozin 300 mg/d. Twelve weeks of ipragliflozin 50 mg/d *vs* placebo reduced glycated hemoglobin and body weight by 0.65% and 0.66 kg, respectively, in Western T2DM patients, and by 1.3% and 1.89 kg, respectively, in Japanese patients. Ipragliflozin (highly selective SGLT2 inhibitor) improves glycemic control and reduces body weight and lowers hypoglycemic risk and abdominal symptoms. Ipragliflozin can be a novel anti-diabetic and anti-obesity agent.

Key words: Sodium-glucose cotransporter 2 inhibitor; Type 2 diabetes mellitus; Ipragliflozin; Japan

Core tip: Ipragliflozin is highly selective for sodium-glucose cotransporter 2 (SGLT2) inhibitor. Twelve weeks of ipragliflozin 50 mg/d *vs* placebo decreased HbA1c and body weight by 0.65% and 0.66 kg, respectively, in Western patients, and by 1.3% and 1.89 kg, respectively, in Japanese patients. The highly selective SGLT2 inhibitor ipragliflozin improves glycemic control and reduces body weight, and lowers hypoglycemic risk and abdominal symptoms. Ipragliflozin has potential as a novel anti-diabetic and anti-obesity agent.

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is characterized by insulin resistance and defective insulin secretion[1]. Hyperglycemia

is caused by glucose influx exceeding glucose outflow from the plasma compartment[2]. In the fasting state, hyperglycemia is related to increased hepatic glucose production[2]. In the postprandial state, further glucose excursions result from insufficient glucose output suppression and defective insulin stimulation of glucose disposal in target tissues[2]. Once the renal tubular transport maximum for glucose exceeds, glycosuria curbs, but does not prevent further hyperglycemia[2].

Oral hypoglycemic agents include insulin secretagogues [sulfonylureas, meglitinides, and dipeptidyl peptidase-4 (DPP-4) inhibitors] and insulin sensitizers [metformin and thiazolidinediones (TZDs)][3]. α-glucosidase inhibitors decrease glucose absorption. The American Diabetes Association (ADA) and the European Association for the Study of Diabetes recom-mend metformin as the first-line oral therapy[2,3]. If the glycated hemoglobin (HbA1c) target is not achieved by 3 mo, either sulfonylurea, TZD, DPP-4 inhibitor, GLP-1 receptor agonist, or basal insulin should be combined with metformin[2].

The ADA recommends lowering HbA1c to < 7.0% to reduce microvascular disease incidence[4]. However, only approximately half of T2DM patients achieve this[3,5]. Oral hypoglycemic agents have side effects: hypoglycemia and weight gain (sulphonylureas)[6]; peripheral edema, weight gain, and fractures (TZDs)[7]; a possible increased risk of bladder cancer (pioglitazone)[8]; and abdominal symptoms (metformin and α-glucosidase inhibitors). Metformin can also cause lactate acidosis.

Few insulin sensitizers and anti-obesity agents exist. Mazindol maintains body weight after obesity therapy and treats obesity-related diseases such as diabetes, hypertension, and hyperlipidemia[9], but has side effects including tremor, nausea, vomiting, and diarrhea. Therefore, novel anti-diabetic and anti-obesity agents are required.

SODIUM-GLUCOSE COTRANSPORTER TYPE 2

The kidney is important in glucose metabolism; it is a target for therapeutic intervention[10]. Sodium-glucose cotransporter 2 (SGLT2) mediates glucose reabsorption from the proximal renal tubule[10]. SGLT2 inhibition induces glucosuria and lowers blood glucose in diabetes, and a lowers hypoglycemic risk[10].

Ipragliflozin is an SGLT2 inhibitor first released in Japan (Figure 1)[3]. Here studies on ipragliflozin and other SGLT2 inhibitors are reviewed.

PHARMACOLOGY, MODE OF ACTION, AND PHARMACOKINETICS

In vitro SGLT inhibition

Two types of SGLT exist: SGLT1 and SGLT2. SGLT1 is expressed in the kidney and intestine; intestinal SGLT1 inhibition causes abdominal symptoms such as diarrhea. It is pivotal for intestinal mass absorption of d-glucose and triggers glucose-induced secretion of gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP-1)[11]. Therefore, SGLT1 inhibition reduces incretin secretion. Miglitol (α-glucosidase inhibitor) suppresses GIP and increases GLP-1, reducing body weight and improving glycemic control[12], but suppression of GLP-1 reduces insulin secretion[13]. Therefore, SGLT2 selectivity is important. The selectivity of currently available SGLT2 inhibitors is presented in Table 1[3,14-19].

Urinary glucose excretion

Healthy Japanese subjects receiving ipragliflozin excreted approximately 70 and 50 g of glucose/24 h after a single 300 mg dose or after multiple 50 or 100 mg doses, respectively[20]. In healthy European subjects, ipragliflozin dose-dependently increased urinary glucose excretion (UGE) to a maximum of approximately 59 g/24 h (327 mmol/24 h) (dose: 5-600 mg/d) without affecting plasma glucose levels[21]. In T2DM, ipragliflozin increased UGE to a maximum of approximately 90 g/24 h after 28 d of treatment with 300 mg/d[22]. Therefore, SGLT2 inhibitors increased UGE more in T2DM patients compared with healthy subjects[3]. Human exfoliated proximal tubular epithelial cells (HEPTECs) from T2DM patients expressed significantly more SGLT2 and the facilitative glucose transporter GLUT2 than cells from healthy individuals[23]. Renal glucose uptake in HEPTECs isolated from T2DM patients was markedly increased compared with that in healthy controls[23]. Therefore, renal glucose transporter expression and activity is increased in T2DM[23]. In T2DM patients, ipragliflozin increases glycosuria directly proportional to the glomerular filtration rate (GFR) and degree of hyperglycemia, so it can be reliably predicted for individuals[24]. Although absolute glycosuria decreases with declining GFR, ipragliflozin efficiency is maintained in patients with severe renal impairment[24].

Effect of ipragliflozin on the pharmacokinetics of other medications

AUCinf or Cmax of single doses of sitagliptin, pioglitazone, or glimepiride[25] were unaffected by multiple doses of ipragliflozin; the combination was well tolerated in healthy subjects[25]. Ipragliflozin (300 mg qd) and metformin together were well tolerated in T2DM patients; the addition of ipragliflozin did not result in a clinically relevant change in the pharmacokinetic properties of metformin[26]. Dose adjustments may not be required when ipragliflozin is administered with other glucose-lowering drugs[25].

Effect of moderate hepatic impairment on the pharmacokinetics of ipragliflozin

Moderate hepatic impairment had no clinically relevant effects on the single-dose pharmacokinetics of ipragliflozin and its major metabolite[27]. A single oral dose of ipragliflozin 100 mg was well tolerated in healthy subjects

Table 1 Sodium-glucose cotransporter 2 selectivity of sodium-glucose cotransporter 2 inhibitors

	Company	IC_{50} for human SGLT1/SGLT2 (nmol/L)	SGLT2 selectivity (fold)
Phlorizin		210/34.6	6
Ipragliflozin	Astellas	1876/7.38	254
canagliflozin	Johnson and Johnson Mitsubishi Tanabe	684/4.4	155
Dapagliflozin	Bristol-Myers Squibb AstraZeneca	1391/1.12	1242
Empagliflozin	Boehringer Ingelheim	8300/3.1	2680
Tofogliflozin	Chugai	8444/2.9	2912
luseogliflozin	Taisho	3990/2.26	1770

SGLT2 selectivity was calculated by using the following formula: IC_{50} value for SGLT1/IC_{50} value for SGLT2. IC_{50}: Half maximal (50%) inhibitory concentration; SGLT: Sodium–glucose cotransporter.

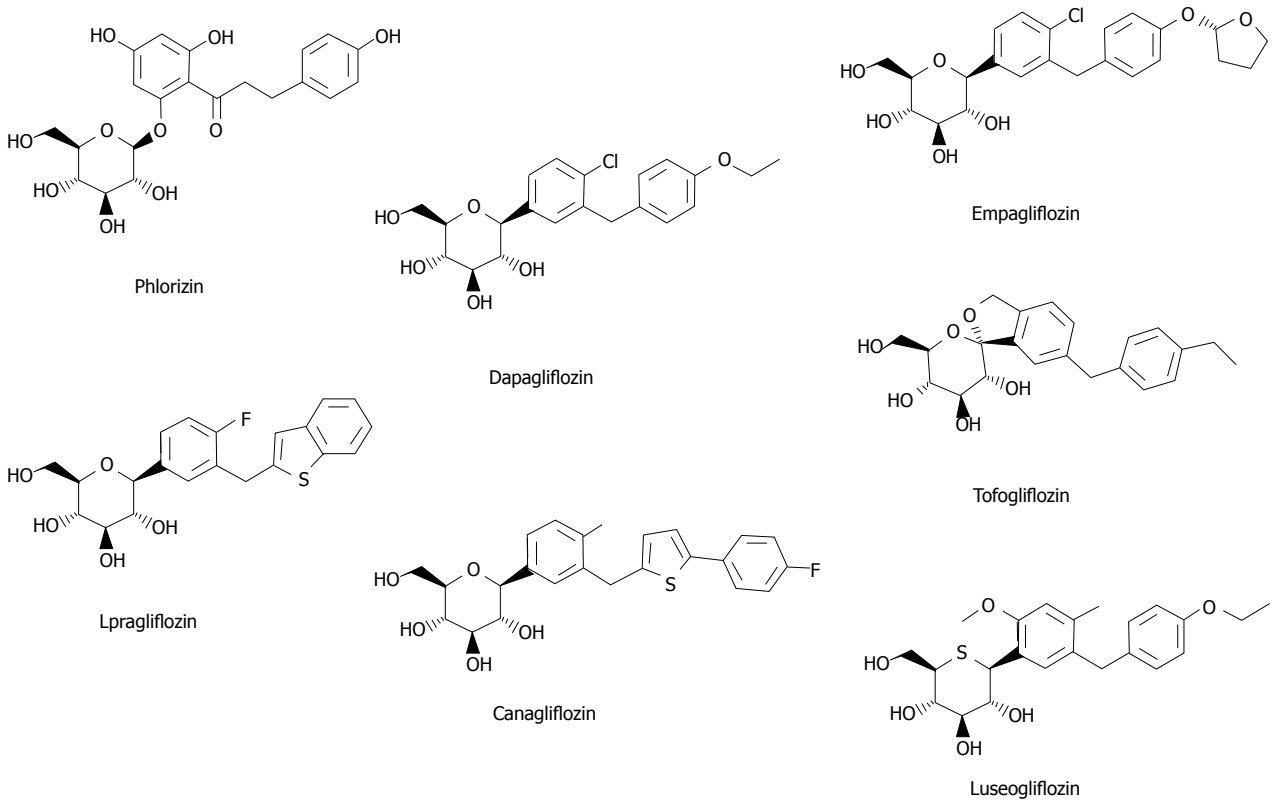

Figure 1 Chemical structure of sodium-glucose cotransporter 2 inhibitors in late-stage clinical trials.

and those with moderate hepatic impairment[27].

EFFICACY AND COMPARATOR STUDIES WITH OTHER SGLT2 INHIBITORS

HbA1c

In Western T2DM patients, a 12-wk treatment with ipragliflozin 12.5, 50, 150, and 300 mg/d reduced HbA1c by 0.49%, 0.65%, 0.73%, and 0.81%, respectively, compared with placebo treatment (Figure 2)[28]. In Japanese patients, 12-wk treatment with ipragliflozin 12.5, 25, 50, and 100 mg/d reduced HbA1c by 0.61%, 0.97%, 1.29%, and 1.31%, respectively, compared with placebo treatment[29].

Canagliflozin 50, 100, 200, 300 mg/d and 300 mg twice daily for 12 wk significantly reduced HbA1C by 0.79%, 0.76%, 0.70%, 0.92%, and 0.95%, respectively, compared with reductions of 0.22% for placebo (all $P < 0.001$), and 0.74% for sitagliptin[30]. The adjusted mean difference in HbA1c between placebo and 100 mg canagliflozin was -0.54%[30] (Figure 2). Dapagliflozin 2.5, 5, and 10 mg reduced HbA1c by 0.67%, 0.70%, and 0.84%, respectively[31]. Empagliflozin 5, 10, and 25 mg for 12 wk reduced HbA1c by 0.4%, 0.5%, and 0.6% compared with placebo (+0.09%)[32]. Ipragliflozin reduced HbA1c levels when added to metformin (-0.87 ± 0.66), pioglitazone (-0.64 ± 0.609), or sulfonylurea (-0.83 ± 0.717)[3,33].

Fasting plasma glucose

In Western T2DM patients, 12-wk of ipragliflozin

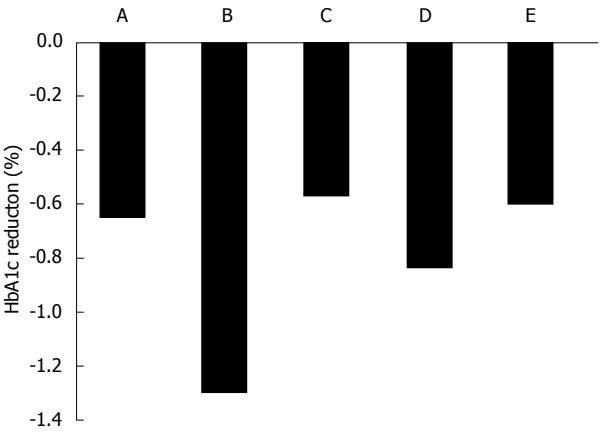

Figure 2 The adjusted mean difference in HbA1c from baseline to 12 wk between placebo and the standard dose of sodium-glucose cotransporter 2 inhibitors. A: 50 mg ipragliflozin in Westerners; B: 50 mg ipragliflozin in Japanese; C: 100 mg canagliflozin in Westerners; D: 10 mg dapagliflozin in Westerners; E: 25 mg empagliflozin in Westerners.

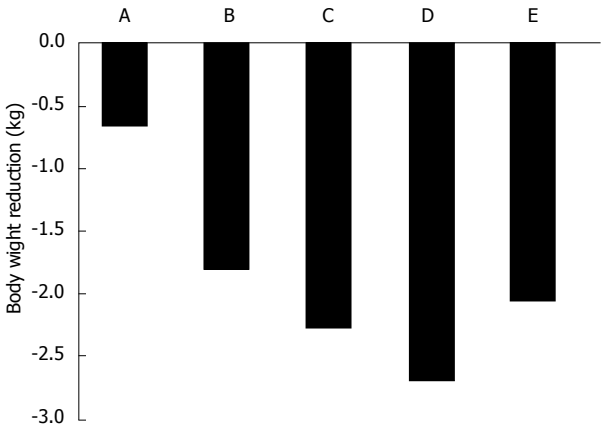

Figure 3 The adjusted mean difference in body weight from baseline to 12 wk between placebo and the standard dose of sodium-glucose cotransporter 2 inhibitors. A: 50 mg ipragliflozin in Westerners; B: 50 mg ipragliflozin in Japanese; C: 100 mg canagliflozin in Westerners; D: 10 mg dapagliflozin in Westerners; E: 25 mg empagliflozin in Westerners.

treatment at 12.5, 50, 150, and 300 mg/d decreased fasting plasma glucose (FPG) by 0.84, 1.10, 1.30, and 1.68 mmol/L, respectively compared with placebo[28]. In Japanese T2DM patients, 12.5, 25, 50, and 100 mg ipragliflozin decreased FPG from baseline by 15.6, 23.7, 34.1, and 46.9 mg/dL (0.87, 1.32, 1.89 and 2.60 mmol/L) compared with +12.0 mg/dL for placebo[29].

Body weight

In T2DM patients, SGLT2 inhibitors ipragliflozin, dapagliflozin, and canagliflozin reduced body weight by approximately 2 kg[3] (Figure 3). In Western individuals, the standard dose of 50-mg ipragliflozin for 12 wk reduced body weight by 0.66 kg[28]. In Japanese T2DM patients, 12-wk of placebo or 12.5-100 mg ipragliflozin treatment reduced body weight by 0.39 kg and 1.46-2.10 kg, respectively[29]. Twelve-weeks of canagliflozin 100 mg[30], dapagliflozin 10 mg[34], or empagliflozin 25 mg[32] reduced body weight by 2.28, 2.7, and 2.06 kg, respectively.

Most weight loss in patients receiving dapagliflozin is related to visceral and subcutaneous fat loss[3,35]. After 24-wk of dapagliflozin treatment at 10 mg/d, placebo-corrected changes were -2.08 kg body weight, -1.52 cm waist circumference, -1.48 kg total body fat mass, -258.4 cm^3 visceral adipose tissue, and -184.9 cm subcutaneous adipose tissue[3,35]. Compared with placebo, 26.2% more patients achieved weight reduction of at least 5%[3,35].

Blood pressure

SGLT2 inhibitors decrease blood pressure *via* osmotic diuresis induced by glucose in the urine earlier during treatment[3]. Ipragliflozin 50 mg for 16 wk reduced systolic blood pressure by 3.2 mmHg and diastolic blood pressure by 2.5 mmHg, without hypotension[36]. Dapagliflozin for 12 wk reduced systolic blood pressure by 2.6-6.4 mmHg, with no clear dose-dependent relationship, but changes in diastolic blood pressure and heart rate were small and inconsistent[34]. Small dose-related increases in 24-h urine volumes were observed (107-470 mL above baseline volumes of 1.8-2.2 L)[34].

Canagliflozin 100 and 300 mg for 26 wk significantly reduced systolic BP by 3.7 and 5.4 mmHg, respectively, compared with placebo (both $P < 0.001$)[37]. Diastolic BP was also reduced by 1.6 and 2.0 mmHg, respectively[37]. Minimal changes in heart rate were observed with canagliflozin 100 and 300 mg compared with placebo (-1.6, -0.5, and +1.4 beats/min, respectively)[37]. Empagliflozin 25 mg for 12 wk decreased systolic blood pressure by 3.4 mmHg, and diastolic blood pressure by 1.7 mmHg, but there was no significant difference compared with placebo[32]. Overall, SGLT2 inhibitors reduced blood pressure by approximately 2-6 mmHg.

Beta-cell function

Chronic hyperglycemia induces β-cell dysfunction and insulin resistance[38]. SGLT2 inhibitors improve glucose toxicity and glycemic control[3]. There are no clinical reports on effect of ipragliflozin on β-cell function but ipragliflozin increased insulin content in the pancreas and suppressed the loss of insulin-positive cells in islets of db/db mice, an animal model of T2DM[3,39].

Compared with placebo, canagliflozin 100 mg/d for 12 wk significantly improved β-cell function as assessed by homeostasis model assessment 2 (HOMA2)-%B (measure of fasting insulin secretion)[30]. Another study reported improvements in β-cell function following 26-wk treatment with canagliflozin 100 and 300 mg compared with placebo, with increases in HOMA2-%B of 12.4 and 22.8, respectively[37].

Proinsulin/insulin (PI/I) ratio reflects β-cell dysfunction associated with the onset and progression of T2DM[40,41]. Mitiglinide improved the postprandial insulin secretion profile, suppressed the postprandial glucose spike, and improved the PI/I ratio in T2DM patients with low insulin resistance and low triglyceride levels[42].

Dose-related decreases in proinsulin/insulin ratio of 0.5 and 0.8 pmol/mIU were observed with canagliflozin at 100 and 300 mg, respectively, compared with placebo, and decreases in proinsulin/C peptide ratio were also seen with both doses of canagliflozin[37]. These results suggest that SGLT2 inhibitors improve β-cell function.

Insulin resistance

To date, there are no clinical reports on effect of ipragliflozin on insulin resistance. However, reductions in HOMA2 insulin resistance after dapagliflozin treatment at 2.5 and 10 mg for 12 wk were significantly larger compared with placebo[43]. The most precise method to assess insulin resistance is the glucose clamp technique[44]. Results of a hyperinsulinemic-euglycemic clamp study demonstrated that within 3 d of completing 2-wk of dapagliflozin treatment, Zucker diabetic fatty rats displayed improved glucose utilization accompanied by reduced glucose production and enhanced glucose influx into liver tissue[16]. In a clamp study of T2DM patients, 12-wk of dapagliflozin treatment increased glucose disposal rates[3,45]. There are few glucose clamp studies of SGLT2 inhibitors because the method is complex and expensive[46]. Recently, a novel insulin resistance index "20/(fasting C-peptide × fasting plasma glucose)," to estimate the insulin resistance index was derived from the glucose clamp method[46]. This index will evaluate insulin resistance in clinical studies.

SAFETY, EFFICACY, AND TOLERABILITY

Genito-urinary tract infections

A meta-analysis of 45 clinical trials indicated that SGLT2 inhibitors increased the risk of urinary and genital tract infections [odds ratios, 1.42 (95%CI: 1.06-1.90) and 5.06 (95%CI: 3.44-7.45)], respectively, probably a result of glucosuria[47].

In ipragliflozin phase 3 trial, treatment-emergent urinary tract infections (UTIs) were reported in 32/412 patients across all treatment groups, including placebo[28]. Infections were symptomatic and asymptomatic in 9 and 23 patients, respectively[28]. A total of 14 patients experienced treatment-emergent genital tract infections but there was no evidence that the frequency was related to the dose of ipragliflozin[28]. All events were treated with antifungal or antibacterial agents and were resolved prior to the final study visit (except three)[28]. In canagliflozin phase 3 trial, the incidence of genital mycotic infections, UTIs, and osmotic diuresis-related adverse events was higher in the treatment group[37]. UTIs were observed in 5%-12% of dapagliflozin-treated patients (with no clear dose relationship) compared with 6% of placebo-treated patients and 9% of metformin-treated patients[34]. Genital infections were observed in 2%-7% of dapagliflozin treated patients, 0% of placebo-treated patients, and 2% of metformin-treated patients[34]. Therefore, STLT2 inhibitors might increase the risk of UTIs.

Hypoglycemia

In a multi-center Japanese study of 361 patients randomized to receive either ipragliflozin (12.5, 25, 50, or 100 mg/d) or a placebo for 12 wk, a single mild symptomatic hypoglycemic event (not confirmed by plasma glucose measurement) occurred in one patient in the 100-mg ipragliflozin group[29]. In ipragliflozin phase 3 trial, only one patient in each of the ipragliflozin 50 mg (67 patients) and 300 mg (68 patients) dose groups experienced treatment-emergent hypoglycemia[29]. In T2DM patients, ipragliflozin did not significantly increase the incidence of hypoglycemic events compared to placebo, even in combination with other hypoglycemic agents[3,48]. Hypoglycemic events were reported in 6%-10% of patients treated with dapagliflozin, with no dose-dependent relationship, compared with 4% and 9% for placebo and metformin, respectively[34]. There were no symptomatic hypoglycemic events with a fingerstick glucose of ⩽ 50 mg/dL[34]. In canagliflozin phase 3 trial, the incidence of hypoglycemia was similar for canagliflozin 100 and 300 mg and placebo (3.6%, 3.0%, and 2.6%, respectively), with no report of severe hypoglycemia[37]. Therefore, these data suggest that SLGT2 inhibitors lowers hypoglycemic risk.

Osmotic diuretic effect

Ipragliflozin caused a mild 1.5%-2.0% increase in hematocrit at all doses[29]. Similarly, blood urea nitrogen (BUN) was also mildly increased by 1.0-2.2 mg/dL compared with placebo[29].

Cancer risk

An increased incidence of bladder and breast cancer was indicated in patients receiving dapagliflozin compared with controls[47]. Data on bladder and breast cancer were retrieved from regulatory databases and other sources to produce a pool of 5501 patients (at least 5000 patient-years of exposure to dapagliflozin), and a total of 3184 patients (at least 2350 patient-years of exposure to placebo or an active comparator)[49,50]. Nine cases of bladder cancer were identified in patients treated with dapagliflozin compared with one case in patients receiving placebo[49,50]. The number of observed cases exceeds the expected number in the general diabetic population[47]. UTIs may increase the risk of bladder cancer. However, early detection after short exposure and potential detection bias related to frequent urinalysis mitigate against a causative relationship[47]. Therefore, no robust conclusions can be drawn, pending accumulation of long-term data[47].

There were 9 cases of breast cancer in the dapagliflozin group (2223 patients) compared with one case in the placebo group (1053 patients), diagnosed within the first year of the study[47]. These figures were higher than the predicted number of 7.1 cases based on the Surveillance Epidemiology and End Results (SEER) program[51]. It remains uncertain whether the use of dapagliflozin is associated with an increased risk of breast cancer and further studies are needed[47]. There are no reports

indicating that other SGLT2 inhibitors are associated with an increased risk of cancer[3].

Safety and tolerability of metformin combination therapy

A meta-analysis of 20 randomized, double-blind studies demonstrated SGLT2 inhibitors administered with metformin significantly decreased the incidence of diarrhea[52]. However, the addition of SGLT2 inhibitors increased the risk of genital infection[52]. Despite some limitations, SGLT2 inhibitors have a favorable safety profile, and combination therapy with metformin is well tolerated[52].

Patient-focused data on quality of life, satisfaction, and acceptability

One study investigated effect of ipragliflozin on quality of life[28]. Outcomes were assessed using the European Quality of Life-5 Dimensions (EQ-5D)[53], Audit of Diabetes-Dependent Quality of Life (ADDQoL)[54], and Diabetes Medication Satisfaction (Diab-MedSat) questionnaires[55]. No differences were observed in EQ-5D domains or ADDQoL scores at week 12[28]. However, mean changes in EQ-5D visual analogue scale scores from baseline to week 12 showed positive changes in the treatment groups, suggesting improvements in perceived health status[28]. Changes in Diab-MedSat scores for burden and symptoms were small and similar across all treatment groups, but changes in the efficacy score from baseline to week 12 were greater for the ipragliflozin groups[28]. Another study reported that changes from baseline to week 12 in EQ-5D domains and ADDQoL scores were small across all treatment groups but with a non-statistically significant trend for improvement in the ipragliflozin treatment groups[33]. These results suggest that the SGLT2 inhibitor ipragliflozin may improve the quality of life in T2DM patients.

Ethnic differences

There are no clinical reports on ethnic differences in effects of ipragliflozin. However, past reports imply that ipragliflozin reduces HbA1c more in Japanese patients compared with Western patients (Figure 2)[28,29]. The mechanism is unclear, but a meta-analysis reported that DPP-4 inhibitors were associated with a reduction in HbA1c of 0.65% in non-Japanese randomized, controlled trials (RCTs; 55 patients), compared with 1.67% in Japanese RCTs[56]. There may be pharmacogenetic or cultural lifestyle differences that contribute to the larger reduction in HbA1c in Japanese patients. Japanese people have a greater amount of abdominal visceral fat relative to abdominal subcutaneous fat compared with Caucasians[57]. Dapagliflozin reduced visceral adipose tissue more than subcutaneous adipose tissue[35]. Therefore, the difference in visceral adipose tissue between Japanese and Western T2DM patients may contribute to the difference in effect of SGLT2 inhibitors.

Japanese and Asian patients often show reduced β-cell function[46] and East Asians may have a limited innate capacity for insulin secretion[58,59]. The body mass index (BMI) of Japanese T2DM patients was significantly correlated with insulin secretion ability in a meal tolerance test; the insulin secretion ability diminished in patients with BMI < 20 kg/m^2[60]. Other reported complications associated with familial renal glucosuria include episodes of ketosis, UTIs, and natriuresis[61]. SGLT2 inhibitors increase blood ketone bodies[62]. Low insulin secretion ability and lean stature in Asian patients receiving SGLT2 inhibitors may increase the risk of ketosis; therefore, caution is required.

CONCLUSIONS AND PLACE IN THERAPY ALONGSIDE OTHER SGLT2 INHIBITORS

SGLT2 inhibitor ipragliflozin improves glycemic control and reduces body weight, especially in Japanese T2DM patients. Furthermore, ipragliflozin lowers hypoglycemic risk and abdominal symptoms and can be safely used with sulphonylureas, metformin, pioglitazone, and DPP4 inhibitors. SGLT2 inhibitors are likely to improve β-cell function and insulin sensitivity. They offer great potential as novel anti-diabetic and anti-obesity agents. Ipragliflozin is particularly effective for Japanese T2DM patients with a greater abdominal visceral fat relative to abdominal subcutaneous fat than Caucasians. Ipragliflozin is a highly selective SGLT2 inhibitor, and lower hypoglycemic risk and abdominal symptoms.

REFERENCES

1 **DeFronzo RA**. Lilly lecture 1987. The triumvirate: beta-cell, muscle, liver. A collusion responsible for NIDDM. *Diabetes* 1988; **37**: 667-687 [PMID: 3289989 DOI: 10.2337/diab.37.6.667]

2 **Inzucchi SE**, Bergenstal RM, Buse JB, Diamant M, Ferrannini E, Nauck M, Peters AL, Tsapas A, Wender R, Matthews DR. Management of hyperglycemia in type 2 diabetes: a patient-centered approach: position statement of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetes Care* 2012; **35**: 1364-1379 [PMID: 22517736 DOI: 10.2337/dc12-0413]

3 **Kurosaki E**, Ogasawara H. Ipragliflozin and other sodium-glucose cotransporter-2 (SGLT2) inhibitors in the treatment of type 2 diabetes: preclinical and clinical data. *Pharmacol Ther* 2013; **139**: 51-59 [PMID: 23563279 DOI: 10.1016/j.pharmthera.2013.04.003]

4 **American Diabetes Association.** Standards of medical care in diabetes--2011. *Diabetes Care* 2011; **34** Suppl 1: S11-S61 [PMID: 21193625 DOI: 10.2337/dc11-S011]

5 **Ong KL**, Cheung BM, Wong LY, Wat NM, Tan KC, Lam KS. Prevalence, treatment, and control of diagnosed diabetes in the U.S. National Health and Nutrition Examination Survey 1999-2004. *Ann Epidemiol* 2008; **18**: 222-229 [PMID: 18201902 DOI: 10.1016/j.annepidem.2007.10.007]

6 **Gallwitz B**, Häring HU. Future perspectives for insulinotropic agents in the treatment of type 2 diabetes-DPP-4 inhibitors and sulphonylureas. *Diabetes Obes Metab* 2010; **12**: 1-11 [PMID: 19788431 DOI: 10.1111/j.1463-1326.2009.01095.x]

7 **Shah P**, Mudaliar S. Pioglitazone: side effect and safety profile. *Expert Opin Drug Saf* 2010; **9**: 347-354 [PMID: 20175701 DOI: 10.1517/14740331003623218]

8 **Lewis JD**, Ferrara A, Peng T, Hedderson M, Bilker WB, Quesenberry CP, Vaughn DJ, Nessel L, Selby J, Strom BL.

Risk of bladder cancer among diabetic patients treated with pioglitazone: interim report of a longitudinal cohort study. *Diabetes Care* 2011; **34**: 916-922 [PMID: 21447663 DOI: 10.2337/dc10-1068]

9 **Inoue S**, Egawa M, Satoh S, Saito M, Suzuki H, Kumahara Y, Abe M, Kumagai A, Goto Y, Shizume K. Clinical and basic aspects of an anorexiant, mazindol, as an antiobesity agent in Japan. *Am J Clin Nutr* 1992; **55**: 199S-202S [PMID: 1728834]

10 **Kim Y**, Babu AR. Clinical potential of sodium-glucose cotransporter 2 inhibitors in the management of type 2 diabetes. *Diabetes Metab Syndr Obes* 2012; **5**: 313-327 [PMID: 22977310 DOI: 10.2147/DMSO.S22545]

11 **Gorboulev V**, Schürmann A, Vallon V, Kipp H, Jaschke A, Klessen D, Friedrich A, Scherneck S, Rieg T, Cunard R, Veyhl-Wichmann M, Srinivasan A, Balen D, Breljak D, Rexhepaj R, Parker HE, Gribble FM, Reimann F, Lang F, Wiese S, Sabolic I, Sendtner M, Koepsell H. Na(+)-D-glucose cotransporter SGLT1 is pivotal for intestinal glucose absorption and glucose-dependent incretin secretion. *Diabetes* 2012; **61**: 187-196 [PMID: 22124465 DOI: 10.2337/db11-1029]

12 **Sumi K,** Ohkura T, Yamamoto N, Fujioka Y, Matsuzawa K, Izawa S, Shiochi H, Kinoshita H, Ohkura H, Kato M, Yamamoto K, Taniguchi S. Long-term miglitol administration suppresses postprandial glucose-dependent insulinotropic polypeptide secretion. *Diabetology International* 2013; **4**: 190-196 [DOI: 10.1007/s13340-013-0116-0]

13 **Schirra J**, Sturm K, Leicht P, Arnold R, Göke B, Katschinski M. Exendin(9-39)amide is an antagonist of glucagon-like peptide-1(7-36)amide in humans. *J Clin Invest* 1998; **101**: 1421-1430 [PMID: 9525985 DOI: 10.1172/JCI1349]

14 **Tahara A**, Kurosaki E, Yokono M, Yamajuku D, Kihara R, Hayashizaki Y, Takasu T, Imamura M, Qun L, Tomiyama H, Kobayashi Y, Noda A, Sasamata M, Shibasaki M. Pharmacological profile of ipragliflozin (ASP1941), a novel selective SGLT2 inhibitor, in vitro and in vivo. *Naunyn Schmiedebergs Arch Pharmacol* 2012; **385**: 423-436 [PMID: 22139434 DOI: 10.1007/s00210-011-0713-z]

15 **Liang Y**, Arakawa K, Ueta K, Matsushita Y, Kuriyama C, Martin T, Du F, Liu Y, Xu J, Conway B, Conway J, Polidori D, Ways K, Demarest K. Effect of canagliflozin on renal threshold for glucose, glycemia, and body weight in normal and diabetic animal models. *PLoS One* 2012; **7**: e30555 [PMID: 22355316 DOI: 10.1371/journal.pone.0030555]

16 **Han S**, Hagan DL, Taylor JR, Xin L, Meng W, Biller SA, Wetterau JR, Washburn WN, Whaley JM. Dapagliflozin, a selective SGLT2 inhibitor, improves glucose homeostasis in normal and diabetic rats. *Diabetes* 2008; **57**: 1723-1729 [PMID: 18356408 DOI: 10.2337/db07-1472]

17 **Grempler R**, Thomas L, Eckhardt M, Himmelsbach F, Sauer A, Sharp DE, Bakker RA, Mark M, Klein T, Eickelmann P. Empagliflozin, a novel selective sodium glucose cotransporter-2 (SGLT-2) inhibitor: characterisation and comparison with other SGLT-2 inhibitors. *Diabetes Obes Metab* 2012; **14**: 83-90 [PMID: 21985634 DOI: 10.1111/j.1463-1326.2011.01517.x]

18 **Ohtake Y**, Sato T, Kobayashi T, Nishimoto M, Taka N, Takano K, Yamamoto K, Ohmori M, Yamaguchi M, Takami K, Yeu SY, Ahn KH, Matsuoka H, Morikawa K, Suzuki M, Hagita H, Ozawa K, Yamaguchi K, Kato M, Ikeda S. Discovery of tofogliflozin, a novel C-arylglucoside with an O-spiroketal ring system, as a highly selective sodium glucose cotransporter 2 (SGLT2) inhibitor for the treatment of type 2 diabetes. *J Med Chem* 2012; **55**: 7828-7840 [PMID: 22889351 DOI: 10.1021/jm300884k]

19 **Kakinuma H**, Oi T, Hashimoto-Tsuchiya Y, Arai M, Kawakita Y, Fukasawa Y, Iida I, Hagima N, Takeuchi H, Chino Y, Asami J, Okumura-Kitajima L, Io F, Yamamoto D, Miyata N, Takahashi T, Uchida S, Yamamoto K. (1S)-1,5-anhydro-1-[5-(4-ethoxybenzyl)-2-methoxy-4-methylphenyl]-1-thio-D-glucitol (TS-071) is a potent, selective sodium-dependent glucose cotransporter 2 (SGLT2) inhibitor for type 2 diabetes treatment. *J Med Chem* 2010; **53**: 3247-3261 [PMID: 20302302 DOI: 10.1021/jm901893x]

20 **Kadokura T**, Saito M, Utsuno A, Kazuta K, Yoshida S, Kawasaki S, Nagase I, Kageyama S. Ipragliflozin (ASP1941), a selective sodium-dependent glucose cotransporter 2 inhibitor, safely stimulates urinary glucose excretion without inducing hypoglycemia in healthy Japanese subjects. *Diabetol Int* 2011; **2**: 172-182 [DOI: 10.1007/s13340-011-0037-8]

21 **Veltkamp SA**, Kadokura T, Krauwinkel WJ, Smulders RA. Effect of Ipragliflozin (ASP1941), a novel selective sodium-dependent glucose co-transporter 2 inhibitor, on urinary glucose excretion in healthy subjects. *Clin Drug Investig* 2011; **31**: 839-851 [PMID: 21877761 DOI: 10.1007/BF03256922]

22 **Schwartz SL**, Akinlade B, Klasen S, Kowalski D, Zhang W, Wilpshaar W. Safety, pharmacokinetic, and pharmacodynamic profiles of ipragliflozin (ASP1941), a novel and selective inhibitor of sodium-dependent glucose co-transporter 2, in patients with type 2 diabetes mellitus. *Diabetes Technol Ther* 2011; **13**: 1219-1227 [PMID: 21854192 DOI: 10.1089/dia.2011.0012]

23 **Rahmoune H**, Thompson PW, Ward JM, Smith CD, Hong G, Brown J. Glucose transporters in human renal proximal tubular cells isolated from the urine of patients with non-insulin-dependent diabetes. *Diabetes* 2005; **54**: 3427-3434 [PMID: 16306358 DOI: 10.2337/diabetes.54.12.3427]

24 **Ferrannini E**, Veltkamp SA, Smulders RA, Kadokura T. Renal glucose handling: impact of chronic kidney disease and sodium-glucose cotransporter 2 inhibition in patients with type 2 diabetes. *Diabetes Care* 2013; **36**: 1260-1265 [PMID: 23359360 DOI: 10.2337/dc12-1503]

25 **Smulders RA**, Zhang W, Veltkamp SA, van Dijk J, Krauwinkel WJ, Keirns J, Kadokura T. No pharmacokinetic interaction between ipragliflozin and sitagliptin, pioglitazone, or glimepiride in healthy subjects. *Diabetes Obes Metab* 2012; **14**: 937-943 [PMID: 22587345 DOI: 10.1111/j.1463-1326.2012.01624.x]

26 **Veltkamp SA**, van Dijk J, Collins C, van Bruijnsvoort M, Kadokura T, Smulders RA. Combination treatment with ipragliflozin and metformin: a randomized, double-blind, placebo-controlled study in patients with type 2 diabetes mellitus. *Clin Ther* 2012; **34**: 1761-1771 [PMID: 22795925 DOI: 10.1016/j.clinthera.2012.06.027]

27 **Zhang W**, Krauwinkel WJ, Keirns J, Townsend RW, Lasseter KC, Plumb L, Kadokura T, Ushigome F, Smulders R. The effect of moderate hepatic impairment on the pharmacokinetics of ipragliflozin, a novel sodium glucose co-transporter 2 (SGLT2) inhibitor. *Clin Drug Investig* 2013; **33**: 489-496 [PMID: 23733389 DOI: 10.1007/s40261-013-0089-6]

28 **Fonseca VA**, Ferrannini E, Wilding JP, Wilpshaar W, Dhanjal P, Ball G, Klasen S. Active- and placebo-controlled dose-finding study to assess the efficacy, safety, and tolerability of multiple doses of ipragliflozin in patients with type 2 diabetes mellitus. *J Diabetes Complications* 2013; **27**: 268-273 [PMID: 23276620 DOI: 10.1016/j.jdiacomp.2012.11.005]

29 **Kashiwagi A,** Kazuta K, Yoshida S, Nagase I. Randomized, placebo-controlled, double-blind glycemic control trial of novel sodium-dependent glucose cotransporter 2 inhibitor ipragliflozin in Japanese patients with type 2 diabetes mellitus. *J Diabetes Invest* 2013; **5**: 382-391 [DOI: 10.1111/jdi.12156]

30 **Rosenstock J**, Aggarwal N, Polidori D, Zhao Y, Arbit D, Usiskin K, Capuano G, Canovatchel W. Dose-ranging effects of canagliflozin, a sodium-glucose cotransporter 2 inhibitor, as add-on to metformin in subjects with type 2 diabetes. *Diabetes Care* 2012; **35**: 1232-1238 [PMID: 22492586 DOI: 10.2337/dc11-1926]

31 **Bailey CJ**, Gross JL, Pieters A, Bastien A, List JF. Effect of dapagliflozin in patients with type 2 diabetes who have inadequate glycaemic control with metformin: a

randomised, double-blind, placebo-controlled trial. *Lancet* 2010; **375**: 2223-2233 [PMID: 20609968 DOI: 10.1016/S0140-6736(10)60407-2]

32 **Ferrannini E**, Seman L, Seewaldt-Becker E, Hantel S, Pinnetti S, Woerle HJ. A Phase IIb, randomized, placebo-controlled study of the SGLT2 inhibitor empagliflozin in patients with type 2 diabetes. *Diabetes Obes Metab* 2013; **15**: 721-728 [PMID: 23398530 DOI: 10.1111/dom.12081]

33 **Wilding JP**, Ferrannini E, Fonseca VA, Wilpshaar W, Dhanjal P, Houzer A. Efficacy and safety of ipragliflozin in patients with type 2 diabetes inadequately controlled on metformin: a dose-finding study. *Diabetes Obes Metab* 2013; **15**: 403-409 [PMID: 23163880 DOI: 10.1111/dom.12038]

34 **List JF**, Woo V, Morales E, Tang W, Fiedorek FT. Sodium-glucose cotransport inhibition with dapagliflozin in type 2 diabetes. *Diabetes Care* 2009; **32**: 650-657 [PMID: 19114612 DOI: 10.2337/dc08-1863]

35 **Bolinder J**, Ljunggren Ö, Kullberg J, Johansson L, Wilding J, Langkilde AM, Sugg J, Parikh S. Effects of dapagliflozin on body weight, total fat mass, and regional adipose tissue distribution in patients with type 2 diabetes mellitus with inadequate glycemic control on metformin. *J Clin Endocrinol Metab* 2012; **97**: 1020-1031 [PMID: 22238392 DOI: 10.1210/jc.2011-2260]

36 **Kashiwagi A,** Takinami Y, Kazuta K, Yoshida S, Utsuno A, Nagase I. Ipragliflozin improved glycaemic control with additional benefits of reductions of body weight and blood pressure in Japanese patients with type 2 diabetes mellitus: BRIGHTEN study. The European Association for the Study of Diabetes. Lisbon, Portugal, 2011: 47th Annual Meeting

37 **Stenlöf K**, Cefalu WT, Kim KA, Alba M, Usiskin K, Tong C, Canovatchel W, Meininger G. Efficacy and safety of canagliflozin monotherapy in subjects with type 2 diabetes mellitus inadequately controlled with diet and exercise. *Diabetes Obes Metab* 2013; **15**: 372-382 [PMID: 23279307 DOI: 10.1111/dom.12054]

38 **Robertson RP**, Harmon J, Tran PO, Poitout V. Beta-cell glucose toxicity, lipotoxicity, and chronic oxidative stress in type 2 diabetes. *Diabetes* 2004; **53** Suppl 1: S119-S124 [PMID: 14749276 DOI: 10.2337/diabetes.53.2007.S119]

39 **Takasu T,** Tahara A, Yokono M, Hayashizaki Y, Kurosaki E, Imamura M, Funatsu T, Li Q. ASP1941, a novel, potent and selective SGLT2 inhibitor, improves hemoglobin A1c and symptoms of diabetes in animal models. American Diabetes Association. Orlando (FL), 2010; 70th Scientific Sessions. Available from: URL: http: //professional.diabetes.org/Abstracts_Display.aspx?TYP=1&CID=79510

40 **Reder ME**, Porte D Jr, Schwartz RS, Kahn SE. Disproportionately elevated proinsulin levels reflect the degree of impaired B cell secretory capacity in patients with noninsulin dependent diabetes mellitus. *J Clin Endocrinol Metab* 1998; **83**: 604-608 [DOI: 10.1210/jcem.83.2.4544]

41 **Røder ME**, Dinesen B, Hartling SG, Houssa P, Vestergaard H, Sodoyez-Goffaux F, Binder C. Intact proinsulin and beta-cell function in lean and obese subjects with and without type 2 diabetes. *Diabetes Care* 1999; **22**: 609-614 [PMID: 10189540 DOI: 10.2337/diacare.22.4.609]

42 **Ohkura T**, Inoue K, Fujioka Y, Nakanishi R, Shiochi H, Sumi K, Yamamoto N, Matsuzawa K, Izawa S, Ohkura H, Kato M, Yamamoto K, Taniguchi S. The proinsulin/insulin (PI/I) ratio is reduced by postprandial targeting therapy in type 2 diabetes mellitus: a small-scale clinical study. *BMC Res Notes* 2013; **6**: 453 [PMID: 24215809 DOI: 10.1186/1756-0500-6-453]

43 **Kaku K**, Inoue S, Matsuoka O, Kiyosue A, Azuma H, Hayashi N, Tokudome T, Langkilde AM, Parikh S. Efficacy and safety of dapagliflozin as a monotherapy for type 2 diabetes mellitus in Japanese patients with inadequate glycaemic control: a phase II multicentre, randomized, double-blind, placebo-controlled trial. *Diabetes Obes Metab* 2013; **15**: 432-440 [DOI: 10.1111/dom.12047]

44 **DeFronzo RA**, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 1979; **237**: 214-223

45 **Mudaliar S**, Henry RR, Boden G, Smith S, Chalamandaris AG, Duchesne D, Iqbal N, List J. Changes in insulin sensitivity and insulin secretion with the sodium glucose cotransporter 2 inhibitor dapagliflozin. *Diabetes Technol Ther* 2014; **16**: 137-144 [PMID: 24237386 DOI: 10.1089/dia.2013.0167]

46 **Ohkura T**, Shiochi H, Fujioka Y, Sumi K, Yamamoto N, Matsuzawa K, Izawa S, Kinoshita H, Ohkura H, Kato M, Taniguchi S, Yamamoto K. 20/(fasting C-peptide × fasting plasma glucose) is a simple and effective index of insulin resistance in patients with type 2 diabetes mellitus: a preliminary report. *Cardiovasc Diabetol* 2013; **12**: 21 [PMID: 23339473 DOI: 10.1186/1475-2840-12-21]

47 **Vasilakou D**, Karagiannis T, Athanasiadou E, Mainou M, Liakos A, Bekiari E, Sarigianni M, Matthews DR, Tsapas A. Sodium-glucose cotransporter 2 inhibitors for type 2 diabetes: a systematic review and meta-analysis. *Ann Intern Med* 2013; **159**: 262-274 [PMID: 24026259 DOI: 10.7326/0003-4819-159-4-201308200-00007]

48 **Goto K,** Kashiwagi A, Kazuta K, Yoshida S, Ueyama E, Utsuno A. Ipragliflozin reduces A1C and body weight in type 2 diabetes patients who have inadequate glycemic control on metformin alone: ILLUMINATE study. Philadelphia (PA): American Diabetes Association, 72th Scientific Sessions, 2012

49 **U.S. Food and Drug Administration.** FDA Briefing Document. NDA 202293. Dapagliflozin Tablets, 5 and 10 mg. Rockville, MD: U.S. Food and Drug Administration, 2011. [updated 2013 April 1]. Available from: URL: http: //www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/drugs/EndocrinologicandMetabolicDrugsAdvisoryCommittee/ucm262994.pdf

50 **European Medicines Agency.** Assessment Report: Forxiga (Dapagliflozin). Procedure no. EMEA/H/C/002322. London: European Medicines Agency, 2012. [updated 2013 April 1]. Available from: URL: http: //www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/002322/WC500136024.pdf

51 **Bhartia M**, Tahrani AA, Barnett AH. SGLT-2 inhibitors in development for type 2 diabetes treatment. *Rev Diabet Stud* 2011; **8**: 348-354 [PMID: 22262072 DOI: 10.1900/RDS.2011.8.348]

52 **Kawalec P**, Mikrut A, Łopuch S. The safety of dipeptidyl peptidase-4 (DPP-4) inhibitors or sodium-glucose cotransporter 2 (SGLT-2) inhibitors added to metformin background therapy in patients with type 2 diabetes mellitus: a systematic review and meta-analysis. *Diabetes Metab Res Rev* 2014; **30**: 269-283 [PMID: 24829965 DOI: 10.1002/dmrr.2494]

53 **EuroQol 5 Dimensions.** [Accessed 13 July 2006]. Available from: URL: http: //www.euroqol.org

54 **McMillan CV**, Honeyford RJ, Datta J, Madge NJ, Bradley C. The development of a new measure of quality of life for young people with diabetes mellitus: the ADDQoL-Teen. *Health Qual Life Outcomes* 2004; **2**: 61 [PMID: 15535888 DOI: 10.1186/1477-7525-2-61]

55 **Brod M**, Skovlund SE, Wittrup-Jensen KU. Measuring the impact of diabetes through patient report of treatment satisfaction, productivity and symptom experience. *Qual Life Res* 2006; **15**: 481-491 [PMID: 16547787 DOI: 10.1007/s11136-005-1624-6]

56 **Park H**, Park C, Kim Y, Rascati KL. Efficacy and safety of dipeptidyl peptidase-4 inhibitors in type 2 diabetes: meta-analysis. *Ann Pharmacother* 2012; **46**: 1453-1469 [PMID: 23136353 DOI: 10.1345/aph.1R041]

57 **Tanaka S**, Horimai C, Katsukawa F. Ethnic differences in abdominal visceral fat accumulation between Japanese, African-Americans, and Caucasians: a meta-analysis. *Acta Diabetol* 2003; **40** Suppl 1: S302-S304 [PMID: 14618500 DOI: 10.1007/s00592-003-0093-z]

58 **Puech A**, Monleaud-Dupy M, Jacob M, Jean M. [Stability

studies of aqueous solutions of hyoscyamine sulphate (author's transl)]. *J Pharm Belg* 1996; **32**: 117-127 [PMID: 894472]

59 **Kodama K**, Tojjar D, Yamada S, Toda K, Patel CJ, Butte AJ. Ethnic differences in the relationship between insulin sensitivity and insulin response: a systematic review and meta-analysis. *Diabetes Care* 2013; **36**: 1789-1796 [PMID: 23704681 DOI: 10.2337/dc12-1235]

60 **Ohkura T**, Fujioka Y, Izawa S, Sumi K, Yamamoto N, Shiochi H, Matsuzawa K, Kinoshita H, Ohkura H, Kato M, Taniguchi S, Yamamoto K. Endogenous insulin secretion ability in meal tolerance test correlated with body mass index (BMI) in Japanese type 2 diabetes patients. *Int J Diabetes Dev Ctries* 2014; Published online [DOI: 10.1007/s13410-013-0181-8]

61 **Santer R**, Calado J. Familial renal glucosuria and SGLT2: from a mendelian trait to a therapeutic target. *Clin J Am Soc Nephrol* 2010; **5**: 133-141 [PMID: 19965550 DOI: 10.2215/CJN.04010609]

62 **Inagaki N**, Kondo K, Yoshinari T, Maruyama N, Susuta Y, Kuki H. Efficacy and safety of canagliflozin in Japanese patients with type 2 diabetes: a randomized, double-blind, placebo-controlled, 12-week study. *Diabetes Obes Metab* 2013; **15**: 1136-1145 [PMID: 23782594 DOI: 10.1111/dom.12149]

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REVIEW

Pathological consequences of C-peptide deficiency in insulin-dependent diabetes mellitus

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Abstract

Diabetes is associated with several complications such as retinopathy, nephropathy, neuropathy and cardiovascular diseases. Currently, insulin is the main used medication for management of insulin-dependent diabetes mellitus (type-1 diabetes). In this metabolic syndrome, in addition to decrease of endogenous insulin, the plasma level of connecting peptide (C-peptide) is also reduced due to beta cell destruction. Studies in the past decade have shown that C-peptide is much more than a byproduct of insulin biosynthesis and possess different biological activities. Therefore, it may be possible that C-peptide deficiency be involved, at least in part, in the development of different complications of diabetes. It has been shown that a small level of remaining C-peptide is associated with significant metabolic benefit. The purpose of this review is to describe beneficial effects of C-peptide replacement on pathological features associated with insulin-dependent diabetes. Also, experimental and clinical findings on the effects of C-peptide on whole-body glucose utilization, adipose tissue metabolism and tissues blood flow are summarized and discussed. The hypoglycemic, antilipolytic and vasodilator effects of C-peptide suggest that it may contribute to fine-tuning of the tissues metabolism under different physiologic or pathologic conditions. Therefore, C-peptide replacement together with the classic insulin therapy may prevent, retard, or ameliorate diabetic complications in patients with type-1 diabetes.

Key words: C-peptide; Diabetes; Insulin; Nephropathy; Neuropathy

Core tip: In type-1 diabetes, in addition to decrease of endogenous insulin, the plasma level of connecting peptide (C-peptide) is also reduced due to beta cell destruction. Therefore, it may be possible that C-peptide deficiency be involved in the development of diabetic complications such as retinopathy, nephropathy, neuropathy and cardiovascular diseases. In this paper, beneficial effects of C-peptide replacement on pathological features associated with type-1 diabetes

are described. Also, experimental and clinical findings that support the hypoglycemic, antilipolytic and vasodilator effects of C-peptide are discussed.

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INTRODUCTION

Diabetes mellitus is still an increasing health problem in both developing and developed countries. World Health Organization reported (August, 2011) that, 346 million people have diabetes worldwide and 3.4 million patients died from diabetes-related complications in the year 2004. Diabetes is generally classified into two main types: insulin-dependent diabetes mellitus [type-1 diabetes (T1D)] which is a state of insulin deficiency because of destruction of islet beta cells, and non-insulin-dependent diabetes mellitus [type-2 diabetes (T2D)] which is characterized by resistance to the action of insulin[1].

Poor control of diabetes is associated with several complications such as nephropathy, retinopathy, neuropathy and cardiovascular diseases[1]. Currently, insulin is the main used medication for management of T1D[2]. Even though early-onset complications may be controlled by insulin therapy, it remains difficult to achieve normal glycemic control and late-onset complications occur in many of diabetic patients[3,4]. In addition to decrease of endogenous insulin, the level of connecting peptide (C-peptide) is also reduced in the plasma of patients with T1D due to autoimmune destruction of beta cell[5]. Although for many years C-peptide has been considered as a byproduct of insulin biosynthesis, data from several lines of studies reveals the beneficial actions of C-peptide replacement in prevention of metabolic changes and structural alterations in T1D[6]. Therefore, one cannot rule out the possibility that C-peptide deficiency may also be involved, at least in part, in the development of some pathological features associated with T1D. This article reviews the current understanding of biological effects of C-peptide and the beneficial actions of C-peptide replacement on preventing or ameliorating the T1D-related complications.

C-PEPTIDE SYNTHESIS AND SECRETION

In pancreatic beta cells, proinsulin is transferred in vesicles from rough endoplasmic reticulum to Golgi apparatus, where the vesicles are directed into a regulated secretion. During this transition of vesicles, three peptidases participate in proinsulin posttranslational processing to generate insulin and C-peptide[7]. First, proinsulin is cleaved by prohormone convertase type 2 at the A-chain/C-peptide junction or by prohormone convertase type 1/3 at the B-chain/C-peptide junction. Then, carboxypeptidases H removes two pairs of amino acids located at both cleaved junctions providing the *des* forms of proinsulin (*des*-31,32 and *des*-64, 65). Finally, endopeptidase type 1/3 and type 2 recognizes des-64,65 proinsulin and des-31, 32 proinsulin, respectively, leading to release of insulin and C-peptide from proinsulin. C-peptide facilitates the correct folding of proinsulin to allow form two disulfide bridges between A- and B-chains of insulin and therefore plays an essential role in biosynthesis of insulin[6,8]. In most species only one form of proinsulin has been described. However, in rats and mice two proinsulin isoforms I and II have been found[9].

Increase of blood glucose leads to secretion of an equimolar amount of insulin and C-peptide into the portal circulation[7]. However, the liver rapidly uptakes insulin because of single pass effect and only 50% of insulin reaches to the systemic circulation with a half-life about 4 min. On the other hand, C-peptide is primarily metabolized by kidney and has a circulating half-life about 30 min which is the reason for its higher plasma concentration than insulin[5,7]. The level of C-peptide in fasting and postprandial conditions varies between 0.3-1 nM and 1.5-2.5 nmol/L, respectively[10].

Since C-peptide and insulin are secreted from beta cells in equimolar concentrations, measuring serum C-peptide is an estimate of residual beta cell function and can be used to differentiate between patients with T1D and T2D[6]. However, the mean C-peptide concentration is higher in diabetic patients with renal diseases insufficiency compared with those have normal renal function. Therefore, in sever renal failure, the serum C-peptide assay is unreliable for assess residual beta cells[6,11,12].

BIOLOGICAL EFFECTS OF C-PEPTIDE

Effects of C-peptide on glucose utilization

Experimental studies on diabetic rats showed that C-peptide prolongs the hypoglycemic effect of insulin[13] and increases whole-body glucose utilization[9,14,15]. The glucose lowering effect of C-peptide was also investigated in human. Hoogwerf *et al*[16] have shown no effect by C-peptide on blood glucose level in healthy subjects or patients with T1D. However, Johansson and coworkers demonstrated that infusion of physiological concentrations of C-peptide to patients with T1D augments whole body glucose utilization by approximately 25%[17]. Also, Oskarsson *et al*[18] showed that C-peptide hasten the insulin-induced hypoglycemia in diabetic patients. Activation of glucose metabolism by short time C-peptide infusion in healthy controls and in patients with T1D was also reported by Wilhelm *et al*[19].

The augmented whole body glucose utilization is most probably a result of increased muscle glucose uptake rather than inhibition of hepatic gluconeogenesis[20]. In normal rats, we observed that adipose tissue glucose consumption was not affected by C-peptide[21]. Direct

examinations under *in vitro* condition confirmed that C-peptide stimulates the rate of glucose transport to muscle strips obtained from healthy subjects or patients with T1D[22]. Also, Zierath *et al*[23] showed that C-peptide dose-dependently increases glucose uptake into human skeletal muscle through a mechanism shared partly with insulin. Although the exact pathway involved in this effect of C-peptide is still unknown, incubation of isolated muscle strips with a cAMP analogue abolishes the C-peptide-stimulated glucose transport.

Regarding metabolic actions of C-peptide, it should be considered that although this peptide at low physiological concentrations mimics insulin effects, however in the presence of high level of insulin (*e.g.,* in the postprandial condition) the concomitant elevated level of C-peptide may blunt the insulin's peripheral effects[21,24]. It is possible that high levels of C-peptide induce a desensitization processes which may be recovered after a period of its absence.

Effect of C-peptide on adipose tissue

Soon after discovery of C-peptide, Solmon *et al*[25] examined the effects of pork and beef C-peptide on adrenocorticotropin-induced lipolysis in rats, but no significant effects were found. Subsequently, Yu and coworkers tested the effect of supraphysiological concentrations of porcine C-peptide on the lipolysis in isolated adipocytes from rats and found an insignificant antilipolytic effect[26]. Using an *ex-vivo* organ culture method, we observed a similar insignificant reduction in basal lipolysis of rat retroperitoneal adipose tissue[21]. Because it has been reported that some effects of C-peptide appear only in diabetes condition[9,27,28], we examined whether C-peptide alters lipolysis in diabetic rats. Our data showed that C-peptide like insulin significantly inhibits isoproterenol-stimulated lipolysis[29]. Therefore C-peptide may act, conditionally, as an antilipolytic hormone and may be involved in fine-tuning of lipid metabolism.

Effects of C-peptide on circulation

Patients with T1D show reduced tissues blood flow despite intensive insulin therapy and good management of glucose control[30]. C-peptide has been shown to enhance blood flow of kidney[17], nerve[31], skeletal muscle[32], myocardium[30,33] and skin[34]. The vasodilator effect of C-peptide is mediated by stimulation of nitric oxide release from endothelial cells[35-37]. Wallerath *et al*[35] reported that physiological postprandial concentration of C-peptide is able to activate endothelial nitric oxide synthase (eNOS) and stimulating nitric oxide production. Forst *et al*[38] showed that intravenous infusion of C-peptide to patients with T1D increases plasma concentration of cGMP, as an index of nitric oxide activity[38]. This finding is in agreement with earlier report that in diabetic rats the C-peptide induced glucose utilization is sensitive to eNOS inhibition[9].

OTHER BIOLOGICAL EFFECTS OF C-PEPTIDE

Interaction with insulin

In the presence of C-peptide, insulin hexamers in solution becomes undetectable. Also, subcutaneous injection of an insulin and C-peptide mixture to diabetic patients accelerates the increase of insulin levels in plasma and in comparison with injection of insulin alone utilizes more glucose. Therefore, it seems that C-peptide increases disaggregation of insulin by binding to insulin oligomers and thereby enhances the availability of monomeric (biologically active form) insulin[39].

Protection of endothelium

It has been reported that C-peptide is able to inhibit leukocyte-endothelium interaction induced by thrombin or by NG-nitro-L-arginine methyl ester. This effect of C-peptide may be important in protection of vasculature against inflammatory disorders such those observed in T1D[40].

EFFECTS OF C-PEPTIDE ON DIABETIC COMPLICATIONS

Effects on diabetic nephropathy

Different aspects of the diabetic renal pathogenic abnormalities can be improved by C-peptide in T1D (Figure 1). In diabetic rats, C-peptide decreases urinary protein excretion[41-43], reduces glomerular hyperfiltration rate and restores the renal functional reserve[42-44]. These beneficial effects have also been demonstrated in insulin-dependent diabetic patients[17,45]. In a clinical study, patients were administered insulin alone or in combination with C-peptide by subcutaneous infusion pump for 4 wk. While combination therapy led to decrease of glomerular filtration rate and protein excretion after 2 wk, the insulin alone was ineffective[45]. Johansson *et al*[46] extended the period of C-peptide therapy to 3 mo and reported a significant decrease in the rate of protein excretion in patients receiving combination of insulin and C-peptide. In line with these findings, Zerbini *et al*[47] found a decreased C-peptide/creatinine ratio in the plasma of T1D patients with nephropathy when compared with those without albuminuria[47]. Microscopic examinations have showed that in diabetic rats, C-peptide reduces the hypertrophy of mesangial matrix in glomeruli of the kidneys[48]. Several mechanisms are postulated for beneficial effects of C-peptide on renal function including inhibition of apoptosis, increase of Na^+, K^+-ATPase activity and interaction with the signaling pathway of growth factors[49,50]. Activation of the key signaling molecules such as phospholipase C and protein kinase C followed by phosphorylation of extracellular-signal-regulated kinase and c-Jun N-terminal kinase have been shown in human renal tubular cells treated with

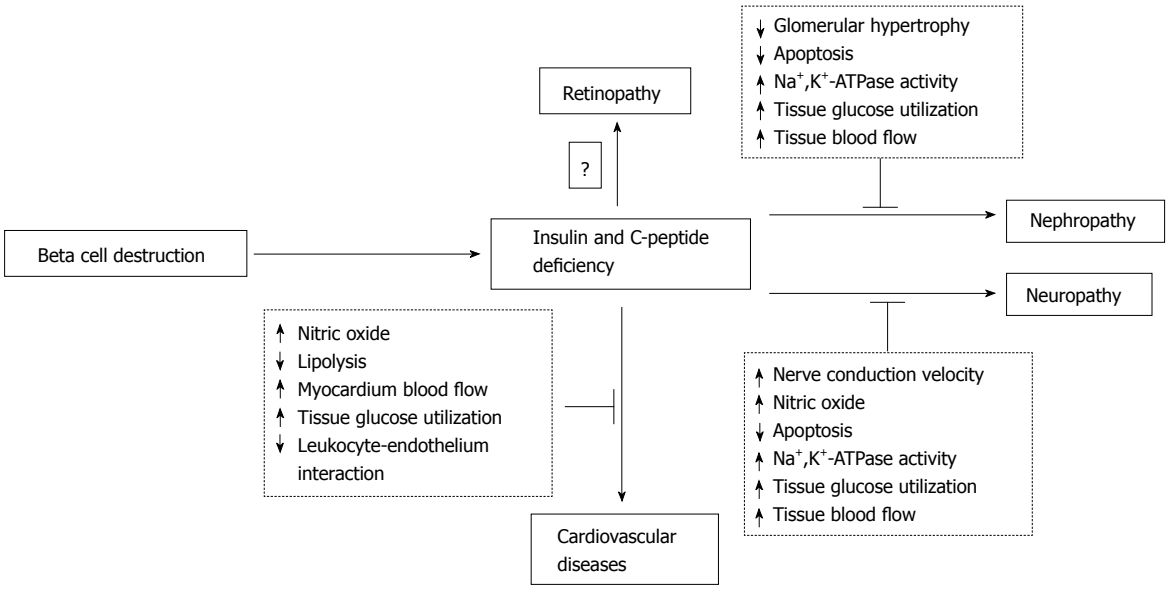

Figure 1 Proposed mechanisms (dashed rectangles) by which C-peptide may prevent, retard, or ameliorate diabetic complications in patient with type-1 diabetes. ↓: Decrease; ↑: Increase.

C-peptide[49]. Regarding beneficial effects of C-peptide, we should emphasis that some of the C-peptide beneficial effects are limited to animals or patients who show very low or missing C-peptide plasma levels[9,27,28]. Therefore, the nephroprotective effect of C-peptide may represent a therapeutic goal for patients with T1D.

Effects on diabetic neuropathy

Accumulating evidence suggests that C-peptide can prevent, retard, or ameliorate neuropathy in T1D (Figure 1)[51-57]. Decreased level of Na^+, K^+-ATPase activity and reduced nitric oxide formation are considered as contributor factors to pathogenesis of diabetic neuropathy. It has been shown that C-peptide prevents the neural Na^+, K^+-ATPase defect and the nerve conduction velocity reduction[52]. In study of Cotter *et al*[31] C-peptide at physiologic doses improved sensory and motor nerve conduction velocity in STZ-induced diabetic rats through increase of nitric oxide release. Ekberg *et al*[58] demonstrated that C-peptide improves vibration perce-ption in patients with T1D[58]. C-peptide also may prevent cognitive dysfunction by its antiapoptotic effect in the brain particularly in the hippocampus[51-54]. The antiapoptotic property was also confirmed by Li *et al*[59] who showed that C-peptide, in the presence of insulin, inhibits high glucose-induced apoptosis in neuroblastoma cells. There are also clinical evidence that autonomic dysfunction can be ameliorated by C-peptide replacement. Infusion of C-peptide to patients with T1D increases the heart rate variability during deep breathing and the heart rate brake index after tilting[55]. In contrast to insulin alone, administration of a combination of C-peptide and insulin improves heart rate during deep breathing in T1D patients[46].

CONCLUSION

According to data presented in this paper, C-peptide is much more than a byproduct of insulin synthesis and has several biological actions such as hypoglycemic, antilipolytic and vasodilator effects. These biological effects suggest that it may act as a hormone to contribute in fine-tuning of the tissues metabolism under different physiologic or pathologic conditions. In T1D diabetes, in particular, it was found that patients who conserve low but sustained secretion of endogenous C-peptide show better metabolic control and less retinopathy, nephropathy and neuropathy than patients who have become fully C-peptide and insulin deficient[56-60]. These beneficial effects are demonstrated only in T1D models. It is possible that in physiological conditions, C-peptide produces its maximum effect and induces some levels of desensitization processes in phosphorylation mediated actions, especially nitric oxide-dependent pathways. Recovering the C-peptide mechanism of action during a period of its absence is in good agreement with the experimental results in T1D models. Therefore, present data suggest the possibility of a clinically applicable role for C-peptide replacement, together with the classic insulin therapy, to prevent, retard, or ameliorate diabetic complications in patient with T1D.

REFERENCES

1 **Deshpande AD,** Harris-Hayes M, Schootman M. Epidemiology of diabetes and diabetes-related complications. *Phys Ther* 2008; **88**: 1254-1264 [PMID: 18801858 DOI: 10.2522/ptj.20080020]
2 **Aathira R,** Jain V. Advances in management of type 1 diabetes mellitus. *World J Diabetes* 2014; **5**: 689-696 [PMID: 25317246 DOI: 10.4239/wjd.v5.i5.689]
3 **Tzoulaki I,** Molokhia M, Curcin V, Little MP, Millett CJ, Ng

A, Hughes RI, Khunti K, Wilkins MR, Majeed A, Elliott P. Risk of cardiovascular disease and all cause mortality among patients with type 2 diabetes prescribed oral antidiabetes drugs: retrospective cohort study using UK general practice research database. *BMJ* 2009; **339**: b4731 [DOI: 10.1136/bmj.b4731]

4 **Palmer JP**, Fleming GA, Greenbaum CJ, Herold KC, Jansa LD, Kolb H, Lachin JM, Polonsky KS, Pozzilli P, Skyler JS, Steffes MW. C-peptide is the appropriate outcome measure for type 1 diabetes clinical trials to preserve beta-cell function: report of an ADA workshop, 21-22 October 2001. *Diabetes* 2004; **53**: 250-264 [PMID: 14693724 DOI: 10.2337/diabetes.53.1.250]

5 **Eisenbarth GS,** Buse JB. Type 1 diabetes mellitus. In: Melmed S, Polonsky KS, Larsen PR. Williams textbook of endocrinology. Philadelphia: Elsevier, 2011: 1436-1453

6 **Marques RG**, Fontaine MJ, Rogers J. C-peptide: much more than a byproduct of insulin biosynthesis. *Pancreas* 2004; **29**: 231-238 [PMID: 15367890 DOI: 10.1097/00006676-200410000-00009]

7 **Steiner DF,** Bell GI, Rubenstein AH. Chemistry and biosynthesis of the islet hormones. In: DeGroot L, Jameson JL. Endocrinology. Philadelphia: Elsevier, 2006: 925-960

8 **Habener JF.** Genetic control of peptide hormone formation. In: Melmed S, Polonsky KS, Larsen PR. Williams textbook of endocrinology. Philadelphia: Elsevier, 2011: 31-43

9 **Li L**, Oshida Y, Kusunoki M, Yamanouchi K, Johansson BL, Wahren J, Sato Y. Rat C peptide I and II stimulate glucose utilization in STZ-induced diabetic rats. *Diabetologia* 1999; **42**: 958-964 [PMID: 10491756 DOI: 10.1007/s001250051254]

10 **Kuzuya H**, Blix PM, Horwitz DL, Rubenstein AH, Steiner DF, Faber OK, Binder C. Heterogeneity of circulating human C-peptide. *Diabetes* 1978; **27** Suppl 1: 184-191 [PMID: 75814 DOI: 10.2337/diab.27.1.S184]

11 **Covic AM**, Schelling JR, Constantiner M, Iyengar SK, Sedor JR. Serum C-peptide concentrations poorly phenotype type 2 diabetic end-stage renal disease patients. *Kidney Int* 2000; **58**: 1742-1750 [PMID: 11012908 DOI: 10.1046/j.1523-1755.2000.00335.x]

12 **DeFronzo RA**, Tobin JD, Rowe JW, Andres R. Glucose intolerance in uremia. Quantification of pancreatic beta cell sensitivity to glucose and tissue sensitivity to insulin. *J Clin Invest* 1978; **62**: 425-435 [PMID: 353075 DOI: 10.1172/JCI109144]

13 **Wójcikowski C**, Maier V, Dominiak K, Fussgänger R, Pfeiffer EF. Effects of synthetic rat C-peptide in normal and diabetic rats. *Diabetologia* 1983; **25**: 288-290 [PMID: 6139319 DOI: 10.1007/BF00279945]

14 **Sato Y**, Oshida Y, Han YQ, Morishita Y, Li L, Ekberg K, Jörnvall H, Wahren J. C-peptide fragments stimulate glucose utilization in diabetic rats. *Cell Mol Life Sci* 2004; **61**: 727-732 [PMID: 15052415 DOI: 10.1007/s00018-003-3460-6]

15 **Wu W**, Oshida Y, Yang WP, Li L, Ohsawa I, Sato J, Iwao S, Johansson BL, Wahren J, Sato Y. Effect of C-peptide administration on whole body glucose utilization in STZ-induced diabetic rats. *Acta Physiol Scand* 1996; **157**: 253-258 [PMID: 8800366 DOI: 10.1046/j.1365-201X.1996.489236000.x]

16 **Hoogwerf BJ**, Bantle JP, Gaenslen HE, Greenberg BZ, Senske BJ, Francis R, Goetz FC. Infusion of synthetic human C-peptide does not affect plasma glucose, serum insulin, or plasma glucagon in healthy subjects. *Metabolism* 1986; **35**: 122-125 [PMID: 3511350]

17 **Johansson BL**, Sjöberg S, Wahren J. The influence of human C-peptide on renal function and glucose utilization in type 1 (insulin-dependent) diabetic patients. *Diabetologia* 1992; **35**: 121-128 [PMID: 1547915]

18 **Oskarsson P**, Johansson BL, Adamson U, Lins PE. Effects of C-peptide on insulin-induced hypoglycaemia and its counterregulatory responses in IDDM patients. *Diabet Med* 1997; **14**: 655-659 [PMID: 9272591]

19 **Wilhelm B,** Weber MM, Ekberg K, Ries C, Kugler M, Pfuetzner A, Kann PH, Wahren J, Forst T. Activation of glucose metabolism by C-peptide in patients with type 1 diabetes mellitus and in healthy control subjects. *Diabetes* 2007; **56**: A337

20 **Johansson BL**, Linde B, Wahren J. Effects of C-peptide on blood flow, capillary diffusion capacity and glucose utilization in the exercising forearm of type 1 (insulin-dependent) diabetic patients. *Diabetologia* 1992; **35**: 1151-1158 [PMID: 1478367]

21 **Ghorbani A**, Omrani GR, Hadjzadeh MA, Varedi M. Effects of rat C-peptide-II on lipolysis and glucose consumption in cultured rat adipose tissue. *Exp Clin Endocrinol Diabetes* 2011; **119**: 343-347 [PMID: 21553365 DOI: 10.1055/s-0031-1275662]

22 **Zierath JR**, Galuska D, Johansson BL, Wallberg-Henriksson H. Effect of human C-peptide on glucose transport in in vitro incubated human skeletal muscle. *Diabetologia* 1991; **34**: 899-901 [PMID: 1778355]

23 **Zierath JR**, Handberg A, Tally M, Wallberg-Henriksson H. C-peptide stimulates glucose transport in isolated human skeletal muscle independent of insulin receptor and tyrosine kinase activation. *Diabetologia* 1996; **39**: 306-313 [PMID: 8721776]

24 **Grunberger G**, Qiang X, Li Z, Mathews ST, Sbrissa D, Shisheva A, Sima AA. Molecular basis for the insulinomimetic effects of C-peptide. *Diabetologia* 2001; **44**: 1247-1257 [PMID: 11692173 DOI: 10.1007/s001250100632]

25 **Solomon SS**, Brush JS, Kitabchi AE. Antilipolytic activity of insulin and proinsulin on ACTH and cyclic nucleotide-induced lipolysis in the isolated adipose cell of rat. *Biochim Biophys Acta* 1970; **218**: 167-169 [PMID: 4319687]

26 **Yu SS**, Kitbachi AE. Biological activity of proinsulin and related polypeptides in the fat tissue. *J Biol Chem* 1973; **248**: 3753-3761 [PMID: 4708090]

27 **Kunt T**, Schneider S, Pfützner A, Goitum K, Engelbach M, Schauf B, Beyer J, Forst T. The effect of human proinsulin C-peptide on erythrocyte deformability in patients with Type I diabetes mellitus. *Diabetologia* 1999; **42**: 465-471 [PMID: 10230651 DOI: 10.1007/s001250051180]

28 **Nordquist L**, Moe E, Sjöquist M. The C-peptide fragment EVARQ reduces glomerular hyperfiltration in streptozotocin-induced diabetic rats. *Diabetes Metab Res Rev* 2007; **23**: 400-405 [PMID: 17103462 DOI: 10.1002/dmrr.704]

29 **Ghorbani A**, Omrani GR, Hadjzadeh MA, Varedi M. Proinsulin C-peptide inhibits lipolysis in diabetic rat adipose tissue through phosphodiestrase-3B enzyme. *Horm Metab Res* 2013; **45**: 221-225 [PMID: 22990990 DOI: 10.1055/s-0032-1323764]

30 **Johansson BL**, Sundell J, Ekberg K, Jonsson C, Seppänen M, Raitakari O, Luotolahti M, Nuutila P, Wahren J, Knuuti J. C-peptide improves adenosine-induced myocardial vasodilation in type 1 diabetes patients. *Am J Physiol Endocrinol Metab* 2004; **286**: E14-E19 [PMID: 12954595]

31 **Cotter MA**, Ekberg K, Wahren J, Cameron NE. Effects of proinsulin C-peptide in experimental diabetic neuropathy: vascular actions and modulation by nitric oxide synthase inhibition. *Diabetes* 2003; **52**: 1812-1817 [PMID: 12829651]

32 **Jensen ME**, Messina EJ. C-peptide induces a concentration-dependent dilation of skeletal muscle arterioles only in presence of insulin. *Am J Physiol* 1999; **276**: H1223-H1228 [PMID: 10199846]

33 **Hansen A**, Johansson BL, Wahren J, von Bibra H. C-peptide exerts beneficial effects on myocardial blood flow and function in patients with type 1 diabetes. *Diabetes* 2002; **51**: 3077-3082 [PMID: 12351450]

34 **Forst T**, Kunt T, Pohlmann T, Goitom K, Engelbach M, Beyer J, Pfützner A. Biological activity of C-peptide on the skin microcirculation in patients with insulin-dependent diabetes mellitus. *J Clin Invest* 1998; **101**: 2036-2041 [PMID: 9593759 DOI: 10.1172/JCI2147]

35 **Wallerath T**, Kunt T, Forst T, Closs EI, Lehmann R, Flohr T, Gabriel M, Schäfer D, Göpfert A, Pfützner A, Beyer J, Förstermann U. Stimulation of endothelial nitric oxide synthase by proinsulin C-peptide. *Nitric Oxide* 2003; **9**: 95-102 [PMID: 14623175 DOI: 10.1016/j.niox.2003.08.004]

36 **Kitamura T**, Kimura K, Makondo K, Furuya DT, Suzuki M, Yoshida T, Saito M. Proinsulin C-peptide increases nitric oxide production by enhancing mitogen-activated protein-kinase-dependent transcription of endothelial nitric oxide synthase in aortic endothelial cells of Wistar rats. *Diabetologia* 2003; **46**: 1698-1705 [PMID: 14586499 DOI: 10.1007/s00125-003-1232-3]

37 **Joshua IG**, Zhang Q, Falcone JC, Bratcher AP, Rodriguez WE, Tyagi SC. Mechanisms of endothelial dysfunction with development of type 1 diabetes mellitus: role of insulin and C-peptide. *J Cell Biochem* 2005; **96**: 1149-1156 [PMID: 16187296 DOI: 10.1002/jcb.20620]

38 **Forst T**, De La Tour DD, Kunt T, Pfützner A, Goitom K, Pohlmann T, Schneider S, Johansson BL, Wahren J, Löbig M, Engelbach M, Beyer J, Vague P. Effects of proinsulin C-peptide on nitric oxide, microvascular blood flow and erythrocyte Na+,K+-ATPase activity in diabetes mellitus type I. *Clin Sci* (Lond) 2000; **98**: 283-290 [PMID: 10677386]

39 **Shafqat J**, Melles E, Sigmundsson K, Johansson BL, Ekberg K, Alvelius G, Henriksson M, Johansson J, Wahren J, Jörnvall H. Proinsulin C-peptide elicits disaggregation of insulin resulting in enhanced physiological insulin effects. *Cell Mol Life Sci* 2006; **63**: 1805-1811 [PMID: 16845606 DOI: 10.1007/s00018-006-6204-6]

40 **Scalia R**, Coyle KM, Levine BJ, Booth G, Lefer AM. C-peptide inhibits leukocyte-endothelium interaction in the microcirculation during acute endothelial dysfunction. *FASEB J* 2000; **14**: 2357-2364 [PMID: 11053258]

41 **Rebsomen L**, Pitel S, Boubred F, Buffat C, Feuerstein JM, Raccah D, Vague P, Tsimaratos M. C-peptide replacement improves weight gain and renal function in diabetic rats. *Diabetes Metab* 2006; **32**: 223-228 [PMID: 16799398]

42 **Sjöquist M**, Huang W, Johansson BL. Effects of C-peptide on renal function at the early stage of experimental diabetes. *Kidney Int* 1998; **54**: 758-764 [PMID: 9734600 DOI: 10.1046/j.1523-1755.1998.00074.x]

43 **Huang DY,** Richter K, Breidenbach A, Vallon V. Human C-peptide acutely lowers glomerular hyperfiltration and proteinuria in diabetic rats a dose-response study. *Naunyn-Schmiedebergs Arch Pharmacol* 2002; **365**: 67-73 [PMID: 11862335 DOI: 10.1007/s00210-001-0502-1]

44 **Samnegård B**, Jacobson SH, Jaremko G, Johansson BL, Sjöquist M. Effects of C-peptide on glomerular and renal size and renal function in diabetic rats. *Kidney Int* 2001; **60**: 1258-1265 [PMID: 11576340 DOI: 10.1046/j.1523-1755.2001.00964.x]

45 **Johansson BL**, Kernell A, Sjöberg S, Wahren J. Influence of combined C-peptide and insulin administration on renal function and metabolic control in diabetes type 1. *J Clin Endocrinol Metab* 1993; **77**: 976-981 [PMID: 8408474 DOI: 10.1210/jcem.77.4.8408474]

46 **Johansson BL**, Pernow J. C-peptide potentiates the vasoconstrictor effect of neuropeptide Y in insulin-dependent diabetic patients. *Acta Physiol Scand* 1999; **165**: 39-44 [PMID: 10072095 DOI: 10.1046/j.1365-201x.1999.00475.x]

47 **Zerbini G**, Mangili R, Luzi L. Higher post-absorptive C-peptide levels in Type 1 diabetic patients without renal complications. *Diabet Med* 1999; **16**: 1048 [PMID: 10656236]

48 **Samnegård B**, Jacobson SH, Jaremko G, Johansson BL, Ekberg K, Isaksson B, Eriksson L, Wahren J, Sjöquist M. C-peptide prevents glomerular hypertrophy and mesangial matrix expansion in diabetic rats. *Nephrol Dial Transplant* 2005; **20**: 532-538 [PMID: 15665028 DOI: 10.1093/ndt/gfh683]

49 **Maezawa Y**, Yokote K, Sonezaki K, Fujimoto M, Kobayashi K, Kawamura H, Tokuyama T, Takemoto M, Ueda S, Kuwaki T, Mori S, Wahren J, Saito Y. Influence of C-peptide on early glomerular changes in diabetic mice. *Diabetes Metab Res Rev* 2006; **22**: 313-322 [PMID: 16389646 DOI: 10.1002/dmrr.612]

50 **Al-Rasheed NM**, Willars GB, Brunskill NJ. C-peptide signals via Galpha i to protect against TNF-alpha-mediated apoptosis of opossum kidney proximal tubular cells. *J Am Soc Nephrol* 2006; **17**: 986-995 [PMID: 16510765]

51 **Li ZG**, Zhang W, Sima AA. C-peptide prevents hippocampal apoptosis in type 1 diabetes. *Int J Exp Diabetes Res* 2002; **3**: 241-245 [PMID: 12546277 DOI: 10.1080/15604280214936]

52 **Sima AA**, Zhang W, Sugimoto K, Henry D, Li Z, Wahren J, Grunberger G. C-peptide prevents and improves chronic Type I diabetic polyneuropathy in the BB/Wor rat. *Diabetologia* 2001; **44**: 889-897 [PMID: 11508275]

53 **Kamiya H**, Zhang W, Ekberg K, Wahren J, Sima AA. C-Peptide reverses nociceptive neuropathy in type 1 diabetes. *Diabetes* 2006; **55**: 3581-3587 [PMID: 17130507]

54 **Sima AA**, Li ZG. The effect of C-peptide on cognitive dysfunction and hippocampal apoptosis in type 1 diabetic rats. *Diabetes* 2005; **54**: 1497-1505 [PMID: 15855338]

55 **Johansson BL,** Borg K, Fernqvist-Forbes E, Odergren T, Remahl S, Wahren J. C-peptide improves autonomic nerve function in IDDM patients. *Diabetologia* 1996; **39**: 687-695 [PMID: 8781764]

56 **Sjöberg S**, Gunnarsson R, Gjötterberg M, Lefvert AK, Persson A, Ostman J. Residual insulin production, glycaemic control and prevalence of microvascular lesions and polyneuropathy in long-term type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 1987; **30**: 208-213 [PMID: 3297896]

57 **Sjöberg S**, Gjötterberg M, Berglund L, Möller E, Ostman J. Residual C-peptide excretion is associated with a better long-term glycemic control and slower progress of retinopathy in type I (insulin-dependent) diabetes mellitus. *J Diabet Complications* 1991; **5**: 18-22 [PMID: 1830314]

58 **Ekberg K**, Brismar T, Johansson BL, Jonsson B, Lindström P, Wahren J. Amelioration of sensory nerve dysfunction by C-Peptide in patients with type 1 diabetes. *Diabetes* 2003; **52**: 536-541 [PMID: 12540632]

59 **Li ZG**, Zhang W, Sima AA. C-peptide enhances insulin-mediated cell growth and protection against high glucose-induced apoptosis in SH-SY5Y cells. *Diabetes Metab Res Rev* 2003; **19**: 375-385 [PMID: 12951645 DOI: 10.1002/dmrr.389]

60 Epidemiology of severe hypoglycemia in the diabetes control and complications trial. The DCCT Research Group. *Am J Med* 1991; **90**: 450-459 [PMID: 2012085]

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REVIEW

Adiponectin: Probe of the molecular paradigm associating diabetes and obesity

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Abstract

Type 2 diabetes is an emerging health challenge all over the world as a result of urbanization, high prevalence of obesity, sedentary lifestyle and other stress related factors compounded with the genetic prevalence. The health consequences and economic burden of the obesity and related diabetes mellitus epidemic are enormous. Different signaling molecules secreted by adipocytes have been implicated in the development of obesity and associated insulin resistance in type 2 diabetes. Human adiponectin, a 244-amino acid collagen-like protein is solely secreted by adipocytes and acts as a hormone with anti-inflammatory and insulin-sensitizing properties. Adiponectin secretion, in contrast to secretion of other adipokines, is paradoxically decreased in obesity which may be attributable to inhibition of adiponectin gene transcription. There are several mechanisms through which adiponectin may decrease the risk of type 2 diabetes, including suppression of hepatic gluconeogenesis, stimulation of fatty acid oxidation in the liver, stimulation of fatty acid oxidation and glucose uptake in skeletal muscle, and stimulation of insulin secretion. To date, no systematic review has been conducted that evaluate the potential importance of adiponectin metabolism in insulin resistance. In this review attempt has been made to explore the relevance of adiponectin metabolism for the development of diabetes mellitus. This article also identifies this novel target for prospective therapeutic research aiming successful management of diabetes mellitus.

Key words: Adiponectin; Obesity; Dyslipidemia; Type 2 diabetes mellitus; Insulin resistance

Core tip: Diabetes mellitus and related metabolic disorders like obesity, dyslipidemia are emerging as major global health challenges in recent era. Adiponectin, an adipokine demands profound importance in the field of metabolomics due to its potential role in all these complications. Plasma adiponectin concentration is remarkably lower in subjects with metabolic disorders predicting its significant role as an important biomarker in disease prognosis. We have attempted to enlighten adiponectin function stretching its role as a modulator associating these metabolic obstacles. We believe, this article will surely contribute to the fundamental and clinical research in the field of diabetes and related complications.

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INTRODUCTION

Rapid urbanization and change in life style has intensified the prevalence of obesity and dyslipidemia which plays crucial role in developing diabetes mellitus across the globe. Diabetes mellitus is a major public health concern with 382 million individuals being affected worldwide in 2013. Type 2 diabetes mellitus (T2DM) constitutes one of the major forms of diabetes disease burden associated with remarkably accelerated rates of microvascular obstacles and macrovascular disorders. Obesity and its association with developing type 2 diabetes is an interesting area of research for scientists in recent years. Insulin resistance is one of the earliest hallmarks of the pre-diabetic state and results from a complex interplay between obesity-favoring environmental factors, such as unrestricted supply of high-caloric foods and markedly increased sedentary lifestyle combined with a permissive genetic background. The high incidence is attributed to a combination of genetic susceptibility plus adoption of a high-calorie, low-activity lifestyle mainly by urban population.

Adipose tissue was long been identified as an energy storage organ but in recent times extensive studies revealed the role of adipose tissue as an important endocrine organ with a number of metabolic activities; thus its function as a storage organ is now far from reality[1]. Adiponectin, an adipose tissue derived hormone, is lower in obese subjects than their lean counterparts[2]. Epidemiological studies revealed that patients with diabetes and cardiovascular disease (CVD) has lower amount of adiponectin in their serum[3,4], and low serum adiponectin level can be an excellent predictor of developing type 2 diabetes and associated CVD in later stage[5-8]. Thus the role of adiponectin hormone as a potential biomarker for predicting the occurrence of type 2 diabetes is evolving as an interesting area in the study of metabolomics. In this review we aimed to highlight the potential beneficiary function of adiponectin in type 2 diabetes, dyslipidemia and obesity considering both genetic and biochemical approach.

OBESITY AND DIABETES: MAJOR GLOBAL THREATS OF THIS MILLENNIUM

In modern times rapid urbanization and change in lifestyle has increased the prevalence of obesity in manifold, especially the young generation has modified their food habits with high calorie junk foods. Furthermore rapid development of technology has increased the tendency of uptaking sedentary lifestyle with less or no work at all, increasing the chances of getting obese. Obesity which is a major global threat virtually affecting both developed and developing countries. In Central America easy access to high calorie food and adoption of sedentary lifestyle has increased the prevalence of both diabetes and obesity[9] where in developing countries like countries in Latin America[10] and East Asia rise in income has shifted the mass from low calorie whole grain diet to high calorie processed foods which is far energy dense affecting not only the adults but the children and adolescents as well. BMI or body mass index and WC or Waist Circumference is two major parameters to measure obesity[11]. Higher value of BMI (30 kg/m^2) and WC increases the risk of type 2 diabetes, high cholesterol, high blood pressure and heart disease.

Type 2 is the most prevalent form of diabetes accounting 90%-95% of the cases, especially in developed countries[12]. According to recent estimates of the International Obesity Task Force, up to 1.7 billion people of the world's population are at a heightened risk of weight-related, non-communicable diseases such as type 2 diabetes which is majorly a lifestyle disorder (International Diabetes Federation, 2004). According to International Diabetes Federation India accounts for the largest number of people (50.8 million) suffering from diabetes in the world, followed by China (43.2 million) and United States (26.8 million). This metabolic syndrome is closely associated with different macro and microvascular disorders[13] (Table 1). The most prevalent diabetic macrovascular complication is Cardiovascular disorder (CVD)[14], which in turn is associated with environmental risk factors as well as genetic predisposition. Antidiabetic drug metformin is particularly useful for overweight and obese diabetic patients. Our earlier report indicates that metformin is particularly useful to restore the antioxidant status of cells hampered in type 2 diabetes stress[15].

Therefore type 2 diabetes and obesity interplays together to exert more deteriorating effect incorporating other metabolic syndromes such as CVD, dyslipidemia and hypertension[16-25].

ADIPONECTIN: STRUCTURE AND RECEPTORS

The correlation between rapidly emerging type 2 diabetes and obesity still remained a major question for researcher. It was hypothesized that metabolic dysfunction may cause to acquire obesity which in turn can develop type 2 diabetes. Adipocyte, the major energy storing cell is a storage site of a number of hormones as well whose prime function remains to govern lipid metabolism. Major adipocyte derived hormones are adiponectin, leptin, resistin and visfatin[26]. Leptin and adiponectin exerts positive effect on lowering blood glucose whereas resistin tends to increase blood glucose levels (Figure 1).

Reported for the first time by Scherer *et al*[27], 1995,

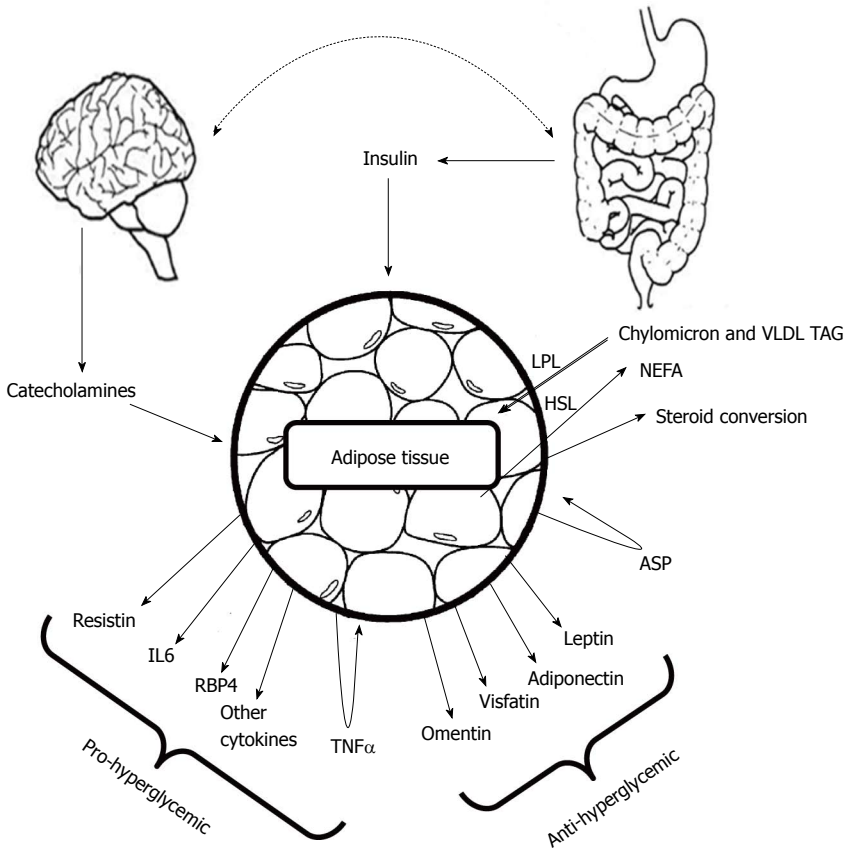

Figure 1 Adipocyte-derived proteins with anti-diabetic actions include leptin, adiponectin, omentin and visfatin; other factors tend to raise blood glucose including resistin, Tumor necrosis factor-α and Retinol-binding protein 4. (Adapted from Mohamed-Ali *et al*[31] and Rosen *et al*[32]). LPL: Lipoprotein lipase; HSL: Hormone-sensitive lipase; NEFA: Non-esterified fatty acids; ASP: Acylation stimulating protein; TAG: Triacylglycerol; TNF-α: Tumor necrosis factor α; RBP4: Retinol-binding protein; IL6: Interleukin 6.

Table 1 Vascular complications in type 2 diabetes

Microvascular complications prevalence	Macrovascular complications prevalence
Retinopathy 23.7%	Cardiovascular disease 11.4%
Background 20.0%	Peripheral vascular disease 4.0%
Proliferative 3.7%	Cerebrovascular accidents 0.9%
Nephropathy 5.5%	Hypertension 38.0%
Peri-neuropathy 27.5%	

Adiponectin, also known as Acrp30 (adipocyte complement-related protein of 30 kDa) is a protein exclusively secreted by adipocyte having huge structural similarity with $C1_q$[27]. Three monomers (30 kDa) associate together at the globular domain to form the adiponectin trimer, where four to six trimers associate through their collagenous domains to form the high order structure. Monomeric adiponectin has not been observed in the plasma and it is believed to remain within adipocyte[28]. Human adiponectin is encoded by the *ADIPOQ* gene on the chromosomal locus 3q27 consisting three exons and two introns[29], involved in regulating glucose levels as well as fatty acid breakdown[1]. Mouse adiponectin is a 247 amino acid long protein where human adiponectin is a protein product of 244 amino acids consisting of four domains, an amino-terminal signal sequence, a variable region, a collagenous domain (cAd) consisting of 22 Gly-X-Y repeats, and a carboxy-terminal globular domain (gAd)[27]. It is the most abundant adipokines with its serum concentration ranging from 5 to 30 μg/mL[30].

Structure of single-chain globular domain adiponectin (sc-gAd) is reported (Figure 2), where globular domain is composed of three part A, B and C respectively[30]. The adiponectin protein can undergo proteolytic cleavage and can form the globular form of adiponectin, where the globular head domain has been reported to increase the fatty acid oxidation[33]. Acrp30 is found in two forms in serum; one is low molecular weight (LMW) trimer-dimer where the other one is high molecular weight complex. Oligomer formation of Acrp30 depends on the formation of disulfide bond mediated by Cys-39. Mutation of Cys-39 results in the trimers which can easily undergo proteolytic cleavage in the collagenous domain[34].

Yamauchi *et al*[35] reported for the first time about the two adiponectin receptors which can successfully increase AMP kinase and PPAR-alpha ligand activities as well as can accelerate fatty acid oxidation and glucose uptake by adiponectin. These receptors are named as AdipoR1 which is abundantly expressed in skeletal muscle and AdipoR2 which is mainly expressed in the liver (Figure 3)[35,36].

They first successfully performed the cloning of complementary DNAs encoding adiponectin receptors 1 and 2 (AdipoR1 and AdipoR2) by expression cloning[35]. AdipoR1 and AdipoR2 mRNA expression in the liver and skeletal muscle increases after fasting and re-feeding can rapidly restore these to levels equal to the original fed state (Figure 4)[35,36]. Both of these receptors contain seven transmembrane domains but they are structurally

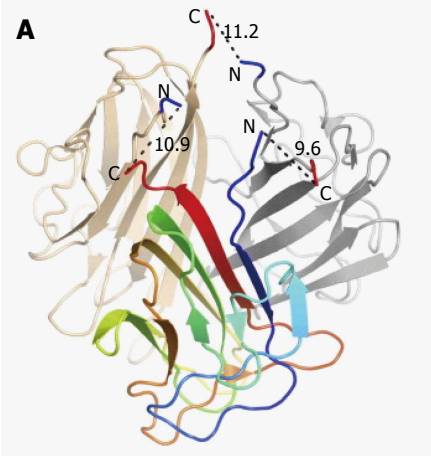

Figure 2 Structure of single-chain globular domain adiponectin (sc-gAd). A: Base region of mouse gAd structure where blue arrow determines the N terminus and red arrow determines the C terminus; B: Domain organization of human adiponectin and the sc-gAd, where there are three domains A, B and C respectively. (Adapted from Min *et al*[30]).

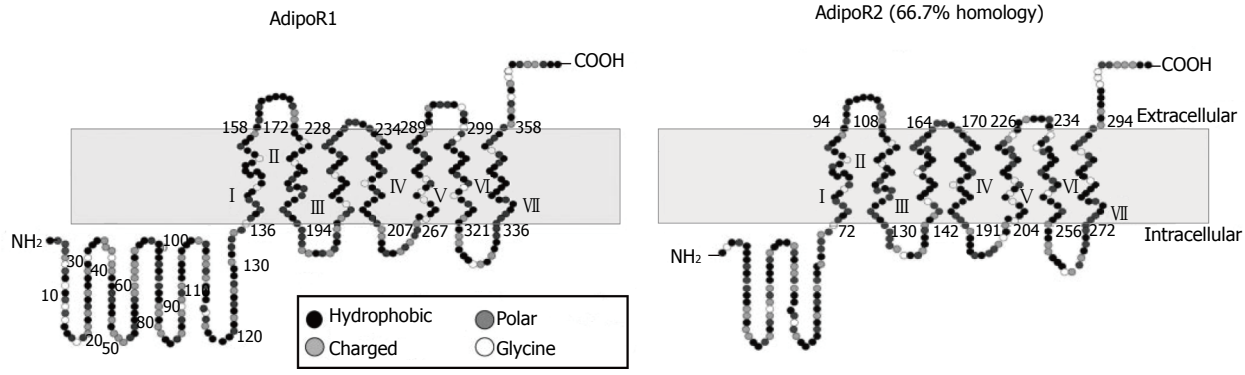

Figure 3 Proposed structure of adiponectin receptors (Adapted from Kadowaki *et al*[36]).

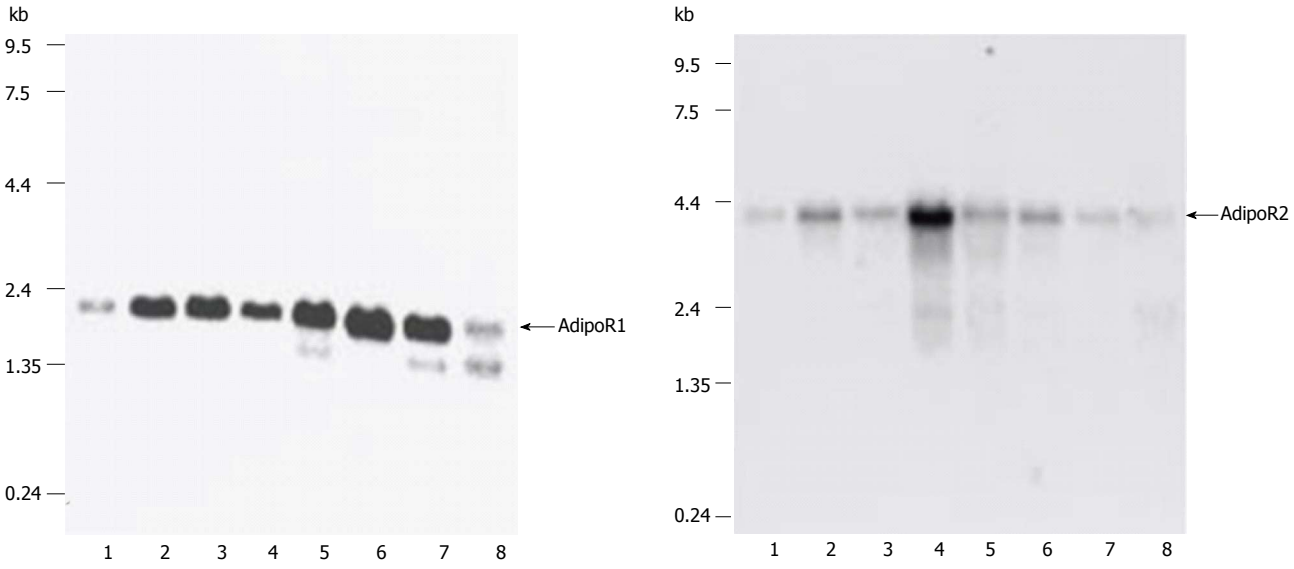

Figure 4 Northern blot analysis of AdipoR1 (top panel) and AdipoR2 (bottom panel) mRNA in mouse tissues (lanes: 1, brain; 2, heart; 3, kidney; 4, liver; 5, lung; 6, skeletal muscle; 7, spleen; 8, testis)[35]. AdipoR: Adiponectin receptors.

and functionally completely distinct from G-protein-coupled receptors. Mild insulin resistance has been observed in both *adipoR1* and *adipoR2* knocked out mice, but complete abolition of adiponectin activity has been observed in *adipoR1/R2* double knockout mice, resulting in increased tissue triglyceride content, inflammation and oxidative stress[37].

It has been observed by one research group (Figure 5) that abolition of AdipoR2 eradicates β cell replication and neogenesis, thus in presence of high energy diet although it shows moderate insulin sensitivity initially and shows moderate body mass, in later state it tends to

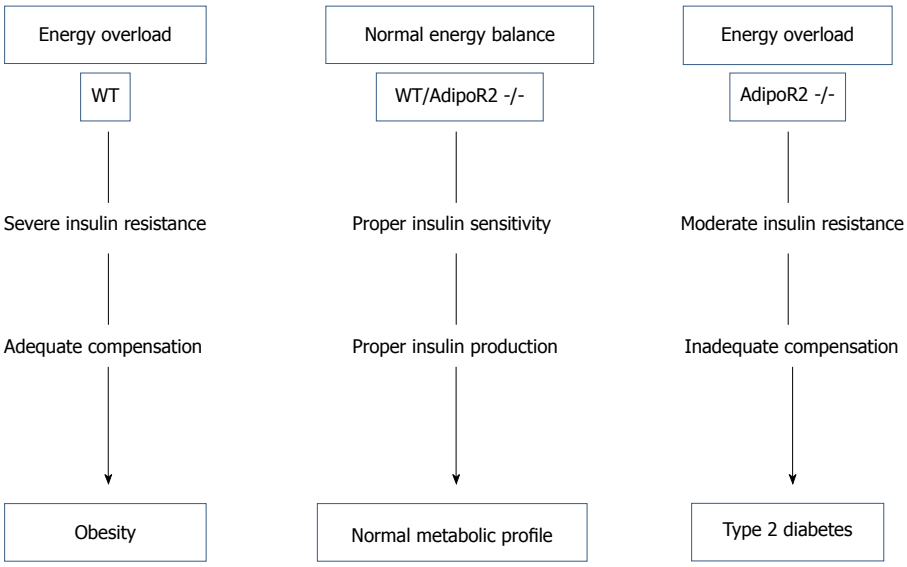

Figure 5 Diagram depicting the metabolic profile of wild type and AdipoR2 -/- mice (Adapted from Liu *et al*[38]). WT: Wild type; AdipoR 2: Adiponectin receptors 2.

develop type 2 diabetes[38]. Insulin resistance consuming high energy diet increases obesity in wild type (WT) mice, where normal energy diet in both WT and AdipoR2 double knocked out mice (AdipoR2 -/-) shows normal metabolism, but AdipoR2 -/- mice with energy overload although shows moderate insulin resistance initially but in later stage develops type 2 diabetes.

In increased oxidation of fatty acids such as in Nonalcoholic steatohepatitis (NASH) the expression levels of AdipoR1/R2 and insulin receptor substrate isoforms 2 (IRS-2) were significantly decreased, whereas IRS-1 was significantly increased[39].

ADIPONECTIN AND ITS ROLE IN OBESITY AND DIABETES

Although it circulates in high concentrations, adiponectin levels are lower in obese subjects than their lean counterparts. Apart from negative correlations with measures of adiposity, adiponectin levels are also reduced in association with insulin resistance and type 2 diabetes[40]. Epidemiological studies in different ethnic groups revealed that low level of plasma adiponectin, especially its HMW form can be an important key factor for type 2 diabetes, hypertension, atherosclerosis and myocardial infarction[41]. Other than preventing insulin resistance and adipose tissue inflammation, adiponectin has been associated to exert several cardioprotective roles through direct actions on heart as well as on other vascular cells (Figure 6)[42]. Adiponectin has negative correlation with insulin resistance, along with it maintains negative correlation with plasma triglyceride and low density lipoproteins (LDLs) where it has positive correlation with high density lipoproteins (HDLs)[43]. In this review we will try to elucidate the role of adiponectin in acquiring adiposity in various aspects, *i.e.*, from the metabolomic view to genetic predisposition.

Adipocyte derived adiponectin can modulate the functions of cardiomyocytes, endothelial cells, endothelial progenitor cells, macrophages, leukocytes, and vascular smooth muscles in both endocrine and paracrine manner (Figure 6). Here we will discuss the possible roles of this adipokine in type 2 diabetes mellitus, obesity and dyslipidemia.

Studies in Japan showed that hypertension has a major effect on atherosclerosis and CVD events in persons with high body mass index with T2DM[16]. Adiponectin and its association with lipid metabolism and increased obesity are studied well in many populations.

Mode of actions of this potential biomarker

Adiponectin serves as a central regulatory protein in many metabolic pathways playing crucial role in many metabolic disorders. Its importance as a potential biomarker in type 2 diabetes is increasing rapidly. The major way to estimate plasma adiponectin is by Sandwich ELISA. Lower plasma adiponectin level (< 5 μg/mL) is associated with increasing obesity and acquiring of metabolic disorders.

As a key factor of the metabolic pathway: Adiponectin has multifunctional roles in metabolic synchronization (Figure 7). Adiponectin (ADIPOQ) an adipocyte derived hormone activates ADIPOR1 and ADIPOR2, the two adiponectin receptors; it also activates PPARγ ultimately increasing the rate of β oxidation which is a major pathway for lipid metabolism. ADIPOR1 increases the action of number of genes including NF-kβ, TNFα, IL1, IL4. NF-kβ[41] furthermore decreases VCAM1, ICAM1 and IL18 levels; these are important genes involved in inflammation. ADIPOR1 also activates p38MAPK, another gene involved in transcriptional machinery. The action of PI3K is indirectly regulated by ADIPOR1. P13K acts on HSP90 which again increases the action of

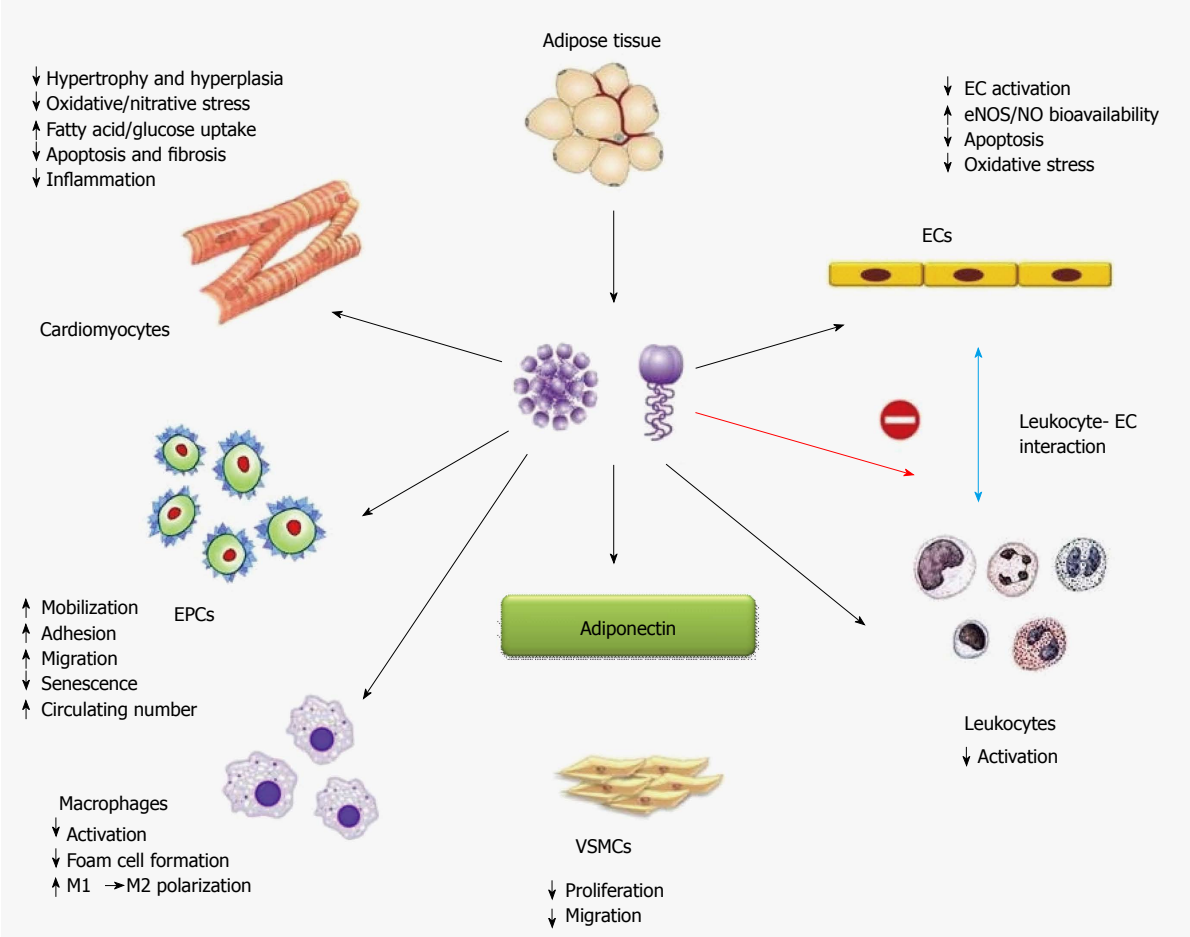

Figure 6 Actions of adiponectin in different cell line (Adapted from Xu *et al*[42]). EC: Endothelial cell; EPCs: Endothelial progenitor cells; VSMC: Vascular smooth muscles; eNOS: Endothelial nitric oxide synthase.

endothelial nitric oxide synthase (eNOS), which is related to oxidative stress. ADIPOR2 activates APPL1 which up regulates AMP-activated protein kinase 1 (AMPK1) which again up regulates eNOS[40,41] increasing the production of nitric oxide. Elevated AMPK also increases the action of PEPCK ultimately increasing gluconeogenesis. APPL1 works on Akt which increases Glut4 translocation ultimately elevating glucose uptake of the cell[42] (Figure 7). Derived from adipocyte it comes in contact with blood plasma and directly acts on Adipo R1/R2 receptors which further activates/inhibits the downstream genes related to oxidative stress and inflammation. Plasma and hemolysate of patients of type 2 diabetes contains elevated level of protein carbonyl content, which indicates increased oxidative stress[44].

T-cadherin (CDH13) localizes adiponectin to the vascular endothelium. It has been reported that T-cadherin deficiency by siRNA knockdown prevented the ability of adiponectin to promote cellular migration and proliferation[45]. T-cadherin protects from stress-induced pathological cardiac remodeling by binding with adiponectin and activating its cardioprotective functions in mice[46].

Mechanisms of action: Adiponectin exhibits two major mechanisms of action by which it inhibits obesity and type 2 diabetes, one by increasing insulin sensitivity and the other way is to increase fatty acid oxidation.

APPL1, stimulated by adiponectin can interact with both adiponectin receptors and can mediate the down-stream events such as lipid oxidation and membrane translocation of glucose transport 4 (GLUT4), thus increasing glucose uptake (Figure 7), providing a platform for increased insulin sensitization[47]. APPL1 also acts as a mediator of adiponectin signaling pathways by interacting directly with ADIPOR1/ADIPOR2 or signaling proteins, thereby playing critical roles in cell proliferation, apoptosis, cell survival, endosomal trafficking, and chromatin remodelling[48]. APPL1 modulates the insulin signalling pathway by acting with Akt and PI3K[49] (Figure 7).

The major form of storing and transporting fatty acids is triglycerides. Adiponectin has been reported to decrease tissue triglyceride content by increasing the expression of CD36, a fatty acid transporter[50]. Increased tissue TG content activates PI3K and Glut4 increasing glucose uptake, elevating insulin resistance[51]. Thus, lowering of tissue triglyceride content promotes insulin sensitivity. Along with adiponectin has been also reported to increase the expression of PPARα which further lowers the tissue triglyceride content[50]. Some researcher

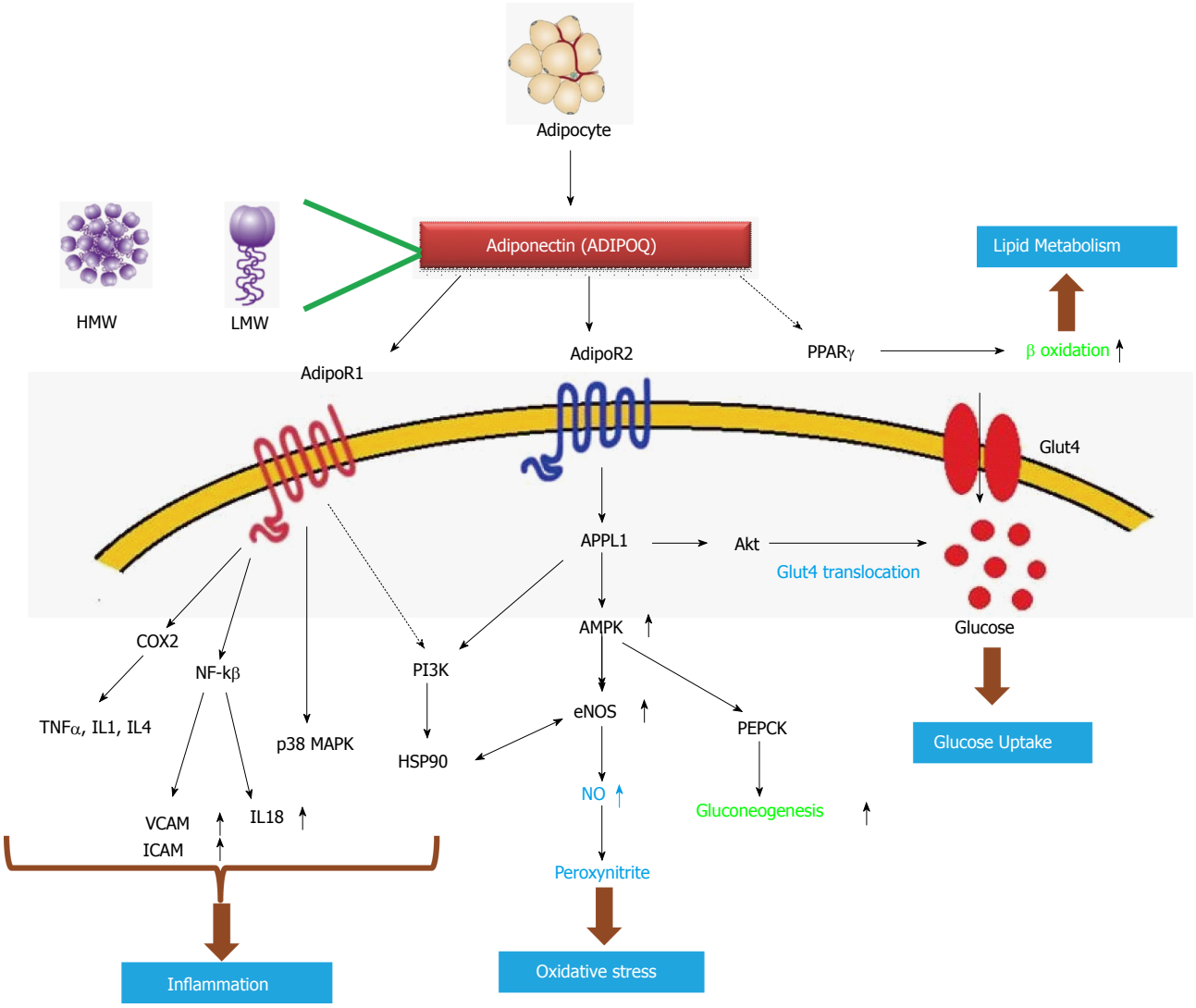

Figure 7 A proposed models of adiponectin metabolic pathway and associated genes. HMW: High molecular weight; LMW: Low molecular weight AdipoR1: Adiponectin receptor 1; PPARγ: Peroxisome proliferator-activated receptor gamma; Glut4: Glucose transporter type 4; APPL1: Adaptor protein, phosphotyrosine interaction, pH domain and leucine zipper containing 1; Akt: Protein kinase B; COX2: Cyclooxygenase 2; AMPK: Adenosine monophosphate-activated protein kinase; PI3K: Phosphoinositide 3-kinase; NF-kβ: Nuclear factor kappa-light-chain-enhancer of activated B cells; TNFα: Tumor necrosis factor alpha; IL: Interleukin; p38 MAPK: p38 mitogen-activated protein kinase; HSP90: Heat shock protein 90; eNOS: Endothelial nitric oxide synthase; PEPCK: Phosphoenolpyruvate carboxykinase; NO: Nitric oxide; VCAM: Vascular cell adhesion protein; ICAM: Intercellular adhesion molecule.

group has demonstrated the role of adiponectin in activating AMPK which can stimulate β oxidation and glucose up taking[52].

It has been established that adiponectin enhances insulin-stimulated IRS-1 tyrosine and Akt phosphorylation. Activation of the LKB1/AMPK/TSC1/2 pathway alleviates the p70S6 kinase-mediated negative regulation of insulin signaling, providing a mechanism by which adiponectin increases insulin sensitivity in cells[53].

Other than playing a crucial role as an insulin sensitizer, adiponectin also defeats obesity and obesity onset type 2 diabetes by increasing fatty acid oxidation. Increased fatty acid oxidation in turn also elevates insulin sensitivity. As stated earlier, adiponectin associated activation of AMPK phosphorylation which in turn implements major role in fatty acid oxidation. In cultured myotubes C2C12, adiponectin treatment has been associated with increased PPARα activity; expression of some downstream genes such as suchasacyl-CoAoxidase and carnitinepalmitoyltransferase1 has been also reported, thus promoting fatty acid oxidation[54]. Adiponectin induces fatty acid oxidation in muscle cells by sequential activation of AMPK, p38 MAPK (mitogen activated protein kinase) and PPARα[54]. It has been studied in humans that LDL activity is correlated positively with plasma adiponectin level, thus LPL may represent a link between low adiponectin levels and dyslipidemia in both nondiabetic individuals and patients with type 2 diabetes[55] where plasma TGs is negatively correlated with LDL activity and positively with diabetic state[56].

It has been well postulated that subjects with type 2 diabetes has reduced mitochondrial content and decreased electron transport chain activity[57]. Adiponectin has been reported to increase mitochondrial biogenesis

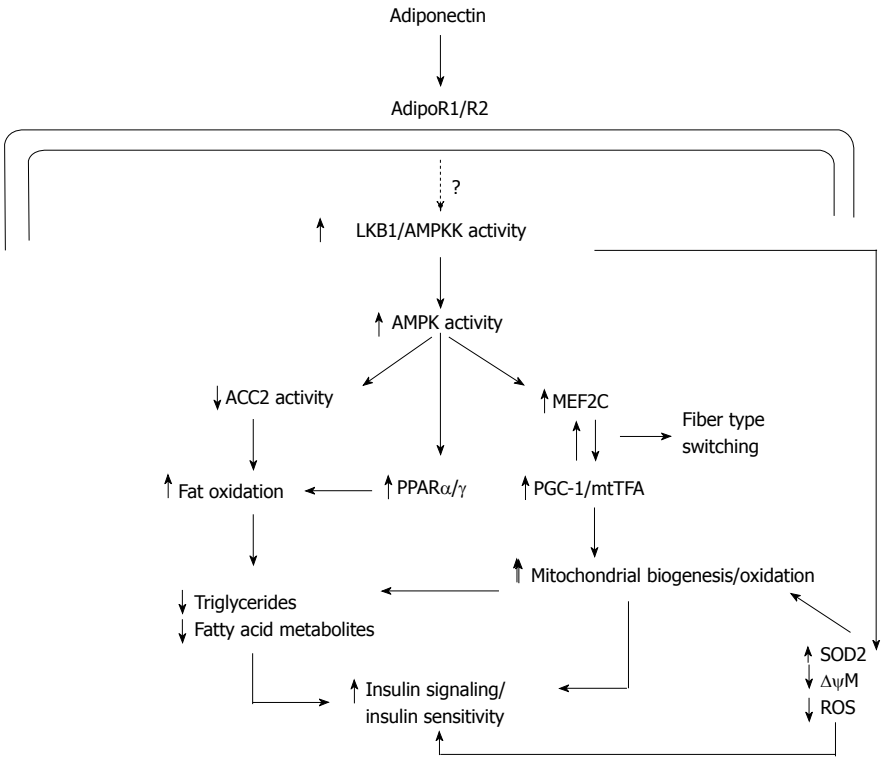

Figure 8 Hypothetical scheme of adiponectin signaling and the regulation of mitochondrial function in skeletal muscle (Adapted from Civitarese *et al*[59]). AdipoR1: Adiponectin receptor 1; PPARγ: Peroxisome proliferator-activated receptor gamma; LKB1: Liver kinase B1; AMPKK: Adenosine monophosphate-activated protein kinase kinase; AMPK: Adenosine monophosphate-activated protein kinase; ACC2: Acetyl-CoA carboxylase 2; MEF2C: Myocyte-specific enhancer factor 2C; PPARα: Peroxisome proliferator-activated receptor alpha; PGC-1: Peroxisome proliferator-activated receptor gamma coactivator 1; mtTFA: Mitochondrial transcription factor A; SOD2: Superoxide dismutase 2; ΔψM: Mitochondrial membrane potential; ROS: Reactive oxygen species.

and oxidative capacity in mice which in turn is favorable for glucose metabolism as well as fatty acid oxidation as mitochondria is the major cellular site of metabolism[58]. In human model also the adiponectin stimulated mitochondrial biogenesis has been observed[59] (Figure 8).

Adiponectin binds to its receptors which activates AMPK and stimulates the phosphorylation of ACC2 which in turn increases fatty-acid oxidation. As previously discussed adiponectin can activate peroxisome proliferative activated receptor-α (PPARα) stimulating transcription of genes in the fatty-acid oxidation pathway and decreasing triglyceride content in muscle, thus promoting fatty acid oxidation (Figure 8) and improving insulin sensitivity[50,54]. Independent of changes in transcription and mitochondrial mass, the improvements in lipid oxidation occur in less than 6 h in mice[52]. Adiponectin activation of AMPK by upstream kinase AMPK kinase activates transcription of myocyte enhancer factor 2C and phosphorylation of peroxisome proliferative activated receptor γ coactivator 1-α (PGC1α), which in turn increases mitochondrial content, oxidative capacity, and oxidative-fibre type composition. Central to the development of mitochondrial dysfunction is reactive oxygen species (ROS) production, which reacts with DNA, protein, and lipids leading to oxidative damage. ROS production is inversely related to mitochondrial content. Activation of the adiponectin pathway reduces the generation of ROS by two processes: (1) Increasing mitochondrial content, which in turn decreases the workload for each mitochondrion leading to reduced membrane potential (←Δψ) and lower ROS production; and (2) adiponectin increases PGC1α activity which increases the transcription/activity of the antioxidant enzyme SOD2 that decreases super oxide radical ($O_2^{\bullet-}$)[60].

Oxidative stress is a major consequence of type 2 diabetes and obesity related disorders. Previously in our laboratory we had established that hyperglycaemic condition increases the oxygen releasing capacity of haemoglobin which in turn boosts the effect of oxidative stress in diabetes and CVDs[44]. Oxidative stress which is a major indicator of inflammation correlates significantly with adiponectin metabolic pathway. Study by a research group demonstrates that lower adiponectin level is significantly associated with higher inflammatory state[61].

Other than decreasing circulating free fatty acid and lowering triglyceride content adiponectin has been also observed to exert anti-inflammatory and anti-atherogenic effects by reducing TNFα-induced monocyte attachment to endothelial cells and inhibiting platelet derived growth factor-BB to minimize vascular smooth muscle cell proliferation[62]. Most adipokines can exert pro inflammatory effects, among which adiponectin is increasing its importance as a potential inflammatory marker. Obesity is characterized by low grade systemic inflammation[63]. Adiponectin inhibits the action of TNFα which is a key pro inflammatory cytokine in both vascular and cardiac tissue[64]. This novel cytokine has been also reported to decrease the secretion of IL 8 from human aortic endothelial cells (HAEC) stimulated with TNFα, along with it also inhibits IL8 mRNA expression induced by TNFα. Phosphorylation of IkBα is decreased by adiponectin, but phosphorylation of ERK, SAPK/JNK, and p38MAPK remains unaffected[65]. Adiponectin also increases intra-cellular cAMP levels in HAEC and increases PKA activity[65]. The inverse relationship of adiponectin with inflammatory marker CRP has been

discussed in the next paragraph of this review.

Thus adiponectin exerts several multitasking roles and combat the prevalence of metabolic disorders like diabetes and obesity. In first step it works as a fascinating insulin sensitizer and in second step it increases fatty acid oxidation. Simultaneously in all above mentioned mode of actions it acts as an important inflammatory marker while playing significant role in minimizing oxidative stress. Thus adiponectin plays affluent role to protect the metabolic harmony of the system through various metabolic pathways and considered as one of the potential biochemical and inflammatory biomarker in metabolic disorders.

Correlation with other adipocyte derived hormones

Adipocyte is involved with the releasing of another three hormones playing some roles in metabolism; these are leptin, resistin and visfatin. Where low plasma adiponectin has been observed in obesity, leptin levels become significantly higher, having an inverse correlation with adiponectin. Increased subcutaneous fat has been a major determinant of leptin levels. The action of leptin remains to decrease appetite, thermogenesis and increase fatty acid oxidation[66]. The leptin signal is transmitted by the Janus kinase, signal transducer; and activator of transcription pathway decrease glucose, and reduce body weight and fat[66]. One research group showed that adiponectin is more influenced by visceral adipose tissue where leptin is by subcutaneous adipose tissue[62] where fasting glucose, insulin, HOMA-IR and triglyceride has an inverse correlation with adiponectin and leptin maintaining a fairly positive association with these parameters[67]. It is reported that leptin/adiponectin ratio alters in type 2 diabetes as this alteration increases insulin resistance[68]. Another research group reported the plasma leptin/adiponectin ratio as an important atherogenic index[69]. Thus it can be concluded that where adiponectin is a proinflammatory adipokine giving proatherogeneic effect, leptin serves as an antiinflammatory molecule giving a direct antiatherogenic effect.

The plasma level of resistin, a cysteine rich adipokine has been observed to increase in type 2 diabetes but this increase in level is not correlated with insulin resistance and adiposity[70]. Another research group found a decrease in serum resistin value in patients with type 2 diabetes[71]. Where adiponectin level is significantly associated with lipid profile, BMI, resistin levels seem to level independent of these attributes in patients with type 2 diabetes mellitus[72]. Thus the association of resistin with type 2 diabetes, obesity and dyslipidemia is still a new field to explore; and the association of this adipokine with adiponectin is poorly understood.

Visfatin, another adipokine maintains a direct relationship between plasma visfatin levels and type 2 diabetes mellitus. Visfatin binds to the insulin receptor at a site distinct from that of insulin and causes hypoglycaemia by reducing glucose release from liver cells and stimulating glucose utilization in adipocytes and myocytes. Visfatin is upregulated by inflammation and hyperglycaemia and downregulated by insulin[73]. Where the association of visfatin with diabetes mellitus has been well studied its correlation with adiponectin is poorly known. Although it has been postulated in one article that adiponectin maintains a fairly inverse relationship with visfatin[74]. Thus activity of other adipokines with adiponectin is still remained a major field to explore in metabolic syndrome.

Association with other important diabetic biomarker

Adiponectin which is increasing its importance as a potential biomarker maintains some association with other diabetic biomarkers such as fasting insulin, C-reactive protein (CRP) and homocysteine. Fasting insulin and CRP has been observed to maintain an inverse correlation with adiponectin level[75]. A data observed on Asian Indian obese men revealed that serum adiponectin level is inversely related with fasting insulin and CRP[76]. Both adiponectin and CRP is strongly associated with insulin sensitivity where CRP is more dependent on adiposity[77]. One study group found no significant correlation between plasma homocysteine level and adiponectin in patients with type 2 diabetes[78]. Although an inverse relationship was found between adiponectin and homocysteine in patients with type 1 diabetes but no significant association has been reported in type 2 diabetes[79].

Genetic variants and expression of genes in adiponectin metabolic pathway

Genetic polymorphisms in ADIPOQ gene and the genes of its receptors has been a major reason for functional defect of this novel adipokine. Genetic polymorphisms of the other genes present in adiponectin metabolic pathway may also alter the functional properties of adiponectin and thus promoting the progression of insulin resistance, dyslipidemia and atherogenesis. These genetic polymorphisms have seen in many ethnic groups. ADIPOQ gene polymorphisms were associated with the risk of T2DM in Chinese Han population[80]. It has been observed that rs2241767AG genotype increases the risk of T2DM in obesity group[80]. A study in south Indian population implies ADIPOQ gene +276 G/T and -3971 A/G polymorphisms are associated with generalized obesity and +349 A/G with central obesity[81].

The polymorphism -1131 T/C in apolipoprotein A5 gene is associated with postprandial hypertriacylglycerolemia, elevated small, dense LDL concentrations and oxidative stress in non-obese Korean men[82] and dyslipidemia in Brazilian subjects[83] (Table 2). A significant association of -11391 G/A adiponectin gene polymorphism with waist circumference in diabetic patients has been observed[84]. In white Europeans, +276 G/T was associated with higher serum adiponectin concentrations where -10066 G/A was associated with lower serum adiponectin concentrations[85]. Genetic polymorphisms of ADIPOR1 and ADIPOR2 are also

Table 2 List of SNPs found in the genes of adiponectin pathway in metabolic disorders such as type 2 diabetes, obesity, dyslipidemia and cardiovascular disorders (coursey to http://www.genecards.org/ for providing the information regarding SNP loacation)

Ref.	Gene	SL No.	Location	Variation	SNP ID
Blech *et al*[96]	*PPARγ*	1	Intron 1	Pro12Ala	rs1801282
Blech *et al*[96];	*ADIPOQ*	1	5′ flanking region	−11365 C/G	rs266729
Ramya *et al*[81]		2	Intron 1	-4522 C/T	rs822393
		3	Intron 1	-3971 A/G	rs822396
		4	Intron 1	+276 G/T	rs1501299
		5	Exon 1 coding synonymous	+45 T/G	rs2241766
		6	Intron 1	+349 A/G	rs2241767
		7	Intron 1	+712 G/A	rs3774261
		8	5′ flanking region	-11391 G/A	rs17300539
		9	Exon 3 splicing enhancers	Y111H T/C	rs17366743
Wang *et al*[97]	*ADIPOR1*	1	Intron 1	+5646 A/G	rs1342386
		2	Intron 1	+5843 A/G	rs1342387
		3	Intron 1	-101 T/G	rs2275737
		4	5′ transcription factor binding site	-8503C/T	rs6666089
Vaxillaire *et al*[98]	*ADIPOR2*	1	Exon 3 splicing enhancers	+33371 C/T	rs12342
		2	Intron 1	+26314 A/G	rs767870
		3	5′ flanking region	-64241 T/G	rs1029629
		4	Intron 1	+8645 G/C	rs1468491
		5	Intron 1	+14645 A/T	rs4766415
		6	Intron 1	-35361 G/A	rs10773982
Thameem *et al*[99]	*eNOS/NOS3*	1	Exon 3 splicing enhancers	Glu298Asp	rs1799983
		2	Intron 1	-786 T/C	rs2070744
Zhang *et al*[100]	*NF-kB*	1	5′ flanking region	-94 insertion/deletion	rs28362491
Rees *et al*[101]	*PEPCK*	1	5′ flanking region	-232C/G	rs2071023
Jang *et al*[82] and Ferreira *et al*[83]	*Apolipoprotein A5 gene (APOA5)*	1	5′ flanking region	-1131T/C	rs662799
Ol *et al*[102]	*COX-2*	1	5′ flanking region	-765G/C	rs20417
Ho *et al*[103]	*IL4*	1	5′ flanking region	-590 C/T	rs2243250

IL-6: Interleukin 6; AdipoR: Adiponectin receptors; eNOS: Endothelial nitric oxide synthase; PPARγ: Peroxisome proliferator-activated receptor gamma; COX2: Cyclooxygenase 2; NF-kβ: Nuclear factor kappa-light-chain-enhancer of activated B cells.

involved in altered function of adiponectin and have been observed by many groups (Table 2). Polymorphisms of other pathway genes like eNOS, NF-kB, PEPCK, IL4 (Table 2) has been also reported to play roles in development of type 2 diabetes, thus they may correlate with adiponectin and regulate its function.

Adiponectin gene function is not solely dependent on gene polymorphisms rather expression levels of certain genes may modulate its function significantly. Both in type 2 diabetic patients and in animal models of insulin resistance it has been observed that the mRNA expression and secretion of adiponectin is significantly decreased[86,87]. Very low calorie diet has been reported to raise adiponectin mRNA level, whereas re-feeding significantly decreases the mRNA level in morbidly obese women[88]. AdipoR2 mRNA expression in subcutaneous tissue is negatively associated with insulin resistance and metabolic parameters independently of obesity may mediate the improvement of insulin resistance in response to exercise[89]. PPARγ agonist thiazolidinedione has been reported to increase adiponectin level in animal models and human patients[90]. A single nucleotide polymorphism in Pro12Ala in PPARγ is reported to be involved in type 2 diabetes. PPARγ has been found to undergo obesity-induced and protein kinase cdk5-mediated phosphorylation at Ser^{273} which mediates obesity-induced down-regulation of adiponectin in white adipose tissue[91].

There are certain evidences that adrenomedullin (ADM) may modulate the expression of adiponectin gene. One group of scientists postulated that a genetic variant in ADM gene (rs182052) alters the expression of adiponectin gene and minimizes plasma adiponectin levels[92]. A variation in CDH13 (rs4783244) showed strong associations with total adiponectin and HMW adiponectin in East Asian population where people with this variation have significantly lower adiponectin plasma level, but adiponectin sensitivity tends to increase, eventually maintaining a better metabolic profile[46].

Glucocorticoids are also reported to regulate adiponectin gene expression in human adipocytes, where TNFα does not seem to directly inhibit adiponectin synthesis in human adipocytes[93]. SIRT1 and Foxo1, two important genes involved in insulin sensitivity whose low expression leads to impaired Foxo1-C/EBPα complex formation, has been reported to decrease adiponectin expression in obesity and type 2 diabetes[94]. CRP has been reported to suppress adiponectin gene expression partially through the PI3K pathway where decreased production of adiponectin might represent a mechanism by which CRP regulates insulin sensitivity[95].

Genetic polymorphisms which supposed to be a screening tool of adiponectin metabolic disorder may be overpowered by the altered gene expression of

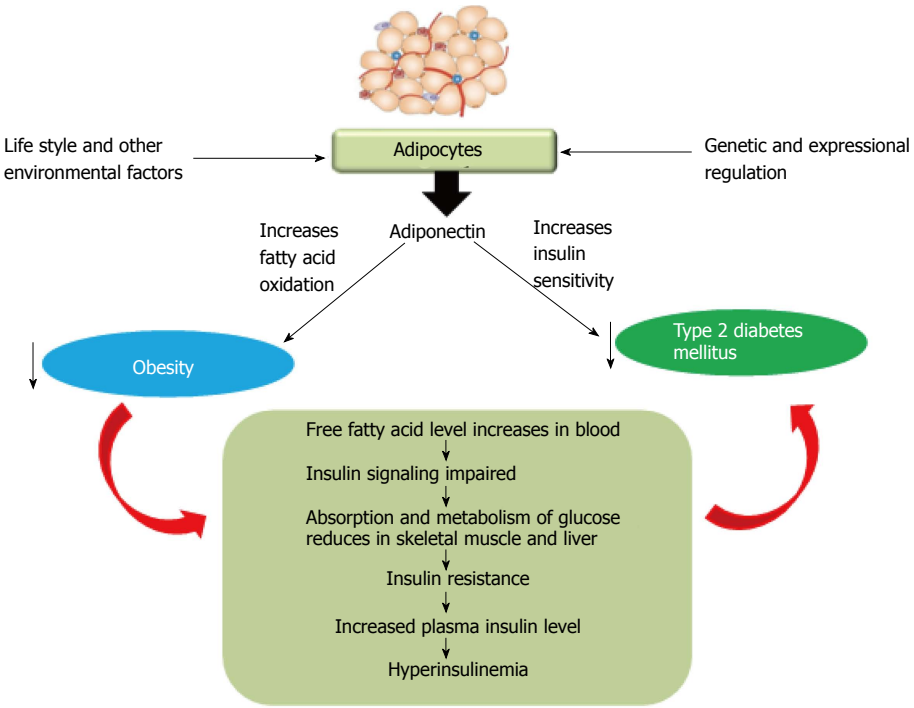

Figure 9 Hypothetical model showing the interrelation between adiponectin, obesity and type 2 diabetes mellitus.

adiponectin and related genes. Both of these actions may significantly be associated with low expression of adiponectin which in turn is positively correlated with insulin resistance increasing the prevalence of diabetes and obesity.

Adiponectin and epigenetics

Epigenetic association of adiponectin expression is remained a big question to answer. DNA methylation can partly explain the link between the early exposures to a detrimental fetal environment, where the mother is hyperglycemic which may in turn increase the risk to develop obesity and diabetes later in life[104]. One group found significant correlation between the mother blood glucose level and placental DNA methylation at cytosines located at *ADIPOQ* gene proximal promoter CpG islands[105]. Expression and methylation of ADIPOR1 gene isolated from skeletal muscle cells has been modified after an exercise period of 6 mo in subjects who are first degree relatives of type 2 diabetes patients[106]. But still there are few evidences of the epigenetic modulation of adiponectin and remains a promising field to explore.

Clinical aspects

Balanced diet with adequate exercise can combat obesity and type 2 diabetes in manifold. Although genetic predisposition is a main key factor of these disorders by still maintaining a well-balanced energy is still a beneficiary supplements in preventing these disorders. Exercise can fairly maintains plasma adiponectin levels and thus promoting insulin sensitivity. One study shows that aerobic exercise increases insulin sensitivity among diabetic patients mediated by adiponectin[107], although drug treatment may be required to normalize plasma adiponectin levels. Adiponectin replenishment therapy is yet not possible as biologically active recombinant adiponectin proteins are inherently unstable and difficult to produce[108]. Certain drug classes such as antidiabetic drugs glitazones and sulfonylureas, and angiotensin receptor blockers, ACE inhibitors and nicotinic acid exert beneficial effects on insulin resistance partly by increasing plasma adiponectin levels. Others such as tetrahydrobiopterin or certain antioxidants are also promising in normalizing plasma adiponectin levels[109]. Omega-3 polyunsaturated fatty acids has been reported to increase plasma adiponectin to leptin ratio in stable coronary artery disease, thus playing a cardioprotective role, might in turn be beneficiary for diabetes and obesity[110]. Thus a healthy life style with some oral supplements may increase adiponectin levels in patients with type 2 diabetes.

CONCLUSION

Adiponectin, the novel adipocyte has been demonstrated well to play crucial role in obesity and type 2 diabetes mellitus (Figure 9). It is increasing its importance as a potential biomarker in above mentioned diseased state as: (1) It increases insulin sensitivity; (2) It increases fatty acid oxidation; (3) It correlates significantly with oxidative stress; and (4) It acts as an important inflammatory biomarker and up/down regulates many genes in various metabolic pathways. Thus adiponectin could be a novel target for the therapeutic approach to treat diabetes mellitus in near future. Recombinant adiponectin is not effective thus altered expression of adiponectin or related

pathway genes could be an effective tool for researchers to mediate its function which in turn may minimize the prevalence of obesity, type 2 diabetes or other metabolic disorders.

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REFERENCES

1 **Maeda K**, Okubo K, Shimomura I, Funahashi T, Matsuzawa Y, Matsubara K. cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (AdiPose Most abundant Gene transcript 1). *Biochem Biophys Res Commun* 1996; **221**: 286-289 [PMID: 8619847 DOI: 10.1006/bbrc.1996.0587]

2 **Cnop M**, Havel PJ, Utzschneider KM, Carr DB, Sinha MK, Boyko EJ, Retzlaff BM, Knopp RH, Brunzell JD, Kahn SE. Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: evidence for independent roles of age and sex. *Diabetologia* 2003; **46**: 459-469 [PMID: 12687327 DOI: 10.1007/s00125-003-1074-z]

3 **Ouchi N**, Kihara S, Arita Y, Maeda K, Kuriyama H, Okamoto Y, Hotta K, Nishida M, Takahashi M, Nakamura T, Yamashita S, Funahashi T, Matsuzawa Y. Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. *Circulation* 1999; **100**: 2473-2476 [PMID: 10604883 DOI: 10.1161/01.CIR.100.25.2473]

4 **Hotta K**, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y, Iwahashi H, Kuriyama H, Ouchi N, Maeda K, Nishida M, Kihara S, Sakai N, Nakajima T, Hasegawa K, Muraguchi M, Ohmoto Y, Nakamura T, Yamashita S, Hanafusa T, Matsuzawa Y. Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. *Arterioscler Thromb Vasc Biol* 2000; **20**: 1595-1599 [PMID: 10845877 DOI: 10.1161/01.ATV.20.6.1595]

5 **Lindsay RS**, Funahashi T, Hanson RL, Matsuzawa Y, Tanaka S, Tataranni PA, Knowler WC, Krakoff J. Adiponectin and development of type 2 diabetes in the Pima Indian population. *Lancet* 2002; **360**: 57-58 [PMID: 12114044 DOI: 10.1016/S0140-6736(02)09335-2]

6 **Spranger J**, Kroke A, Möhlig M, Bergmann MM, Ristow M, Boeing H, Pfeiffer AF. Adiponectin and protection against type 2 diabetes mellitus. *Lancet* 2003; **361**: 226-228 [PMID: 12547549 DOI: 10.1016/S0140-6736(03)12255-6]

7 **Pischon T**, Girman CJ, Hotamisligil GS, Rifai N, Hu FB, Rimm EB. Plasma adiponectin levels and risk of myocardial infarction in men. *JAMA* 2004; **291**: 1730-1737 [PMID: 15082700 DOI: 10.1001/jama.291.14.1730]

8 **Nakamura Y**, Shimada K, Fukuda D, Shimada Y, Ehara S, Hirose M, Kataoka T, Kamimori K, Shimodozono S, Kobayashi Y, Yoshiyama M, Takeuchi K, Yoshikawa J. Implications of plasma concentrations of adiponectin in patients with coronary artery disease. *Heart* 2004; **90**: 528-533 [PMID: 15084551 DOI: 10.1136/hrt.2003.011114]

9 **Barcelo A**, Gregg EW, Gerzoff RB, Wong R, Perez Flores E, Ramirez-Zea M, Cafiero E, Altamirano L, Ascencio Rivera M, de Cosio G, de Maza MD, del Aguila R, Emanuel E, Gil E, Gough E, Jenkins V, Orellana P, Palma R, Palomo R, Pastora M, Peña R, Pineda E, Rodriguez B, Tacsan L, Thompson L, Villagra L. Prevalence of diabetes and intermediate hyperglycemia among adults from the first multinational study of noncommunicable diseases in six Central American countries: the Central America Diabetes Initiative (CAMDI). *Diabetes Care* 2012; **35**: 738-740 [PMID: 22323417 DOI: 10.2337/dc11-1614]

10 **Uauy R**, Albala C, Kain J. Obesity trends in Latin America: transiting from under- to overweight. *J Nutr* 2001; **131**: 893S-899S [PMID: 11238781]

11 **WHO Expert Consultation.** Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet* 2004; **363**: 157-163 [PMID: 14726171 DOI: 10.1016/S0140-6736(03)15268-3]

12 **Chakraborty A,** Bhattacharyya M. Diabetes, Hypertension and Cardiovascular Disease-An Unsolved Enigma. *Phytotherapy in the Management of Diabetes and Hypertension* 2012: 85-119

13 **Ramachandran A**. Socio-economic burden of diabetes in India. *J Assoc Physicians India* 2007; **55** Suppl: 9-12 [PMID: 17927005]

14 **Cade WT**. Diabetes-related microvascular and macrovascular diseases in the physical therapy setting. *Phys Ther* 2008; **88**: 1322-1335 [PMID: 18801863 DOI: 10.2522/ptj.20080008]

15 **Chakraborty A**, Chowdhury S, Bhattacharyya M. Effect of metformin on oxidative stress, nitrosative stress and inflammatory biomarkers in type 2 diabetes patients. *Diabetes Res Clin Pract* 2011; **93**: 56-62 [PMID: 21146883 DOI: 10.1016/j.diabres.2010.11.030]

16 **Saito I**. Epidemiological evidence of type 2 diabetes mellitus, metabolic syndrome, and cardiovascular disease in Japan. *Circ J* 2012; **76**: 1066-1073 [PMID: 22453006 DOI: 10.1253/circj.CJ-11-1519]

17 **Coelho VG**, Caetano LF, Liberatore Júnior Rdel R, Cordeiro JA, Souza DR. [Lipid profile and risk factors for cardiovascular diseases in medicine students]. *Arq Bras Cardiol* 2005; **85**: 57-62 [PMID: 16041456]

18 **Rizk NM**, Yousef M. Association of lipid profile and waist circumference as cardiovascular risk factors for overweight and obesity among school children in Qatar. *Diabetes Metab Syndr Obes* 2012; **5**: 425-432 [PMID: 23277742 DOI: 10.2147/DMSO.S39189]

19 **Fletcher B**, Berra K, Ades P, Braun LT, Burke LE, Durstine JL, Fair JM, Fletcher GF, Goff D, Hayman LL, Hiatt WR, Miller NH, Krauss R, Kris-Etherton P, Stone N, Wilterdink J, Winston M. Managing abnormal blood lipids: a collaborative approach. *Circulation* 2005; **112**: 3184-3209 [PMID: 16286609 DOI: 10.1161/CIRCULATIONAHA.105.169180]

20 **Wilson PW**, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB. Prediction of coronary heart disease using risk factor categories. *Circulation* 1998; **97**: 1837-1847 [PMID: 9603539 DOI: 10.1161/01.CIR.97.18.1837]

21 **Brezinka V**, Padmos I. Coronary heart disease risk factors in women. *Eur Heart J* 1994; **15**: 1571-1584 [PMID: 7835374]

22 **Grundy SM**, Benjamin IJ, Burke GL, Chait A, Eckel RH, Howard BV, Mitch W, Smith SC, Sowers JR. Diabetes and cardiovascular disease: a statement for healthcare professionals from the American Heart Association. *Circulation* 1999; **100**: 1134-1146 [PMID: 10477542 DOI: 10.1161/01.CIR.100.10.1134]

23 **Stone PH**, Muller JE, Hartwell T, York BJ, Rutherford JD, Parker CB, Turi ZG, Strauss HW, Willerson JT, Robertson T. The effect of diabetes mellitus on prognosis and serial left ventricular function after acute myocardial infarction: contribution of both coronary disease and diastolic left ventricular dysfunction to the adverse prognosis. The MILIS Study Group. *J Am Coll Cardiol* 1989; **14**: 49-57 [PMID: 2661630 DOI: 10.1016/0735-1097(89)90053-3]

24 **Singer DE**, Moulton AW, Nathan DM. Diabetic myocardial infarction. Interaction of diabetes with other preinfarction risk factors. *Diabetes* 1989; **38**: 350-357 [PMID: 2917699 DOI: 10.2337/diabetes.38.3.350]

25 **Smith JW**, Marcus FI, Serokman R. Prognosis of patients with diabetes mellitus after acute myocardial infarction. *Am J Cardiol* 1984; **54**: 718-721 [PMID: 6385680 DOI: 10.1016/

S0002-9149(84)80196-4]
26 **Rondinone CM**. Adipocyte-derived hormones, cytokines, and mediators. *Endocrine* 2006; **29**: 81-90 [PMID: 16622295 DOI: 10.1385/ENDO: 29: 1: 181]
27 **Scherer PE**, Williams S, Fogliano M, Baldini G, Lodish HF. A novel serum protein similar to C1q, produced exclusively in adipocytes. *J Biol Chem* 1995; **270**: 26746-26749 [PMID: 7592907 DOI: 10.1074/jbc.270.45.26746]
28 **Chandran M**, Phillips SA, Ciaraldi T, Henry RR. Adiponectin: more than just another fat cell hormone? *Diabetes Care* 2003; **26**: 2442-2450 [PMID: 12882876 DOI: 10.2337/diacare.26.8.2442]
29 **Siitonen N**, Pulkkinen L, Lindström J, Kolehmainen M, Eriksson JG, Venojärvi M, Ilanne-Parikka P, Keinänen-Kiukaanniemi S, Tuomilehto J, Uusitupa M. Association of ADIPOQ gene variants with body weight, type 2 diabetes and serum adiponectin concentrations: the Finnish Diabetes Prevention Study. *BMC Med Genet* 2011; **12**: 5 [PMID: 21219602 DOI: 10.1186/1471-2350-12-5]
30 **Min X**, Lemon B, Tang J, Liu Q, Zhang R, Walker N, Li Y, Wang Z. Crystal structure of a single-chain trimer of human adiponectin globular domain. *FEBS Lett* 2012; **586**: 912-917 [PMID: 22449980 DOI: 10.1016/j.febslet.2012.02.024]
31 **Mohamed-Ali V**, Pinkney JH, Coppack SW. Adipose tissue as an endocrine and paracrine organ. *Int J Obes Relat Metab Disord* 1998; **22**: 1145-1158 [PMID: 9877249 DOI: 10.1038/sj.ijo.0800770]
32 **Rosen ED**, Spiegelman BM. Adipocytes as regulators of energy balance and glucose homeostasis. *Nature* 2006; **444**: 847-853 [PMID: 17167472 DOI: 10.1038/nature05483]
33 **Fruebis J**, Tsao TS, Javorschi S, Ebbets-Reed D, Erickson MR, Yen FT, Bihain BE, Lodish HF. Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. *Proc Natl Acad Sci USA* 2001; **98**: 2005-2010 [PMID: 11172066 DOI: 10.1073/pnas.041591798]
34 **Pajvani UB**, Du X, Combs TP, Berg AH, Rajala MW, Schulthess T, Engel J, Brownlee M, Scherer PE. Structure-function studies of the adipocyte-secreted hormone Acrp30/adiponectin. Implications fpr metabolic regulation and bioactivity. *J Biol Chem* 2003; **278**: 9073-9085 [PMID: 12496257 DOI: 10.1074/jbc.M207198200]
35 **Yamauchi T**, Kamon J, Ito Y, Tsuchida A, Yokomizo T, Kita S, Sugiyama T, Miyagishi M, Hara K, Tsunoda M, Murakami K, Ohteki T, Uchida S, Takekawa S, Waki H, Tsuno NH, Shibata Y, Terauchi Y, Froguel P, Tobe K, Koyasu S, Taira K, Kitamura T, Shimizu T, Nagai R, Kadowaki T. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. *Nature* 2003; **423**: 762-769 [PMID: 12802337 DOI: 10.1038/nature01705]
36 **Kadowaki T**, Yamauchi T. Adiponectin and adiponectin receptors. *Endocr Rev* 2005; **26**: 439-451 [PMID: 15897298 DOI: 10.1210/er.2005-0005]
37 **Yamauchi T**, Nio Y, Maki T, Kobayashi M, Takazawa T, Iwabu M, Okada-Iwabu M, Kawamoto S, Kubota N, Kubota T, Ito Y, Kamon J, Tsuchida A, Kumagai K, Kozono H, Hada Y, Ogata H, Tokuyama K, Tsunoda M, Ide T, Murakami K, Awazawa M, Takamoto I, Froguel P, Hara K, Tobe K, Nagai R, Ueki K, Kadowaki T. Targeted disruption of AdipoR1 and AdipoR2 causes abrogation of adiponectin binding and metabolic actions. *Nat Med* 2007; **13**: 332-339 [PMID: 17268472 DOI: 10.1038/nm1557]
38 **Liu Y**, Michael MD, Kash S, Bensch WR, Monia BP, Murray SF, Otto KA, Syed SK, Bhanot S, Sloop KW, Sullivan JM, Reifel-Miller A. Deficiency of adiponectin receptor 2 reduces diet-induced insulin resistance but promotes type 2 diabetes. *Endocrinology* 2007; **148**: 683-692 [PMID: 17068142 DOI: 10.1210/en.2006-0708]
39 **Matsunami T**, Sato Y, Ariga S, Sato T, Shimomura T, Kashimura H, Hasegawa Y, Yukawa M. Regulation of synthesis and oxidation of fatty acids by adiponectin receptors (AdipoR1/R2) and insulin receptor substrate isoforms (IRS-1/-2) of the liver in a nonalcoholic steatohepatitis animal model. *Metabolism* 2011; **60**: 805-814 [PMID: 20846698 DOI: 10.1016/j.metabol.2010.07.032]
40 **Lihn AS**, Pedersen SB, Richelsen B. Adiponectin: action, regulation and association to insulin sensitivity. *Obes Rev* 2005; **6**: 13-21 [PMID: 15655035]
41 **Zhu W**, Cheng KK, Vanhoutte PM, Lam KS, Xu A. Vascular effects of adiponectin: molecular mechanisms and potential therapeutic intervention. *Clin Sci* (Lond) 2008; **114**: 361-374 [PMID: 18230060]
42 **Xu A**, Vanhoutte PM. Adiponectin and adipocyte fatty acid binding protein in the pathogenesis of cardiovascular disease. *Am J Physiol Heart Circ Physiol* 2012; **302**: H1231-H1240 [PMID: 22210749 DOI: 10.1152/ajpheart.00765.2011]
43 **Yamamoto Y**, Hirose H, Saito I, Tomita M, Taniyama M, Matsubara K, Okazaki Y, Ishii T, Nishikai K, Saruta T. Correlation of the adipocyte-derived protein adiponectin with insulin resistance index and serum high-density lipoprotein-cholesterol, independent of body mass index, in the Japanese population. *Clin Sci* (Lond) 2002; **103**: 137-142 [PMID: 12149104]
44 **Saha A**, Adak S, Chowdhury S, Bhattacharyya M. Enhanced oxygen releasing capacity and oxidative stress in diabetes mellitus and diabetes mellitus-associated cardiovascular disease: a comparative study. *Clin Chim Acta* 2005; **361**: 141-149 [PMID: 16098498 DOI: 10.1016/j.cccn.2005.05.018]
45 **Parker-Duffen JL**, Nakamura K, Silver M, Kikuchi R, Tigges U, Yoshida S, Denzel MS, Ranscht B, Walsh K. T-cadherin is essential for adiponectin-mediated revascularization. *J Biol Chem* 2013; **288**: 24886-24897 [PMID: 23824191 DOI: 10.1074/jbc.M113.454835]
46 **Denzel MS**, Scimia MC, Zumstein PM, Walsh K, Ruiz-Lozano P, Ranscht B. T-cadherin is critical for adiponectin-mediated cardioprotection in mice. *J Clin Invest* 2010; **120**: 4342-4352 [PMID: 21041950 DOI: 10.1172/JCI43464]
47 **Mao X**, Kikani CK, Riojas RA, Langlais P, Wang L, Ramos FJ, Fang Q, Christ-Roberts CY, Hong JY, Kim RY, Liu F, Dong LQ. APPL1 binds to adiponectin receptors and mediates adiponectin signalling and function. *Nat Cell Biol* 2006; **8**: 516-523 [PMID: 16622416 DOI: 10.1038/ncb1404]
48 **Deepa SS**, Dong LQ. APPL1: role in adiponectin signaling and beyond. *Am J Physiol Endocrinol Metab* 2009; **296**: E22-E36 [PMID: 18854421 DOI: 10.1152/ajpendo.90731.2008]
49 **Manning BD**, Cantley LC. AKT/PKB signaling: navigating downstream. *Cell* 2007; **129**: 1261-1274 [PMID: 17604717 DOI: 10.1016/j.cell.2007.06.009]
50 **Yamauchi T**, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, Mori Y, Ide T, Murakami K, Tsuboyama-Kasaoka N, Ezaki O, Akanuma Y, Gavrilova O, Vinson C, Reitman ML, Kagechika H, Shudo K, Yoda M, Nakano Y, Tobe K, Nagai R, Kimura S, Tomita M, Froguel P, Kadowaki T. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat Med* 2001; **7**: 941-946 [PMID: 11479627 DOI: 10.1038/90984]
51 **Shulman GI**. Cellular mechanisms of insulin resistance. *J Clin Invest* 2000; **106**: 171-176 [PMID: 10903330 DOI: 10.1172/JCI10583]
52 **Yamauchi T**, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S, Yamashita S, Noda M, Kita S, Ueki K, Eto K, Akanuma Y, Froguel P, Foufelle F, Ferre P, Carling D, Kimura S, Nagai R, Kahn BB, Kadowaki T. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat Med* 2002; **8**: 1288-1295 [PMID: 12368907]
53 **Wang C**, Mao X, Wang L, Liu M, Wetzel MD, Guan KL, Dong LQ, Liu F. Adiponectin sensitizes insulin signaling by reducing p70 S6 kinase-mediated serine phosphorylation of IRS-1. *J Biol Chem* 2007; **282**: 7991-7996 [PMID: 17244624 DOI: 10.1074/jbc.M700098200]

54 **Yoon MJ**, Lee GY, Chung JJ, Ahn YH, Hong SH, Kim JB. Adiponectin increases fatty acid oxidation in skeletal muscle cells by sequential activation of AMP-activated protein kinase, p38 mitogen-activated protein kinase, and peroxisome proliferator-activated receptor alpha. *Diabetes* 2006; **55**: 2562-2570 [PMID: 16936205 DOI: 10.2337/db05-1322]

55 **von Eynatten M**, Schneider JG, Humpert PM, Rudofsky G, Schmidt N, Barosch P, Hamann A, Morcos M, Kreuzer J, Bierhaus A, Nawroth PP, Dugi KA. Decreased plasma lipoprotein lipase in hypoadiponectinemia: an association independent of systemic inflammation and insulin resistance. *Diabetes Care* 2004; **27**: 2925-2929 [PMID: 15562208 DOI: 10.2337/diacare.27.12.2925]

56 **De Vries R**, Wolffenbuttel BH, Sluiter WJ, van Tol A, Dullaart RP. Post-heparin plasma lipoprotein lipase, but not hepatic lipase activity, is related to plasma adiponectin in type 2 diabetic patients and healthy subjects. *Clin Lab* 2005; **51**: 403-409 [PMID: 16122151]

57 **Kelley DE**, He J, Menshikova EV, Ritov VB. Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. *Diabetes* 2002; **51**: 2944-2950 [PMID: 12351431 DOI: 10.2337/diabetes.51.10.2944]

58 **Iwabu M**, Yamauchi T, Okada-Iwabu M, Sato K, Nakagawa T, Funata M, Yamaguchi M, Namiki S, Nakayama R, Tabata M, Ogata H, Kubota N, Takamoto I, Hayashi YK, Yamauchi N, Waki H, Fukayama M, Nishino I, Tokuyama K, Ueki K, Oike Y, Ishii S, Hirose K, Shimizu T, Touhara K, Kadowaki T. Adiponectin and AdipoR1 regulate PGC-1alpha and mitochondria by Ca(2+) and AMPK/SIRT1. *Nature* 2010; **464**: 1313-1319 [PMID: 20357764 DOI: 10.1038/nature08991]

59 **Civitarese AE**, Ukropcova B, Carling S, Hulver M, DeFronzo RA, Mandarino L, Ravussin E, Smith SR. Role of adiponectin in human skeletal muscle bioenergetics. *Cell Metab* 2006; **4**: 75-87 [PMID: 16814734 DOI: 10.1016/j.cmet.2006.05.002]

60 **Maassen JA**, Janssen GM, Lemkes HH. Mitochondrial diabetes mellitus. *J Endocrinol Invest* 2002; **25**: 477-484 [PMID: 12035948]

61 **Chen SJ**, Yen CH, Huang YC, Lee BJ, Hsia S, Lin PT. Relationships between inflammation, adiponectin, and oxidative stress in metabolic syndrome. *PLoS One* 2012; **7**: e45693 [PMID: 23029185 DOI: 10.1371/journal.pone.0045693]

62 **Park KG**, Park KS, Kim MJ, Kim HS, Suh YS, Ahn JD, Park KK, Chang YC, Lee IK. Relationship between serum adiponectin and leptin concentrations and body fat distribution. *Diabetes Res Clin Pract* 2004; **63**: 135-142 [PMID: 14739054]

63 **Ouchi N**, Walsh K. Adiponectin as an anti-inflammatory factor. *Clin Chim Acta* 2007; **380**: 24-30 [PMID: 17343838 DOI: 10.1016/j.cca.2007.01.026]

64 **Ouchi N**, Walsh K. A novel role for adiponectin in the regulation of inflammation. *Arterioscler Thromb Vasc Biol* 2008; **28**: 1219-1221 [PMID: 18565846 DOI: 10.1161/ATVBAHA.108.165068]

65 **Kobashi C**, Urakaze M, Kishida M, Kibayashi E, Kobayashi H, Kihara S, Funahashi T, Takata M, Temaru R, Sato A, Yamazaki K, Nakamura N, Kobayashi M. Adiponectin inhibits endothelial synthesis of interleukin-8. *Circ Res* 2005; **97**: 1245-1252 [PMID: 16269654 DOI: 10.1161/01.RES.0000194328.57164.36]

66 **Yadav A**, Kataria MA, Saini V, Yadav A. Role of leptin and adiponectin in insulin resistance. *Clin Chim Acta* 2013; **417**: 80-84 [PMID: 23266767 DOI: 10.1016/j.cca.2012.12.007]

67 **Matsubara M**, Maruoka S, Katayose S. Inverse relationship between plasma adiponectin and leptin concentrations in normal-weight and obese women. *Eur J Endocrinol* 2002; **147**: 173-180 [PMID: 12153737 DOI: 10.1530/eje.0.1470173]

68 **Oda N**, Imamura S, Fujita T, Uchida Y, Inagaki K, Kakizawa H, Hayakawa N, Suzuki A, Takeda J, Horikawa Y, Itoh M. The ratio of leptin to adiponectin can be used as an index of insulin resistance. *Metabolism* 2008; **57**: 268-273 [PMID: 18191059 DOI: 10.1016/j.metabol.2007.09.011]

69 **Satoh N**, Naruse M, Usui T, Tagami T, Suganami T, Yamada K, Kuzuya H, Shimatsu A, Ogawa Y. Leptin-to-adiponectin ratio as a potential atherogenic index in obese type 2 diabetic patients. *Diabetes Care* 2004; **27**: 2488-2490 [PMID: 15451921 DOI: 10.2337/diacare.27.10.2488]

70 **Hasegawa G**, Ohta M, Ichida Y, Obayashi H, Shigeta M, Yamasaki M, Fukui M, Yoshikawa T, Nakamura N. Increased serum resistin levels in patients with type 2 diabetes are not linked with markers of insulin resistance and adiposity. *Acta Diabetol* 2005; **42**: 104-109 [PMID: 15944845 DOI: 10.1007/s00592-005-0187-x]

71 **Yang J**, Li M, Wu CY, Wang H, Xu QS, Deng JY. [Reduced resistin levels in patients with type 2 diabetes mellitus]. *Zhonghua Yixue Zazhi* 2003; **83**: 1471-1474 [PMID: 14521723]

72 **Shetty GK**, Economides PA, Horton ES, Mantzoros CS, Veves A. Circulating adiponectin and resistin levels in relation to metabolic factors, inflammatory markers, and vascular reactivity in diabetic patients and subjects at risk for diabetes. *Diabetes Care* 2004; **27**: 2450-2457 [PMID: 15451915]

73 **Adeghate E**. Visfatin: structure, function and relation to diabetes mellitus and other dysfunctions. *Curr Med Chem* 2008; **15**: 1851-1862 [PMID: 18691043 DOI: 10.2174/092986708785133004]

74 **El-Hini SH**, Mohamed FI, Hassan AA, Ali F, Mahmoud A, Ibraheem HM. Visfatin and adiponectin as novel markers for evaluation of metabolic disturbance in recently diagnosed rheumatoid arthritis patients. *Rheumatol Int* 2013; **33**: 2283-2289 [PMID: 23471745 DOI: 10.1007/s00296-013-2714-3]

75 **Adam FM**, Nara MG, Adam JM. Fasting insulin, adiponectin, hs-CRP levels, and the components of metabolic syndrome. *Acta Med Indones* 2006; **38**: 179-184 [PMID: 17132879 DOI: 10.1267/science.040579197]

76 **Vikram NK**, Misra A, Pandey RM, Dwivedi M, Luthra K. Adiponectin, insulin resistance, and C-reactive protein in postpubertal Asian Indian adolescents. *Metabolism* 2004; **53**: 1336-1341 [PMID: 15375791 DOI: 10.1016/j.metabol.2004.05.010]

77 **Putz DM**, Goldner WS, Bar RS, Haynes WG, Sivitz WI. Adiponectin and C-reactive protein in obesity, type 2 diabetes, and monodrug therapy. *Metabolism* 2004; **53**: 1454-1461 [PMID: 15536601 DOI: 10.1016/j.metabol.2004.06.013]

78 **Sakuta H**, Suzuki T, Yasuda H, Ito T. Adiponectin levels and cardiovascular risk factors in Japanese men with type 2 diabetes. *Endocr J* 2005; **52**: 241-244 [PMID: 15863955 DOI: 10.1507/endocrj.52.241]

79 **Heilman K**, Zilmer M, Zilmer K, Kool P, Tillmann V. Elevated plasma adiponectin and decreased plasma homocysteine and asymmetric dimethylarginine in children with type 1 diabetes. *Scand J Clin Lab Invest* 2009; **69**: 85-91 [PMID: 18830896 DOI: 10.1080/00365510802419454]

80 **Du W**, Li Q, Lu Y, Yu X, Ye X, Gao Y, Ma J, Cheng J, Cao Y, Du J, Shi H, Zhou L. Genetic variants in ADIPOQ gene and the risk of type 2 diabetes: a case-control study of Chinese Han population. *Endocrine* 2011; **40**: 413-422 [PMID: 21594755 DOI: 10.1007/s12020-011-9488-8]

81 **Ramya K**, Ayyappa KA, Ghosh S, Mohan V, Radha V. Genetic association of ADIPOQ gene variants with type 2 diabetes, obesity and serum adiponectin levels in south Indian population. *Gene* 2013; **532**: 253-262 [PMID: 24055485 DOI: 10.1016/j.gene.2013.09.012]

82 **Jang Y**, Kim JY, Kim OY, Lee JE, Cho H, Ordovas JM, Lee JH. The -1131T--& gt; C polymorphism in the apolipoprotein A5 gene is associated with postprandial hypertriacylglycerolemia; elevated small, dense LDL concentrations; and oxidative stress in nonobese Korean men. *Am J Clin Nutr* 2004; **80**: 832-840 [PMID: 15447887]

83 **Ferreira CN**, Carvalho MG, Fernandes AP, Santos IR, Rodrigues KF, Lana AM, Almeida CR, Loures-Vale AA, Gomes KB, Sousa MO. The polymorphism -1131T& gt; C in

apolipoprotein A5 gene is associated with dyslipidemia in Brazilian subjects. *Gene* 2013; **516**: 171-175 [PMID: 23266809 DOI: 10.1016/j.gene.2012.12.016]

84 **Hasani-Ranjbar S**, Amoli MM, Tabatabaei-Malazy O, Rumi Y, Tavakkoly-Bazzaz J, Samimi H, Abbasifarid E. Effect of adiponectin gene polymorphisms on waist circumference in patients with diabetes. *J Diabetes Metab Disord* 2012; **11**: 14 [PMID: 23497697 DOI: 10.1186/2251-6581-11-14]

85 **AlSaleh A**, O'Dell SD, Frost GS, Griffin BA, Lovegrove JA, Jebb SA, Sanders TA. Single nucleotide polymorphisms at the ADIPOQ gene locus interact with age and dietary intake of fat to determine serum adiponectin in subjects at risk of the metabolic syndrome. *Am J Clin Nutr* 2011; **94**: 262-269 [PMID: 21562092 DOI: 10.3945/ajcn.111.014209]

86 **Weyer C**, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y, Pratley RE, Tataranni PA. Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. *J Clin Endocrinol Metab* 2001; **86**: 1930-1935 [PMID: 11344187 DOI: 10.1210/jc.86.5.1930]

87 **Yang WS**, Lee WJ, Funahashi T, Tanaka S, Matsuzawa Y, Chao CL, Chen CL, Tai TY, Chuang LM. Weight reduction increases plasma levels of an adipose-derived anti-inflammatory protein, adiponectin. *J Clin Endocrinol Metab* 2001; **86**: 3815-3819 [PMID: 11502817 DOI: 10.1210/jc.86.8.3815]

88 **Liu YM**, Lacorte JM, Viguerie N, Poitou C, Pelloux V, Guy-Grand B, Coussieu C, Langin D, Basdevant A, Clément K. Adiponectin gene expression in subcutaneous adipose tissue of obese women in response to short-term very low calorie diet and refeeding. *J Clin Endocrinol Metab* 2003; **88**: 5881-5886 [PMID: 14671185 DOI: 10.1210/jc.2003-030886]

89 **Blüher M**, Williams CJ, Klöting N, Hsi A, Ruschke K, Oberbach A, Fasshauer M, Berndt J, Schön MR, Wolk A, Stumvoll M, Mantzoros CS. Gene expression of adiponectin receptors in human visceral and subcutaneous adipose tissue is related to insulin resistance and metabolic parameters and is altered in response to physical training. *Diabetes Care* 2007; **30**: 3110-3115 [PMID: 17878241 DOI: 10.2337/dc07-1257]

90 **Maeda N**, Takahashi M, Funahashi T, Kihara S, Nishizawa H, Kishida K, Nagaretani H, Matsuda M, Komuro R, Ouchi N, Kuriyama H, Hotta K, Nakamura T, Shimomura I, Matsuzawa Y. PPARgamma ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. *Diabetes* 2001; **50**: 2094-2099 [PMID: 11522676 DOI: 10.2337/diabetes.50.9.2094]

91 **Choi JH**, Banks AS, Estall JL, Kajimura S, Boström P, Laznik D, Ruas JL, Chalmers MJ, Kamenecka TM, Blüher M, Griffin PR, Spiegelman BM. Anti-diabetic drugs inhibit obesity-linked phosphorylation of PPARgamma by Cdk5. *Nature* 2010; **466**: 451-456 [PMID: 20651683 DOI: 10.1038/nature09291]

92 **Wong HK**, Ong KL, Leung RY, Cheung TT, Xu A, Lam TH, Lam KS, Cheung BM. Plasma level of adrenomedullin is influenced by a single nucleotide polymorphism in the adiponectin gene. *PLoS One* 2013; **8**: e70335 [PMID: 23936408 DOI: 10.1371/journal.pone.0070335]

93 **Degawa-Yamauchi M**, Moss KA, Bovenkerk JE, Shankar SS, Morrison CL, Lelliott CJ, Vidal-Puig A, Jones R, Considine RV. Regulation of adiponectin expression in human adipocytes: effects of adiposity, glucocorticoids, and tumor necrosis factor alpha. *Obes Res* 2005; **13**: 662-669 [PMID: 15897474 DOI: 10.1038/oby.2005.74]

94 **Qiao L**, Shao J. SIRT1 regulates adiponectin gene expression through Foxo1-C/enhancer-binding protein alpha transcriptional complex. *J Biol Chem* 2006; **281**: 39915-39924 [PMID: 17090532 DOI: 10.1074/jbc.M607215200]

95 **Yuan G**, Chen X, Ma Q, Qiao J, Li R, Li X, Li S, Tang J, Zhou L, Song H, Chen M. C-reactive protein inhibits adiponectin gene expression and secretion in 3T3-L1 adipocytes. *J Endocrinol* 2007; **194**: 275-281 [PMID: 17641277 DOI: 10.1677/JOE-07-0133]

96 **Blech I**, Katzenellenbogen M, Katzenellenbogen A, Wainstein J, Rubinstein A, Harman-Boehm I, Cohen J, Pollin TI, Glaser B. Predicting diabetic nephropathy using a multifactorial genetic model. *PLoS One* 2011; **6**: e18743 [PMID: 21533139 DOI: 10.1371/journal.pone.0018743]

97 **Wang H**, Zhang H, Jia Y, Zhang Z, Craig R, Wang X, Elbein SC. Adiponectin receptor 1 gene (ADIPOR1) as a candidate for type 2 diabetes and insulin resistance. *Diabetes* 2004; **53**: 2132-2136 [PMID: 15277397 DOI: 10.2337/diabetes.53.8.2132]

98 **Vaxillaire M**, Dechaume A, Vasseur-Delannoy V, Lahmidi S, Vatin V, Leprêtre F, Boutin P, Hercberg S, Charpentier G, Dina C, Froguel P. Genetic analysis of ADIPOR1 and ADIPOR2 candidate polymorphisms for type 2 diabetes in the Caucasian population. *Diabetes* 2006; **55**: 856-861 [PMID: 16505255 DOI: 10.2337/diabetes.55.03.06.db05-0665]

99 **Thameem F**, Puppala S, Arar NH, Stern MP, Blangero J, Duggirala R, Abboud HE. Endothelial nitric oxide synthase (eNOS) gene polymorphisms and their association with type 2 diabetes-related traits in Mexican Americans. *Diab Vasc Dis Res* 2008; **5**: 109-113 [PMID: 18537098 DOI: 10.3132/dvdr.2008.018]

100 **Zhang D**, Li L, Zhu Y, Zhao L, Wan L, Lv J, Li X, Huang P, Wei L, Ma M. The NFKB1 -94 ATTG insertion/deletion polymorphism (rs28362491) contributes to the susceptibility of congenital heart disease in a Chinese population. *Gene* 2013; **516**: 307-310 [PMID: 23299027 DOI: 10.1016/j.gene.2012.12.078]

101 **Rees SD**, Britten AC, Bellary S, O'Hare JP, Kumar S, Barnett AH, Kelly MA. The promoter polymorphism -232C/G of the PCK1 gene is associated with type 2 diabetes in a UK-resident South Asian population. *BMC Med Genet* 2009; **10**: 83 [PMID: 19725958 DOI: 10.1186/1471-2350-10-83]

102 **Ol KK**, Agachan B, Gormus U, Toptas B, Isbir T. Cox-2 gene polymorphism and IL-6 levels in coronary artery disease. *Genet Mol Res* 2011; **10**: 810-816 [PMID: 21574137 DOI: 10.4238/vol10-2gmr967]

103 **Ho KT**, Shiau MY, Chang YH, Chen CM, Yang SC, Huang CN. Association of interleukin-4 promoter polymorphisms in Taiwanese patients with type 2 diabetes mellitus. *Metabolism* 2010; **59**: 1717-1722 [PMID: 20580039 DOI: 10.1016/j.metabol.2010.04.010]

104 **Houde AA,** Hivert MF, Bouchard L. Fetal epigenetic programming of adipokines. *Adipocyte* 2013; **2**: 41-46 [PMID: 23700551]

105 **Bouchard L**, Hivert MF, Guay SP, St-Pierre J, Perron P, Brisson D. Placental adiponectin gene DNA methylation levels are associated with mothers' blood glucose concentration. *Diabetes* 2012; **61**: 1272-1280 [PMID: 22396200 DOI: 10.2337/db11-1160]

106 **Nitert MD**, Dayeh T, Volkov P, Elgzyri T, Hall E, Nilsson E, Yang BT, Lang S, Parikh H, Wessman Y, Weishaupt H, Attema J, Abels M, Wierup N, Almgren P, Jansson PA, Rönn T, Hansson O, Eriksson KF, Groop L, Ling C. Impact of an exercise intervention on DNA methylation in skeletal muscle from first-degree relatives of patients with type 2 diabetes. *Diabetes* 2012; **61**: 3322-3332 [PMID: 23028138 DOI: 10.2337/db11-1653]

107 **Yokoyama H**, Emoto M, Araki T, Fujiwara S, Motoyama K, Morioka T, Koyama H, Shoji T, Okuno Y, Nishizawa Y. Effect of aerobic exercise on plasma adiponectin levels and insulin resistance in type 2 diabetes. *Diabetes Care* 2004; **27**: 1756-1758 [PMID: 15220262 DOI: 10.2337/diacare.27.7.1756]

108 **Gu W**, Li Y. The therapeutic potential of the adiponectin pathway. *BioDrugs* 2012; **26**: 1-8 [PMID: 22050309 DOI: 10.2165/11594790-000000000-00000]

109 **Antonopoulos AS**, Lee R, Margaritis M, Antoniades C. Adiponectin as a regulator of vascular redox state: therapeutic implications. *Recent Pat Cardiovasc Drug Discov* 2011; **6**: 78-88 [PMID: 21453253 DOI: 10.2174/157489011795933837]

110 **Mostowik M**, Gajos G, Zalewski J, Nessler J, Undas A.

Omega-3 polyunsaturated fatty acids increase plasma adiponectin to leptin ratio in stable coronary artery disease. *Cardiovasc Drugs Ther* 2013; **27**: 289-295 [PMID: 23584593 DOI: 10.1007/s10557-013-6457-x]

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MINIREVIEWS

Rare complications of pediatric diabetic ketoacidosis

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Abstract

The incidence of type 1 diabetes (T1D) among youth is steadily increasing across the world. Up to a third of pediatric patients with T1D present with diabetic ketoacidosis, a diagnosis that continues to be the leading cause of death in this population. Cerebral edema is the most common rare complication of diabetic ketoacidosis in children. Accordingly, treatment and outcome measures of cerebral edema are vastly researched and the pathophysiology is recently the subject of much debate. Nevertheless, cerebral edema is not the only sequela of diabetic ketoacidosis that warrants close monitoring. The medical literature details various other complications in children with diabetic ketoacidosis, including hypercoagulability leading to stroke and deep vein thrombosis, rhabdomyolysis, pulmonary and gastrointestinal complications, and long-term memory dysfunction. We review the pathophysiology, reported cases, management, and outcomes of each of these rare complications in children. As the incidence of T1D continues to rise, practitioners will care for an increasing number of pediatric patients with diabetic ketoacidosis and should be aware of the various systems that may be affected in both the acute and chronic setting.

Key words: Type 1 diabetes; Diabetic ketoacidosis; Complications; Pediatric; Review

Core tip: Diabetic ketoacidosis is highly prevalent in pediatric patients with both newly diagnosed and established type 1 diabetes. The most common rare complication is cerebral edema, which is the leading cause of death in youth with diabetes. However, several other complications involving multiple systems have been described and can cause significant morbidity in cases of pediatric diabetic ketoacidosis, thus warranting awareness and targeted monitoring.

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INTRODUCTION

Approximately 1 in 300 youth have type 1 diabetes (T1D)[1], and the incidence in the pediatric population is

increasing by almost 3% each year in the United States[2] and worldwide[3]. Despite the burgeoning statistics and awareness, the prevalence of diabetic ketoacidosis (DKA) remains as high as 30% in children presenting with T1D[4]. DKA is defined by the American Diabetes Association[5], the European Society for Paediatric Endocrinology, and the Pediatric Endocrine Society[6] as hyperglycemia (plasma glucose > 200 mg/dL or approximately 11 mmol/L) and venous pH < 7.3 and/or bicarbonate < 15 mmol/L. DKA is the most common cause of death in children with T1D[7,8], and the most common rare and primary fatal complication of DKA is cerebral edema[7]. The treatment and prevention of cerebral edema is, therefore, the subject of extensive medical research and attention. However, cerebral edema is not the only complication of DKA worthy of close monitoring during patient care. In this review article we will examine cerebral edema as well as the vascular, musculoskeletal, pulmonary, gastrointestinal, and cognitive complications of pediatric DKA, which are less common but can result in acute and long-term morbidity.

CEREBRAL EDEMA

Many children who present with DKA have some degree of altered mental status. Typically the altered status is due to acidosis or hyperosmolarity, although some studies show that subclinical cerebral edema occurs in the majority of patients in DKA[9,10]. Approximately 0.5%-1% of children in DKA develop frank cerebral edema[11-13]. Morbidity related to cerebral edema is approximately 13%-35% and mortality 24%-28%[12,14]. Risk factors for the development of cerebral edema during DKA include new onset T1DM, low bicarbonate, low partial pressure of CO_2, and high BUN[13,15].

Conventional thinking attributes the mechanism of injury in cerebral edema to swelling from an influx of fluid into the brain[15-17]. This influx is thought to be due to the rapidly declining serum osmolarity caused by overly aggressive fluid resuscitation; however, data reveals the only treatment-related risk factor to be administration of bicarbonate[15]. The association between high fluid infusion rates and development of cerebral edema trends toward, but does not reach, statistical significance[13]. Radiographic confirmation of cerebral edema in patients with DKA prior to initiation of fluid therapy further discredits the association[13,15]. Also, many children have normal brain imaging at the onset of clinical cerebral edema and do not develop radiographic signs of edema until hours or days later, suggesting that edema is a consequence rather than the cause of injury[11].

A more plausible hypothesis is that cerebral edema is caused by cerebral hypoperfusion, which leads to cytotoxic edema (cell swelling and death) at presentation followed by vasogenic edema (breakdown of the blood brain barrier leading to capillary leakage) during treatment[9]. There is supporting evidence for this mechanism, including the association between cerebral hypoperfusion and the risk factors associated with the development of cerebral edema, including high BUN, low bicarbonate, and low partial pressure of CO_2[13,15]. Additionally, Lam *et al*[18] show that untreated DKA in rats is associated with changes on diffusion-weighted Imaging Magnetic Resonance (DWI MR) consistent with cytotoxic edema. When the DKA is treated, the DWI MR images demonstrate slight changes that suggest advancement to vasogenic cerebral edema. MR DWI changes consistent with vasogenic edema have also been shown in children during treatment of DKA[16]. These studies support the model that DKA-related cerebral edema stems from early ischemic brain damage followed by reperfusion injury during treatment.

COAGULOPATHIC COMPLICATIONS

Abnormalities of hemostasis have been identified in patients with poorly controlled diabetes, although the mechanism is not entirely understood[19,20]. Likewise, clinical studies of both adult and pediatric patients with T1D with DKA have described a variety of transient changes in coagulation factors, such as increased platelet activation, fibrinolytic activity, and endothelial activation[21,22]. A prospective study of adolescents with T1D and DKA demonstrated low levels of free protein S, which facilitates activated protein C in inactivating von Willebrand factor[23]. Accordingly, the levels of von Willebrand factor activity were increased. Protein C activity was decreased in DKA but normalized following treatment.

DKA is also characterized by elevated levels of inflammatory markers (CRP), cytokines (IL6, IL1beta, TNF alpha), and complement activation[24]. This inflammatory state, combined with the disruption of the normal coagulation cascade, can place patients at increased risk of thrombosis and stroke during acute episodes of DKA.

Deep vein thrombosis

Deep vein thrombosis (DVT) is not uncommon in critically ill children who require central venous catheter placement as they introduce a foreign body, cause endothelial damage, and impair blood flow[25]. Children and adolescents with DKA, however, appear to be at increased risk of DVT when they undergo placement of a central venous catheter[26,27]. This increased risk of thrombosis likely stems from shock compounded by DKA, as severe dehydration activates the coagulation cascade and causes venous stasis and DKA itself confers a hypercoagulable state. Gutierrez *et al*[26] published the first report to describe this observation in a retrospective case-matched control series. It details that 4 of 8 children with DKA who underwent placement of a femoral central venous catheter developed DVT compared to 0 of the 16 of control patients who underwent central venous catheter placement without diabetes or DKA[26]. A retrospective cohort study published by Worly *et al*[27] found similar observations with evidence of femoral

DVT on Doppler ultrasound within 48 h of the central catheter placement for treatment of DKA. Patients in that series with DKA and DVT had significantly higher serum glucose, corrected sodium concentrations, and lower pH and serum bicarbonate than their age-matched cohorts with shock and central venous catheters. DVT in children with DKA and catheter placement is also more common in those less than 3 years of age, which may be due to smaller vessel diameter and greater severity of illness at presentation[26].

Children with DKA and DVT require low-molecular weight heparin until ultrasound confirmation of DVT resolution, which can take up to 6 mo[27]. Given the increased risk of DVT and associated morbidity, use of central venous catheters should be avoided in children with DKA when possible. If placement is required, the central venous catheters should be removed as soon as possible and use of prophylactic anticoagulation therapy should be considered in cases of prolonged use.

Cerebral venous thrombosis

In general, the incidence of cerebral sinovenous thrombosis is 0.67 cases per 100000 children per year[28]. Central venous thrombosis in association with pediatric DKA is reported twice in the medical literature[29,30]. The first published case report is a 5-year-old girl with known T1D who presented with emesis, lethargy, and mild DKA who then neurologically decompensated 12 h into treatment, as evidenced by unconsciousness, response to painful stimuli only and limb rigidity[29]. A CT scan demonstrated a thrombosis in the straight sinus and the vein of Galen with ischemic changes in the thalamus. She was anticoagulated with Heparin for 48 h followed by Warfarin for three months, and her baseline neurological status two years later was remarkably normal aside from mild learning difficulties.

The second case reported was in an 8 years old boy on first presentation of T1D with severe DKA with hyperosmolar state with serum glucose of 1668 mg/dL[30]. Two hours into treatment he became unconscious and with sluggish pupillary response. A CT demonstrated thrombosis in the superior sagittal sinus and vein of Galen, as well as large infarctions in both cerebral hemispheres. Long-term follow-up information is not available for this case.

Stroke

The overall incidence of pediatric stroke is estimated at 2-13 per 100000 children[31]. Hemorrhagic or ischemic brain infarction accounts for approximately 10% of intracerebral complications of DKA, and not all cases of stroke in DKA are associated with cerebral edema[32]. The procoagulant state of DKA places patients at increased risk of ischemic brain injury as well as subsequent hemorrhagic conversion arising from hypoxia and vascular injury[24]. Diagnosis of stroke during an episode of acute DKA is difficult as there is considerable overlap of signs, symptoms, and laboratory data[33]. Early signs and symptoms of CNS injury include nonspecific findings such as headache, confusion, lethargy, and unexpected changes in heart rate, respiratory rate, or blood pressure[34]. Focal neurological signs allow clinicians to rapidly identify stroke victims; however, less than 30% of patients with DKA-associated stroke have characteristic focal neurologic deficits[24]. It is also often difficult to differentiate whether cerebral edema in DKA is the cause or the effect of acute cerebral infarction, as stroke itself may cause cerebral edema. Arterial ischemic and hemorrhagic strokes have been documented in children and youth with DKA in a wide variety of cerebral locations, including single or multiple infarctions or thrombi over unilateral or bilateral lobes. The pathologic tissue findings of acute cerebral infarction related to DKA are not expected to be different from those of a nondiabetic child who has suffered a stroke.

Management and outcomes of pediatric stroke associated with DKA

Treatment guidelines for children and adults with diabetic ketoacidosis and stroke are lacking, including the optimal rehydration rate, parameters for use of thrombolytics and other medications, and monitoring schedules[35]. In general, pediatric patients with suspected stroke should receive prompt neurological imaging and neurologic consultation while managed in an intensive care setting. Thrombolysis for the treatment of pediatric stroke remains controversial without supportive data, although children have achieved successful outcomes when administered intravenous tissue plasminogen activator for acute treatment of ischemic stroke[36,37].

The first large-scale prospective outcome study on children with ischemic stroke or sinovenous thrombosis found 41% to have moderate or severe deficits on neurologic examination after a mean of 2.1 years[38]. A recent cross-sectional outcome study of pediatric patients with ischemic stroke and cerebral sinovenous thrombosis a mean of 10.8 years after onset found that 37% were normal and 15% suffered severe deficits[39]. The authors found a strong predictor of long-term outcomes to be functional status at 1 year post-stroke.

Few data are available regarding the long-term effects of pediatric stroke secondary to DKA, and the cases available are largely dependent on the anatomic site affected. Foster *et al*[24] reviewed the outcomes of 28 case reports of arterial ischemic stroke, cerebral venous thrombotic stroke, and hemorrhagic stroke associated with DKA in youth and noted full recovery in only 14%. The majority of patients were left with varying degrees of residual neurologic deficit and 29% of cases resulted in death or persistent vegetative state. These grim outcomes highlight the need for large, randomized clinical trials of pediatric stroke during DKA treatment in order to help achieve the most positive outcomes.

RHABDOMYOLYSIS

Rhabdomyolysis is the breakdown of skeletal muscle leading to leakage of cell contents and resulting in muscle

pain, weakness, and potential acute renal injury[40,41]. Biochemical changes include elevated creatinine kinase and myoglobinuria. The most common causes of rhabdomyolysis in children are viral myositis, trauma, medications, and underlying metabolic diseases. While rhabdomyolysis is more frequently described in patients with hyperosmolar hyperglycemic syndrome (HHS), it is also a well-documented phenomenon in DKA[42]. Rhabdomyolysis in the setting of diabetes is often subclinical, with risk factors being low pH and high serum glucose, BUN, creatinine, sodium, and osmolarity[42-45].

The mechanism by which rhabdomyolysis occurs is unclear, although is thought to be secondary to the changes in electrolyte and glucose concentration across the muscle cell combined with the presence of insulin[42,46,47]. These changes may lead to increased intracellular calcium which, in turn, can activate proteases and lead to muscle cell leakage.

The incidence of rhabdomyolysis in adults with DKA is approximately 10%[47]. A study of children presenting with new onset T1DM found urine myoglobinuria in 10%[44]. Several case reports detail rhabdomyolysis in pediatric DKA[42,44,48,49]. These patients, who ranged in age from 15 mo to 12 years, all presented with a mixed HHS and DKA picture as they had acidosis, a blood glucose > 600 mg/dL, and hyperosmolarity. They were also significantly dehydrated with elevated BUN or creatinine, consistent with the risk factors for developing rhabdomyolysis during DKA.

The presence of rhabdomyolysis in adults greatly increases mortality, likely secondary to decreased renal function[43]. While there are no studies looking at the morbidity and mortality of rhabdomyolysis in children presenting in DKA, the incidence of acute renal failure in all children with rhabdomyolysis is 5%[40]. Other serious complications include severe hyperkalemia and hypocalcemia, which can both lead to cardiac arrest[50,51]. Fluid therapy and bicarbonate administration to alkalinize the urine are the gold standard treatment to prevent kidney injury.

PULMONARY COMPLICATIONS

Pneumomediastinum

Pneumomediastinum is a rare event that occurs secondary to alveolar rupture after a change in pressure gradients in the alveoli[52]. These changes can occur secondary to mechanical ventilation, vomiting, coughing, and the valsalva maneuver[52-54]. Patients with DKA are at increased risk of developing pneumomediastinum in the presence of emesis and Kussmaul breathing, which can generate alveolar pressures of 20-30 mmHg[55-57]. There are over 50 documented cases of pneumomediastinum in the setting of DKA[54,55,58] and analysis of the series found a male preponderance (71% male), an average age of 20 years old, and an average blood glucose of 638 mg/dL[55]. All patients had significant acidosis with respiratory compensation, supporting hyperpnea as a mechanism for the development of pneumomediastinum. Complications include pneumothorax as well as pneumopericardium, which can lead to cardiac tamponade.

Pneumomediastinum classically presents with chest pain and/or dyspnea. Patients often have a positive Hamman's sign, which is crepitus over the precordium that is synchronized with systole[53,59,60], and subcutaneous emphysema may also develop. However, many patients are asymptomatic and pneumomediastinum is only found incidentally[55,59]. Additional treatment is not usually required for cases of pneumomediastinum as the leaked air is often reabsorbed without incident[53,56].

Pulmonary edema

Pulmonary edema is another rare complication of DKA found in both children and adults[44,61,62]. While the edema can be subclinical, some children develop hypoxemia requiring supplemental oxygenation or intubation. To determine the incidence of pulmonary edema in the setting of DKA, Hoffman *et al*[63] performed CT scans on children on presentation of DKA, 6-8 h into treatment, and on discharge. They found increased pulmonary density on presentation that worsened during treatment and self-resolved by discharge. While none developed hypoxemia, P02 values trended low during treatment in the majority of patients.

The edema is thought to be secondary to a decrease in capillary colloid osmotic pressure during intravenous fluid treatment with 0.45% normal saline[61,64,65]. A concomitant fall in the hematocrit with fall in colloid pressure supports fluid administration rather than increased capillary permeability and leakage as the cause of edema. Hoffman *et al*[63] also found a negative correlation between lung density and hematocrit, supporting this mechanism.

The development of pulmonary edema in the setting of DKA can be difficult to manage, as it often requires fluid restriction while DKA requires substantial fluid administration to correct total body water losses[61]. Pulmonary edema in pediatric DKA is rare and general outcomes are not well described, although all of the children in case reports recovered without significant pulmonary sequelae[44,61,62].

GASTROINTESTINAL COMPLICATIONS

Pancreatitis

Acute pancreatitis occurs in 2% of children and 11% of adults with DKA[66,67]. It can be difficult to diagnose with concomitant DKA as abdominal pain is a common complaint and non-specific elevation of both lipase and amylase are noted with DKA. Nair *et al*[66] conducted CT scans on 100 adult patients admitted with DKA and found 11 to have acute pancreatitis, as evidenced by pancreatic enlargement, necrosis, or fluid collections. Elevated serum amylase had a positive predictive value of 69%, elevated lipase 52%, and abdominal pain only 30%.

Haddad *et al*[67] conducted a prospective study looking at pancreatic enzyme levels of children with new onset

Table 1 Incidence of complications of pediatric diabetic ketoacidosis

System	Dysfunction	Incidence
Vascular	Deep vein thrombosis	50% with central venous catheter placement[26,27]
Neurological	Cerebral edema	0.5%-1%[11-13]
	Cerebral venous thrombosis	Rare (2 known cases)
	Hemorrhagic or ischemic brain infarction	10% of intracerebral complications[32]
Musculoskeletal	Rhabdomyolysis	Unavailable; 10% of adults with DKA[43]
Respiratory	Pneumomediastinum	Unavailable; 50 documented cases over pediatric and adult populations[54,55,58]
	Pulmonary edema	Unavailable; described in study of 7 pediatric patients with DKA[63]
Gastrointestinal	Pancreatitis	2%[67]
	GI Bleed	No documented cases in children; 9% in adults with DKA[74]
Neurological	Memory dysfunction	Unavailable; described in study of 33 pediatric patients with remote history of DKA[79]

DKA: Diabetic ketoacidosis.

T1DM with and without DKA. Of those with DKA, 40% had elevated amylase and/or lipase levels and 40% had hypertriglyceridemia. Conversely, only 1 of 12 patients (8%) without DKA had mildly elevated lipase. Thirteen percent of patients with DKA had a lipase level that was elevated more than 3 times normal range and reported persistent abdominal pain after the DKA resolved, although their CT scans remained negative. Only one patient's symptoms recurred with increasing enzyme levels and her repeat imaging was positive for pancreatitis. This study demonstrates that non-specific enzyme elevation is common in children with DKA.

The etiology of non-specific elevation of lipase and amylase during DKA may be secondary to non-pancreatic sources of the enzymes, an insult to the pancreas itself causing enzyme leakage, and decreased renal clearance[67-70]. In cases of acute pancreatitis during DKA, transient hypertriglyceridemia is postulated to be the primary etiology[68,71] through both increased blood viscosity and increased levels of free fatty acids in the pancreas secondary to triglyceride lipolysis, ultimately leading to pancreatic ischemia and injury[72,73]. The child who developed pancreatitis in Haddad's study did not have hypertriglyceridemia, however, leading the authors to propose that severe acidosis may play a role in the development of acute pancreatitis[67].

Management of acute pancreatitis during DKA involves aggressive fluid administration, as pancreatitis can worsen intravascular dehydration[66]. Care must be taken when resuming oral intake as it may exacerbate pancreatitis. The cases of pancreatitis described were mild and all resolved without complications[67,68,72].

Upper gastrointestinal bleeding

There is a 9% incidence of upper gastrointestinal (GI) bleeding in adults with DKA[74], although there are no documented reports in children. The most common manifestation is coffee ground emesis, with hematemesis or melena also noted. Faigel *et al* conducted a retrospective review of 25 patients who developed upper GI bleeding during DKA; all 8 who underwent endoscopy were found to have esophagitis. Additionally, 63% had esophageal erosions or ulcerations and one (13%) had a Mallory-Weiss tear. Conversely, only 33% of patients with DKA without bleeding had evidence of esophagitis on endoscopy and only 11% had erosions. Acute esophageal necrosis, which is characterized by a black-appearance of the distal esophageal mucosa, is a rare cause of upper GI bleeding in DKA that was not found in Faigel's study[75-77].

Use of ulcer medications, including proton pump inhibitors and H2 receptor antagonists, longer duration of diabetes, and diabetic complications including nephropathy, retinopathy and gastroparesis are clinical risk factors associated with upper GI hemorrhage[74]. Laboratory values associated with an increased risk of hemorrhage include elevated BUN, creatinine, and glucose; arterial pH and coagulation tests did not differ between the two groups. Acute hyperglycemia in particular has been shown to delay gastric emptying[78], which causes esophageal mucosal damage secondary to acid reflux and ultimately leads to a GI bleed[74].

Only 32% of those with upper GI bleeding in Faigel's study underwent endoscopy. While 27% of patients with hemorrhage required blood transfusions, none required invasive therapy and GI bleeding did not directly result in mortality. However, those with GI bleeds had a mortality rate of 15% from other causes, compared to 4% in those without GI bleeds. This higher mortality rate is attributed to greater illness severity with greater likelihood of being admitted to the ICU.

COGNITIVE COMPLICATIONS

Even in the absence of symptoms suggesting cerebral injury, children with diabetic ketoacidosis can exhibit long-term cognitive complications. Ghetti *et al*[79] assessed for memory deficits in 33 children with T1D who had suffered at least one episode of DKA and 29 children with T1D who had never experienced DKA. Interestingly, the children with DKA history had a significantly lower ability to recall events in association with specific details, as tested by event-color and event-spatial position associations. The average time since the last episode of DKA was 2.54 years, although varied from 0.11 to 14.54 years, and memory performance

was worse in children whose DKA was in the more distant past. This retrospective study also demonstrated that, aside from DKA, reduced memory performance was associated with male sex, young age at onset of diabetes, and severe hypoglycemia. The authors hypothesize that cerebral edema-related hypoxic/ischemic injury to the hippocampus is responsible for these specific, long-term cognitive deficits, as similar outcomes are observed in both clinical and animal studies of hypoxic injury[80,81].

Animal models allow for a more controlled assessment of DKA-related cognitive dysfunction. Rats with streptozotocin-induced diabetes who are subjected to only one episode of DKA have longer mean latency times on maze testing after DKA recovery compared to rats with streptozotocin-induced diabetes without DKA[82]. This measurable decrease in neurocognitive function raises concern for similar effects in people with DKA, although the underlying mechanism was not examined further *via* imaging or gross dissection. A recent prospective study of patients ages 6-18 years with and without DKA at diagnosis of T1D demonstrated cerebral white matter changes on MRI that, despite resolution over the first week, resulted in persistent alterations in attention and memory for up to 6 mo later[83]. The greatest risk factors for these changes in cerebral structure were degree of acidosis and younger age at presentation, further highlighting the need for improved DKA prevention.

CONCLUSION

The most common cause of acute deterioration in children with DKA is cerebral edema, the pathogenesis of which remains under active investigation and discussion. Other rare complications of pediatric DKA include acute changes in coagulation, pulmonary function, musculoskeletal and gastrointestinal health as well as long-term cognitive outcomes (Table 1). These findings are rare and require a high index of clinical suspicion, but early recognition and treatment may help avoid permanent deficits. More data related to the presentation, treatment and outcomes of these complications in pediatric DKA patients is still needed, therefore, avoidance of DKA in children and adolescents through public and professional awareness is paramount to preventing these acute and chronic complications.

REFERENCES

1 **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]

2 **Lawrence JM**, Imperatore G, Dabelea D, Mayer-Davis EJ, Linder B, Saydah S, Klingensmith GJ, Dolan L, Standiford DA, Pihoker C, Pettitt DJ, Talton JW, Thomas J, Bell RA, D'Agostino RB. Trends in incidence of type 1 diabetes among non-Hispanic white youth in the u.s., 2002-2009. *Diabetes* 2014; **63**: 3938-3945 [PMID: 24898146 DOI: 10.2337/db13-1891]

3 **DIAMOND Project Group.** Incidence and trends of childhood Type 1 diabetes worldwide 1990-1999. *Diabet Med* 2006; **23**: 857-866 [PMID: 16911623 DOI: 10.1111/j.1464-5491.2006.01925.x]

4 **Dabelea D**, Rewers A, Stafford JM, Standiford DA, Lawrence JM, Saydah S, Imperatore G, D'Agostino RB, Mayer-Davis EJ, Pihoker C. Trends in the prevalence of ketoacidosis at diabetes diagnosis: the SEARCH for diabetes in youth study. *Pediatrics* 2014; **133**: e938-e945 [PMID: 24685959 DOI: 10.1542/peds.2013-2795]

5 **Wolfsdorf J**, Glaser N, Sperling MA; American Diabetes Association. Diabetic ketoacidosis in infants, children, and adolescents: A consensus statement from the American Diabetes Association. *Diabetes Care* 2006; **29**: 1150-1159 [PMID: 16644656 DOI: 10.2337/diacare.2951150]

6 **Dunger DB**, Sperling MA, Acerini CL, Bohn DJ, Daneman D, Danne TP, Glaser NS, Hanas R, Hintz RL, Levitsky LL, Savage MO, Tasker RC, Wolfsdorf JI. European Society for Paediatric Endocrinology/Lawson Wilkins Pediatric Endocrine Society consensus statement on diabetic ketoacidosis in children and adolescents. *Pediatrics* 2004; **113**: e133-e140 [PMID: 14754983]

7 **Edge JA**, Ford-Adams ME, Dunger DB. Causes of death in children with insulin dependent diabetes 1990-96. *Arch Dis Child* 1999; **81**: 318-323 [PMID: 10490436]

8 **DiLiberti JH**, Lorenz RA. Long-term trends in childhood diabetes mortality: 1968-1998. *Diabetes Care* 2001; **24**: 1348-1352 [PMID: 11473068]

9 **Glaser N**. Cerebral injury and cerebral edema in children with diabetic ketoacidosis: could cerebral ischemia and reperfusion injury be involved? *Pediatr Diabetes* 2009; **10**: 534-541 [PMID: 19821944 DOI: 10.1111/j.1399-5448.2009.00511.x]

10 **Glaser NS**, Wootton-Gorges SL, Buonocore MH, Marcin JP, Rewers A, Strain J, DiCarlo J, Neely EK, Barnes P, Kuppermann N. Frequency of sub-clinical cerebral edema in children with diabetic ketoacidosis. *Pediatr Diabetes* 2006; **7**: 75-80 [PMID: 16629712 DOI: 10.1111/j.1399-543X.2006.00156.x]

11 **Muir AB**, Quisling RG, Yang MC, Rosenbloom AL. Cerebral edema in childhood diabetic ketoacidosis: natural history, radiographic findings, and early identification. *Diabetes Care* 2004; **27**: 1541-1546 [PMID: 15220225]

12 **Edge JA**, Hawkins MM, Winter DL, Dunger DB. The risk and outcome of cerebral oedema developing during diabetic ketoacidosis. *Arch Dis Child* 2001; **85**: 16-22 [PMID: 11420189]

13 **Lawrence SE**, Cummings EA, Gaboury I, Daneman D. Population-based study of incidence and risk factors for cerebral edema in pediatric diabetic ketoacidosis. *J Pediatr* 2005; **146**: 688-692 [PMID: 15870676 DOI: 10.1016/j.jpeds.2004.12.041]

14 **Marcin JP**, Glaser N, Barnett P, McCaslin I, Nelson D, Trainor J, Louie J, Kaufman F, Quayle K, Roback M, Malley R, Kuppermann N. Factors associated with adverse outcomes in children with diabetic ketoacidosis-related cerebral edema. *J Pediatr* 2002; **141**: 793-797 [PMID: 12461495]

15 **Glaser N**, Barnett P, McCaslin I, Nelson D, Trainor J, Louie J, Kaufman F, Quayle K, Roback M, Malley R, Kuppermann N; Pediatric Emergency Medicine Collaborative Research Committee of the American Academy of Pediatrics. Risk factors for cerebral edema in children with diabetic ketoacidosis. The Pediatric Emergency Medicine Collaborative Research Committee of the American Academy of Pediatrics. *N Engl J Med* 2001; **344**: 264-269 [PMID: 11172153 DOI: 10.1056/NEJM200101253440404]

16 **Glaser NS**, Marcin JP, Wootton-Gorges SL, Buonocore MH, Rewers A, Strain J, DiCarlo J, Neely EK, Barnes P, Kuppermann N. Correlation of clinical and biochemical findings with diabetic ketoacidosis-related cerebral edema in children using magnetic resonance diffusion-weighted imaging. *J Pediatr* 2008; **153**: 541-546 [PMID: 18589447 DOI: 10.1016/j.jpeds.2008.04.048]

17 **Carlotti AP**, Bohn D, Halperin ML. Importance of timing of risk factors for cerebral oedema during therapy for diabetic ketoacidosis. *Arch Dis Child* 2003; **88**: 170-173 [PMID:

12538330]
18 **Lam TI**, Anderson SE, Glaser N, O'Donnell ME. Bumetanide reduces cerebral edema formation in rats with diabetic ketoacidosis. *Diabetes* 2005; **54**: 510-516 [PMID: 15677509]
19 **Carr ME**. Diabetes mellitus: a hypercoagulable state. *J Diabetes Complications* 2005; **15**: 44-54 [PMID: 11259926]
20 **Fattah MA**, Shaheen MH, Mahfouz MH. Disturbances of haemostasis in diabetes mellitus. *Dis Markers* 2003; **19**: 251-258 [PMID: 15258325]
21 **Ileri NS**, Büyükaşik Y, Karaahmetoğlu S, Ozatli D, Sayinalp N, Ozcebe OI, Kirazli S, Müftüoğlu O, Dündar SV. Evaluation of the haemostatic system during ketoacidotic deterioration of diabetes mellitus. *Haemostasis* 1999; **29**: 318-325 [PMID: 10844405]
22 **Bilici M**, Tavil B, Dogru O, Davutoglu M, Bosnak M. Diabetic ketoasidosis is associated with prothrombotic tendency in children. *Pediatr Hematol Oncol* 2011; **28**: 418-424 [PMID: 21615248 DOI: 10.3109/08880018.2011.558568]
23 **Carl GF**, Hoffman WH, Passmore GG, Truemper EJ, Lightsey AL, Cornwell PE, Jonah MH. Diabetic ketoacidosis promotes a prothrombotic state. *Endocr Res* 2003; **29**: 73-82 [PMID: 12665320]
24 **Foster JR**, Morrison G, Fraser DD. Diabetic ketoacidosis-associated stroke in children and youth. *Stroke Res Treat* 2011; **2011**: 219706 [PMID: 21423557 DOI: 10.4061/2011/219706]
25 **Beck C**, Dubois J, Grignon A, Lacroix J, David M. Incidence and risk factors of catheter-related deep vein thrombosis in a pediatric intensive care unit: a prospective study. *J Pediatr* 1998; **133**: 237-241 [PMID: 9709712]
26 **Gutierrez JA**, Bagatell R, Samson MP, Theodorou AA, Berg RA. Femoral central venous catheter-associated deep venous thrombosis in children with diabetic ketoacidosis. *Crit Care Med* 2003; **31**: 80-83 [PMID: 12544997 DOI: 10.1097/01.CCM.0000037163.14383.D0]
27 **Worly JM**, Fortenberry JD, Hansen I, Chambliss CR, Stockwell J. Deep venous thrombosis in children with diabetic ketoacidosis and femoral central venous catheters. *Pediatrics* 2004; **113**: e57-e60 [PMID: 14702496]
28 **deVeber G**, Andrew M, Adams C, Bjornson B, Booth F, Buckley DJ, Camfield CS, David M, Humphreys P, Langevin P, MacDonald EA, Gillett J, Meaney B, Shevell M, Sinclair DB, Yager J. Cerebral sinovenous thrombosis in children. *N Engl J Med* 2001; **345**: 417-423 [PMID: 11496852 DOI: 10.1056/NEJM200108093450604]
29 **Keane S**, Gallagher A, Ackroyd S, McShane MA, Edge JA. Cerebral venous thrombosis during diabetic ketoacidosis. *Arch Dis Child* 2002; **86**: 204-205 [PMID: 11861244]
30 **Sasiadek MJ**, Sosnowska-Pacuszko D, Zielinska M, Turek T. Cerebral venous thrombosis as a first presentation of diabetes. *Pediatr Neurol* 2006; **35**: 135-138 [PMID: 16876012 DOI: 10.1016/j.pediatrneurol.2006.01.010]
31 **Lynch JK**, Hirtz DG, DeVeber G, Nelson KB. Report of the National Institute of Neurological Disorders and Stroke workshop on perinatal and childhood stroke. *Pediatrics* 2002; **109**: 116-123 [PMID: 11773550]
32 **Rosenbloom AL**. Intracerebral crises during treatment of diabetic ketoacidosis. *Diabetes Care* 1990; **13**: 22-33 [PMID: 2105195]
33 **Ho J**, Pacaud D, Hill MD, Ross C, Hamiwka L, Mah JK. Diabetic ketoacidosis and pediatric stroke. *CMAJ* 2005; **172**: 327-328 [PMID: 15684112 DOI: 10.1503/cmaj.1032013]
34 **Singhal AB**, Biller J, Elkind MS, Fullerton HJ, Jauch EC, Kittner SJ, Levine DA, Levine SR. Recognition and management of stroke in young adults and adolescents. *Neurology* 2013; **81**: 1089-1097 [PMID: 23946297 DOI: 10.1212/WNL.0b013e3182a4a451]
35 **Jovanovic A**, Stolic RV, Rasic DV, Markovic-Jovanovic SR, Peric VM. Stroke and diabetic ketoacidosis--some diagnostic and therapeutic considerations. *Vasc Health Risk Manag* 2014; **10**: 201-204 [PMID: 24748799 DOI: 10.2147/VHRM.S59593]
36 **Belvís R**. Thrombolysis for acute stroke in pediatrics. *Stroke* 2007; **38**: 1722-1723 [PMID: 17431202 DOI: 10.1161/STROKEAHA.107.487116]
37 **Shuayto MI**, Lopez JI, Greiner F. Administration of intravenous tissue plasminogen activator in a pediatric patient with acute ischemic stroke. *J Child Neurol* 2006; **21**: 604-606 [PMID: 16970853]
38 **deVeber GA**, MacGregor D, Curtis R, Mayank S. Neurologic outcome in survivors of childhood arterial ischemic stroke and sinovenous thrombosis. *J Child Neurol* 2000; **15**: 316-324 [PMID: 10830198]
39 **Elbers J**, deVeber G, Pontigon AM, Moharir M. Long-Term Outcomes of Pediatric Ischemic Stroke in Adulthood. *J Child Neurol* 2013; **29**: 782-788 [PMID: 23589374 DOI: 10.1177/0883073813484358]
40 **Mannix R**, Tan ML, Wright R, Baskin M. Acute pediatric rhabdomyolysis: causes and rates of renal failure. *Pediatrics* 2006; **118**: 2119-2125 [PMID: 17079586 DOI: 10.1542/peds.2006-1352]
41 **Luck RP**, Verbin S. Rhabdomyolysis: a review of clinical presentation, etiology, diagnosis, and management. *Pediatr Emerg Care* 2008; **24**: 262-268 [PMID: 18418269 DOI: 10.1097/PEC.0b013e31816bc7b7]
42 **Casteels K**, Beckers D, Wouters C, Van Geet C. Rhabdomyolysis in diabetic ketoacidosis. *Pediatr Diabetes* 2003; **4**: 29-31 [PMID: 14655521 DOI: 10.1034/j.1399-5448.2003.00026.x]
43 **Wang LM**, Tsai ST, Ho LT, Hu SC, Lee CH. Rhabdomyolysis in diabetic emergencies. *Diabetes Res Clin Pract* 1994; **26**: 209-214 [PMID: 7736901]
44 **Buckingham BA**, Roe TF, Yoon JW. Rhabdomyolysis in diabetic ketoacidosis. *Am J Dis Child* 1981; **135**: 352-354 [PMID: 6782859]
45 **Singhal PC**, Abramovici M, Ayer S, Desroches L. Determinants of rhabdomyolysis in the diabetic state. *Am J Nephrol* 1991; **11**: 447-450 [PMID: 1819210]
46 **Zierler KL**. Increased muscle permeability to aidolase produced by insulin and by albumin. *Am J Physiol* 1958; **192**: 283-286 [PMID: 13508870]
47 **Finberg L**, Luttrell C, Redd H. Pathogenesis of lesions in the nervous system in hypernatremic states. II. Experimental studies of gross anatomic changes and alterations of chemical composition of the tissues. *Pediatrics* 1959; **23**: 46-53 [PMID: 13613863]
48 **Koh CT**, Cowley DM, Savage MO. Rhabdomyolysis in diabetic ketoacidosis. *Am J Dis Child* 1981; **135**: 1079 [PMID: 6794360]
49 **Al-Matrafi J**, Vethamuthu J, Feber J. Severe acute renal failure in a patient with diabetic ketoacidosis. *Saudi J Kidney Dis Transpl* 2009; **20**: 831-834 [PMID: 19736483]
50 **Watemberg N**, Leshner RL, Armstrong BA, Lerman-Sagie T. Acute pediatric rhabdomyolysis. *J Child Neurol* 2000; **15**: 222-227 [PMID: 10805187]
51 **Zeitler P**, Haqq A, Rosenbloom A, Glaser N. Hyperglycemic hyperosmolar syndrome in children: pathophysiological considerations and suggested guidelines for treatment. *J Pediatr* 2011; **158**: 9-14, 14.e1-2 [PMID: 21035820 DOI: 10.1016/j.jpeds.2010.09.048]
52 **Schulman A**, Fataar S, Van der Spuy JW, Morton PC, Crosier JH. Air in unusual places: some causes and ramifications of pneumomediastinum. *Clin Radiol* 1982; **33**: 301-306 [PMID: 7075135]
53 **Munsell WP**. Pneumomediastinum. A report of 28 cases and review of the literature. *JAMA* 1967; **202**: 689-693 [PMID: 6072511]
54 **Steenkamp DPV,** Minkin R. A Case of Pneumomediastinum: A Rare Complication of Diabetic Ketoacidosis. *Clinical Diabetes* 2011; **29:** 2
55 **Pooyan P**, Puruckherr M, Summers JA, Byrd RP, Roy TM. Pneumomediastinum, pneumopericardium, and epidural pneumatosis in DKA. *J Diabetes Complications* 2004; **18**: 242-247

[PMID: 15207845 DOI: 10.1016/S1056-8727(03)00059-X]
56 **Bullaboy CA**, Jennings RB, Johnson DH, Coulson JD, Young LW, Wood BP. Radiological case of the month. Pneumomediastinum and subcutaneous emphysema caused by diabetic hyperpnea. *Am J Dis Child* 1989; **143**: 93-94 [PMID: 2910052]
57 **McNicholl B**, Murray JP, Egan B, McHugh P. Pneumomediastinum and diabetic hyperpnoea. *Br Med J* 1968; **4**: 493-494 [PMID: 5697667]
58 **Escobar E**, Mullenix PS, Sapp JE. Imaging presentation of complicated diabetic ketoacidosis: a case report. *Emerg Radiol* 2012; **19**: 561-563 [PMID: 22684306 DOI: 10.1007/s10140-012-1056-x]
59 **Weathers LS**, Brooks WG, DeClue TJ. Spontaneous pneumomediastinum in a patient with diabetic ketoacidosis: a potentially hidden complication. *South Med J* 1995; **88**: 483-484 [PMID: 7716607]
60 **Hamman L.** A Note on the Mechanism of Spontaneous Pneumothorax. *Ann Intern Med* 1939; **13:** 5 [DOI: 10.7326/0003-4819-13-6-923]
61 **Breidbart S**, Singer L, St Louis Y, Saenger P. Adult respiratory distress syndrome in an adolescent with diabetic ketoacidosis. *J Pediatr* 1987; **111**: 736-738 [PMID: 3117997]
62 **Perez Rueda C**, Obando Santaella I, Mongil Ruiz I, Fernandez Gomez E, Gonzalez de Castro A. Noncardiogenic pulmonary edema associated with diabetic ketoacidosis. *J Pediatr* 1988; **113**: 161 [PMID: 3133457]
63 **Hoffman WH**, Locksmith JP, Burton EM, Hobbs E, Passmore GG, Pearson-Shaver AL, Deane DA, Beaudreau M, Bassali RW. Interstitial pulmonary edema in children and adolescents with diabetic ketoacidosis. *J Diabetes Complications* 1998; **12**: 314-320 [PMID: 9877465]
64 **Leonard RC**, Asplin C, McCormick CV, Hockaday TD. Acute respiratory distress in diabetic ketoacidosis: possible contribution of low colloid osmotic pressure. *Br Med J* (Clin Res Ed) 1983; **286**: 760-762 [PMID: 6402236]
65 **Fein IA**, Rachow EC, Sprung CL, Grodman R. Relation of colloid osmotic pressure to arterial hypoxemia and cerebral edema during crystalloid volume loading of patients with diabetic ketoacidosis. *Ann Intern Med* 1982; **96**: 570-575 [PMID: 6803635]
66 **Nair S**, Yadav D, Pitchumoni CS. Association of diabetic ketoacidosis and acute pancreatitis: observations in 100 consecutive episodes of DKA. *Am J Gastroenterol* 2000; **95**: 2795-2800 [PMID: 11051350 DOI: 10.1111/j.1572-0241.2000.03188.x]
67 **Haddad NG**, Croffie JM, Eugster EA. Pancreatic enzyme elevations in children with diabetic ketoacidosis. *J Pediatr* 2004; **145**: 122-124 [PMID: 15238920 DOI: 10.1016/j.jpeds.2004.03.050]
68 **Nair S**, Pitchumoni CS. Diabetic ketoacidosis, hyperlipidemia, and acute pancreatitis: the enigmatic triangle. *Am J Gastroenterol* 1997; **92**: 1560-1561 [PMID: 9317089]
69 **Vinicor F**, Lehrner LM, Karn RC, Merritt AD. Hyperamylasemia in diabetic ketoacidosis: sources and significance. *Ann Intern Med* 1979; **91**: 200-204 [PMID: 111594]
70 **Warshaw AL**, Feller ER, Lee KH. On the cause of raised serum-amylase in diabetic ketoacidosis. *Lancet* 1977; **1**: 929-931 [PMID: 67388]
71 **Fulop M**, Eder H. Severe hypertriglyceridemia in diabetic ketosis. *Am J Med Sci* 1990; **300**: 361-365 [PMID: 2124781]
72 **Wolfgram PM,** Macdonald MJ. Severe Hypertriglyceridemia Causing Acute Pancreatitis in a Child with New Onset Type I Diabetes Mellitus Presenting in Ketoacidosis. *J Pediatr Intensive Care* 2013; **2**: 77-80 [PMID: 24455446 DOI: 10.3233/PIC-13053]
73 **Tsuang W**, Navaneethan U, Ruiz L, Palascak JB, Gelrud A. Hypertriglyceridemic pancreatitis: presentation and management. *Am J Gastroenterol* 2009; **104**: 984-991 [PMID: 19293788 DOI: 10.1038/ajg.2009.27]
74 **Faigel DO**, Metz DC. Prevalence, etiology, and prognostic significance of upper gastrointestinal hemorrhage in diabetic ketoacidosis. *Dig Dis Sci* 1996; **41**: 1-8 [PMID: 8565740]
75 **Gurvits GE**. Black esophagus: acute esophageal necrosis syndrome. *World J Gastroenterol* 2010; **16**: 3219-3225 [PMID: 20614476]
76 **Gurvits GE**, Shapsis A, Lau N, Gualtieri N, Robilotti JG. Acute esophageal necrosis: a rare syndrome. *J Gastroenterol* 2007; **42**: 29-38 [PMID: 17322991 DOI: 10.1007/s00535-006-1974-z]
77 **Yasuda H**, Yamada M, Endo Y, Inoue K, Yoshiba M. Acute necrotizing esophagitis: role of nonsteroidal anti-inflammatory drugs. *J Gastroenterol* 2006; **41**: 193-197 [PMID: 16699852 DOI: 10.1007/s00535-005-1741-6]
78 **Fraser RJ**, Horowitz M, Maddox AF, Harding PE, Chatterton BE, Dent J. Hyperglycaemia slows gastric emptying in type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 1990; **33**: 675-680 [PMID: 2076799]
79 **Ghetti S**, Lee JK, Sims CE, Demaster DM, Glaser NS. Diabetic ketoacidosis and memory dysfunction in children with type 1 diabetes. *J Pediatr* 2010; **156**: 109-114 [PMID: 19833353 DOI: 10.1016/j.jpeds.2009.07.054]
80 **Yonelinas AP**, Kroll NE, Quamme JR, Lazzara MM, Sauvé MJ, Widaman KF, Knight RT. Effects of extensive temporal lobe damage or mild hypoxia on recollection and familiarity. *Nat Neurosci* 2002; **5**: 1236-1241 [PMID: 12379865 DOI: 10.1038/nn961]
81 **Raman L**, Tkac I, Ennis K, Georgieff MK, Gruetter R, Rao R. In vivo effect of chronic hypoxia on the neurochemical profile of the developing rat hippocampus. *Brain Res Dev Brain Res* 2005; **156**: 202-209 [PMID: 16099307 DOI: 10.1016/j.devbrainres.2005.02.013]
82 **Glaser N**, Anderson S, Leong W, Tancredi D, O'Donnell M. Cognitive dysfunction associated with diabetic ketoacidosis in rats. *Neurosci Lett* 2012; **510**: 110-114 [PMID: 22266599 DOI: 10.1016/j.neulet.2012.01.014]
83 **Cameron FJ**, Scratch SE, Nadebaum C, Northam EA, Koves I, Jennings J, Finney K, Neil JJ, Wellard RM, Mackay M, Inder TE. Neurological consequences of diabetic ketoacidosis at initial presentation of type 1 diabetes in a prospective cohort study of children. *Diabetes Care* 2014; **37**: 1554-1562 [PMID: 24855156 DOI: 10.2337/dc13-1904]

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MINIREVIEWS

Transcriptional factors, Mafs and their biological roles

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Abstract

The Maf family of transcription factors is characterized by a typical bZip structure; these transcription factors act as important regulators of the development and differentiation of many organs and tissues, including the kidney. The Maf family consists of two subgroups that are characterized according to their structure: large Maf transcription factors and small Maf transcription factors. The large Maf subgroup consists of four proteins, designated as MAFA, MAFB, c-MAF and neural retina-specific leucine zipper. In particular, MAFA is a distinct molecule that has been attracting the attention of researchers because it acts as a strong transactivator of insulin, suggesting that Maf transcription factors are likely to be involved in systemic energy homeostasis. In this review, we focused on the regulation of glucose/energy balance by Maf transcription factors in various organs.

Key words: Cell; Insulin; MAFA; Microarray; siRNA

Core tip: This manuscript demonstrates that Maf transcription factors are likely to be involved in the regulation of hormonal systems related to glucose metabolism, with regulation by Maf transcription factors likely occurring near the start of the cascade or acting directly on the expression of genes in coordination with other factors in multiple organs and tissues. The Maf family plays diverse roles as transcription factors in the establishment of energy balance in peripheral organs, such as the pancreas, liver, and adipose tissue.

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INTRODUCTION

Maf is a family of oncogenes that were first discovered in the genome of the avian transforming retrovirus, AS42[1,2]. Maf-related proteins have been identified in many species and exhibit a universally recognized DNA binding site, enabling the proteins to act as transcription

factors. These Maf transcription factors are well known to play active roles in many organs, tissues, and cells for the development, differentiation and establishment of specific functions, including effects in the pancreas[3], lens[4], myeloma cells[5], and cartilage[6,7].

The Maf family has two distinct subgroups that are categorized according to their molecular size: small Maf transcription factors (150-160 amino acids: MAFF, MAFG, and MAFK), and large Maf transcription factors (240-340 amino acids: MAFA, MAFB, c-MAF, and NRL). Small Maf transcription factors lack a transactivation domain, and these protein products form homodimers or heterodimers within the Maf family or with other transcription factors, inducing transactivation factors[2,8]. A complex regulatory network is known to link small Maf transcription factors with other regulatory proteins[9,10]. On the other hand, large Maf proteins consist of a family of transcription factors characterized by a typical bZip structure, which is a motif for protein dimerization and DNA binding[11,12]. Several reports have revealed that Maf proteins are involved in the essential functions of developing, differentiating and establishing the function of cells, tissues and organs. These transcription factors reportedly regulate several distinct developmental processes, cell differentiation, and the establishment of cell functions; for example, the mouse *Mafb* gene is responsible for the segmentation of the hindbrain[13], while *c-Maf* has been identified in the liver, renal tubules[14], adipocytes, and muscle. In this review, we will mainly focus on large Maf transcription factors and their roles in the regulation of various organs, as well as their effects on energy balance.

MAF TRANSCRIPTION FACTORS AND PANCREATIC β CELLS

One of the large Maf transcription factors, transcription factor MAFA, is an interesting molecule among the Maf family members since it promotes the differentiation of pancreatic β cells[15,16]. Several reports have also indicated that *Mafa* activates the insulin gene C1 element, contributing to β cell function and differentiation[17,18]. The formation of β cells has been described in detail in several reports and has been summarized in reviews. Two types of large Maf transcription factors, MAFA and transcription factor MAFB, are known to coordinate with each other and with other transcription factors and related genes to induce the generation and differentiation of β cells[3,19]. MAFB is known to function as a transcription factor in many tissues and organs and has been detected in the pancreas. MAFB was initially identified as a transactivator of β cells, acting on the glucagon gene G1 element. Further studies subsequently revealed that MAFB can be detected in both α and β cells during the early phase of development, followed by a reduction in expression and then a switch to mainly MAFA expression[20-22]. An additional study has demonstrated that the loss of *Mafa* causes a decrease in insulin gene expression in glucotoxic β cells[23], while MAFA deficient mice could not activate insulin transcription, even though the insulin content of the β cells was not significantly diminished[24]. Recently, Hang *et al*[25] described the collaboration of MAFA and MAFB in the development of pancreatic β cells in greater detail[25,26]. As for the transcription factor c-MAF, which is known to play a role in hematopoietic cell differentiation, its expression has been confirmed in the pancreas and is thought to be involved in α cell differentiation and function[27].

Previously, Maf transcriptional factors could be shown to stained in premature and mature pancreas tissue in our report[28]. Cells that stained positive for Maf transcription factors were diffusely localized in premature pancreas tissue, with some cells exhibiting double staining. The staining pattern for each Maf protein was different: unlike, MAFA-positive cells, which exhibited a diffuse staining pattern, MAFB and c-MAF were stained prominently in the branching ducts and acinar buds. Subsequently, MAFA and MAFB were stained more intensely in the islet areas of adult pancreas tissue, suggesting that Maf transcription factors are involved in the differentiation and acquisition of pancreatic endocrine cells, coordinating with each other in some situations (Figure 1). In contrast, non-endocrine composite cells of the pancreas, such as acinar cells and ductal cells, may also be affected by several Maf transcriptional factors during their maturation and differentiation process. More interesting observation is that cells positive stained for Maf transcription factor continued to be detected not only in the islets but around the ductal and interstitial area after maturation.

ACTIVITIES OF MAF TRANSCRIPTION FACTORS BEYOND β CELLS

Despite the details that have been revealed regarding the activities of Maf transcription factors in β-cell function and differentiation, the precise mechanism and coordination of Mafs and other transcriptional factors regarding the regulation of insulin production and its activity remain unknown. The precursors of pancreatic endocrine cells and the mechanism of β cell replication in the islets have been reported[29-31]. However, several types of Maf transcription factors are likely to be implicated in both the pancreatic endocrine cell lineage and interaction with other transcription factors. Each Maf transcriptional factor were often co-stained in one endocrine cell in immature pancreas.

The network of targeted genes and transcription factors, including several Maf transcription factors, needs to be clarified as part of efforts to accelerate β cell regeneration or preparation for cell therapy. Maf transcriptional factors are reportedly expressed in other tissues and cells, for example, epithelial cells and lymphocytes[32,33], where they accelerate specific cell function and differentiation. Lumelsky *et al*[34] reported the development of embryonic stem cells into insulin-producing cells in the pancreas[34], while Kawai *et al*[1] described the mechanism of β cell replication in islets.

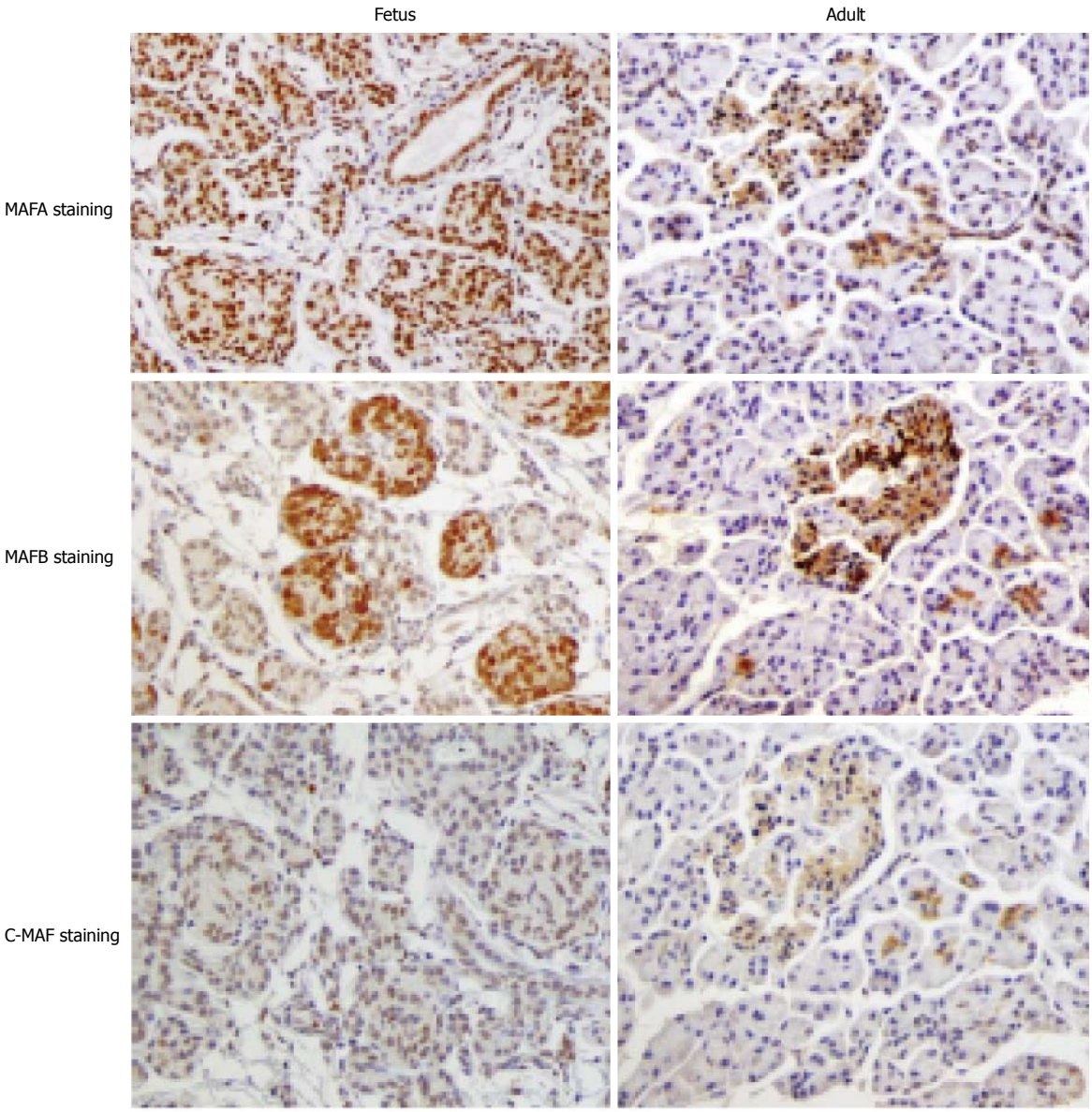

Figure 1 Immunostaining for MAFA, MAFB, and c-MAF in fetal and adult human pancreas tissues. An immunohistochemical analysis was performed using primary antibodies against MAFA (BL1069; Bethyl Laboratories, Inc.), MAFB (P20; Santa Cruz Biotechnology, Inc.), and c-MAF (M153; Santa Cruz Biotechnology, Inc.). The details are described in reference[28]. Samples of human normal fetal tissue (female, 20 wk, catalog No. T2244188, Lot No. A607380) and adult pancreas tissue (male, 23 years, catalog No. T2234188, Lot No. A604382) were purchased from BioChain. The fetal pancreas tissues were diffusely stained for the Maf transcription factors, and characteristic histological differences were observed between the fetal and adult tissues, with a more intense staining pattern observed in the islet areas of the adult pancreas tissue.

In addition, several reports have described the existence of tissue-specific stem cells in the pancreas[35,36]. Recently, several reports have discussed the more efficient production of β cells (glucose-sensitive and insulin-secreting cells) through the introduction of a combination of transcription factors, including Maf transcription factors, or the use of induced pluripotent stem cells[37,38].

Large Maf transcription factors have been identified during the development of the pancreas, and the expressions of these large Maf transcription factors exhibited different localizations in newborn and adult pancreas tissues, which differ in their endocrine characteristics. Thus, Maf transcription factors may contribute to establish all the cells in pancreatic tissue, including cells involved in endocrine cell differentiation, such as α and β cells, exocrine cells, and ductal cells.

MAF TRANSCRIPTION FACTORS AND THE KIDNEY

In the kidney, large Maf transcription factors may be implicated in both normal development and pathophysiological processes responsible for kidney disease. We previously reported the expression profiles of large Maf transcription factors in the kidney. We have reported the expression of *c-Maf* mRNA levels in mouse kidney tissue from embryonic day 12 (E12) until 1 or 4 wk after birth.

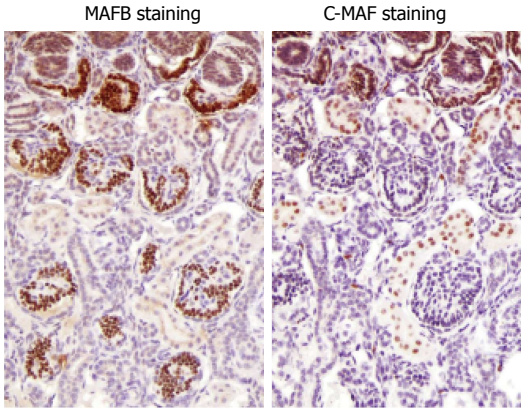

Figure 2 Immunostaining for MAFB and c-MAF in fetal human kidney tissue. An immunohistochemical analysis was performed using primary antibodies against MAFA (BL1069; Bethyl Laboratories, Inc.), MAFB (P20; Santa Cruz Biotechnology, Inc.), and MAF c-Maf (M153; Santa Cruz Biotechnology, Inc.). The details are described in reference[40]. A sample of human normal fetal kidney tissue (male, 25 wk) was purchased from BioChain (catalog No. T8244431, Lot No. A606275). Glomerular podocyte lesions stained positive for MAFB, and while the proximal tubules stained positive for c-MAF.

c-Maf mRNA was firstly expressed at E16 in the proximal tubules and continued to be expressed until 4 wk after birth. Meanwhile, MAFB expression has been identified in the glomeruli (Figure 2)[39,40].

The *Mafb* mouse gene (also known as Kreisler and *Krml*) is known for its role in hindbrain patterning. Sadl *et al*[41] showed that mice homozygous for the kr (enu) mutation develop renal disease, in which the glomerular podocytes are affected, resulting in nephrotic syndrome. The fusion and effacement of the podocyte foot processes were observed histologically, and MAFB was shown to be essential for the cellular differentiation of the podocytes. Since the podocytes of the kr (enu) homozygotes differentiated abnormally, the homozygotes exhibited proteinuria, as is observed in nephrotic syndrome. The authors speculated that MAFB acted during the final stages of glomerular development, *i.e.*, the transition between the capillary loop and the mature stages, and downstream of the Pod1 basic domain helix-loop-helix transcription factor[41]. In *Mafb*-knockout mice, renal dysgenesis with abnormal podocyte differentiation and tubular apoptosis were prominent, accompanied by the suppression of F4/80 expression in mature macrophages[42].

A prominent phenotypic feature of *c-Maf* - knockout mice is a small cell volume of the kidney proximal tubules and hepatocytes[40]. The precise mechanism underlying this dysregulation of cell structure formation has not been clarified, but the *c-Maf* transcriptional factor has been suggested to contribute to the embryonic cell development and differentiation of at least the proximal tubules and hepatocytes. The mRNA expression profile in kidney tissue from *Maf*-knockout mice, as evaluated using a DNA microarray, showed that the plasma level of glutathione peroxidase 3 (GPx-3) was predominantly downregulated. Since GPx-3 is an antioxidant enzyme, C-MAF may be related to the antioxidant system mediating the modulation of GPx-3 in the kidney[14]. Recently, c-Maf-inducing protein (also known as c-Mip; protein designation, CMIP), a pleckstrin homology (PH) and leucine-rich repeat (LRR)-domain-containing protein, has been identified; CMIP inactivates GSKbeta and interacts with RelA, a key member of the NF-kappaB family. Interestingly, the expression of CMIP (c-Maf-inducing protein) was increased in the podocytes of patients with idiopathic nephrotic syndromes[43]. Membrane nephropathy is characterized by nephrotic-range proteinuria in clinical and subepithelial deposits of immune complex in the basement membrane of the glomerulus. The primary cause of the disease has not been clarified, but antibodies against podocytes located on the outer layer of the basement membrane of the glomeruli form complexes that lead to deposits. A recent report has shown that CMIP was overexpressed in podocytes in an experimental glomerulonephritis rat model exhibiting heavy proteinuria and membranous nephropathy in human. This overexpression was suppressed by immunological treatment resulting in a reduction of proteinuria; thus, while the role and significance of CMIP in podocytes and how it induces massive proteinuria have not yet been elucidated, CMIP or c-MAF-related transcriptional activities may deregulate podocyte function and cause proteinuria[44].

The expression of MAFA in the kidney is uncertain; however, based on the UniGene databank, human MAFA is also expressed in the kidney, lung, and blood. One interesting report described transgenic mice with a disease in which the hybridized gene complex resulted in MAFA deficiency and MAFK overproduction in pancreatic β cells. The phenotype of these transgenic mice was severe diabetes with large amount of proteinuria. A histological examination showed a reduction in β cells in the pancreas and typical histological diabetic nephropathy accompanied by a characteristic nodular lesion in the glomeruli.

The combination of several Maf dysfunctions generate diabetes with diabetic nephropathy, suggesting one possible mechanism for the onset of diabetic nephropathy. Consequently, these mouse models mice may suggest a mechanism for disease onset and could be useful in investigations of treatments for diabetic nephropathy[45].

MAF TRANSCRIPTION FACTORS AND THE CENTRAL NERVOUS SYSTEM

Energy balance in humans is well regulated by multiple organized systems, and the central nervous system (CNS) is likely to be involved and to play important roles in these systems[46]. The CNS contributes to the maintenance of energy balance in part by controlling feeding behavior and also by changing biological conditions and the homeostasis of intra-body conditions. Systemic adjustments of the metabolic state are achieved by the coordination of

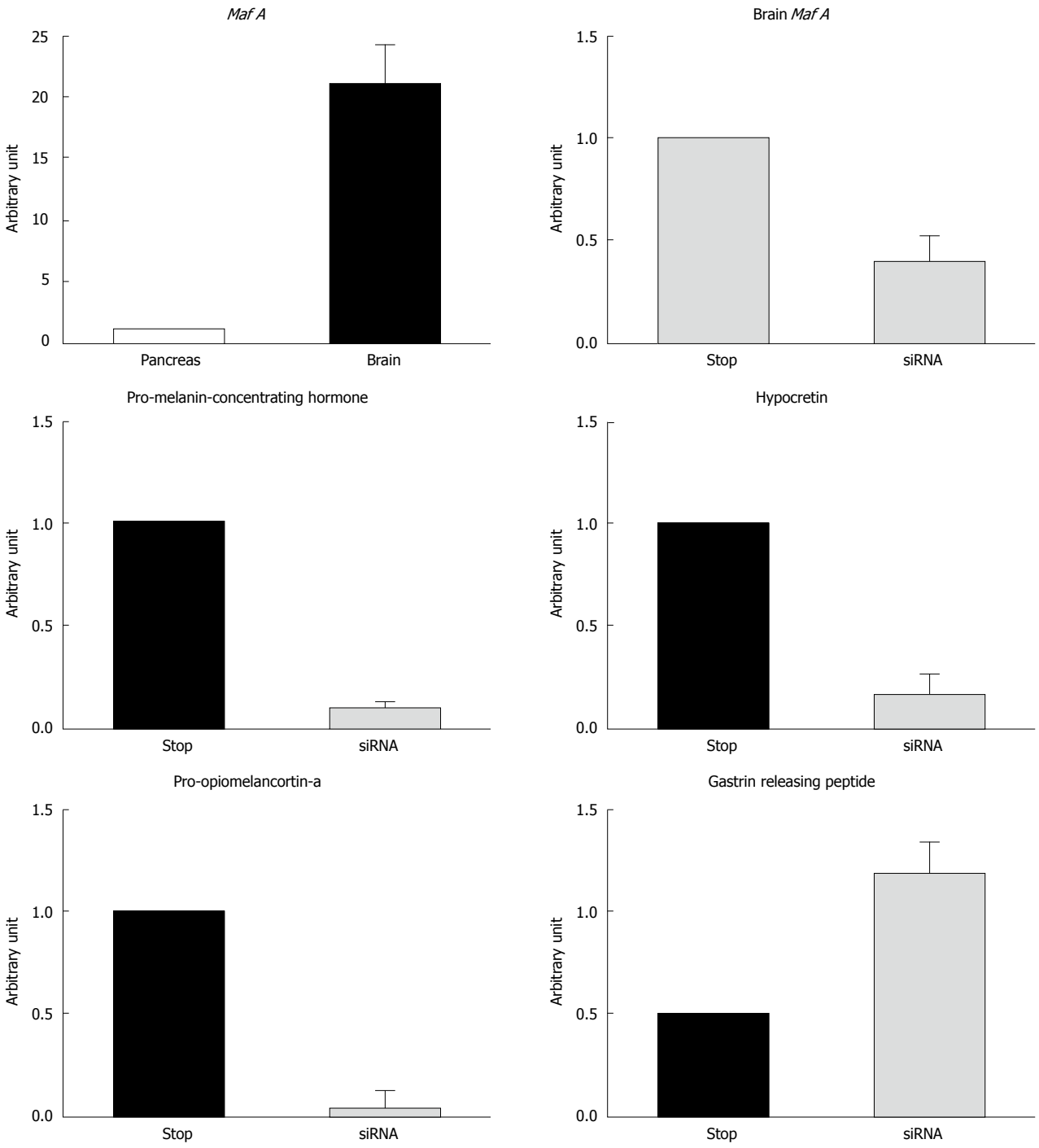

Figure 3 Suppression of *MafA* mRNA by siRNA in the brain and resulting alternation of related genes. A designed small interfering RNA (siRNA) oligomer for mouse *Mafa* was intravenously injected using the hydrodynamic method according to a procedure described by Hamar *et al*[58]. A DNA microarray analysis was then performed using Affymetrix GeneChip technology. The mRNA levels were quantified using real-time PCR. The details of the experiment have been described previously. Expression level of *Mafa* mRNA in the brain. The expression level of *Mafa* mRNA in the brain was 20 times higher than that of *Mafa* mRNA in the pancreas, as assessed using real-time PCR. Suppression of *Mafa* in mice using siRNA in the brain. The mRNA expression level take out in the brain tissue are shown. The *Mafa* mRNA expression level was significantly downregulated by the siRNA. Pro-melanin-concentrating hormone, Hypocretin, and Pro-opiomelanocortin-a were downregulated, and Gastrin-releasing peptide was upregulated, as assessed using real-time PCR with specific primers.

the CNS and peripheral effector organs. In the CNS, transcription factors are involved in the regulation of behavior and intra-body biological conditions[47]. Calorie intake is sensed in the CNS, altering the expression of signal transduction-mediating transcription factors. These responses are then translated into intra-CNS hormones (resulting in changes in eating behavior) and peripheral hormones take out including insulin and leptin, which function to regulate energy balance by direct effects on peripheral organs in coordination with calorie intake and consumption balance.

In our previous mouse study, the *Mafa* mRNA level

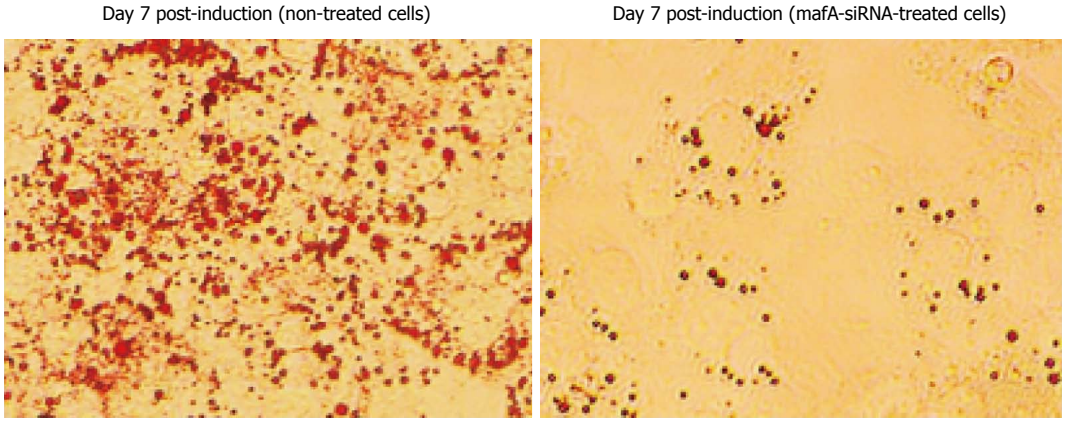

Figure 4 Comparison between histological changes and the Oil-Red-O staining of stop-mafA-siRNA- and mafA-siRNA-treated cells. Mouse 3T3-L1 pre-adipocytes were induced to differentiate, and *Mafa* SiRNA was transfected using a transfection reagent. The morphological appearances of the pre-adipocyte culture before induction and 7 d after induction were then compared. The morphology of the 3T3-L1 cells was directly observed, and lipid droplets were stained using Oil Red O. Oil Red O staining was compared between untreated and *Mafa*-siRNA-treated cells. Intracellular lipid staining was not observed in the *Mafa*-siRNA-treated cells.

was significantly downregulated in brain tissue *in vivo*, as observed using a siRNA technique, and changes in the gene profile in the CNS were screened[48] (Figure 3). The results showed distinct effects on gene expressions in brain tissue, and some of the affected genes were related to eating behavior and energy consumption, such as growth hormone and arginine vasopressin. In addition, several interesting genes and related gene products were identified in this profiling. Pro-melanin-concentrating hormone (Pro-MCH) regulates body weight[49], and changes in its expression can alter the susceptibility to fat metabolism[50,51]. Orexin is a topical neuronal peptide that regulates arousal and sleep[52], and defective orexin producing neuronal cells cause narcolepsy. Orexin may work in the brain-gut network, which regulates appetite during wakefulness[53]. In addition, pro-opiomelanocortin-alpha (an alpha-melanocyte stimulating hormone) and gastrin-releasing peptide, which are important neuropeptides regulating eating behavior and modifying the excretion of several hormones required for food digestion, have also been implicated in the above-mentioned network.

Thus, MAFA is a strong transactivator of insulin in peripheral organs and pancreas, while the modulation of *MAFA* mRNA expression in the CNS induces change in related genes resulting in upregulation and downregulation of neuropeptides that influence appetite, behavior, arousal, and sleep.

MAF TRANSCRIPTION FACTORS AND ADIPOCYTES

Adipocytes develop from mesenchymal stem cells in adipose tissue and various other tissues. Mesenchymal stem cells destined to become adipocytes develop and differentiate into mature adipocytes as a result of transcriptional regulation. PPARγ is known to coordinate with members of the C/EBP family to exert well-documented and important functions at different time points during adipocyte differentiation[54]. Siersbæk *et al*[55] also reported transcriptional networks for adipogenesis take out in which two waves of transcriptional cascades composed the adipogenetic pathway. Maf transcription factors are likely to be involved in this process, and Serria *et al*[33] reported that the expression of c-MAF is downregulated during 3T3-L1 cell differentiation and proliferation. Furthermore, an age-related decrease in the expression of c-MAF in mesenchymal cells has been reported, and present evidence indicates that c-MAF regulates mesenchymal cell bifurcation into osteoblasts and adipocytes. A role of c-MAF in osteogenesis and adipogenesis was also observed in *c-Maf*-knockout mice[56].

As discussed previously, MAFA may be involved in the differentiation of both adipocytes and pancreatic β cells. To explore the role of MAFA in adipose tissue, alterations in the expressions of MAFA-related genes in a cultured adipocyte cell line, 3T3-L1, were observed after *Mafa* mRNA interference had been induced[57]. *Mafa* mRNA suppression induced morphological changes in 3T3-L1 cells during differentiation. As shown in Figure 4, the cytoplasm of spindle-shape cells expanded after differentiation and lipid droplets formed in mature adipocytes, as revealed by the presence of red droplets of Oil Red O stain in the cytoplasm. This morphological change was not observed during *Mafa* siRNA suppression, and no expansion of the cytoplasm was observed. Since lipid droplet formation is essential for adipocyte differentiation, MAFA may play a critical role in the process of adipocyte differentiation. The expression levels of peroxisome proliferator-activated receptor (PPARγ2) and CCAAT/enhancer-binding protein (C/EBPα) were recognized as being essential for the differentiation and function of 3T3-L1 cells. PPARγ 2 plays a leading role in the synthesis and accumulation of lipid droplets in adipocytes, and C/EBPα is critical for the establishment of insulin sensitivity[54]. At the molecular level, the mRNA expression levels of the *PPARγ* gene or the *C/EBP* gene, which encode master adipogenic transcription factors, were markedly suppressed by *Mafa*-siRNA treatment, *i.e.,* by the suppression of MAFA expression. In conclusion,

adiopocyte differentiation and formation is regulated by a network of multiple transcription factors, and Maf transcription factors are likely to be involved, in coordination with other transcription factors.

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REFERENCES

1 **Kawai S**, Goto N, Kataoka K, Saegusa T, Shinno-Kohno H, Nishizawa M. Isolation of the avian transforming retrovirus, AS42, carrying the v-maf oncogene and initial characterization of its gene product. *Virology* 1992; **188**: 778-784 [PMID: 1585647 DOI: 10.1016/0042-6822(92)90532-T]

2 **Nishizawa M**, Kataoka K, Goto N, Fujiwara KT, Kawai S. v-maf, a viral oncogene that encodes a "leucine zipper" motif. *Proc Natl Acad Sci USA* 1989; **86**: 7711-7715 [PMID: 2554284 DOI: 10.1073/pnas.86.20.7711]

3 **Matsuoka TA**, Zhao L, Artner I, Jarrett HW, Friedman D, Means A, Stein R. Members of the large Maf transcription family regulate insulin gene transcription in islet beta cells. *Mol Cell Biol* 2003; **23**: 6049-6062 [PMID: 12917329 DOI: 10.1128/MCB.23.17.6049-6062.2003]

4 **Sakai M**, Imaki J, Yoshida K, Ogata A, Matsushima-Hibaya Y, Kuboki Y, Nishizawa M, Nishi S. Rat maf related genes: specific expression in chondrocytes, lens and spinal cord. *Oncogene* 1997; **14**: 745-750 [PMID: 9038383 DOI: 10.1038/sj.onc.1200869]

5 **Chesi M**, Bergsagel PL, Shonukan OO, Martelli ML, Brents LA, Chen T, Schröck E, Ried T, Kuehl WM. Frequent dysregulation of the c-maf proto-oncogene at 16q23 by translocation to an Ig locus in multiple myeloma. *Blood* 1998; **91**: 4457-4463 [PMID: 9616139]

6 **MacLean HE**, Kim JI, Glimcher MJ, Wang J, Kronenberg HM, Glimcher LH. Absence of transcription factor c-maf causes abnormal terminal differentiation of hypertrophic chondrocytes during endochondral bone development. *Dev Biol* 2003; **262**: 51-63 [PMID: 14512017 DOI: 10.1016/S0012-1606(03)00324-5]

7 **Omoteyama K**, Ikeda H, Imaki J, Sakai M. Activation of connective tissue growth factor gene by the c-Maf and Lc-Maf transcription factors. *Biochem Biophys Res Commun* 2006; **339**: 1089-1097 [PMID: 16343439 DOI: 10.1016/j.bbrc.2005.11.119]

8 **Fujiwara KT**, Kataoka K, Nishizawa M. Two new members of the maf oncogene family, mafK and mafF, encode nuclear b-Zip proteins lacking putative trans-activator domain. *Oncogene* 1993; **8**: 2371-2380 [PMID: 8361754]

9 **Blank V**. Small Maf proteins in mammalian gene control: mere dimerization partners or dynamic transcriptional regulators? *J Mol Biol* 2008; **376**: 913-925 [PMID: 18201722 DOI: 10.1016/j.jmb.2007.11.074]

10 **Kannan MB**, Solovieva V, Blank V. The small MAF transcription factors MAFF, MAFG and MAFK: current knowledge and perspectives. *Biochim Biophys Acta* 2012; **1823**: 1841-1846 [PMID: 22721719 DOI: 10.1016/j.bbamcr.2012.06.012]

11 **Kataoka K**, Nishizawa M, Kawai S. Structure-function analysis of the maf oncogene product, a member of the b-Zip protein family. *J Virol* 1993; **67**: 2133-2141 [PMID: 8383235]

12 **Kataoka K**. Multiple mechanisms and functions of maf transcription factors in the regulation of tissue-specific genes. *J Biochem* 2007; **141**: 775-781 [PMID: 17569705 DOI: 10.1093/jb/mvm105]

13 **Giudicelli F**, Gilardi-Hebenstreit P, Mechta-Grigoriou F, Poquet C, Charnay P. Novel activities of Mafb underlie its dual role in hindbrain segmentation and regional specification. *Dev Biol* 2003; **253**: 150-162 [PMID: 12490204 DOI: 10.1006/dbio.2002.0864]

14 **Shirota S**, Yoshida T, Sakai M, Kim JI, Sugiura H, Oishi T, Nitta K, Tsuchiya K. Correlation between the expression level of c-maf and glutathione peroxidase-3 in c-maf -/- mice kidney and c-maf overexpressed renal tubular cells. *Biochem Biophys Res Commun* 2006; **348**: 501-506 [PMID: 16890189 DOI: 10.1016/j.bbrc.2006.07.111]

15 **Olbrot M**, Rud J, Moss LG, Sharma A. Identification of beta-cell-specific insulin gene transcription factor RIPE3b1 as mammalian MafA. *Proc Natl Acad Sci USA* 2002; **99**: 6737-6742 [PMID: 12011435 DOI: 10.1073/pnas.102168499]

16 **Kataoka K**, Han SI, Shioda S, Hirai M, Nishizawa M, Handa H. MafA is a glucose-regulated and pancreatic beta-cell-specific transcriptional activator for the insulin gene. *J Biol Chem* 2002; **277**: 49903-49910 [PMID: 12368292 DOI: 10.1074/jbc.M206796200]

17 **Matsuoka TA**, Artner I, Henderson E, Means A, Sander M, Stein R. The MafA transcription factor appears to be responsible for tissue-specific expression of insulin. *Proc Natl Acad Sci USA* 2004; **101**: 2930-2933 [PMID: 14973194 DOI: 10.1073/pnas.0306233101]

18 **Zhao L**, Guo M, Matsuoka TA, Hagman DK, Parazzoli SD, Poitout V, Stein R. The islet beta cell-enriched MafA activator is a key regulator of insulin gene transcription. *J Biol Chem* 2005; **280**: 11887-11894 [PMID: 15665000 DOI: 10.1074/jbc.M409475200]

19 **Kataoka K**, Shioda S, Ando K, Sakagami K, Handa H, Yasuda K. Differentially expressed Maf family transcription factors, c-Maf and MafA, activate glucagon and insulin gene expression in pancreatic islet alpha- and beta-cells. *J Mol Endocrinol* 2004; **32**: 9-20 [PMID: 14765989 DOI: 10.1677/jme.0.0320009]

20 **Artner I**, Le Lay J, Hang Y, Elghazi L, Schisler JC, Henderson E, Sosa-Pineda B, Stein R. MafB: an activator of the glucagon gene expressed in developing islet alpha- and beta-cells. *Diabetes* 2006; **55**: 297-304 [PMID: 16443760 DOI: 10.2337/diabetes.55.02.06.db05-0946]

21 **Artner I**, Blanchi B, Raum JC, Guo M, Kaneko T, Cordes S, Sieweke M, Stein R. MafB is required for islet beta cell maturation. *Proc Natl Acad Sci USA* 2007; **104**: 3853-3858 [PMID: 17360442 DOI: 10.1073/pnas.0700013104]

22 **Hang Y**, Stein R. MafA and MafB activity in pancreatic β cells. *Trends Endocrinol Metab* 2011; **22**: 364-373 [PMID: 21719305 DOI: 10.1016/j.tem.2011.05.003]

23 **Harmon JS**, Stein R, Robertson RP. Oxidative stress-mediated, post-translational loss of MafA protein as a contributing mechanism to loss of insulin gene expression in glucotoxic beta cells. *J Biol Chem* 2005; **280**: 11107-11113 [PMID: 15664999 DOI: 10.1074/jbc.M410345200]

24 **Zhang C**, Moriguchi T, Kajihara M, Esaki R, Harada A, Shimohata H, Oishi H, Hamada M, Morito N, Hasegawa K, Kudo T, Engel JD, Yamamoto M, Takahashi S. MafA is a key regulator of glucose-stimulated insulin secretion. *Mol Cell Biol* 2005; **25**: 4969-4976 [PMID: 15923615 DOI: 10.1128/MCB.25.12.4969-4976.2005]

25 **Hang Y**, Yamamoto T, Benninger RK, Brissova M, Guo M, Bush W, Piston DW, Powers AC, Magnuson M, Thurmond DC, Stein R. The MafA transcription factor becomes essential to islet β-cells soon after birth. *Diabetes* 2014; **63**: 1994-2005 [PMID: 24520122 DOI: 10.2337/db13-1001]

26 **Hu He K**, Juhl K, Karadimos M, El Khattabi I, Fitzpatrick C, Bonner-Weir S, Sharma A. Differentiation of pancreatic endocrine progenitors reversibly blocked by premature induction of MafA. *Dev Biol* 2014; **385**: 2-12 [PMID: 24183936 DOI: 10.1016/j.ydbio.2013.10.024]

27 **Gosmain Y**, Marthinet E, Cheyssac C, Guérardel A, Mamin A, Katz LS, Bouzakri K, Philippe J. Pax6 controls the expression of critical genes involved in pancreatic {alpha} cell differentiation and function. *J Biol Chem* 2010; **285**:

33381-33393 [PMID: 20592023 DOI: 10.1074/jbc.M110.147215]
28 **Tsuchiya M**, Taniguchi S, Yasuda K, Nitta K, Maeda A, Shigemoto M, Tsuchiya K. Potential roles of large mafs in cell lineages and developing pancreas. *Pancreas* 2006; **32**: 408-416 [PMID: 16670624]
29 **Dor Y**, Brown J, Martinez OI, Melton DA. Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation. *Nature* 2004; **429**: 41-46 [PMID: 15129273 DOI: 10.1038/nature02520]
30 **Ramiya VK**, Maraist M, Arfors KE, Schatz DA, Peck AB, Cornelius JG. Reversal of insulin-dependent diabetes using islets generated in vitro from pancreatic stem cells. *Nat Med* 2000; **6**: 278-282 [PMID: 10700229 DOI: 10.1038/73128]
31 **Soria B**, Roche E, Berná G, León-Quinto T, Reig JA, Martín F. Insulin-secreting cells derived from embryonic stem cells normalize glycemia in streptozotocin-induced diabetic mice. *Diabetes* 2000; **49**: 157-162 [PMID: 10868930 DOI: 10.2337/diabetes.49.2.157]
32 **Agnello D**, Lankford CS, Bream J, Morinobu A, Gadina M, O'Shea JJ, Frucht DM. Cytokines and transcription factors that regulate T helper cell differentiation: new players and new insights. *J Clin Immunol* 2003; **23**: 147-161 [PMID: 12797537 DOI: 10.1023/A:1023381027062]
33 **Serria MS**, Ikeda H, Omoteyama K, Hirokawa J, Nishi S, Sakai M. Regulation and differential expression of the c-maf gene in differentiating cultured cells. *Biochem Biophys Res Commun* 2003; **310**: 318-326 [PMID: 14521912 DOI: 10.1016/j.bbrc.2003.08.144]
34 **Lumelsky N**, Blondel O, Laeng P, Velasco I, Ravin R, McKay R. Differentiation of embryonic stem cells to insulin-secreting structures similar to pancreatic islets. *Science* 2001; **292**: 1389-1394 [PMID: 11326082 DOI: 10.1126/science.1058866]
35 **Yalniz M**, Pour PM. Are there any stem cells in the pancreas? *Pancreas* 2005; **31**: 108-118 [PMID: 16024996 DOI: 10.1097/01.mpa.0000174939.97438.9f]
36 **Soria B**, Bedoya FJ, Martin F. Gastrointestinal stem cells. I. Pancreatic stem cells. *Am J Physiol Gastrointest Liver Physiol* 2005; **289**: G177-G180 [PMID: 16014979 DOI: 10.1152/ajpgi.00116.2005]
37 **Ham DS**, Shin J, Kim JW, Park HS, Cho JH, Yoon KH. Generation of functional insulin-producing cells from neonatal porcine liver-derived cells by PDX1/VP16, BETA2/NeuroD and MafA. *PLoS One* 2013; **8**: e79076 [PMID: 24260156 DOI: 10.1371/journal.pone.0079076]
38 **Wang L**, Huang Y, Guo Q, Fan X, Lu Y, Zhu S, Wang Y, Bo X, Chang X, Zhu M, Wang Z. Differentiation of iPSCs into insulin-producing cells via adenoviral transfection of PDX-1, NeuroD1 and MafA. *Diabetes Res Clin Pract* 2014; **104**: 383-392 [PMID: 24794627 DOI: 10.1016/j.diabres.2014.03.017]
39 **Imaki J**, Onodera H, Tsuchiya K, Imaki T, Mochizuki T, Mishima T, Yamashita K, Yoshida K, Sakai M. Developmental expression of maf-1 messenger ribonucleic acids in rat kidney by in situ hybridization histochemistry. *Biochem Biophys Res Commun* 2000; **272**: 777-782 [PMID: 10860830 DOI: 10.1006/bbrc.2000.2865]
40 **Imaki J**, Tsuchiya K, Mishima T, Onodera H, Kim JI, Yoshida K, Ikeda H, Sakai M. Developmental contribution of c-maf in the kidney: distribution and developmental study of c-maf mRNA in normal mice kidney and histological study of c-maf knockout mice kidney and liver. *Biochem Biophys Res Commun* 2004; **320**: 1323-1327 [PMID: 15249232 DOI: 10.1016/j.bbrc.2004.05.222]
41 **Sadl V**, Jin F, Yu J, Cui S, Holmyard D, Quaggin S, Barsh G, Cordes S. The mouse Kreisler (Krml1/MafB) segmentation gene is required for differentiation of glomerular visceral epithelial cells. *Dev Biol* 2002; **249**: 16-29 [PMID: 12217315 DOI: 10.1006/dbio.2002.0751]
42 **Moriguchi T**, Hamada M, Morito N, Terunuma T, Hasegawa K, Zhang C, Yokomizo T, Esaki R, Kuroda E, Yoh K, Kudo T, Nagata M, Greaves DR, Engel JD, Yamamoto M, Takahashi S. MafB is essential for renal development and F4/80 expression in macrophages. *Mol Cell Biol* 2006; **26**: 5715-5727 [PMID: 16847325 DOI: 10.1128/MCB.00001-06]
43 **Zhang SY**, Kamal M, Dahan K, Pawlak A, Ory V, Desvaux D, Audard V, Candelier M, BenMohamed F, Matignon M, Christov C, Decrouy X, Bernard V, Mangiapan G, Lang P, Guellaën G, Ronco P, Sahali D. c-mip impairs podocyte proximal signaling and induces heavy proteinuria. *Sci Signal* 2010; **3**: ra39 [PMID: 20484117 DOI: 10.1126/scisignal.2000678]
44 **Sendeyo K**, Audard V, Zhang SY, Fan Q, Bouachi K, Ollero M, Rucker-Martin C, Gouadon E, Desvaux D, Bridoux F, Guellaën G, Ronco P, Lang P, Pawlak A, Sahali D. Upregulation of c-mip is closely related to podocyte dysfunction in membranous nephropathy. *Kidney Int* 2013; **83**: 414-425 [PMID: 23302718 DOI: 10.1038/ki.2012.426]
45 **Shimohata H**, Yoh K, Fujita A, Morito N, Ojima M, Tanaka H, Hirayama K, Kobayashi M, Kudo T, Yamagata K, Takahashi S. MafA-deficient and beta cell-specific MafK-overexpressing hybrid transgenic mice develop human-like severe diabetic nephropathy. *Biochem Biophys Res Commun* 2009; **389**: 235-240 [PMID: 19715672 DOI: 10.1016/j.bbrc.2009.08.124]
46 **Arch JR**. Central regulation of energy balance: inputs, outputs and leptin resistance. *Proc Nutr Soc* 2005; **64**: 39-46 [PMID: 15877921 DOI: 10.1079/PNS2004407]
47 **Coppari R**, Ramadori G, Elmquist JK. The role of transcriptional regulators in central control of appetite and body weight. *Nat Clin Pract Endocrinol Metab* 2009; **5**: 160-166 [PMID: 19229236 DOI: 10.1038/ncpendmet1070]
48 **Tsuchiya M**, Tsuchiya K, Yasuda K, Fujita M, Takinishi A, Furukawa M, Nitta K, Maeda A. MafA is a Key Molecule in Glucose and Energy Balance in the Central Nervous System and Peripheral Organs. *Int J Biomed Sci* 2011; **7**: 19-26 [PMID: 23675216]
49 **Ludwig DS**, Tritos NA, Mastaitis JW, Kulkarni R, Kokkotou E, Elmquist J, Lowell B, Flier JS, Maratos-Flier E. Melanin-concentrating hormone overexpression in transgenic mice leads to obesity and insulin resistance. *J Clin Invest* 2001; **107**: 379-386 [PMID: 11160162 DOI: 10.1172/JCI10660]
50 **Qu D**, Ludwig DS, Gammeltoft S, Piper M, Pelleymounter MA, Cullen MJ, Mathes WF, Przypek R, Kanarek R, Maratos-Flier E. A role for melanin-concentrating hormone in the central regulation of feeding behaviour. *Nature* 1996; **380**: 243-247 [PMID: 8637571 DOI: 10.1038/380243a0]
51 **Shimada M**, Tritos NA, Lowell BB, Flier JS, Maratos-Flier E. Mice lacking melanin-concentrating hormone are hypophagic and lean. *Nature* 1998; **396**: 670-674 [PMID: 9872314 DOI: 10.1038/25341]
52 **Sakurai T**, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, Williams SC, Richardson JA, Kozlowski GP, Wilson S, Arch JR, Buckingham RE, Haynes AC, Carr SA, Annan RS, McNulty DE, Liu WS, Terrett JA, Elshourbagy NA, Bergsma DJ, Yanagisawa M. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell* 1998; **92**: 573-585 [PMID: 9491897 DOI: 10.1016/S0092-8674(00)80949-6]
53 **Kirchgessner AL**. Orexins in the brain-gut axis. *Endocr Rev* 2002; **23**: 1-15 [PMID: 11844742 DOI: 10.1210/edrv.23.1.0454]
54 **Farmer SR**. Transcriptional control of adipocyte formation. *Cell Metab* 2006; **4**: 263-273 [PMID: 17011499 DOI: 10.1016/j.cmet.2006.07.001]
55 **Siersbæk R**, Mandrup S. Transcriptional networks controlling adipocyte differentiation. *Cold Spring Harb Symp Quant Biol* 2011; **76**: 247-255 [PMID: 21900150 DOI: 10.1101/sqb.2011.76.010512]
56 **Nishikawa K**, Nakashima T, Takeda S, Isogai M, Hamada M, Kimura A, Kodama T, Yamaguchi A, Owen MJ, Takahashi S, Takayanagi H. Maf promotes osteoblast differentiation in mice by mediating the age-related switch in mesenchymal cell differentiation. *J Clin Invest* 2010; **120**: 3455-3465 [PMID: 20877012 DOI: 10.1172/JCI42528]

57 **Tsuchiya M**, Maeda A, Suzuki A, Yasuda K, Yoshida T, Nitta K, Tsuchiya K. Suppression of MafA mRNA with siRNA prevents adipose cell differentiation in 3T3-L1 cells. *Int J Mol Med* 2009; **23**: 725-732 [PMID: 19424598 DOI: 10.3892/ijmm_00000186]

58 **Hamar P**, Song E, Kökény G, Chen A, Ouyang N, Lieberman J. Small interfering RNA targeting Fas protects mice against renal ischemia-reperfusion injury. *Proc Natl Acad Sci USA* 2004; **101**: 14883-14888 [PMID: 15466709 DOI: 10.1073/pnas.0406421101]

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MINIREVIEWS

Coronary atherosclerosis is already ongoing in pre-diabetic status: Insight from intravascular imaging modalities

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Abstract

Diabetes mellitus is a powerful risk factor of coronary artery disease (CAD), leading to death and disability. In recent years, given the accumulating evidence that prediabetes is also related to increasing risk of CAD including cardiovascular events, a new guideline has been proposed for the treatment of blood cholesterol for primary prevention of cardiovascular events. This guideline recommends aggressive lipid-lowering statin therapy for primary prevention in diabetes and other patients. The ultimate goal of patient management is to inhibit progression of systemic atherosclerosis and prevent fatal cardiovascular events such as acute coronary syndrome (ACS). Because disruption of atherosclerotic coronary plaques is a trigger of ACS, the high-risk atheroma is called a vulnerable plaque. Several types of novel diagnostic imaging technologies have been developed for identifying the characteristics of coronary atherosclerosis before the onset of ACS, especially vulnerable plaques. According to coronary angioscopic evaluation, atherosclerosis severity and plaque vulnerability were more advanced in prediabetic than in nondiabetic patients and comparable to that in diabetic patients. In addition, pharmacological intervention by statin therapy changed plaque color and complexity, and the dynamic changes in plaque features are considered plaque stabilization. In this article, we review the findings of atherosclerosis in prediabetes, detected by intravascular imaging modalities, and the therapeutic implications.

Key words: Diabetes; Prediabetes; Statin therapy; Coronary artery disease; Intravascular imaging modality

Core tip: Coronary artery disease is the principal cause of death and disability in not only diabetes but also prediabetes patients. Aggressive statin therapy is an established method of primary prevention of cardiovascular disease events in diabetic patients. According to the findings of coronary imaging modalities detecting atherosclerotic lesions, statin therapy in prediabetes may be beneficial for reducing atherosclerotic cardiovascular risk.

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INTRODUCTION

Diabetes is categorized as a metabolic disease char-acterized by hyperglycemia arising from abnormal insulin secretion from the pancreas and/or lack (or absence) of insulin action. Diabetes causes damage, dysfunction, or failure of various organs involving heart and blood vessels[1]. It is well known that diabetes promotes atherosclerotic disease of systemic and coronary arteries and increases the mortality rate from cardiovascular disease[2,3]. However, myocardial ischemia owing to coronary artery disease (CAD) is occasionally absent from the typical symptoms in patients with diabetes[4]. As a result, severe multivessel disease of the coronary arteries can manifest as silent myocardial ischemia before treatment is begun. A delayed recognition of CAD can worsen the prognosis in many diabetic patients[5]. Moreover, a recent study showed that impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) are also causes of adverse cardiovascular events[6,7].

A goal of CAD management is to prevent cardiovascular diseases such as acute coronary syndrome (ACS). The principal pathogenesis of ACS is disruption of atheromatous coronary plaques and subsequent flow-limiting thrombus formation[8,9]. Plaque disruption, the trigger of this serious event, is pathologically classified as a rupture, and shallower intimal injury is termed erosion. Additionally, previous pathological studies showed that the majority of disrupted plaques have a large lipid core under a thin fibrous cap, hence the term thin-cap fibroatheroma (TCFA). Not only plaque rupture but also superficial calcified nodules are possible origins of ACS[10-12]. A vulnerable plaque is defined as a future high-risk plaque provoking ACS, and TCFA is representative of a vulnerable plaque. In recent years, novel intraco-ronary imaging modalities have been developed for detecting such vulnerable plaques.

Coronary angiography remains the gold standard for diagnosis of CAD and the following catheter intervention therapy. However, an angiogram represents a 2-dimensional silhouette of the coronary artery, and the angiogram does not supply certain information about the vessel wall components or the atherosclerotic plaque. Therefore, a coronary angiogram is not capable of detecting vulnerable plaques, including TCFA. Thus, supplemental CAD diagnostic modalities, including various intravascular imaging devices such as intravascular ultrasound (IVUS), coronary angioscopy (CAS), and optical coherence tomography (OCT), have been developed to discriminate each component of the plaque and to identify the presence of vulnerable plaques.

INTRACORONARY INVASIVE IMAGING MODALITIES

IVUS

IVUS is an intravascular imaging modality that supplies cross-sectional images of the coronary artery including the lumen and vessel wall. High-frequency (20-40 MHz) IVUS visualizes 3 layers of the vessel wall: the intima, media, and adventitia. IVUS allows *in vivo* qualitative measurements of the lumen and plaque area (and volume). Conventional grayscale IVUS images have major limitation of precise tissue characterization except calcification. Although a large plaque burden and microcalcifications, factors of plaque vulnerability, are detected by grayscale IVUS, this imaging system is not able to identify TCFA[13]. Because of these limitations, 3 modalities using radiofrequency analysis, virtual histology IVUS (VH-IVUS; Volcano Therapeutics, Rancho Cordova, CA, United States)[14], iMAP-IVUS (Boston Scientific, Santa Clara, CA, United States)[15], and integrated backscatter IVUS (IB-IVUS; YD Co., Nara, Japan)[16] are now available in clinical settings.

VH-IVUS takes into account detailed qualitative and quantitative assessment of the vessel wall components. The axial resolution of VH-IVUS is just about 150-250 μm. *Ex vivo* studies have shown that power spectrum-related parameters from raw backscattered ultrasound signals permit discrimination of plaque components[17]. These parameters are used in a classification scheme to yield a tissue color map for each plaque characteristic as follows: dark green indicates fibrous, yellow-green indicates fibrofatty, red indicates necrotic core, and white indicates dense calcium. VH-derived TCFA was defined as at least 3 consecutive frames with a plaque burden of at least 40% and without overlying fibrous tissue[18]. A recent prospective study using VH-IVUS, the PROSPET trial, has shown that the VH-derived TCFA with a minimal luminal area $\leqslant$ 4 mm^2 and a plaque burden $\geqslant$ 70% was the highest-risk plaque type leading to adverse cardiovascular events[19].

CAS

CAS using optic fibers is a technology that permits direct visualization of the lumen surface of the coronary artery and provides detailed information about plaque morphology and the presence of a thrombus with high resolution (50 μm). CAS clearly identifies irregularities of the lumen surface, such as ulceration, fissures, and tears. Disrupted plaques are involved in these plaques with irregularities (or complexity). In addition, CAS is an extremely sensitive detector of a thrombus. Angiographic stenosis of the lesion progresses despite healing of the silent plaque disruption in the nonculprit lesions[20].

Based on angioscopic analysis, an atherosclerotic plaque is defined as a nonmobile, protruding structure that can be clearly delimited from the adjacent vessel wall. Although a normal vessel wall appears glistening white, plaques can be yellow or white according to the surface color. The color of the plaque is classified semiquantitatively: (1) grade 0 is white; (2) grade 1 is

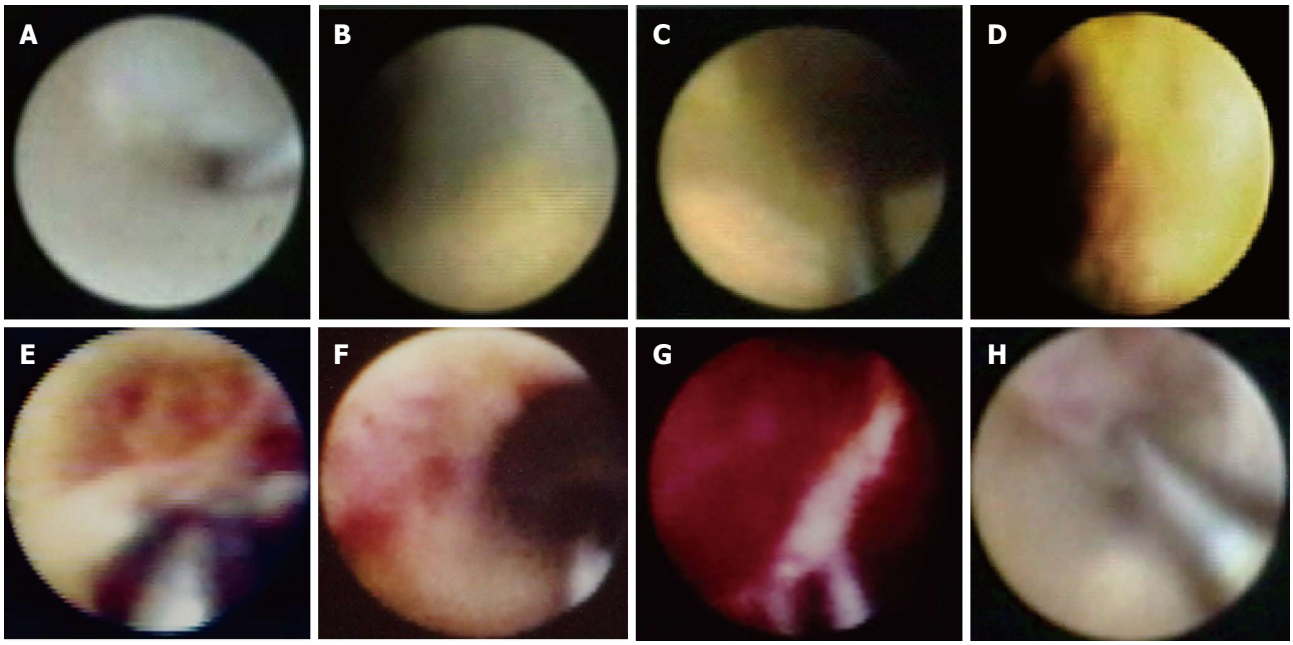

Figure 1 Classification of coronary angioscopic images. A: White plaque (yellow grade 0); B: Light yellow plaque (grade 1); C: Yellow plaque (grade 2); D: Intense yellow plaque (grade 3); E: Plaque rupture; F: Plaque erosion; G: Red thrombus; H: White thrombus.

light yellow; (3) grade 2 is medium yellow; and (4) grade 3 is intense yellow. The majority of yellow plaques contain lipid-rich tissue or a necrotic core according to comparative validation using OCT and IVUS. The grade of yellow plaques is affected by the thickness of the fibrous cap covering lipidic tissue. A high-intensity yellow plaque is identical to a TCFA. On the contrary, white plaques contain fibrous tissue or a thick fibrous cap covering the lipidic plaque[21-24]. Yellow plaques are detected not only in the culprit lesion but also in the nonculprit lesions of ACS[25-29]. Representative CAS images of plaques and thrombi are shown in Figure 1.

Prospective studies demonstrated that the incidence of ACS is higher in patients with intense yellow or multiple yellow plaques than in patients without yellow plaques[30,31]. These findings indicate that intense yellow or multiple yellow plaques detected by CAS might be vulnerable and could cause future coronary events.

OCT

OCT imaging employs a near-infrared range light source, at approximately 1300 nm. OCT has a 10-fold higher image resolution (10-15 μm) than IVUS, and its image quality is superior to that of other imaging devices. In addition, OCT provides accurate tissue characterization of the plaque. Normal vessel walls appear as a 3-layer structure on OCT images as well as IVUS. The vascular media is seen as a dark band delineated by the internal elastic lamina and external elastic lamina. Fibrous plaques consist of homogeneous and low-attenuation areas. Lipid-rich plaques exhibit a high-attenuation mass with a diffuse border. A calcified plaque is presented as high-attenuation mass with a clear border[32-36].

OCT is the only intravascular imaging technology with high spatial resolution that can measure the fibrous cap thickness[37,38].

CORONARY ATHEROSCLEROSIS INDUCED BY GLUCOSE METABOLISM DISORDER

Diabetes-associated coronary atherosclerosis as determined by imaging modalities

In diabetic patients, coronary angiograms characteristically reveal diffuse long lesions in multiple small vessels[39,40]. In an IVUS study, plaques in diabetic patients were characterized by an increased amount of dense calcium, a necrotic core, and a high frequency of VH-TCFA[41]. In addition, IVUS studies showed that the levels of hemoglobin A1c (HbA1c) was associated with atheroma volume and the severity of coronary atherosclerosis[42,43]. CAS showed that diabetes was an independent predictor of plaque disruption in the nonculprit vessel[44]. In an OCT study, patients with diabetes had large lipid plaque volumes and a high prevalence of calcified plaque and thrombus[45].

Possible mechanism of hyperglycemia-induced coronary atherosclerosis

Free fatty acids and insulin resistance, which are elevated by hyperglycemia, stimulate molecular mechanisms and alter the function and structure of blood vessels, including increased oxidative stress and activation of protein kinase C and the receptor for advanced glycation end products. Consequently, hyperglycemia decreases the availability of nitric oxide, increases the production of endothelin, and activates transcription factors such as

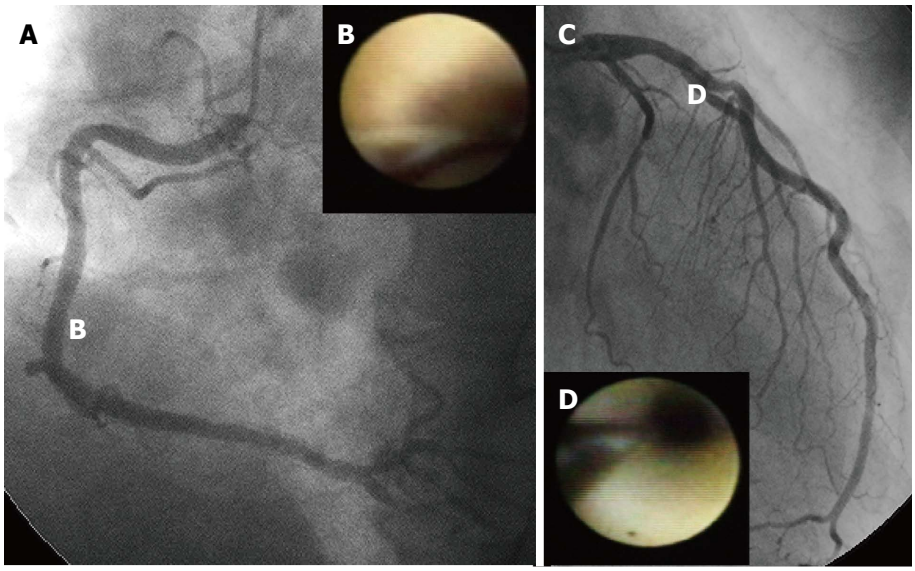

Figure 2 Representative images of nondiabetic patients. A and B: No angiographic stenosis was observed in the right coronary artery (A), whereas 1 yellow plaque was identified on angioscopy (B). The yellow intensity of these plaques was defined as grade 2; C: The left circumflex artery was too small to observe by coronary angioscopy, and a 75% stenosis was identified on angiography in the middle part of the left ascending artery; D: According to angioscopic findings, this lesion was evaluated as a grade 1 yellow plaque. In this case, the average number of yellow plaques per vessel was 1 (2 yellow plaques in 2 vessels), and the maximum yellow grade per coronary artery was 2.

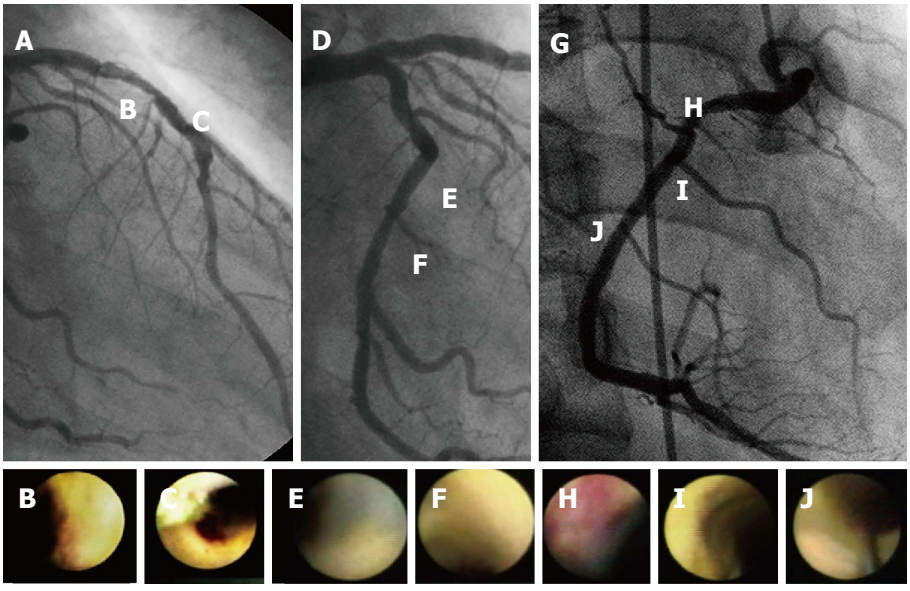

Figure 3 Representative images of prediabetes patients. A: A 50% stenosis and a 90% stenosis were identified on angiography in the middle part of the left ascending artery; B and C: According to angioscopic findings, both of these lesions were evaluated as grade 3 yellow plaques; D-F: No angiographic stenosis was observed in the left circumflex artery (D), whereas 2 yellow plaques were defined as grades 1 and 2, respectively (E and F); G-J: Significant stenosis was not observed in the right coronary artery (G), whereas an intramural red thrombus was observed at the proximal site (H), and 3 yellow plaques were identified on angioscopy (H–J). The yellow intensity of these plaques was defined as grades 1, 2, and 1, respectively. In this case, the average number of yellow plaques per vessel was 2.33 (7 yellow plaques in 3 vessels), and the maximum yellow grade per coronary artery was 3.

nuclear factor-κB and activator protein-1. These factors bring about systemic vasoconstriction and inflammation and promote systemic atherosclerosis[46-48]. A similar glucose metabolism disorder occurs in the prediabetic state[49,50].

The American Diabetes Association defines prediabetes as IFG, IGT, and HbA1c values ranging 5.7%-6.4%[1]. The patients with IFG and IGT should be informed of their increased risk for diabetes as well as CAD. The HbA1c value is more commonly used to diagnose diabetes, and an HbA1c level of 5.7%-6.4% also indicates a relatively high risk for future diabetes and CAD[1].

The low concordance in the relationships between IFG, IGT, and HbA1c, as well as the diagnoses of prediabetes using these parameters, accentuates the various dysfunctions of glucose metabolism. A dys-

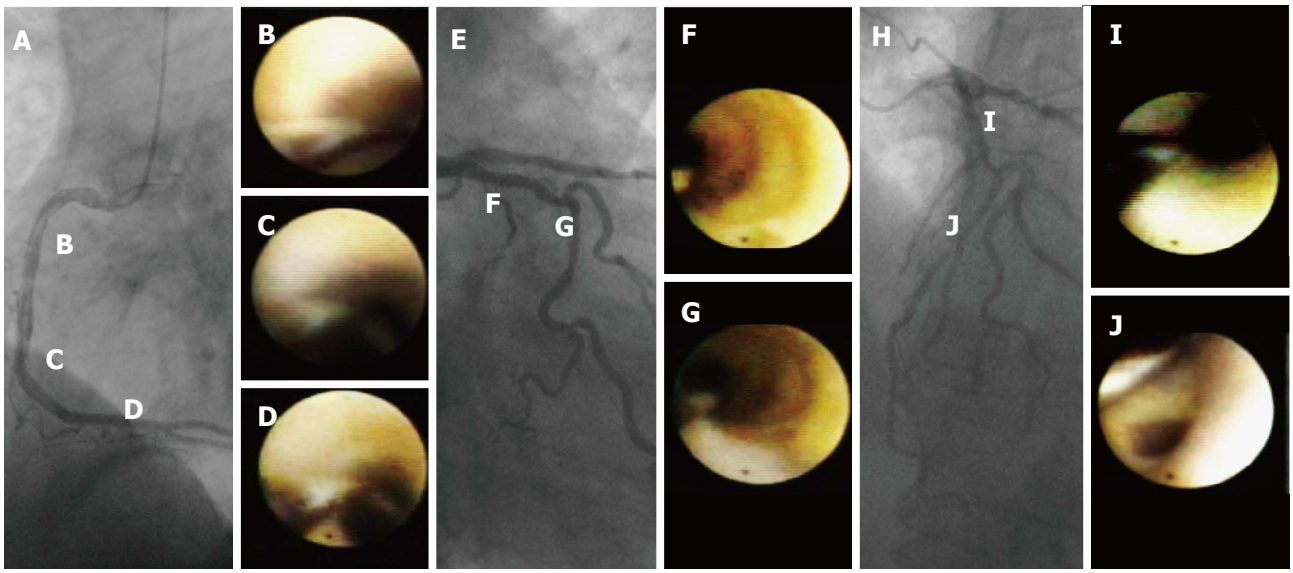

Figure 4 Representative images of diabetes patients. A-D: No angiographic stenosis was observed in the right coronary artery (A), whereas 3 yellow plaques were identified on angioscopy (B-D). The yellow intensity of these plaques was defined as grades 2, 1, and 2, respectively; E-G: Significant stenosis was not observed in the left circumflex artery (E), whereas 2 yellow plaques were identified on angioscopy (F and G). The yellow intensity of both of these plaques was evaluated as grade 3 (intense yellow); H: A 50% stenosis and a 90% stenosis were identified on angiography in the middle part of the left ascending artery; I and J: According to angioscopic findings, both these lesions were evaluated as light yellow plaques (grade 1). In this case, the average number of yellow plaques per vessel was 2.33 (7 yellow plaques in 3 vessels), and the maximum yellow grade per coronary artery was 3.

function in hepatic insulin resistance manifests as IFG, whereas muscle insulin resistance represents IGT[51]. Although data for IFG and IGT are provided by the daily glucose snapshot, HbA1c levels after chronic exposure (over 60-90 d) to basal and postprandial hyperglycemia reflects a combination of IFG and IGT[52].

Prediabetes-associated coronary atherosclerosis as determined by imaging modalities

An angiographic study revealed that atherosclerosis of coronary arteries developed not only in patients with diabetes but also in those with IGT[53]. An IVUS study showed that even patients with a prediabetic status detected by IGT and IFG exhibited abundant lipid-rich plaques in their coronary arteries[54]. Recently, we used CAS to identify yellow vulnerable plaques in the coronary arteries of patients with prediabetes and diabetes compared with controls. Representative images of patients without diabetes and with prediabetes and diabetes are shown in Figures 2-4. Our findings indicate that both the degree of coronary atherosclerosis and the plaque vulnerability were more advanced in patients with prediabetes than in those without diabetes, and were comparable to patients with diabetes. We showed that the number of yellow plaques (0.80 ± 0.64 *vs* 1.45 ± 0.81 *vs* 1.63 ± 0.99; $P = 0.011$) and yellow grade (1.44 ± 1.03 *vs* 2.00 ± 0.86 *vs* 2.30 ± 0.70; $P = 0.047$) in patients with prediabetes were greater than those in patients without diabetes, but similar to those in patients with diabetes (Figure 5)[55].

Prevention of atherosclerotic cardiovascular diseases

Recently, the American College of Cardiology and the American Heart Association proposed a new guideline for the treatment of hyperlipidemia to reduce the risk of cardiovascular events and recommended aggressive statin therapy for both primary and secondary prevention of atherosclerotic cardiovascular disease (ACVD) events in diabetes patients[56]. Moreover, for patients with diabetes without preexisting CAD, the American Diabetes Association currently recommends starting pharmacological therapy at a low-density lipoprotein cholesterol (LDL-C) level of ⩾ 130 mg/dL with a goal of < 100 mg/dL[57]. In an angioscopic investigation, lowering LDL-C by statin induces reduction of color intensity in yellow plaques, and the phenomenon is regarded as its effect on plaque stabilization[58].

Four major groups were identified that would benefit from statin therapy to reduce ACVD risk: (1) patients with clinical ACVD (secondary prevention); (2) patients with primary elevations of LDL-C ⩾ 190 mg/dL (primary prevention); (3) patients 40-75 years of age who have diabetes and LDL-C 70-189 mg/dL (primary prevention); and (4) patients up to 75 years of age without diabetes and with an estimated 10-year ACVD risk ⩾ 7.5% and LDL-C 70-189 mg/dL (primary prevention). Selected patients with < 5% 10-year ACVD risk who are < 40 or > 75 years of age may also benefit from statin therapy[56]. The 10-year ACVD risk was estimated from age, sex, race, blood cholesterol level, history of hypertension and diabetes, smoking habits, *etc*. In the group with 10-year ACVD risk < 7.5%, a benefit of statin therapy was not completely established. Regarding prediabetes, we should pay attention to this group and consider the benefit of statin therapy.

Because coronary atherosclerosis is already present

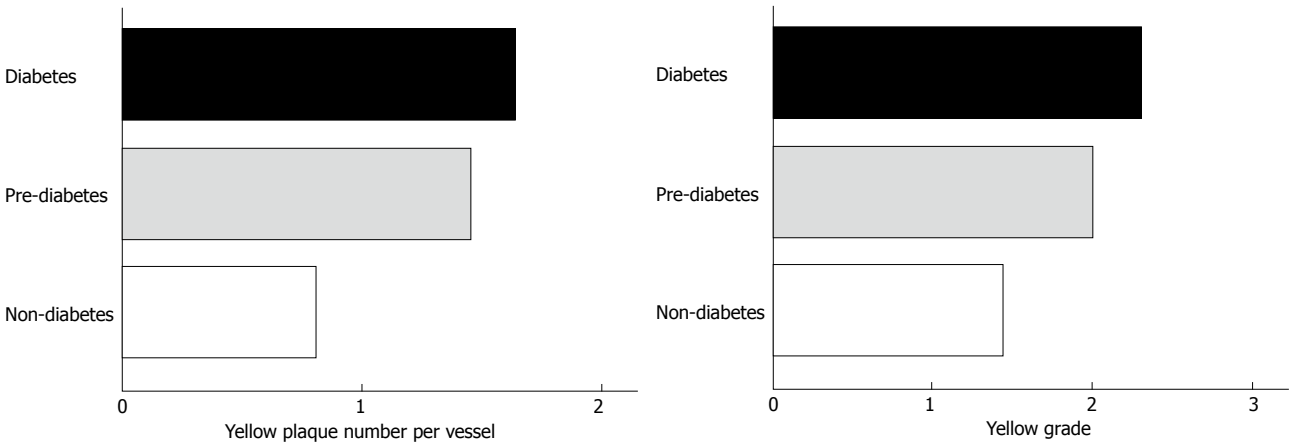

Figure 5 Comparisons of yellow plaque detected by coronary angioscopy among the 3 groups. A: Average number of yellow plaques per vessel; B: Average maximum yellow grade per coronary artery (MYG). The number of yellow plaques per vessel and maximum yellow grade per coronary artery in the prediabetic group were greater than those in the nondiabetic group ($P = 0.017$ and $P = 0.040$, respectively), whereas they were similar to those in the diabetic group ($P = 0.44$ and $P = 0.21$, respectively).

in prediabetes and our angioscopic examination revealed that the level of coronary atherosclerosis with prediabetes is almost equal to that in patients with diabetes, even for patients with prediabetes, earlier pharmacological therapy should be recommended.

CONCLUSION

We should consider the risk of CAD in the prediabetic state with mild glucose metabolism disorder, and further clinical investigations are required to establish an exact risk stratification and prevent future cardiovascular events in patients with prediabetes.

REFERENCES

1 **American Diabetes Association.** Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2010; **33** Suppl 1: S62-S69 [PMID: 20042775 DOI: 10.2337/dc10-S062]

2 **Stamler J**, Vaccaro O, Neaton JD, Wentworth D. Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial. *Diabetes Care* 1993; **16**: 434-444 [PMID: 8432214 DOI: 10.2337/diacare.16.2.434]

3 **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]

4 **Wingard DL**, Barrett-Connor EL, Scheidt-Nave C, McPhillips JB. Prevalence of cardiovascular and renal complications in older adults with normal or impaired glucose tolerance or NIDDM. A population-based study. *Diabetes Care* 1993; **16**: 1022-1025 [PMID: 8359095 DOI: 10.2337/diacare.16.7.1022]

5 **Grundy SM**, Benjamin IJ, Burke GL, Chait A, Eckel RH, Howard BV, Mitch W, Smith SC, Sowers JR. Diabetes and cardiovascular disease: a statement for healthcare professionals from the American Heart Association. *Circulation* 1999; **100**: 1134-1146 [PMID: 10477542 DOI: 10.1161/01.CIR.100.10.1134]

6 **Unwin N**, Shaw J, Zimmet P, Alberti KG. Impaired glucose tolerance and impaired fasting glycaemia: the current status on definition and intervention. *Diabet Med* 2002; **19**: 708-723 [PMID: 12207806 DOI: 10.1046/j.1464-5491.2002.00835.x]

7 **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]

8 **Fuster V**, Badimon L, Badimon JJ, Chesebro JH. The pathogenesis of coronary artery disease and the acute coronary syndromes (1). *N Engl J Med* 1992; **326**: 242-250 [PMID: 1727977 DOI: 10.1056/NEJM199201233260406]

9 **Fuster V**, Badimon L, Badimon JJ, Chesebro JH. The pathogenesis of coronary artery disease and the acute coronary syndromes (2). *N Engl J Med* 1992; **326**: 310-318 [PMID: 1728735 DOI: 10.1056/NEJM199201303260506]

10 **Naghavi M**, Libby P, Falk E, Casscells SW, Litovsky S, Rumberger J, Badimon JJ, Stefanadis C, Moreno P, Pasterkamp G, Fayad Z, Stone PH, Waxman S, Raggi P, Madjid M, Zarrabi A, Burke A, Yuan C, Fitzgerald PJ, Siscovick DS, de Korte CL, Aikawa M, Juhani Airaksinen KE, Assmann G, Becker CR, Chesebro JH, Farb A, Galis ZS, Jackson C, Jang IK, Koenig W, Lodder RA, March K, Demirovic J, Navab M, Priori SG, Rekhter MD, Bahr R, Grundy SM, Mehran R, Colombo A, Boerwinkle E, Ballantyne C, Insull W, Schwartz RS, Vogel R, Serruys PW, Hansson GK, Faxon DP, Kaul S, Drexler H, Greenland P, Muller JE, Virmani R, Ridker PM, Zipes DP, Shah PK, Willerson JT. From vulnerable plaque to vulnerable patient: a call for new definitions and risk assessment strategies: Part I. *Circulation* 2003; **108**: 1664-1672 [PMID: 14530185 DOI: 10.1161/01.CIR.0000087480.94275.97]

11 **Naghavi M**, Libby P, Falk E, Casscells SW, Litovsky S, Rumberger J, Badimon JJ, Stefanadis C, Moreno P, Pasterkamp G, Fayad Z, Stone PH, Waxman S, Raggi P, Madjid M, Zarrabi A, Burke A, Yuan C, Fitzgerald PJ, Siscovick DS, de Korte CL, Aikawa M, Airaksinen KE, Assmann G, Becker CR, Chesebro JH, Farb A, Galis ZS, Jackson C, Jang IK, Koenig W, Lodder RA, March K, Demirovic J, Navab M, Priori SG, Rekhter MD, Bahr R, Grundy SM, Mehran R, Colombo A, Boerwinkle E, Ballantyne C, Insull W, Schwartz RS, Vogel R, Serruys PW, Hansson GK, Faxon DP, Kaul S, Drexler H, Greenland P, Muller JE, Virmani R, Ridker PM, Zipes DP, Shah PK, Willerson JT. From vulnerable plaque to vulnerable patient: a call for new definitions and risk assessment strategies: Part II. *Circulation* 2003; **108**: 1772-1778 [PMID: 14557340 DOI:

10.1161/01.CIR.0000087481.55887.C9]
12 **Virmani R**, Kolodgie FD, Burke AP, Finn AV, Gold HK, Tulenko TN, Wrenn SP, Narula J. Atherosclerotic plaque progression and vulnerability to rupture: angiogenesis as a source of intraplaque hemorrhage. *Arterioscler Thromb Vasc Biol* 2005; **25**: 2054-2061 [PMID: 16037567 DOI: 10.1161/01.ATV.0000178991.71605.18]
13 **Schmermund A**, Erbel R. Unstable coronary plaque and its relation to coronary calcium. *Circulation* 2001; **104**: 1682-1687 [PMID: 11581149 DOI: 10.1161/hc3901.093339]
14 **Nair A**, Kuban BD, Tuzcu EM, Schoenhagen P, Nissen SE, Vince DG. Coronary plaque classification with intravascular ultrasound radiofrequency data analysis. *Circulation* 2002; **106**: 2200-2206 [PMID: 12390948 DOI: 10.1161/01.CIR.0000035654.18341.5E]
15 **Sathyanarayana S**, Carlier S, Li W, Thomas L. Charact-erisation of atherosclerotic plaque by spectral similarity of radiofrequency intravascular ultrasound signals. *EuroIntervention* 2009; **5**: 133-139 [PMID: 19577995 DOI: 10.4244/EIJV5I1A21]
16 **Kawasaki M**, Takatsu H, Noda T, Ito Y, Kunishima A, Arai M, Nishigaki K, Takemura G, Morita N, Minatoguchi S, Fujiwara H. Noninvasive quantitative tissue characterization and two-dimensional color-coded map of human atherosclerotic lesions using ultrasound integrated backscatter: comparison between histology and integrated backscatter images. *J Am Coll Cardiol* 2001; **38**: 486-492 [PMID: 11499742 DOI: 10.1016/S0735-1097(01)01393-6]
17 **Moore MP**, Spencer T, Salter DM, Kearney PP, Shaw TR, Starkey IR, Fitzgerald PJ, Erbel R, Lange A, McDicken NW, Sutherland GR, Fox KA. Characterisation of coronary atherosclerotic morphology by spectral analysis of radiofrequency signal: in vitro intravascular ultrasound study with histological and radiological validation. *Heart* 1998; **79**: 459-467 [PMID: 9659192 DOI: 10.1136/hrt.79.5.459]
18 **Rodriguez-Granillo GA**, García-García HM, Mc Fadden EP, Valgimigli M, Aoki J, de Feyter P, Serruys PW. In vivo intravascular ultrasound-derived thin-cap fibroatheroma detection using ultrasound radiofrequency data analysis. *J Am Coll Cardiol* 2005; **46**: 2038-2042 [PMID: 16325038 DOI: 10.1016/j.jacc.2005.07.064]
19 **Stone GW**, Maehara A, Lansky AJ, de Bruyne B, Cristea E, Mintz GS, Mehran R, McPherson J, Farhat N, Marso SP, Parise H, Templin B, White R, Zhang Z, Serruys PW. A prospective natural-history study of coronary atherosclerosis. *N Engl J Med* 2011; **364**: 226-235 [PMID: 21247313 DOI: 10.1056/NEJMoa1002358]
20 **Burke AP**, Kolodgie FD, Farb A, Weber DK, Malcom GT, Smialek J, Virmani R. Healed plaque ruptures and sudden coronary death: evidence that subclinical rupture has a role in plaque progression. *Circulation* 2001; **103**: 934-940 [PMID: 11181466 DOI: 10.1161/01.CIR.103.7.934]
21 **Kawasaki M,** Takatsu H, Noda T, Sano K, Ito Y, Hayakawa K, Tsuchiya K, Arai M, Nishigaki K, Takemura G, Minatoguchi S, Fujiwara T, Fujiwara H. In vivo quantitative tissue characterization of human coronary arterial plaques by use of integrated backscatter intravascular ultrasound and comparison with angioscopic findings. *Circulation* 2002; **105**: 2487-2492 [PMID: 12034654 DOI: 10.1161/01.CIR.0000017200.47342.10]
22 **Takano M**, Jang IK, Inami S, Yamamoto M, Murakami D, Okamatsu K, Seimiya K, Ohba T, Mizuno K. In vivo comparison of optical coherence tomography and angioscopy for the evaluation of coronary plaque characteristics. *Am J Cardiol* 2008; **101**: 471-476 [PMID: 18312760 DOI: 10.1016/j.amjcard.2007.09.106]
23 **Kubo T**, Imanishi T, Takarada S, Kuroi A, Ueno S, Yamano T, Tanimoto T, Matsuo Y, Masho T, Kitabata H, Tanaka A, Nakamura N, Mizukoshi M, Tomobuchi Y, Akasaka T. Implication of plaque color classification for assessing plaque vulnerability: a coronary angioscopy and optical coherence tomography investigation. *JACC Cardiovasc Interv* 2008; **1**: 74-80 [PMID: 19393149 DOI: 10.1016/j.jcin.2007.11.001]
24 **Yamamoto M**, Takano M, Okamatsu K, Murakami D, Inami S, Xie Y, Seimiya K, Ohba T, Seino Y, Mizuno K. Relationship between thin cap fibroatheroma identified by virtual histology and angioscopic yellow plaque in quantitative analysis with colorimetry. *Circ J* 2009; **73**: 497-502 [PMID: 19151504 DOI: 10.1253/circj.CJ-08-0762]
25 **Sakai S**, Mizuno K, Yokoyama S, Tanabe J, Shinada T, Seimiya K, Takano M, Ohba T, Tomimura M, Uemura R, Imaizumi T. Morphologic changes in infarct-related plaque after coronary stent placement: a serial angioscopy study. *J Am Coll Cardiol* 2003; **42**: 1558-1565 [PMID: 14607438 DOI: 10.1016/j.jacc.2003.06.003]
26 **Okamatsu K**, Takano M, Sakai S, Ishibashi F, Uemura R, Takano T, Mizuno K. Elevated troponin T levels and lesion characteristics in non-ST-elevation acute coronary syndromes. *Circulation* 2004; **109**: 465-470 [PMID: 14732748 DOI: 10.1161/01.CIR.0000109696.92474.92]
27 **Mizuno K**, Satomura K, Miyamoto A, Arakawa K, Shibuya T, Arai T, Kurita A, Nakamura H, Ambrose JA. Angioscopic evaluation of coronary-artery thrombi in acute coronary syndromes. *N Engl J Med* 1992; **326**: 287-291 [PMID: 1728732 DOI: 10.1056/NEJM199201303260502]
28 **Mizuno K**, Miyamoto A, Satomura K, Kurita A, Arai T, Sakurada M, Yanagida S, Nakamura H. Angioscopic coronary macromorphology in patients with acute coronary disorders. *Lancet* 1991; **337**: 809-812 [PMID: 1672912 DOI: 10.1016/0140-6736(91)92514-3]
29 **Takano M**, Inami S, Ishibashi F, Okamatsu K, Seimiya K, Ohba T, Sakai S, Mizuno K. Angioscopic follow-up study of coronary ruptured plaques in nonculprit lesions. *J Am Coll Cardiol* 2005; **45**: 652-658 [PMID: 15734606 DOI: 10.1016/j.jacc.2004.09.077]
30 **Uchida Y**, Nakamura F, Tomaru T, Morita T, Oshima T, Sasaki T, Morizuki S, Hirose J. Prediction of acute coronary syndromes by percutaneous coronary angioscopy in patients with stable angina. *Am Heart J* 1995; **130**: 195-203 [PMID: 7631596 DOI: 10.1016/0002-8703(95)90429-8]
31 **Ohtani T**, Ueda Y, Mizote I, Oyabu J, Okada K, Hirayama A, Kodama K. Number of yellow plaques detected in a coronary artery is associated with future risk of acute coronary syndrome: detection of vulnerable patients by angioscopy. *J Am Coll Cardiol* 2006; **47**: 2194-2200 [PMID: 16750684 DOI: 10.1016/j.jacc.2006.01.064]
32 **Yabushita H**, Bouma BE, Houser SL, Aretz HT, Jang IK, Schlendorf KH, Kauffman CR, Shishkov M, Kang DH, Halpern EF, Tearney GJ. Characterization of human atherosclerosis by optical coherence tomography. *Circulation* 2002; **106**: 1640-1645 [PMID: 12270856 DOI: 10.1161/01.CIR.0000029927.92825.F6]
33 **Jang IK**, Bouma BE, Kang DH, Park SJ, Park SW, Seung KB, Choi KB, Shishkov M, Schlendorf K, Pomerantsev E, Houser SL, Aretz HT, Tearney GJ. Visualization of coronary atherosclerotic plaques in patients using optical coherence tomography: comparison with intravascular ultrasound. *J Am Coll Cardiol* 2002; **39**: 604-609 [PMID: 11849858 DOI: 10.1016/S0735-1097(01)01799-5]
34 **Kubo T**, Imanishi T, Takarada S, Kuroi A, Ueno S, Yamano T, Tanimoto T, Matsuo Y, Masho T, Kitabata H, Tsuda K, Tomobuchi Y, Akasaka T. Assessment of culprit lesion morphology in acute myocardial infarction: ability of optical coherence tomography compared with intravascular ultrasound and coronary angioscopy. *J Am Coll Cardiol* 2007; **50**: 933-939 [PMID: 17765119 DOI: 10.1016/j.jacc.2007.04.082]
35 **Jang IK**, Tearney GJ, MacNeill B, Takano M, Moselewski F, Iftima N, Shishkov M, Houser S, Aretz HT, Halpern EF, Bouma BE. In vivo characterization of coronary atherosclerotic plaque by use of optical coherence tomography. *Circulation* 2005; **111**: 1551-1555 [PMID: 15781733 DOI: 10.1161/01.

CIR.0000159354.43778.69]
36 **Kume T**, Akasaka T, Kawamoto T, Watanabe N, Toyota E, Neishi Y, Sukmawan R, Sadahira Y, Yoshida K. Assessment of coronary arterial plaque by optical coherence tomography. *Am J Cardiol* 2006; **97**: 1172-1175 [PMID: 16616021 DOI: 10.1016/j.amjcard.2005.11.035]
37 **Virmani R**, Kolodgie FD, Burke AP, Farb A, Schwartz SM. Lessons from sudden coronary death: a comprehensive morphological classification scheme for atherosclerotic lesions. *Arterioscler Thromb Vasc Biol* 2000; **20**: 1262-1275 [PMID: 10807742 DOI: 10.1161/01.ATV.20.5.1262]
38 **Burke AP**, Farb A, Malcom GT, Liang YH, Smialek J, Virmani R. Coronary risk factors and plaque morphology in men with coronary disease who died suddenly. *N Engl J Med* 1997; **336**: 1276-1282 [PMID: 9113930 DOI: 10.1056/NEJM199705013361802]
39 **Malmberg K**, Yusuf S, Gerstein HC, Brown J, Zhao F, Hunt D, Piegas L, Calvin J, Keltai M, Budaj A. Impact of diabetes on long-term prognosis in patients with unstable angina and non-Q-wave myocardial infarction: results of the OASIS (Organization to Assess Strategies for Ischemic Syndromes) Registry. *Circulation* 2000; **102**: 1014-1019 [PMID: 10961966 DOI: 10.1161/01.CIR.102.9.1014]
40 **Kip KE**, Faxon DP, Detre KM, Yeh W, Kelsey SF, Currier JW. Coronary angioplasty in diabetic patients. The National Heart, Lung, and Blood Institute Percutaneous Transluminal Coronary Angioplasty Registry. *Circulation* 1996; **94**: 1818-1825 [PMID: 8873655 DOI: 10.1161/01.CIR.94.8.1818]
41 **Nasu K**, Tsuchikane E, Katoh O, Fujita H, Surmely JF, Ehara M, Kinoshita Y, Tanaka N, Matsubara T, Asakura Y, Asakura K, Terashima M, Suzuki T. Plaque characterisation by Virtual Histology intravascular ultrasound analysis in patients with type 2 diabetes. *Heart* 2008; **94**: 429-433 [PMID: 17646194 DOI: 10.1136/hrt.2007.118950]
42 **Nicholls SJ**, Tuzcu EM, Kalidindi S, Wolski K, Moon KW, Sipahi I, Schoenhagen P, Nissen SE. Effect of diabetes on progression of coronary atherosclerosis and arterial remodeling: a pooled analysis of 5 intravascular ultrasound trials. *J Am Coll Cardiol* 2008; **52**: 255-262 [PMID: 18634979 DOI: 10.1016/j.jacc.2008.03.051]
43 **Berry C**, Noble S, Grégoire JC, Ibrahim R, Levesquie S, Lavoie MA, L'Allier PL, Tardif JC. Glycaemic status influences the nature and severity of coronary artery disease. *Diabetologia* 2010; **53**: 652-658 [PMID: 20225394 DOI: 10.1007/s00125-009-1651-x]
44 **Wang Z**, Inami S, Kirinoki S, Yamamoto H, Takagi G, Aoki S, Kato K, Takano H, Asai K, Yasutake M, Takano M, Yamamoto M, Ohba T, Mizuno K. Angioscopic study of silent plaque disruption in nonischemic related coronary artery in patients with stable ischemic heart disease. *Int Heart J* 2010; **51**: 383-387 [PMID: 21173512 DOI: 10.1536/ihj.51.383]
45 **Kato K**, Yonetsu T, Kim SJ, Xing L, Lee H, McNulty I, Yeh RW, Sakhuja R, Zhang S, Uemura S, Yu B, Mizuno K, Jang IK. Comparison of nonculprit coronary plaque characteristics between patients with and without diabetes: a 3-vessel optical coherence tomography study. *JACC Cardiovasc Interv* 2012; **5**: 1150-1158 [PMID: 23174639 DOI: 10.1016/j.jcin.2012.06.019]
46 **Brownlee M**. Advanced protein glycosylation in diabetes and aging. *Annu Rev Med* 1995; **46**: 223-234 [PMID: 7598459 DOI: 10.1146/annurev.med.46.1.223]
47 **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 DOI: 10.1073/pnas.89.22.11059]
48 **Baynes JW**. Role of oxidative stress in development of complications in diabetes. *Diabetes* 1991; **40**: 405-412 [PMID: 2010041 DOI: 10.2337/diab.40.4.405]
49 **Grundy SM**. Pre-diabetes, metabolic syndrome, and cardiovascular risk. *J Am Coll Cardiol* 2012; **59**: 635-643 [PMID: 22322078 DOI: 10.1016/j.jacc.2011.08.080]
50 **Ferrannini E**, Gastaldelli A, Iozzo P. Pathophysiology of prediabetes. *Med Clin North Am* 2011; **95**: 327-339, vii-viii [PMID: 21281836 DOI: 10.1016/j.mcna.2010.11.005]
51 **Nathan DM**, Davidson MB, DeFronzo RA, Heine RJ, Henry RR, Pratley R, Zinman B. Impaired fasting glucose and impaired glucose tolerance: implications for care. *Diabetes Care* 2007; **30**: 753-759 [PMID: 17327355 DOI: 10.2337/dc07-9920]
52 **Goldstein DE**, Little RR, Lorenz RA, Malone JI, Nathan D, Peterson CM, Sacks DB. Tests of glycemia in diabetes. *Diabetes Care* 2004; **27**: 1761-1773 [PMID: 15220264 DOI: 10.2337/diacare.27.7.1761]
53 **Kataoka Y**, Yasuda S, Morii I, Otsuka Y, Kawamura A, Miyazaki S. Quantitative coronary angiographic studies of patients with angina pectoris and impaired glucose tolerance. *Diabetes Care* 2005; **28**: 2217-2222 [PMID: 16123493 DOI: 10.2337/diacare.28.9.2217]
54 **Amano T**, Matsubara T, Uetani T, Nanki M, Marui N, Kato M, Yoshida T, Arai K, Yokoi K, Ando H, Kumagai S, Ishii H, Izawa H, Hotta N, Murohara T. Abnormal glucose regulation is associated with lipid-rich coronary plaque: relationship to insulin resistance. *JACC Cardiovasc Imaging* 2008; **1**: 39-45 [PMID: 19356403 DOI: 10.1016/j.jcmg.2007.09.003]
55 **Kurihara O**, Takano M, Yamamoto M, Shirakabe A, Kimata N, Inami T, Kobayashi N, Munakata R, Murakami D, Inami S, Okamatsu K, Ohba T, Ibuki C, Hata N, Seino Y, Mizuno K. Impact of prediabetic status on coronary atherosclerosis: a multivessel angioscopic study. *Diabetes Care* 2013; **36**: 729-733 [PMID: 23223344 DOI: 10.2337/dc12-1635]
56 **Stone NJ**, Robinson JG, Lichtenstein AH, Bairey Merz CN, Blum CB, Eckel RH, Goldberg AC, Gordon D, Levy D, Lloyd-Jones DM, McBride P, Schwartz JS, Shero ST, Smith SC, Watson K, Wilson PW, Eddleman KM, Jarrett NM, LaBresh K, Nevo L, Wnek J, Anderson JL, Halperin JL, Albert NM, Bozkurt B, Brindis RG, Curtis LH, DeMets D, Hochman JS, Kovacs RJ, Ohman EM, Pressler SJ, Sellke FW, Shen WK, Smith SC, Tomaselli GF. 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *Circulation* 2014; **129**: S1-S45 [PMID: 24222016 DOI: 10.1161/01.cir.0000437738.63853.7a]
57 **Haffner SM**. Dyslipidemia management in adults with diabetes. *Diabetes Care* 2004; **27** Suppl 1: S68-S71 [PMID: 14693930 DOI: 10.2337/diacare.27.2007.S68]
58 **Takano M**, Mizuno K, Yokoyama S, Seimiya K, Ishibashi F, Okamatsu K, Uemura R. Changes in coronary plaque color and morphology by lipid-lowering therapy with atorvastatin: serial evaluation by coronary angioscopy. *J Am Coll Cardiol* 2003; **42**: 680-686 [PMID: 12932601 DOI: 10.1016/S0735-1097(03)00770-8]

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MINI REVIEWS

Literature review of type 2 diabetes mellitus among minority Muslim populations in Israel

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Abstract

This review surveys the literature published on the characteristics and implications of pre-diabetes and type 2 diabetes mellitus (T2DM) for the Arab and Bedouin populations of Israel. T2DM is a global health problem. The rapid rise in its prevalence in the Arab and Bedouin populations in Israel is responsible for their lower life expectancy compared to Israeli Jews. The increased prevalence of T2DM corresponds to increased rates of obesity in these populations. A major risk group is adult Arab women aged 55-64 years. In this group obesity reaches 70%. There are several genetic and nutritional explanations for this increase. We found high hospitalization rates for micro and macrovascular complications among diabetic patients of Arab and Bedouin origin. Despite the high prevalence of diabetes and its negative health implications, there is evidence that care and counseling relating to nutrition, physical activity and self-examination of the feet are unsatisfactory. Economic difficulties are frequently cited as the reason for inadequate medical care. Other proposed reasons include faith in traditional therapy and misconceptions about drugs and their side effects. In Israel, the quality indicators program is based on one of the world's leading information systems and deals with the management of chronic diseases such as diabetes. The program's baseline data pointed to health inequality between minority populations and the general population in several areas, including monitoring and control of diabetes. Based on these data, a pilot intervention program was planned, aimed at minority populations. This program led to a decrease in inequality and served as the basis for a broader, more comprehensive intervention that has entered the implementation stage. Interventions that were shown to be effective in other Arabic countries may serve as models for diabetes management in the Arab and Bedouin populations in Israel.

Key words: Type 2 diabetes mellitus; Pre-diabetes; Risk factors for diabetes; Muslims; Bedouins; Arabs; Ethnic differences

Core tip: Type 2 diabetes mellitus is a global health problem and its rapid rise in prevalence in the Arab and Bedouin populations in Israel is responsible for the lower life expectancy among Israeli Arabs compared to Israeli Jews. An important high-risk group is adult Arab women in the 55 to 64 year age range where obesity rates approach 70%. Our review found high hospitalization rates for micro and macrovascular complications among diabetic patients of Arab and Bedouin origin. There is evidence that care and counseling relating to nutrition, physical activity and self-examination of the feet are unsatisfactory in these populations. In Israel, data from the quality indicators program demonstrated inequality in health and served as the basis for an intervention program in minority populations to improve the monitoring and control of diabetics in these populations. Preliminary data indicated that this program has a significant potential to reduce health inequality between the Jewish and Arab populations.

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INTRODUCTION

Once considered a disease of western society, type 2 diabetes mellitus (T2DM) has now spread to every country in the world, with Asia accounting for 60% of the world's diabetic population[1]. Obesity and T2DM have become a central medical problem among immigrants and minorities[2].

There are three patterns of increase in diabetes prevalence: gradual, rapid and accelerated. The prevalence rates today are 4%-9% in Europeans and reach 14%-20% among Asian immigrants to India, Arabs, Chinese, individuals of African descent and Hispanics. Particularly high rates of diabetes, up to about 50%, are found in native populations in the United States, Canada, Australia and the Pacific region. Explanations for the increasing prevalence of diabetes in Europe include changes in lifestyle and obesity[3]. Several hypotheses have been proposed over recent years to explain the rapid and accelerated rise in diabetes among developing nations. One of the explanations, known as the "thrifty phenotype" or "fetal origins of disease", assumes that malnutrition during pregnancy and infancy can lead to a process of adaptation and more "efficient" metabolic production that facilitates the anabolic processing of energy sources when the individual has an unrestricted intake of calories later on[2,4]. Conversely, the "drifty genotype" hypothesis contends that the prevalence of thrifty genes is attributable to a genetic drift resulting from the disappearance of predative selection pressures[5].

SETTING

According the Central Bureau of Statistics, the State of Israel had a population of 8102000 in March 2013, with close to 75% Jews, about 21% Arabs and about 4% others[6].

Bedouins are one of the ethnic groups in Israeli society. They comprise 3.5% of the total population with about 280000 individuals, most living in the Negev region in southern Israel. They are Semitic tribes that originated in the Arabian Peninsula from where they began to disperse to the north to find areas worthy of grazing and living. They are Muslim Arabs that live in accordance with the unique customs of their society[7].

The Bedouins in the Negev are at the bottom of the socioeconomic scale in Israel[8]. Phenomena such as polygamy, in-marriage (marriage within one's own tribe or group as required by custom or law) and high birth rates (in 2009 the average Bedouin family numbered 6.8 children) are common in Bedouin society[7].

These characteristics, intermingling with elements of modern life, have brought about a condition in which a significant proportion of their society is in a phase of "population in transition".

Today, about 61% of the Negev Bedouins live in permanent towns and 39% live in unauthorized villages. There is a large difference in living conditions between these two groups. The latter live in huts or tents without official supplies of water or electricity. Houses are heated in the winter primarily by burning wood over open fires. Cooking is done on gas stoves or open fires. The sanitation level is very low with no central sewerage or garbage removal. These conditions affect morbidity, adherence to treatment and access to healthcare services[9].

Israel has a national health insurance law. In accordance with this law, the population receives medical care through non-profit medical organizations. The work principle of these medical organizations is based on the patient-oriented model based on primary care in the community by a team of doctors and nurses with specialist consultants, if required[10]. Although the Arab and Bedouin populations of Israel live in the same geographical area as the Jews and have at their disposal the same broad basket of healthcare services, they are separate ethnic groups embracing a different lifestyle, nutritional habits and environmental exposures. Furthermore, in recent years, the Arab population of Israel has experienced a rapid change towards a westernized lifestyle.

We surveyed the literature on pre-diabetic states and T2DM and their consequences among the Arab and Bedouin populations of Israel. The search was conducted in the PubMed database using the search terms "nutrition and obesity", "diabetes" and "Arabs and Bedouins in Israel". Thirty six relevant articles were found, 30 in English and six in Hebrew.

ISRAELI ARABS

Genetic background, obesity and pre-diabetes in the Arab population of Israel

While the life expectancy of Israeli Arabs was lower

than Israeli Jews from 1975-2004, the gap decreased between 1975 and 1998. However, since 1998 the gap has increased again and the difference in 2004 was 3.2 years more for Israeli Jewish men and 4 years more for Israeli Jewish women. The main causes of death that lead to the gap in life expectancy are chronic diseases, especially ischemic heart disease and diabetes[11]. The Arab community of Israel is characterized by a high rate of consanguinity. One study investigated the effect of consanguinity on multifactorial common adult morbidity, including T2DM. There was no significant difference in T2DM between patients with consanguinity and those without[12]. Another study that investigated the existence of a direct genetic association that affects the development of diabetes demonstrated that distinct genetic backgrounds are responsible for the development of beta-cell dysfunction and insulin resistance among Arabs[13]. Obesity comprises a central element in the development of T2DM and the risk of diabetes increases substantially with increased body mass index (BMI)[14]. A study from central Israel showed that the mean BMI of 18-year-old Jews and Arabs is similar. This finding changes with age so that 52% of Arab women are classified as obese compared with 31% of Jewish women and 25% of Arab men compared to 23% of Jewish men. A central group pointed to in this study was Arab women aged 55-64 years where the rate of obesity reaches 70%[16]. A study of randomly recruited healthy, overweight Arabs (BMI > 27) attending a primary healthcare clinic in Israel revealed that 27% of them had undiagnosed T2DM, 42% had impaired glucose tolerance (IGT), and only 31% had a normal OGCT. The metabolic syndrome was diagnosed in 48%[17]. There is evidence from various populations that IGT and impaired fasting glucose (IFG) often are associated with different groups of patients[18]. The study from Israel assessed insulin resistance and impaired pancreatic function among overweight Arab patients with IFG only, IGT only or IFG and IGT (combined glucose intolerance-CGT) compared to those with a normal response to glucose (NGT). Patients with IFG and CGT were more obese and had higher values of insulin resistance compared to those with IGT only or normal fasting glucose. There was no statistically significant difference in insulin resistance between patients with IGT only and those with NGT. Beta-cell function was depressed in patients with IGT only and CGT compared to those with IFG and NGT, while beta-cell function indices in patients with IFG were similar to those with NGT[19].

DIABETES MELLITUS IN ISRAELI ARABS

Studies show that in recent decades the incidence rate of diabetes in the Israeli Arab population has increased by 9.1 per 1000 persons annually[20]. A study of the urban Jewish and Arabs population from the central area of Israel found that the prevalence rates for adult-onset diabetes were 21% among Arabs and 12% among Jews. The Arabs presented with diabetes at a younger age than the Jews and 25% of the Arab population was diagnosed with diabetes by age 57 compared to age 68 in the Jewish population[21]. An alarmingly high prevalence of diabetes was found in Israeli Arab women over 50 years, reaching 50%[22]. Another study found that the prevalence of diabetes among women younger than 65 years old was significantly higher compared to men. The mean age of diabetic women was 48.3 compared to 59.5 among men and women had a higher BMI (34.5 *vs* 30.04, respectively) at diagnosis. The age of diagnosis of diabetes correlated significantly with BMI[14]. Despite the high prevalence of obesity, metabolic syndrome and overt diabetes among Arabs, there is evidence of inadequate care for diabetes in this population. More than a third of respondents reported that they did not receive any counseling on issues such as foot care or the effects of smoking on diabetes. Misconceptions, attributable to social norms, are common and more than a third forgo taking medications because they cannot afford them[23]. Arab diabetics received less nutritional counseling (OR = 0.46), less counseling on physical activity (OR = 0.42) and less advice on self-testing of the feet than Jewish patients (OR = 0.55)[24]. There is poor diabetes control and sub-optimal follow-up care among Arab patients with diabetes[25].

The results of studies on the prevalence of obesity, pre-diabetes and diabetes type 2 in the Arab Israeli population are summarized in Table 1.

DIABETES AS A RISK FACTOR IN ISRAELI ARABS

In a five country observational study that determined the incidence of hypoglycemia during the holiday of Ramadan among Muslim subjects with T2DM treated with a sulphonylurea, the highest incidence of hypoglycemia was reported by patients from Israel (40%)[26].

In a recent study among patients in hospitals that had predominantly Arab patients (more than 90%), the proportion of diabetics was 39%. There was a female preponderance among patients admitted with diabetes (52.9%), while only 45% of hospitalized patients without diabetes were women ($P = 0.0003$).

A difference was found in the reasons for hospitalization between patients with diabetes and those without. In the diabetic group, there were more hospitalizations (37% *vs* 27% respectively, $P < 0.001$) and urinary tract infections (7.7% *vs* 6.9%, respectively). The authors recommended that the prevention of cardiovascular disease and urinary tract infections among the diabetic population should be a priority, especially for Arab women over 40 who have a high risk for morbidity and a high rate of hospitalizations[27].

A study that evaluated risk factors among Arab and Jewish patients who underwent rehabilitation for a first stroke revealed that a high percentage of Arab patients have hypertension and T2DM. The prevalence of diabetes

Table 1 Results of studies on the prevalence of obesity, pre-diabetes and diabetes type 2 in the Arab Israeli population

Ref.	Date	Subjects	Study results
Keinan-Boker *et al*[16]	2005	Representative sample of 3246 individuals from the general Israeli population	In the subgroup of older Arab women, aged 55-64 yr, obesity reached 70%
Abdul-Ghani *et al*[17]	2005	95 randomly recruited Arab subjects who were overweight and over the age of 40	27% had undiagnosed DM, 42% impaired fasting glucose or impaired glucose tolerance, 48% metabolic syndrome
Abdul-Ghani *et al*[14]	2005	7434 patients from an outpatient clinic in an Arab village	The prevalence of diabetes type 2 in Arab patients younger than the age of 65 was significantly higher among women than men. Diabetic women were younger than men at diagnosis (48 yr *vs* 59 yr) and had a higher BMI
Kalter-Leibovici *et al*[15]	2007	880 randomly selected Arab and Jewish patients	The prevalence of obesity was 52% in Arab women compared to 31% in Jewish women and 25% in Arab men compared to 23% in Jewish men
Idilbi *et al*[20]	2012	Review of official health statistics	The incidence rate of diabetes in the Israeli Arab population increased by 9.1 per 1000 persons annually. In contrast, it decreased among Jews
Kalter-Leibovici *et al*[21]	2012	1100 Arab and Jewish patients older than the age of 20	The prevalence of diabetes was 21% among Arabs and 12% among Jews. Arabs developed diabetes 11 years earlier than Jews

DM: Diabetes mellitus.

among Arabs was 51.4%, among non-immigrant Jews 38.5%, and among immigrant Jews 39.1% ($P < 0.001$)[28]. In another study that evaluated ethnic disparities between patients with a first episode of primary intracerebral hemorrhage in northern Israel, the Arabs were found to be younger and to have a higher prevalence of diabetes[29]. A national survey among 28 hospitals in Israel that assessed ethnic variations in acute ischemic stroke showed that the mean age of Arab patients was nine years younger than Jewish patients (63 ± 11 years *vs* 72 ± 12 years, respectively), Arabs were more likely to be obese (OR = 1.72) and to have diabetes (OR = 1.41)[30]. A higher prevalence of diabetes among Arabs than Jews was also found in a study that compared ethnic differences in ischemic stroke in patients of working age (≤ 65 years)[31].

In two studies that examined risk factors in hospitalized Arab and Jewish women with coronary heart disease who underwent cardiac catheterization, a higher prevalence of diabetes was found among the Arab women[32,33].

These differences should be addressed when developing stroke and coronary artery disease preventative strategies, planning healthcare services and designing culturally relevant public education programs.

The results of studies on diabetes as a risk factor among Israeli Arabs appear in Table 2.

NUTRITION AND OBESITY AMONG BEDOUINS IN THE NEGEV

Similar to other Arab Israeli populations, the prevalence of obesity was also higher among Bedouins compared to Jews (27.9% *vs* 20%, respectively)[34]. A study of Bedouin women of childbearing age found a high prevalence of obesity associated with nutritional deficits[35]. In order to investigate possible dietary causes for the discrepancy in obesity rates between adult Jews and Bedouins, researchers from southern Israel compared eating patterns in the two populations. Bedouin men and women reported a lower intake of fat and protein and a higher intake of carbohydrates than Jews[34]. Another study demonstrated that the nutrition of Bedouin women who lead a semi-traditional lifestyle had a caloric value that was 50% higher than that of Jewish women. The mean BMI of the Bedouin women was 30[36]. To evaluate the importance of modern food and drink in their daily diet, the nutrition of Bedouin women living in permanent towns and non-permanent settlements was compared. Residents in non-permanent settlements, where there are no means to preserve food, ate more traditional dairy products while those in permanent towns ate more meat. Both population groups based their two main meals on traditional food, but processed foods and drink were consumed as snacks. These processed products are calorie-rich and can be a factor in the rising rate of diabetes[37].

T2DM AMONG BEDOUINS IN THE NEGEV

An epidemiological survey conducted among Bedouins about half a century ago reported that only a few patients had hypertension and diabetes and none had ischemic heart disease[38,39]. Later, evidence accumulated that cardiovascular risk factors among the Bedouins were on the rise and that this increase was more pronounced among Bedouin living in settled settings compared to the traditional tribal groups. A study performed in 1990 demonstrated that among Bedouins who lived in permanent towns, 15% were obese and 23% were overweight compared to Bedouins who did not live in permanent towns, where there were no obese individuals and 23% were overweight. This difference was particularly apparent in the younger age group. No difference was found between the groups regarding fasting blood glucose[40]. A study from 2005 found a difference in diabetes prevalence in urban compared to rural settlements (5.5% *vs* 3.9%, respectively, $P < 0.001$). In this study, diabetes control was less successful among Bedouin diabetes patients. Only 29.3% had their diabetes under control compared to 46.7% among non-Bedouin

Table 2 Results of studies on diabetes as a risk factor among Israeli Arabs and Bedouins

Ref.	Date	Subjects	Study results
Jabara *et al*[32]	2007	546 women (102 Arabs) after cardiac catheterization	Arab women had a higher prevalence rate for diabetes (61% *vs* 46% in Jews)
Salameh *et al*[33]	2008	40 Arab and 179 Jewish women hospitalized with coronary artery disease	More Arab patients had diabetes (73% *vs* 40%)
Telman *et al*[31]	2010	727 Arab and Jewish patients of working age (< 65 yr) with stroke	There was a higher prevalence of diabetes in the Arab patients
Telman *et al*[29]	2010	546 patients with a first episode of primary intracerebral hemorrhage	Diabetes was more frequent among the Arab patients.
Aravind *et al*[26]	2011	1378 Muslim patients from five countries who were treated with sulfonylurea during Ramadan	The highest percentage of hypoglycemia (40%) was reported in patients from Israel
Greenberg *et al*[28]	2011	2000 patients with a first stroke (237 Arabs)	A high percentage of Arabs had diabetes (51.4% *vs* 35.8% in Jews)
Gross *et al*[30]	2011	1540 patients with acute ischemic stroke, 169 Arabs	Arab patients were more likely to have diabetes (OR 1.41)
Chorny *et al*[46]	2011	523 diabetic patients (Jews and Bedouins) who were examined by an ophthalmologist	The prevalence of maculopathy and retinopathy was higher among the Bedouins (22% *vs* 13.4%)
Nseir *et al*[27]	2013	3784 patients from hospitals with predominantly Arab patients	39% of the hospitalized patients were diabetics. The diabetics had more hospitalizations due to atherosclerotic disease
Rabaev *et al*[45]	2014	220 patients admitted with diabetic ketoacidosis (19% Bedouins)	There was no difference in outcomes (in-hospital mortality, 30-d mortality) between Jews and Bedouins

diabetes patients[41]. A study at the largest urban Bedouin outpatient clinic in 2002 revealed that the prevalence of diabetes was 7.3% among men and 9.9% among women. Women had significantly higher BMI levels than men but lower levels of HbA1c and microalbuminuria[42]. Prescribed oral medicines were purchased by 69% of the women compared to 76% of the men. Insulin was purchased by 19% of the women compared to 15% of the men[42]. The study from 2007 showed an age-adjusted prevalence rate for diabetes of 12% in the Bedouin urban population compared to 8% among Jews. The prevalence rate was especially notable among Bedouins in the 40-49 year age group where it was three times higher than in the Jewish population of the same age. The adherence rate to diabetes treatment was 27% among the Bedouins compared to 42% in the Jewish population. The Bedouin population was also less compliant with follow-up blood tests: 22% of the Bedouin patients had no HbA1C measurements over the course of the previous year, compared to 13% of the Jews. The rates of controlled diabetic patients were lower among the Bedouins than the Jews (29.5% *vs* 57%, respectively)[43]. The results of studies on the prevalence of obesity and diabetes type 2 among Bedouins in the Negev are summarized in Table 3.

A recent study evaluated the reasons for non-treatment of cardiovascular disease and its risk factors in the Bedouin population. Structured interviews on knowledge and attitudes relating to chronic diseases and their treatment were conducted among patients with T2DM, hypertension and lipid metabolic disorders. Ninety-nine high and 101 low-adherent patients were interviewed. More patients in the low-adherence group believed that traditional folk treatment was an alternative to prescription drugs for the treatment of T2DM, hypertension and hyperlipidemia and 10% took traditional drugs only. Patients in the group that was classified as undertreated believed that adverse drug effects were more harmful than the disease itself (65% *vs* 47%, respectively) and this was also the reason for the cessation of treatment among 47% who were classified as low-adherent[44].

In a retrospective analysis of the clinical characteristics and outcomes of diabetic ketoacidosis in the Jewish and Bedouin populations that included patients with both type 1 and type 2 diabetes, no differences were found for in-hospital mortality, 30 d mortality or complication rates in Jewish and Bedouin patients[45]. Damage to the eye as a result of microvascular injury is a common complication in diabetes patients. Among diabetic patients referred to ophthalmologists in southern Israel, significantly more diabetic complications (damage to the retina and the macula) were found among the Bedouins than among the Jews (22% *vs* 13.4%, respectively), although the Bedouin patients were younger than the Jews (average age 58.6 ± 12 years *vs* 64 ± 10.3 years). The predicting factors for diabetic eye complications among Bedouins were the duration of the diabetes, high levels of HbA1c, insulin treatment and smoking[46]. The results of studies on diabetes as a risk factor among Israeli Arabs appear in Table 2.

THE PROGRAM FOR HEALTH QUALITY INDICATORS AND ITS EFFECT ON THE CONTROL OF DIABETES IN MINORITY POPULATIONS

Health quality indicators were introduced at the inception of the process of departmentalization of clinics and was part of the process of assessment of the clinics. The first indicator that was chosen in this process was the rate of influenza inoculation in the target population. Over the years the number of indicators increased so that today there are 70 indicators in 11 primary areas. A significant

Table 3 Results of studies on the prevalence of obesity and diabetes type 2 among Bedouins in the Negev

Ref.	Date	Subjects	Study results
Ben Assa[38]	1961	2000 examined Bedouins	10 diabetic patients
Fraser *et al*[40]	1990	Tribal and settled Bedouin males	Among settled Bedouins, 15% were obese and 35% were overweight. Among tribal Bedouins, none were obese and 23% were overweight
Abou-Rbiah *et al*[42]	2002	3115 patients from an urban Bedouin clinic	The prevalence of diabetes was 7.3% in males and 9.9% in females. The mean BMI was 30 in females and 29 in males
Cohen *et al*[41]	2005	Population of the Negev area	The prevalence of diabetes was 5.1% in Bedouins, 3.7% in non-Bedouins, 5.5% in urban Bedouins, 3.9% in rural Bedouins. Diabetes was well controlled in 29.3% of the Bedouins and 46.7% of the non-Bedouins
Tamir *et al*[43]	2007	28449 Bedouins, 14012 Jews, older than 20	The prevalence of diabetes was 12% in the Bedouins compared to 8% in the Jews) The non-compliance rate for treatment was 72.9% among diabetic Bedouins
Fraser *et al*[34]	2008	793 Jews and 169 Bedouins aged 35-64 yr	The obesity rate was 27.9% among Bedouins and 20% among Jews
Leshem *et al*[36]	2008	31 encampment Bedouin women	The mean BMI was 30.3
Abu-Saad *et al*[35]	2012	683 pregnant Bedouin women	42% were either overweight or obese (based on their pre-pregnancy BMI)

BMI: Body mass index.

proportion of these indicators relates to the implementation of preventive medicine and monitoring and control of patients with diabetes. The program's data pointed to a serious disparity in the monitoring and control of diabetes in the Arab sector as well as other Israeli sub-populations, including Ethiopian Jews, compared to the general population[47,48]. In 2008, the Clalit Health Services, which serve 70% of the Israeli population, reached an organizational decision that the reduction of these disparities was a strategic goal, so a dedicated intervention program to address them was designed. Between 2008-2010, a "pilot" program was conducted aimed at 55 clinics serving 400000 clients from "difficult" populations, including Arab and Bedouin communities in the Negev. The program focused on seven quality indicators, including the monitoring and control of diabetes. The strategy included a concerted effort aimed at providing medical solutions to loci of inequality in health quality indicators. Language facilitators were introduced into the clinics and efforts were made to incorporate religious leaders into the program, including lectures by the local Kadi on the religious importance of maintaining a healthy body, the reading of prayers on the importance of preventive medicine and physical activity in mosques on Fridays, and discussions with the village sheikh to grant permission and consent to women to carry out physical activity in the form of walks. As a result of this intervention in key clinics, a reduction of 67% in health quality disparity (including measurements related to monitoring and control of diabetes) were achieved within less than two years. There was an increased risk at baseline of 8% in the key clinics in emergency room visits and hospitalizations, which was reduced to that of other population sectors as a result of the intervention. In light of the success of the pilot intervention, in a relatively short period of time a separate program was developed that was broader and more comprehensive. This program was designed to bring about a reduction in inequality among socioeconomic levels and different sectors of the population. This program is now in the implementation stage[48] and its results have not been reported to date.

CONCLUSION

T2DM is a global health problem and the rapid rise in its prevalence in Arab and Bedouin populations in Israel is a cause of the difference in life expectancy between Jews and Arabs. The increased prevalence of T2DM corresponds with increased obesity rates in these populations. The primary at-risk group is Arab women aged 55 to 64 years who have an obesity rate that approaches 70%. There are several genetic and nutritional explanations for the increase. In this review, we found evidence for high rates of hospitalizations and micro and macrovascular complications among diabetic patients of Arab and Bedouin origin. Despite the high prevalence of diabetes and its negative health implications, there is evidence of a lack of appropriate care and counseling about nutrition, physical activity and self-examination of feet. Financial difficulties are frequently cited as reasons for inadequate healthcare and belief in traditional therapy and misconceptions about drugs and their side effects are also significant factors.

Although these overall data are troubling, recent findings are more encouraging.

A quality indicators program in the community has been in existence in Israel for the last 15 years. It is based on some of the world's leading information systems with data regarding sociodemographic factors, drug therapy, healthcare services, laboratory and imaging data, and recording of chronic diseases. It consists of several domains, including preventive medicine and management of chronic diseases. The program has led to an improvement in the quality of medical care, including diabetes control.

Since the program's data indicated inequality in different health quality indicators, including indicators relating to the monitoring and control of diabetes,

between the general population and several population sectors including minorities, a pilot intervention program was conducted to reduce these inequalities in selected clinics from 2008-2010.

This intervention program has contributed to a narrowing of health-related gaps and has reduced inequalities between the Arab and Jewish populations as well as between socioeconomic levels. The program demonstrated that the healthcare system is capable of reducing health inequalities, even if they are the result of variables for which they are not directly responsible, such as disparities in income, educational level, culture differences and isolated residential areas. It appears that an evidence-based, dedicated intervention is the key to success. In the wake of this success, a broader intervention was planned and has now entered into the implementation phase. The results of this program which involves the entire minority population have not been reported yet.

Interventions that are based on empowerment for medical care, cultural elements presented in Arabic terms and concepts, nutritional habits and lifestyle were shown to be effective in other Arabic countries[49] and can serve as models for diabetes management in the Arab and Bedouin populations in Israel.

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REFERENCES

1 **Hu FB**. Globalization of diabetes: the role of diet, lifestyle, and genes. *Diabetes Care* 2011; **34**: 1249-1257 [PMID: 21617109 DOI: 10.2337/dc11-0442]
2 **Candib LM**. Obesity and diabetes in vulnerable populations: reflection on proximal and distal causes. *Ann Fam Med* 2007; **5**: 547-556 [PMID: 18025493 DOI: 10.1370/afm.754]
3 **Bhattarai MD**. Three patterns of rising type 2 diabetes prevalence in the world: need to widen the concept of prevention in individuals into control in the community. *JNMA J Nepal Med Assoc* 2009; **48**: 173-179 [PMID: 20387365]
4 **Vaag AA**, Grunnet LG, Arora GP, Brøns C. The thrifty phenotype hypothesis revisited. *Diabetologia* 2012; **55**: 2085-2088 [PMID: 22643933 DOI: 10.1007/s00125-012-2589-y]
5 **Sellayah D**, Cagampang FR, Cox RD. On the evolutionary origins of obesity: a new hypothesis. *Endocrinology* 2014; **155**: 1573-1588 [PMID: 24605831 DOI: 10.1210/en.2013-2103]
6 **Treister-Goltzman Y**, Peleg R. Health and morbidity among Bedouin women in southern Israel: a descriptive literature review of the past two decades. *J Community Health* 2014; **39**: 819-825 [PMID: 24492991 DOI: 10.1007/s10900-014-9832-z]
7 **Ben-Rabi D**, Amiel S, Nijim F, Dolev T. Bedouin Children in the Negev: Characteristics, Needs and Patterns of Service Use. Myers-JDC-Brookdale Institute, 2009 Available from: URL: http: //www.bjpa.org/Publications/details.cfm?PublicationID=13488
8 **Bar-Yakov M,** Cohen A. New Statistical Activities and Publications in Israel: Population and Demographics. Jerusalem, Israel: Israel Central Bureau of Statistics, 2010 Report No. 153. Available from: URL: http: //www.cbs.gov.il/q153_eng.htm
9 **Treister-Goltzman Y**, Peleg R. What is Known About Health and Morbidity in the Pediatric Population of Muslim Bedouins in Southern Israel: A Descriptive Review of the Literature from the Past Two Decades. *J Immigr Minor Health* 2014 [PMID: 24585250 DOI: 10.1007/s10903-014-0001-3]
10 **Peleg R**, Press Y, Asher M, Pugachev T, Glicensztain H, Lederman M, Biderman A. An intervention program to reduce the number of hospitalizations of elderly patients in a primary care clinic. *BMC Health Serv Res* 2008; **8**: 36 [PMID: 18254972 DOI: 10.1186/1472-6963-8-36]
11 **Na'amnih W**, Muhsen K, Tarabeia J, Saabneh A, Green MS. Trends in the gap in life expectancy between Arabs and Jews in Israel between 1975 and 2004. *Int J Epidemiol* 2010; **39**: 1324-1332 [PMID: 20534651 DOI: 10.1093/ije/dyq073]
12 **Jaber L**, Shohat T, Rotter JI, Shohat M. Consanguinity and common adult diseases in Israeli Arab communities. *Am J Med Genet* 1997; **70**: 346-348 [PMID: 9182771]
13 **Shalata A**, Jazmawi W, Aslan O, Badarni K, Dabbah K, Sawaed S, Cohen Castel O, Borochowitz ZU, Karkabi K, Defronzo R, Abdul-Ghani M. Early metabolic defects in Arab subjects with strong family history of Type 2 diabetes. *J Endocrinol Invest* 2013; **36**: 417-421 [PMID: 23211319 DOI: 10.3275/8762]
14 **Abdul-Ghani MA**, Kher J, Abbas N, Najami T. Association of high body mass index with low age of disease onset among Arab women with type 2 diabetes in a primary care clinic. *Isr Med Assoc J* 2005; **7**: 360-363 [PMID: 15984376]
15 **Kalter-Leibovici O**, Atamna A, Lubin F, Alpert G, Keren MG, Murad H, Chetrit A, Goffer D, Eilat-Adar S, Goldbourt U. Obesity among Arabs and Jews in Israel: a population-based study. *Isr Med Assoc J* 2007; **9**: 525-530 [PMID: 17710784]
16 **Keinan-Boker L**, Noyman N, Chinich A, Green MS, Nitzan-Kaluski D. Overweight and obesity prevalence in Israel: findings of the first national health and nutrition survey (MABAT). *Isr Med Assoc J* 2005; **7**: 219-223 [PMID: 15847200]
17 **Abdul-Ghani MA**, Sabbah M, Muati B, Dakwar N, Kashkosh H, Minuchin O, Vardi P, Raz I. High frequency of pre-diabetes, undiagnosed diabetes and metabolic syndrome among overweight Arabs in Israel. *Isr Med Assoc J* 2005; **7**: 143-147 [PMID: 15792256]
18 **Bardini G**, Barbaro V, Romano D, Rotella CM, Giannini S. Different distribution of phenotypes and glucose tolerance categories associated with two alternative proposed cutoffs of insulin resistance. *Acta Diabetol* 2014; **51**: 321-324 [PMID: 23797705 DOI: 10.1007/s00592-013-0495-5]
19 **Abdul-Ghani MA**, Sabbah M, Kher J, Minuchin O, Vardi P, Raz I. Different contributions of insulin resistance and beta-cell dysfunction in overweight Israeli Arabs with IFG and IGT. *Diabetes Metab Res Rev* 2006; **22**: 126-130 [PMID: 16187399 DOI: 10.1002/dmrr.595]
20 **Idilbi NM**, Barhana M, Milman U, Carel RS. [Diabetes mellitus and cancer: the different expression of these diseases in Israeli Arabs and Jews]. *Harefuah* 2012; **151**: 625-628, 654 [PMID: 23367733]
21 **Kalter-Leibovici O**, Chetrit A, Lubin F, Atamna A, Alpert G, Ziv A, Abu-Saad K, Murad H, Eilat-Adar S, Goldbourt U. Adult-onset diabetes among Arabs and Jews in Israel: a population-based study. *Diabet Med* 2012; **29**: 748-754 [PMID: 22050554 DOI: 10.1111/j.1464-5491.2011.03516.x]
22 **Kalter-Leibovici O**, Younis-Zeidan N, Atamna A, Lubin F, Alpert G, Chetrit A, Novikov I, Daoud N, Freedman LS. Lifestyle intervention in obese Arab women: a randomized controlled trial. *Arch Intern Med* 2010; **170**: 970-976 [PMID: 20548010 DOI: 10.1001/archinternmed.2010.103]
23 **Khatib M**, Efrat S, Deeb D. Knowledge, beliefs, and economic barriers to healthcare: a survey of diabetic patients in an Arab-Israeli town. *J Ambul Care Manage* 2007; **30**: 79-85 [PMID: 17170641]
24 **Tirosh A**, Calderon-Margalit R, Mazar M, Stern Z. Differences

in quality of diabetes care between Jews and Arabs in Jerusalem. *Am J Med Qual* 2008; **23**: 60-65 [PMID: 18187592 DOI: 10.1177/1062860607307998]
25 **Wilf-Miron R**, Peled R, Yaari E, Shem-Tov O, Weinner VA, Porath A, Kokia E. Disparities in diabetes care: role of the patient's socio-demographic characteristics. *BMC Public Health* 2010; **10**: 729 [PMID: 21108780 DOI: 10.1186/1471-2458-10-729]
26 **Aravind SR**, Al Tayeb K, Ismail SB, Shehadeh N, Kaddaha G, Liu R, Balshaw R, Lesnikova N, Heisel O, Girman CJ, Musser BJ, Davies MJ, Katzeff HL, Engel SS, Radican L. Hypoglycaemia in sulphonylurea-treated subjects with type 2 diabetes undergoing Ramadan fasting: a five-country observational study. *Curr Med Res Opin* 2011; **27**: 1237-1242 [PMID: 21506631 DOI: 10.1185/03007995.2011.578245]
27 **Nseir W**, Haj S, Beshara B, Mograbi J, Cohen O. Seeking out high risk population: the prevalence characteristics and outcome of diabetic patients of arab ethnicity hospitalized in internal medical and acute coronary units in Israel. *Int J Endocrinol* 2013; **2013**: 371608 [PMID: 23861680 DOI: 10.1155/2013/371608]
28 **Greenberg E**, Treger I, Schwarz J. Age, gender and risk factor disparities in first-stroke Jewish and Arab patients in Israel undergoing rehabilitation. *Isr Med Assoc J* 2011; **13**: 680-683 [PMID: 22279702]
29 **Telman G**, Hlebtovsky A, Sprecher E, Zaaroor M, Kouperberg E. Ethnic disparities in first primary intracerebral hemorrhage in northern Israel. *Neuroepidemiology* 2010; **34**: 208-213 [PMID: 20197704 DOI: 10.1159/000289352]
30 **Gross B**, Feldman-Idov Y, Molshatzki N, Azrilin O, Goldbourt U, Bornstein NM, Tanne D. Ethnic variations in acute ischemic stroke: findings from the National Acute Stroke Israeli Survey (NASIS). *Cerebrovasc Dis* 2011; **31**: 506-510 [PMID: 21411992 DOI: 10.1159/000324527]
31 **Telman G**, Kouperberg E, Sprecher E, Yarnitsky D. Ethnic differences in ischemic stroke of working age in northern Israel. *J Stroke Cerebrovasc Dis* 2010; **19**: 376-381 [PMID: 20472467 DOI: 10.1016/j.jstrokecerebrovasdis.2009.06.004]
32 **Jabara R**, Namouz S, Kark JD, Lotan C. Risk characteristics of Arab and Jewish women with coronary heart disease in Jerusalem. *Isr Med Assoc J* 2007; **9**: 316-320 [PMID: 17491229]
33 **Salameh S**, Hochner-Celnikier D, Chajek-Shaul T, Manor O, Bursztyn M. Ethnic gap in coronary artery disease: comparison of the extent, severity, and risk factors in Arab and Jewish middle-aged women. *J Cardiometab Syndr* 2008; **3**: 26-29 [PMID: 18326974]
34 **Fraser D**, Bilenko N, Vardy H, Abu-Saad K, Shai I, Abu-Shareb H, Shahar DR. Differences in food intake and disparity in obesity rates between adult Jews and Bedouins in southern Israel. *Ethn Dis* 2008; **18**: 13-18 [PMID: 18447093]
35 **Abu-Saad K**, Shahar DR, Fraser D, Vardi H, Friger M, Bolotin A, Freedman LS. Adequacy of usual dietary intake and nutritional status among pregnant women in the context of nutrition transition: the DEPOSIT Study. *Br J Nutr* 2012; **108**: 1874-1883 [PMID: 22264559 DOI: 10.1017/S000711451100729X]
36 **Leshem M**, Saadi A, Alem N, Hendi K. Enhanced salt appetite, diet and drinking in traditional Bedouin women in the Negev. *Appetite* 2008; **50**: 71-82 [PMID: 17606311 DOI: 10.1016/j.appet.2007.05.010]
37 **Fraser D**, Abu-Saad K, Abu-Shareb H. The relative importance of traditional and "modern" foods for Israeli Negev Bedouins. A population in transition. *Nutr Metab Cardiovasc Dis* 2001; **11**: 66-69 [PMID: 11894757]
38 **Ben Assa S**. Observations on 2000 Bedouin patients. *Harefuah* 1961; **67:** 450-453
39 **Ben Assa S.** The medical work among the Bedouin in the Negev. *Harefuah* 1961; **67:** 211-212
40 **Fraser D**, Weitzman S, Blondheim S, Shany S, Abou-Rbiah Y. The prevalence of cardiovascular risk factors among male Bedouins: a population in transition. *Eur J Epidemiol* 1990; **6**: 273-278 [PMID: 2253732]
41 **Cohen AD**, Gefen K, Ozer A, Bagola N, Milrad V, Cohen L, Abu-Hammad T, Abu-Rabia Y, Hazanov I, Vardy DA. Diabetes control in the Bedouin population in southern Israel. *Med Sci Monit* 2005; **11**: CR376-CR380 [PMID: 16049379]
42 **Abou-Rbiah Y**, Weitzman S. Diabetes among Bedouins in the Negev: the transition from a rare to a highly prevalent condition. *Isr Med Assoc J* 2002; **4**: 687-689 [PMID: 12440231]
43 **Tamir O**, Peleg R, Dreiher J, Abu-Hammad T, Rabia YA, Rashid MA, Eisenberg A, Sibersky D, Kazanovich A, Khalil E, Vardy D, Shvartzman P. Cardiovascular risk factors in the Bedouin population: management and compliance. *Isr Med Assoc J* 2007; **9**: 652-655 [PMID: 17939626]
44 **Yoel U**, Abu-Hammad T, Cohen A, Aizenberg A, Vardy D, Shvartzman P. Behind the scenes of adherence in a minority population. *Isr Med Assoc J* 2013; **15**: 17-22 [PMID: 23484233]
45 **Rabaev E**, Sagy I, Zaid EA, Nevzorov R, Harman-Boehm I, Zeller L, Barski L. [Differences in clinical characteristics and outcomes of diabetic ketoacidosis (DKA) in Jewish and Bedouin patients]. *Harefuah* 2014; **153**: 134-138, 241 [PMID: 24791549]
46 **Chorny A**, Lifshits T, Kratz A, Levy J, Golfarb D, Zlotnik A, Knyazer B. [Prevalence and risk factors for diabetic retinopathy in type 2 diabetes patients in Jewish and Bedouin populations in southern Israel]. *Harefuah* 2011; **150**: 906-910, 935 [PMID: 22352283]
47 **Cohen AD**, Dreiher J, Regev-Rosenberg S, Yakovson O, Lieberman N, Goldfracht M, Balicer RD. [The quality indigators program in Clalit Health Services: the first decade]. *Harefuah* 2010; **149**: 204-209, 265 [PMID: 20812490]
48 **Balicer RD**, editor. Dealing of Clalit Health Services with inequalities in health]. [Hebrew]. Israel: Proceeding of the conference: Dealing with inequalities in health, 2010
49 **Mohamed H**, Al-Lenjawi B, Amuna P, Zotor F, Elmahdi H. Culturally sensitive patient-centred educational programme for self-management of type 2 diabetes: a randomized controlled trial. *Prim Care Diabetes* 2013; **7**: 199-206 [PMID: 23830727 DOI: 10.1016/j.pcd.2013.05.002]

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ORIGINAL ARTICLE

Retrospective Study

Obesity and cardiometabolic disease risk factors among US adolescents with disabilities

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Informed consent: Health information collected in the National Health and Nutrition Examination Survey is kept in strictest confidence. During the informed consent process, survey participants are assured that data collected will be used only for stated purposes and will not be disclosed or released to others without the consent of the individual or the establishment in accordance with section 308(d) of the Public Health Service Act (42 U.S.C. 242 m).
Conflict-of-interest: There are no conflicts of interest to report for any study authors.
Data sharing: Technical appendix, statistical code, and dataset available from the corresponding author at smessiah@med.miami.edu. The presented data cannot be linked to individuals and risk of personal identification is minimal as such.

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Abstract

AIM: To generate prevalence estimates of weight status and cardiometabolic disease risk factors among adolescents with and without disabilities.

METHODS: Analysis of the 1999-2010 National Health and Nutrition Examination Survey data was conducted among 12-18 years old with (n = 256) and without disabilities (n = 5020). Mean values of waist circumference, fasting glucose, high-density-lipoprotein cholesterol, triglycerides, systolic and diastolic blood pressure and metabolic syndrome (MetS, $\geqslant$ 3 risk factors present) were examined by the following standardized body mass index (BMI) categories for those with and without disabilities; overweight (BMI $\geqslant$ 85th - < 95th percentile for age and sex), obesity (BMI $\geqslant$ 95th percentile) and severe obesity (BMI $\geqslant$35 kg/m^2). Linear regression models were fit with each cardiometabolic disease risk factor independently as continuous outcomes to show relationships with disability status.

RESULTS: Adolescents with disabilities were significantly

more likely to be overweight (49.3%), obese (27.6%) and severely obese (12%) *vs* their peers without disabilities (33.1%, 17.5% and 3.6%, respectively, $P \leq 0.01$ for all). A higher proportion of overweight, obese and severely obese children with disabilities had abnormal SBP, fasting lipids and glucose as well as MetS (18.9% of overweight, 32.3% of obese, 55% of severely obese) *vs* their peers without disabilities (9.7%, 16.8%, 36.3%, respectively). US adolescents with disabilities are over three times as likely to have MetS (OR = 3.45, 95%CI: 1.08-10.99, $P = 0.03$) *vs* their peers with no disabilities.

CONCLUSION: Results show that adolescents with disabilities are disproportionately affected by obesity and poor cardiometabolic health *vs* their peers with no disabilities. Health care professionals should monitor the cardiometabolic health of adolescents with disabilities.

Key words: Adolescents; Children; Disability; Obesity; Cardiometabolic; Disease risk

Core tip: Our results here show that US adolescents with disabilities are disproportionately affected by obesity and are over three times as likely to have the metabolic syndrome vs their peers with no disabilities. Half of all adolescents with disabilities are overweight, obese or severely obese. In addition to the metabolic syndrome, obese adolescents with disabilities are significantly more likely than their normal weight counterparts to have increased or abnormal systolic blood pressure, lipid and fasting glucose levels, placing them at risk for cardiovascular disease and/or type 2 diabete. Health care professionals should monitor the cardiometabolic health of adolescents with disabilities.

Messiah SE, Vidot DC, Somarriba G, Haney K, Aytur S, Natale RA, Brosco JP, Arheart KL. Obesity and cardiometabolic disease risk factors among US adolescents with disabilities. *World J Diabetes* 2015; 6(1): 200-207 Available from: URL: http://www.wjgnet.com/1948-9358/full/v6/i1/200.htm DOI: http://dx.doi.org/10.4239/wjd.v6.i1.200

INTRODUCTION

In 2011 an estimated 5.1% (2.3 million) of 5-15 years old and 5.6% (1.2 million) of 16-20 years old in the United States reported a disability (physical, sensory, and cognitive or developmental disabilities)[1]. Even more troubling, obesity is 38% higher in children with disabilities and mobility limitations compared to their peers without disabilities[2]. Similarly, 57% of adults who are disabled are obese compared to 35.7% of peers without disabilities[3]. Healthy people 2020 reports that not only are individuals with disabilities more likely to be overweight or obese, they are also less likely to engage in outdoor physical activities[4,5], less likely to have social support to do so, and have worse overall health status *vs* their non-disabled counterparts[6].

These above stated prevalence statistics are important because obesity is strongly linked to hypertension, hyperlipidemia, type 2 diabetes mellitus, respiratory and musculoskeletal problems, liver disease, psycho-social problems, low self-esteem, which all lead to increased healthcare costs[7,8]. As such, it has been estimated that life expectancy will decrease due to obesity-related health issues alone[9]. Many studies have shown that the current childhood obesity epidemic has resulted in poor cardiometabolic health consequences including the components of metabolic syndrome (MetS)-elevated blood pressure and glucose concentrations, hypertriglyceridemia, low high density lipoprotein (HDL) cholesterol concentrations, and central adiposity (elevated waist circumference) - and the syndrome itself (three or more of these components in the same individual)[10-13]. Cardiometabolic disease risk factors present during the pediatric years predicts chronic diseases such as cancer, stroke, type 2 diabetes and cardiovascular disease in adults[14,15]. While previous studies have documented that youth with the MetS are at high risk for cardiometabolic disease and atherosclerosis as adults, there are few population-based studies examining the prevalence of cardiometabolic risk among adolescents with disabilities despite their increased prevalence of obesity *vs* their peers without disabilities. Therefore, the purpose of the current analysis is to estimate the prevalence of cardiometabolic disease risk, including the MetS, among the United States adolescent population with and without developmental physical and/or learning disabilities by weight status (normal weight, overweight, obese, severely obese). It was hypothesized that obese adolescents with disabilities would be significantly more likely to have the metabolic syndrome *vs* obese adolescents without disabilities.

MATERIALS AND METHODS

Study population

Participant data from the National Health and Nutrition Examination Survey (NHANES) were analyzed. Six cycles of NHANES data (1999-2000, 2001-2002, 2003-2004, 2005-2006, 2007-2008, and 2009-2010) were combined to ensure adequate sample size and statistical reliability[16]. The NHANES sampling design to obtain a nationally representative sample of the United States population is described in detail elsewhere[16].

Eligibility criteria

We selected all adolescents ages 12-18 years old from the combined 1999-2010 NHANES data who had the following variables available for analysis: waist circumference, body mass index (BMI), high density lipoprotein (HDL) cholesterol, systolic and diastolic blood pressure,

and fasting glucose and triglycerides. Because we chose to only analyze those who had data available on the cardiometabolic disease risk factors collected for their age group, the sample size was reduced from a total sample size of 10173 to 5276. There were no baseline significant differences between adolescents included in the sample (n = 5276) and those excluded (n = 4897) in terms of gender, ethnicity, education, income, or disability status. The mean age in the group included was 15.1 years compared to 14.9 years for those not included (P = 0.01).

Children were excluded from the analysis if they were known to have diabetes (n = 51), used medication that altered blood pressure, lipid metabolism, or blood glucose such as insulin, androgens, anabolic steroids, or adrenal corticosteroids (n = 42), or self-reported and/or tested positive *via* urine test as pregnant (n = 117).

Disability status

Individual physical functioning data were compiled from the NHANES Physical Function questionnaires[17] to determine disability status. A participant was categorized as having a disability (yes/no) if they answered yes to any of the following questions: (1) "Do you/does child have an impairment or health problem that limits (your/his/her) ability to crawl, walk, run, or play?"; (2) "Is this an impairment or health problem that has lasted, or is expected to last 12 mo or longer?"; and (3) "Is (child) limited in the kind or amount of play activities he/she can do because of a physical, mental, or emotional problem?" Participants who did not report a disability were placed in the no disability category, which constituted the reference group for the analyses. Information on specific category of disability (autism, Down's syndrome) is not available for NHANES participants under the age of 19.

Individual cardiometabolic disease risk factors

The criteria used to estimate the prevalence of abnormal or elevated (or low in the case of HDL cholesterol) individual cardiometabolic disease risk factors were modified to pediatric-specific criteria based on the National Cholesterol Education Program's Adult Treatment Panel (ATP Ⅲ) MetS definition for adults[18]. The threshold values used in this study to define each pediatric-specific abnormal risk factor are described below.

Waist circumference: Abnormal waist circumference was defined as above the 90th percentile of the NHANES Ⅲ (1988-1994) prevalence estimates adjusted for age, sex and ethnicity[19].

Systolic and diastolic blood pressure: Blood pressure was considered to be abnormal if systolic and/or diastolic values were greater than standardized 90th percentile values adjusted for age and sex[20].

HDL cholesterol: NHANES Ⅲ values[21] for cholesterol less than the 10th percentile were used to define abnormal or low HDL-cholesterol for the current study.

Triglyceride: NHANES Ⅲ[21] findings for triglyceride greater than the 90th percentile values adjusted for sex and ethnicity were used to define elevated levels in the current study.

Fasting glucose: A fasting glucose level of 100 mg/dL or higher was classified as abnormal[22]. The fasting glucose-specific, 4-year weights were applied for analysis.

Metabolic syndrome

An adolescent met criteria for the MetS if they had ⩾ 3 of the following risk factors: elevated waist circumference, triglycerides, fasting glucose, systolic and/or diastolic blood pressure, and low HDL cholesterol[11-13].

BMI percentile categories

Comparison of abnormal cardiometabolic disease risk factors were examined by the following standardized BMI categories for those with and without disabilities; (1) normal weight = BMI < 85th percentile for age and sex; (2) overweight = BMI ⩾ 85th - < 95th percentile for age and sex; (3) obese = BMI ⩾ 95th percentile for age and sex[23]; and (4) severely obese = absolute BMI ⩾ 35 kg/m^2[24].

Measures and data collection

People who were selected and consented to participate in the NHANES completed an in-home survey collected *via* Computer Assisted Personal Interviewing (CAPI) procedures. Demographic, socioeconomic, dietary, and health-related information was collected during this process. After the in-home interview, participants were asked to undergo a physical exam at a Medical Examination Center (MEC).

All laboratory methods used at the MEC are reported in detail in the NHANES Laboratory/Medical Technologists Procedures Manual[25,26]. Heights and circumferences were recorded to the nearest 0.1 cm.

Covariates

Demographic data including age in years, gender, ethnicity (Non-Hispanic White, Non-Hispanic Black, Mexican American, Other Hispanic and Other) and education level were used in analysis as covariates. Mexican American and Other Hispanic categories were combined to create a "Hispanic" classification.

Statistical methods

All data were analyzed using SAS survey procedures (SAS version 9.3, SAS Institute, Cary, NC). Sample weights (created to generate estimates for an entire sampling frame) were readjusted to account for the combined survey cycles. Weighting takes into account the specific probabilities of selection for the individual domains that were over-sampled (for example, in the 1999-2000 and 2001-2002 surveys both Mexican Americans and blacks were over-sampled), as well as non-response and differences between the sample and the total population. The correct sampling weights must be used to produce unbiased estimates when multiple surveys/

Table 1 Demographic and anthropometric characteristics of of those 12-18 years old with and without disabilities in the United States, 1999-2010 National Health and Nutrition Examination Surveys *n* (%)

	Overall *n* = 5276	Disability *n* = 256	No disability *n* = 5020	*P*-value[a]
Gender				0.31
Boys	2674 (50.8)	126 (2.5)	2548 (48.3)	
Girls	2602 (49.2)	130 (2.6)	2472 (46.6)	
Race/ethnicity				0.78
Non-hispanic white	1477 (61.1)	80 (64.3)	1397 (60.9)	
Non-hispanic black	1414 (13.5)	64 (12.1)	1350 (13.6)	
Hispanic	2146 (18.7)	97 (17.9)	2049 (18.7)	
Other	239 (6.7)	15 (5.7)	224 (6.8)	
Education level				0.22
Grade School	295 (5.2)	19 (6.1)	276 (5.1)	
Middle School	2247 (41.5)	101 (34.7)	2146 (41.9)	
High School	2264 (44.5)	117 (52.6)	2147 (44.1)	
High School graduate/GED	292 (6.4)	9 (3.5)	283 (6.6)	
More than High School	111 (2.3)	6 (2.9)	105 (2.3)	
Household income				0.17
< $10000	236 (5.3)	22 (9.9)	214 (5.1)	
$10000-$19999	595 (13.0)	32 (17.1)	563 (12.7)	
$20000-$34999	692 (15.8)	32 (21.4)	660 (15.5)	
$35000-$54999	644 (20.2)	24 (14.0)	620 (20.5)	
$55000-$74999	375 (14.0)	16 (11.1)	359 (14.1)	
> $75000	679 (31.7)	26 (26.4)	653 (32.0)	
Body mass index percentile group[b]				
Normal weight	3281 (66.1)	135 (50.7)	3146 (66.9)	< 0.001
Overweight	1995 (33.9)	121 (49.3)	1874 (33.1)	< 0.001
Obese	1099 (18.0)	67 (27.6)	1032 (17.5)	0.01
Severely obese	261 (4.2)	16 (5.8)	245 (4.1)	0.26
Body mass index	Mean (SE)	Mean (SE)	Mean (SE)	
Percentile	64.4 (0.6)	71.3 (2.4)	63.9 (0.6)	< 0.01
Z score	0.53 (0.02)	0.83 (0.1)	0.52 (0.0)	0.01
Age	15.1 (0.04)	15.1 (0.2)	15.1 (0.04)	0.68

[a]Represents mean difference between those with and without disabilities; [b]Normal weight = body mass index < 85th percentile for age and sex, overweight = body mass index ≥ 85th - < 95th percentile for age and sex, obese = body mass index ≥ 95th percentile for age and sex (Kuczmarski *et al*[23], 2000) severely obese = absolute body mass index ≥ 35 kg/m^2 (Kelly *et al*[24], 2000).

years are combined.

Survey frequencies were used to summarize demographic descriptive characteristics of the sample, and the SAS survey means procedure was used to obtain descriptive characteristics of anthropometric measurements. A binary variable for disability status was created for comparison and analysis purposes. The prevalence of each cardiometabolic disease risk factor was estimated for all 4 BMI categories for those with and without disabilities.

Linear regression models were fit with each cardiometabolic disease risk factor independently as continuous outcomes to show relationships with disability status. Logistic regression models were fit with a cluster of ≥ 3 abnormal cardiometabolic disease risk factor (MetS) as a binary outcome [Y = ≥ 3 abnormal factors; N = ≤ 3 abnormal factors. Adjustments were made in a step-wise procedure for Model (1) age, gender, ethnicity; Model (2) age, gender, ethnicity, education level; and Model (3) (Full Mode)] age, gender, ethnicity, education level, and annual household income. Adjusted odds ratios were reported with corresponding 95%CIs.

Statistical analysis

The statistical review of the study was performed by senior author Dr. Kristopher Arheart, a biomedical statistician and a leading expert on NHANES data and analysis. His approval of the methods are documented *via* his senior authorship inclusion on the manuscript.

RESULTS

Demographic characteristics of the sample (*n* = 5276, weighted *n* = 15942916) are presented in Table 1. Five percent (5.1%) of the sample (*n* = 256, weighted *n* = 812061) was classified as having a disability. There were no statistically significant differences in gender, ethnicity, education level, or annual household income between disabled and no-disability groups. Adolescents with disabilities were significantly less likely to be normal weight *vs* their peers with no disabilities (50.7% *vs* 66.9%, $P < 0.001$), and were significantly more likely to be overweight (49.3% *vs* 33.1%, $P < 0.001$) and obese (27.6% *vs* 17.5%, $P = 0.01$). Adolescents with disabilities had a significantly higher mean BMI percentile (71.3%ile), and *Z*-score (0.83) *vs* children without disabilities (64.4%ile; 0.53, respectively).

No significant differences between adolescents with and without disabilities were found for all cardiometabolic disease risk factors mean values among overweight, obese and severely obese sub-groups with the exception of

Table 2 Mean values of cardiometabolic disease risk factors among those 12-18 years old with and without disabilities in the United States by body mass index weight category[a], 1999-2010 National Health and Nutrition Examination Surveys

Cardiometabolic disease risk factors	Disability mean (SE)	No disability mean (SE)	*P*-value
Waist circumference, cm			
Normal weight	74.7 (0.61)	73.1 (0.18)	0.01
Overweight	96.5 (1.63)	94.7 (0.38)	0.31
Obese	105.1 (1.59)	102.2 (0.50)	0.10
Severely Obese	122.2 (3.30)	116.6 (0.71)	0.09
Systolic blood pressure, mmHg			
Normal weight	106.7 (1.22)	107.3 (0.26)	0.61
Overweight	114.2 (1.46)	112.2 (0.36)	0.19
Obese	115.7 (1.85)	113.7 (0.38)	0.31
Severely Obese	120.9 (3.49)	116.2 (0.65)	0.18
Diastolic blood pressure, mmHg			
Normal weight	60.8 (0.45)	61.2 (1.72)	0.82
Overweight	59.8 (0.59)	61.7 (1.45)	0.19
Obese	59.7 (0.67)	60.8 (2.17)	0.59
Severely obese	61.1 (1.46)	60.8 (3.75)	0.94
High density lipoprotein, mg/dL			
Normal weight	50.3 (1.23)	52.9 (0.29)	0.05
Overweight	44.9 (1.66)	45.9 (0.37)	0.53
Obese	41.0 (2.10)	43.9 (0.45)	0.19
Severely Obese	37.2 (3.07)	41.1 (0.92)	0.24
Triglycerides, mg/dL			
Normal weight	92.3 (10.77)	78.4 (1.14)	0.21
Overweight	105.4 (11.05)	100.3 (3.27)	0.63
Obese	115.9 (15.40)	113.2 (4.53)	0.85
Severely Obese	173.0 (23.62)	131.0 (12.76)	0.12
Glucose, mg/dL			
Normal weight	92.8 (1.32)	92.4 (0.37)	0.80
Overweight	96.7 (1.16)	94.0 (0.35)	0.03
Obese	96.8 (1.59)	95.2 (0.47)	0.32
Severely Obese	94.5 (1.10)	95.9 (1.00)	0.39

[a]Normal weight = body mass index < 85th percentile for age and sex, overweight = body mass index ⩾ 85th - < 95th percentile for age and sex, obese = body mass index ⩾ 95th percentile for age and sex (Kuczmarski *et al*[23], 2000) severely obese = absolute body mass index ⩾ 35 kg/m^2 (Kelly *et al*[24], 2000).

fasting glucose; among those who were overweight, mean values were significantly higher in those with disabilities (96.7 mg/dL) *vs* those without disabilities (94.0 mg/dL, $P = 0.03$). Normal weight adolescents with disabilities were significantly more likely to have an elevated waist circumference *vs* those children without disabilities (74.7 cm *vs* 73.1 cm, $P = 0.01$) (Table 2).

With the exception of diastolic blood pressure and triglycerides, overweight, obese and severely obese adolescents with and without disabilities were significantly more likely to have abnormal or elevated levels of waist circumference, systolic blood pressure, HDL cholesterol, triglycerides, fasting glucose, and MetS *vs* their normal weight counterparts. A higher proportion of overweight, obese and severely obese children with disabilities had abnormal SBP, fasting lipids and glucose as well as MetS (15.7% of overweight, 28.1% of obese, 61.3% of severely obese) *vs* their peers without disabilities (9.1%, 15.4%, 31.2%, respectively) (Table 3).

Adjusted logistic regression analysis showed that disabled adolescents are more than 3 times as likely as their nondisabled peers to have the MetS (AOR = 3.45, 95%CI: 1.08-11.0, $P = 0.04$). Females were significantly less likely to have MetS *vs* males (OR = 0.33, 95%CI: 0.21-0.53, $P < 0001$) (Table 4).

DISCUSSION

Our results here show that US adolescents with disabilities are disproportionately affected by obesity and are over three times as likely to have the MetS *vs* their peers with no disabilities. Half of all adolescents with disabilities are overweight, obese or severely obese. In addition to the MetS, obese adolescents with disabilities are significantly more likely than their normal weight counterparts to have increased or abnormal systolic blood pressure, lipid and fasting glucose levels, placing them at risk for cardiovascular disease and/or type 2 diabetes.

The findings in this study are consistent with previous literature describing higher rates of obesity and obesity related conditions in adults with disabilities[3]. Specifically, Froehlich-Grobe *et al*[3] reported that the prevalence those with disabilities have a significantly higher prevalence of obesity and extreme obesity (41.6% and 9.3%, respectively) compared to individuals without disabilities (29.2% and 3.9%, respectively). Additionally, those with disabilities at all weight categories were significantly more likely to have cardiometabolic risk factors and overt disease risk present. Furthermore, when comparing level of physical activity among disabled and nondisabled adolescents the literature consistently shows that adolescents with disabilities are less

Table 3 Prevalence of abnormal cardiometabolic disease risk factors among those who are overweight, obese and severely obese and 12-18 years old with and without disabilities in the United States compared to those of normal weight[a], 1999-2010 National Health and Nutrition Examination Surveys *n* (%)

Cardiometabolic disease risk factors	Normal weight[a]	Overweight	*P*-value	Obese	*P*-value	Severely obese	*P*-value
Waist circumference, cm[b]							
Disability	0 (0)	33 (29.7)	< 0.0001	33 (53.0)	< 0.0001	15 (90.7)	< 0.0001
No disability	0 (0)	441 (23.8)	< 0.0001	432 (43.6)	< 0.0001	225 (94.6)	< 0.0001
Systolic blood pressure, mmHg[c]							
Disability	5 (4.2)	20 (17.6)	0.02	16 (24.2)	0.01	7 (37.4)	0.004
No disability	145 (3.7)	251 (12.8)	< 0.0001	169 (14.5)	< 0.0001	57 (21.5)	< 0.0001
Diastolic blood pressure, mmHg[c]							
Disability	15 (11.6)	17 (16.5)	0.49	8 (16.0)	0.69	2 (16.2)	0.86
No disability	367 (11.2)	194 (11.4)	0.83	121 (12.5)	0.35	45 (19.6)	0.0002
High density lipoprotein, mg/dL[d]							
Disability	23 (15.7)	42 (37.8)	< 0.001	30 (47.0)	0.001	11 (62.0)	0.006
No disability	370 (13.2)	583 (33.7)	< 0.0001	390 (41.0)	< 0.0001	113 (56.0)	< 0.0001
Triglycerides, mg/dL[d]							
Disability	13 (23.3)	21 (34.2)	0.82	16 (45.8)	0.06	7 (67.1)	0.007
No disability	224 (16.6)	271 (31.4)	< 0.0001	182 (40.8)	< 0.0001	46 (50.1)	< 0.0001
Glucose, mg/dL[e]							
Disability	9 (6.1)	13 (15.5)	0.03	8 (13.9)	0.5	0 (0)	-
No disability	232 (7.4)	171 (8.5)	0.35	107 (10.7)	0.02	26 (12.1)	0.05
Metabolic syndrome (≥ 3 risk factors)							
Disability	0 (0)	17 (15.7)	< 0.0001	17 (28.1)	< 0.0001	11 (61.3)	< 0.0001
No disability	8 (0.20)	153 (9.1)	< 0.0001	144 (15.4)	< 0.0001	71 (31.2)	< 0.0001

[a]Normal weight = body mass index < 85th percentile for age and sex, overweight = body mass index ≥ 85th-< 95th percentile for age and sex, obese = body mass index ≥ 95th percentile for age and sex (Kuczmarski *et al*[23], 2000) severely obese = absolute body mass index ≥ 35 kg/m^2 (Kelly *et al*[24], 2000); [b]> 90th percentile for age and sex (Fernandez *et al*[18], 2004); [c]> 90th percentile for age and sex (National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004); [d]> 90th percentile for age and sex for triglycerides, < 10th percentile for age and sex for HDL cholesterol (Hickman *et al*[20], 1998); [e]> 100 mg/dL (American Diabetes Association, 2006).

likely to participate in sports or regular physical activity and are thus exposed to more inactivity *via* screen time such as TV, computer and video games[27].

Qualitative research has identified various barriers to facilitate participation in fitness and recreation programs and facilities among those with disabilities. These barriers include but are not limited to the built and natural environment, equipment, interpretation of guidelines, regulations, and laws, professional knowledge, education, and training issues; and facility- and community-level policies and procedures[28]. Research conducted in urban areas suggests that three out of five individuals with disabilities do not have sidewalks between their residences and the nearest bus stop, and over 70% lack curb cuts and bus shelters[29].

Thus, a Healthy People 2020 recommendation is to include those with disabilities in health promotion programs that include both healthy eating and active living components to help decrease their health risks[1]. Inclusion of persons with disabilities in urban planning and transportation planning processes, and promoting the principles of Universal Design[30] are also recognized as an important strategies. Similarly, the National Prevention Strategy, whose aim is to improve the health of each American at every stage of life and eliminate all health disparities, has formulated a plan that includes improving social inclusion of those with disabilities with mental and emotional well-being, healthy eating and active living with all citizens[31]. The combination of healthy eating and active living programs have a positive health effect on people with disabilities, including a decrease in weight and BMI, becoming more fit[32], higher fruit and vegetable intake and self-reported activity levels, and decreased health risks.

We report that half of all United States adolescents with disabilities are either overweight, obese or severely obese, which has strong implications for adult health. Previous studies have documented the importance of childhood obesity as one of the strongest risk factors for adult obesity and cardiometabolic disease[14,15]. We also found that adolescents with disabilities are at over triple the risk for the MetS *vs* their peers with no disabilities, which also has direct implications for their adult health. Previous studies have shown that if MetS is present in the childhood years, that individual has an almost 10 fold risk for cardiovascular disease, and 4 fold risk for type 2 diabetes as an adult[7,14,15]. Our findings here suggest that adolescents with disabilities who are concomitantly challenged with unhealthy weight should be closely monitored for associated cardiometabolic risk to prevent chronic disease onset.

Limitations

A few study limitations should be noted. First, because NHANES is a cross-sectional design, causality cannot be inferred (*e.g.*, whether disability causes obesity or vice versa). Second, the prevalence of obesity in this subpopulation of NHANES data may be underestimated because those with the most severe disabilities may not be able to participate. Additionally, height and weight was

Table 4 Odds Ratios to predict the metabolic syndrome by selected covariates among those 12-18 years old with and without disabilities in the United States, 1999-2010 National Health and Nutrition Examination Survey

	OR (95%CI)	*P*-value
Disability status		
No disability (ref)	1	-
Disability	3.45 (1.08-10.99)	0.03
Age		
12 years old (ref)	1	-
> 12 years old	1.22 (1.03-1.44)	0.02
Sex		
Male (ref)	1	-
Female	0.33 (0.21-0.53)	< 0.0001
Ethnicity		
Non-Hispanic white (ref)	1	-
Race/Ethnicity	0.77 (0.59-1.00)	0.05
Education		
< High School (ref)		
Education level	1.01 (0.97-1.05)	0.70
Household income		
> $75000		
Household Income	1.02 (1.00-1.03)	0.05

not recorded in those participants who could not stand independently. However, our analysis only included those participants who had all cardiometabolic disease risk factors available, including BMI and waist circumference. Finally, information on specific category of disability (autism, Down's syndrome) was not available for NHANES participants under the age of 19.

Conclusion

Recently, the American Medical Association (AMA) officially labeled obesity as a disease "requiring a range of medical interventions to advance obesity treatment and prevention"[33]. This statement has direct implications for our finding here that half of all US adolescents with disabilities are either overweight, obese or severely obese. As adolescents, those with disabilities are already more than three times as likely as their peers without disabilities to have the MetS. Future research efforts should focus on the etiology of the disproportionate prevalence of both obesity and cardiometabolic disease risk in those with developmental disabilities. Our findings suggest that overweight and obese adolescents with disabilities should be clinically monitored for elevated weight and concomitant cardiometabolic disease risk factors throughout their teenage years.

COMMENTS

Background

The prevalence of obesity is 38% higher in children with disabilities and mobility limitations compared to their peers without disabilities. Similarly, 57% of adults who are disabled are obese compared to 35.7% of peers without disabilities. Healthy People 2020 reports that not only are individuals with disabilities more likely to be overweight or obese, they are also less likely to engage in outdoor physical activities, less likely to have social support to do so, and have worse overall health status *vs* their non-disabled counterparts. There are few population-based studies examining the prevalence of cardiometabolic risk among adolescents with disabilities despite their increased prevalence of obesity *vs* their peers without disabilities. Therefore, the purpose of the current analysis is to estimate the prevalence of cardiometabolic disease risk, including the metabolic syndrome, among the United States adolescent population with and without developmental physical and/or learning disabilities by weight status (normal weight, overweight, obese, severely obese).

Research frontiers

The purpose of the current analysis is to estimate the prevalence of cardiometabolic disease risk, including the metabolic syndrome, among the United States adolescent population with and without developmental physical and/or learning disabilities by weight status (normal weight, overweight, obese, severely obese).

Innovations and breakthroughs

The results here show that United States adolescents with disabilities are disproportionately affected by obesity and are over three times as likely to have the metabolic syndrome *vs* their peers with no disabilities. Half of all adolescents with disabilities are overweight, obese or severely obese. In addition to the metabolic syndrome, obese adolescents with disabilities are significantly more likely than their normal weight counterparts to have increased or abnormal systolic blood pressure, lipid and fasting glucose levels, placing them at risk for cardiovascular disease and/or type 2 diabetes.

Applications

The findings suggest that overweight and obese adolescents with disabilities should be clinically monitored for elevated weight and concomitant cardiometabolic disease risk factors throughout their teenage years.

Terminology

The metabolic syndrome is defined as having $\geqslant$ 3 of the following cardiometabolic disease risk factors present simultaneously - elevated blood pressure, elevated glucose concentrations, hypertriglyceridemia, low high density lipoprotein cholesterol concentrations, and central adiposity (elevated waist circumference.

Peer review

This is a very interesting and well written manuscript.

REFERENCES

1 **Cornell University Employment and Disability Institute.** U.S. Census Bureau's 2011 American Community Survey (ACS) Public Use Microdata Sample (PUMS) data. Available from: URL: http: //www.disabilitystatistics.org/reports/acs.cfm?statistic=1

2 **Rimmer JH**, Yamaki K, Davis BM, Wang E, Vogel LC. Obesity and overweight prevalence among adolescents with disabilities. *Prev Chronic Dis* 2011; **8**: A41 [PMID: 21324255]

3 **Froehlich-Grobe K**, Lee J, Washburn RA. Disparities in obesity and related conditions among Americans with disabilities. *Am J Prev Med* 2013; **45**: 83-90 [PMID: 23790992 DOI: 10.1016/j.amepre.2013.02.021]

4 **Coombes E**, Jones AP, Hillsdon M. The relationship of physical activity and overweight to objectively measured green space accessibility and use. *Soc Sci Med* 2010; **70**: 816-822 [PMID: 20060635 DOI: 10.1016/j.socscimed.2009.11.020]

5 **Hillsdon M**, Panter J, Foster C, Jones A. The relationship between access and quality of urban green space with population physical activity. *Public Health* 2006; **120**: 1127-1132 [PMID: 17067646 DOI: 10.1016/j.puhe.2006.10.007]

6 **U.S. Department of Health and Human Services**. Healthy People 2020. Available from: URL: http: //www.healthypeople.gov/2020/topicsobjectives2020/overview.aspx?topicid=9

7 **Lipshultz SE,** Messiah SE, Miller TL, editors. Pediatric Metabolic Syndrome: Comprehensive Clinical Review and Related Health Issues. London: Springer, 2012. Available from: URL: http: //books.google.com/books?hl=en&lr=&id=hI1qVQEqik0C&oi=fnd&pg=PR4&dq=Pediatric Metabolic Syndrome: Comprehensive Clinical Review and Related Health Issues. London: Springer; 2012.&ots=od0V0eC0mM&sig=OrqXUtIvnWuS-O9STep0yK_gVWo#v=onepage&q=Pediatric Metabolic Syndrome: Comprehensive Clinical Review and Related Health Issues. London: Springer; 2012.&f=false

8 **Finkelstein EA**, Trogdon JG, Cohen JW, Dietz W. Annual

medical spending attributable to obesity: payer-and service-specific estimates. *Health Aff* (Millwood) 2009; **28**: w822-w831 [PMID: 19635784 DOI: 10.1377/hlthaff.28.5.w822]

9 **Olshansky SJ**, Passaro DJ, Hershow RC, Layden J, Carnes BA, Brody J, Hayflick L, Butler RN, Allison DB, Ludwig DS. A potential decline in life expectancy in the United States in the 21st century. *N Engl J Med* 2005; **352**: 1138-1145 [PMID: 15784668 DOI: 10.1056/NEJMsr043743]

10 **Doak CM**, Visscher TL, Renders CM, Seidell JC. The prevention of overweight and obesity in children and adolescents: a review of interventions and programmes. *Obes Rev* 2006; **7**: 111-113 [PMID: 16436107 DOI: 10.1111/j.1467-789X.2006.00234.x]

11 **Cook S**, Weitzman M, Auinger P, Nguyen M, Dietz WH. Prevalence of a metabolic syndrome phenotype in adolescents: findings from the third National Health and Nutrition Examination Survey, 1988-1994. *Arch Pediatr Adolesc Med* 2003; **157**: 821-827 [PMID: 12912790 DOI: 10.1001/archpedi.157.8.821]

12 **Messiah SE**, Arheart KL, Luke B, Lipshultz SE, Miller TL. Relationship between body mass index and metabolic syndrome risk factors among US 8- to 14-year-olds, 1999 to 2002. *J Pediatr* 2008; **153**: 215-221 [PMID: 18534237 DOI: 10.1016/j.jpeds.2008.03.002]

13 **de Ferranti SD**, Gauvreau K, Ludwig DS, Neufeld EJ, Newburger JW, Rifai N. Prevalence of the metabolic syndrome in American adolescents: findings from the Third National Health and Nutrition Examination Survey. *Circulation* 2004; **110**: 2494-2497 [PMID: 15477412 DOI: 10.1161/01.CIR.0000145117.40114.C7]

14 **Morrison JA**, Friedman LA, Gray-McGuire C. Metabolic syndrome in childhood predicts adult cardiovascular disease 25 years later: the Princeton Lipid Research Clinics Follow-up Study. *Pediatrics* 2007; **120**: 340-345 [PMID: 17671060 DOI: 10.1542/peds.2006-1699]

15 **Sun SS**, Liang R, Huang TT, Daniels SR, Arslanian S, Liu K, Grave GD, Siervogel RM. Childhood obesity predicts adult metabolic syndrome: the Fels Longitudinal Study. *J Pediatr* 2008; **152**: 191-200 [PMID: 18206688 DOI: 10.1016/j.jpeds.2007.07.055]

16 **Centers for Disease Control and Prevention**. National Center for Health Statistics. National Health and Nutrition Examination Survey Data. Hyattsville, MD: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, 2010. Available from: URL: http: //www.cdc.gov/nchs/about/major/nhanes/nhanes2009-2010/questexam03_04.htm

17 **Centers for Disease Control and Prevention.** National Center for Health Statistics. National Health and Nutrition Examination Survey. 2009 - 2010 Data Documentation, Codebook, and Frequencies. Physical Functioning (PFQ_F). Available from: URL: http: //www.cdc.gov/nchs/nhanes/nhanes2009-2010/PFQ_F.htm

18 **National Institutes of Health.** The Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Bethesda, MD: National Institutes of Health; NIH Publication 01-3670, 2001

19 **Fernández JR**, Redden DT, Pietrobelli A, Allison DB. Waist circumference percentiles in nationally representative samples of African-American, European-American, and Mexican-American children and adolescents. *J Pediatr* 2004; **145**: 439-444 [PMID: 15480363 DOI: 10.1016/j.jpeds.2004.06.044]

20 **National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents.** The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. *Pediatrics* 2004; **114**: 555-576 [PMID: 15286277]

21 **Hickman TB**, Briefel RR, Carroll MD, Rifkind BM, Cleeman JI, Maurer KR, Johnson CL. Distributions and trends of serum lipid levels among United States children and adolescents ages 4-19 years: data from the Third National Health and Nutrition Examination Survey. *Prev Med* 1998; **27**: 879-890 [PMID: 9922071 DOI: 10.1006/pmed.1998.0376]

22 **American Diabetes Association.** American Diabetes Association: clinical practice recommendations 2002. *Diabetes Care* 2002; **25** Suppl 1: S1-147 [PMID: 11788484 DOI: 10.2337/diacare.25.2007.S1]

23 **Kuczmarski RJ**, Ogden CL, Grummer-Strawn LM, Flegal KM, Guo SS, Wei R, Mei Z, Curtin LR, Roche AF, Johnson CL. CDC growth charts: United States. *Adv Data* 2000; **(314)**: 1-27 [PMID: 11183293]

24 **Kelly AS**, Barlow SE, Rao G, Inge TH, Hayman LL, Steinberger J, Urbina EM, Ewing LJ, Daniels SR. Severe obesity in children and adolescents: identification, associated health risks, and treatment approaches: a scientific statement from the American Heart Association. *Circulation* 2013; **128**: 1689-1712 [PMID: 24016455 DOI: 10.1161/CIR.0b013e3182a5cfb3]

25 **The Centers for Disease Control and Prevention.** National Health and Nutrition Examination Survey: Laboratory Procedures Manual. 2004. Available from: URL: http: //www.cdc.gov/nchs/data/nhanes/lab1-6.pdf

26 **Chumlea NC**, Kuczmarski RJ. Using a bony landmark to measure waist circumference. *J Am Diet Assoc* 1995; **95**: 12 [PMID: 7798573]

27 **Rimmer JH**, Riley B, Wang E, Rauworth A, Jurkowski J. Physical activity participation among persons with disabilities: barriers and facilitators. *Am J Prev Med* 2004; **26**: 419-425 [PMID: 15165658 DOI: 10.1016/j.amepre.2004.02.002]

28 **Centers for Disease Control and Prevention.** Disability and Health. Available from: URL: http: //www.naccho.org/topics/environmental/landuseplanning/upload/DisabilityFocusGroupReport_000.pdf

29 **Clarke P**, Ailshire JA, Bader M, Morenoff JD, House JS. Mobility disability and the urban built environment. *Am J Epidemiol* 2008; **168**: 506-513 [PMID: 18667526 DOI: 10.1093/aje/kwn185]

30 **Mobility International USA.** Universal Design/Accessibility Standards Resources. Available from: URL: http: //www.miusa.org/ncde/tools/universaldesign.

31 **United States Department of Health and Human Services.** National Prevention Strategy. Washington, DC. [Accessed 2013 September 13]. Available from: URL: http: //www.surgeongeneral.gov/initiatives/prevention/strategy/

32 **Naaldenberg J**, Kuijken N, van Dooren K, van Schrojenstein Lantman de Valk H. Topics, methods and challenges in health promotion for people with intellectual disabilities: a structured review of literature. *Res Dev Disabil* 2013; **34**: 4534-4545 [PMID: 24161461 DOI: 10.1016/j.ridd.2013.09.029]

33 **American Medical Association.** Report of the Council on Science and Public Health. CSAPH Report 3-A-13. Is obesity a disease? Chicago: American Medical Association, 2013. Available from: URL: http: //www.ama-assn.org/assets/meeting/2013a/a13-addendum-refcomm-d.pdf

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ORIGINAL ARTICLE

Observational Study

Glycaemic control, treatment satisfaction and quality of life in type 2 diabetes patients in Greece: The PANORAMA study Greek results

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Data sharing: Technical appendix, statistical code, and dataset available from the corresponding author at avramopoulos@medweb.gr. Consent from participants was not obtained but the presented data are anonymized and risk of identification is low.

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Abstract

AIM: To provide an update on glycaemic control in European patients with type 2 diabetes mellitus (T2DM). We present the Greek population data of the study.

METHODS: An observational multicenter, cross-sectional study evaluating glycaemic control and a range of other clinical and biological measures as well as quality of life (QoL) and treatment satisfaction in 375 patients with T2DM enrolled by 25 primary care sites from Greece.

RESULTS: The mean age of the patients was 63.5 years and the male/female ratio 48.9%/51.1%. 79.7% of the patients exerted none or light physical activity, 82.4% were overweight or obese and 32.9% did not meet HbA1c target of less than 7.0% (53 mmol/mol). Patients reported high satisfaction to continue with treatment, high satisfaction with administered treatment and increased willingness to recommend treatment to others (mean Diabetes Treatment Satisfaction Questionnaire score 29.1 ± 5.6). However, 80% of the patients reported that their QoL would be better without diabetes. Finally, the most challenging parameter reported was the lack of freedom to eat and drink.

CONCLUSION: This analysis of the Greek Panorama study results showed that a considerable percentage of T2DM patients in Greece do not achieve glycaemic target levels, despite the favourably reported patient satisfaction from administered therapy. Additionally, the majority of primary care T2DM patients in Greece depict the negative effect of the disease in their QoL.

Key words: Quality of life; Treatment satisfaction; Type 2 diabetes mellitus

Core tip: Diabetes is a common, chronic disease with serious complications. Despite the multiple antidiabetic

treatment options and the clear treatment guidelines, a significant proportion of type 2 diabetes patients do not achieve the glycaemic goals. Few studies have examined the quality of life in these patients. PANORAMA was a Pan-European multinational study that provided an update of the glycaemic control and quality of life in patients with diabetes. The Greek results of this study showed that a significant proportion of Greek patients were not under glycaemic control despite the high satisfaction that they had from their treatment. A negative impact of the disease in quality of life was also noted.

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic and complex metabolic disease characterized by hyperglycaemia, as a result of insulin resistance, impaired insulin secretion and excessive or abnormal glucagon release. It is well established that its prevalence increases globally especially in the developed countries, and this increased prevalence is associated with deleterious changes in lifestyle, unhealthy eating patterns and reduced physical activity[1]. Epidemiological studies in the Greek population have shown that diabetes prevalence is also on the rise, increasing from 5.7% in 2001 to 10.4% in 2006[2]. At the same time cardiovascular (CV) risk factors, tightly related to T2DM, such as obesity, hypertension and hypercholesterolemia demonstrated even a greater increase[3]. Many effective pharmacological treatments for diabetes are now available that can be initiated after the behavioural modifications of exercise and diet. However, despite the progress in treatment strategies, many patients still face difficulties in achieving or maintaining HbA1c target levels. Moreover, diabetes is often accompanied by complications, stemming from various reasons including non-adherence to treatment and delayed adjustment of treatment regimen leading to progressive loss of b-cell function[4,5]. These complications have a negative impact on patients' satisfaction with treatment as well as patients' quality of life (QoL)[6-8]. Moreover, living with diabetes, reduces health related QoL which is often manifested as loss of functional ability, restrictions and barriers to everyday activities, limitations to work capacity and poor general health, while on the other hand may propagate psychological disorders such as anxiety and depression[7,9-12]. Although the advantages and disadvantages of treatment intensification on glycaemic control and various clinical measures have been the focus of several recent investigations[13-16], the data available on patients' QoL and treatment satisfaction, especially in primary care, are still sparse. The Pan-European study PANORAMA has attempted to satisfy the need for a more up-to-date national and European data on glycaemic control from a pool of T2DM patients treated with diet, oral anti-diabetes drugs (OAD) and/or injectables. Given the alarming reports of increased prevalence of T2DM in Greece, the present study aimed at investigating the level of glycaemic control in T2DM patients and describing diabetes treatment satisfaction, QoL and fear of hypoglycaemic episodes in the Greek population of the PANORAMA study.

MATERIALS AND METHODS

Study design

The objectives, design and methodology of the study have recently been published by Bradley *et al*[17]. PANORAMA was an observational multicentre, multinational (Belgium, France, Germany, Greece, Italy, The Netherlands, Spain, Turkey and United Kingdom), cross-sectional study (NCT00916513), evaluating glycaemic control and a range of other clinical and biological measures as well as health-related QoL and treatment satisfaction in patients with T2DM. In Italy and Turkey, physicians were recruited both from hospitals and primary care practices due to country-specific healthcare systems, whereas the physicians from the other participating countries were recruited from the primary care setting only. The group of participating investigators in Greece (n = 25) included both diabetologists/endocrinologists (n = 10) and internists (n = 15).

Study population

Eligible patients enrolled in the study were aged ⩾ 40 years, diagnosed with T2DM at least one year prior to study initiation and had at least 1-year of available medical records at the participating site. Patients were given dietary and exercise advice, and could have been treated with OADs with or without insulin as well as with GLP-1 receptor agonists, with treatment unchanged within the previous 3 mo. The study excluded patients with type 1 diabetes and/or history of diabetic ketoacidosis, secondary diabetes and pregnant women. Also, excluded from the study were patients treated with systemic corticosteroids other than replacement therapy, patients already participating in a clinical trial and patients unable to complete the questionnaires. The PANORAMA study accommodated two methods of enrolling patients, a randomized and a sequential method[17]. In Greece, patient enrolment followed the sequential method, where each participating physician sequentially enrolled patients that attended the participating centre for a routine visit.

Study procedures

Once patients had signed the informed consent, data from their medical records were collected during a single study visit (index visit). These data included patient's socio-

demographic and anthropometric characteristics (age, gender, weight, height, educational level, socioeconomic status, alcohol consumption, smoking status, physical activity), biological measures [blood glucose, HbA1c levels, lipids: LDL-C, HDL-C, triglycerides (TG), total cholesterol (TC)] and disease-related variables (duration of diabetes, current and past diabetes treatment regimens, hypoglycaemic episodes, macrovascular and microvascular complications). The HbA1c levels of each patient were recorded by the physician at a single index visit using Bayer's A1CNow® device (certified test by the United States National Glycohemoglobin Standardization Program). This HbA1c measurement determined whether treatment goals had been achieved.

Patient reported outcomes

Patients' reported outcomes (PROs), using validated translations of standard and widely used assessment tools, were recorded *via* the DTSQ (Diabetes Treatment Satisfaction Questionnaire), ADDQoL (Audit of Diabetes Dependent QoL), worry subscale of HSF-Ⅱ (Hypoglycaemia Fear Survey-Ⅱ) and EQ5D (EuroQoL health utility questionnaire). Composite scores were calculated according to defined algorithms for each instrument.

DTSQ, is a self-administered instrument that has demonstrated validity and reliability in diabetes populations and is recommended by the World Health Organization (WHO) and the International Diabetes Federation (IDF). The DTSQ assesses treatment satisfaction over the few weeks before its completion. The treatment satisfaction score is the sum of six of the items of the DTSQ for each respondent. Each of the treatment satisfaction scale item is scored from 6 to 0 with a higher score indicating greater satisfaction. The treatment satisfaction score can range between 36 (very satisfied) and 0 (very dissatisfied). The two additional items measuring perceived frequency of hypo - and hyperglycaemia are scored from 0 (none of the time) to 6 (most of the time)[18,19].

ADDQoL is an individualized measure of the impact of diabetes on QoL. It is a self-administered questionnaire with 21 items. The first 19 items concern specific life domains such as social and work life and are scored on a 5-point impact scale, accompanied by a related importance rating scale for each domain used to assess the importance of each aspect of life for the individual's QoL. Weight impact scores can range from +3 (maximum positive impact of diabetes) to -9 (maximum negative impact of diabetes). The 2 remaining overview items are scored separately and include a single diabetes-specific QoL item measuring the impact of diabetes on QoL that is scored from +1 (maximum positive impact of diabetes) to -3 (maximum negative impact of diabetes) and a single item, present QoL, that is scored from +3 (excellent) to -3 (extremely bad) to measure overall QoL[20,21].

The worry subscale of HSF-II consists of 18 items, rated by patients using a 5-point Likert scale ranging from 0 (never) to 4 (almost always). The 18 items are preceded by the statement "'Because my blood sugar could drop, I worried about …". Scores on the "worry" subscale range from 0 to 72, with 0 representing "least worry"[22,23].

Statistical analysis

Statistical analysis was performed using the SAS system (SAS for Windows v8.2) according to Statistical Analysis Plan prepared prior to database lock. Data were summarised by standard summary statistics. The continuous variables of age, duration of T2DM and HbA1c at index visit were expressed as mean ± SD. Additionally, the categorical variables of demographics, disease characteristics, treatment regimens, physicians' perceptions for not reaching HbA1c goals and corrective actions taken, blood and lipid profiles and microvascular and macrovascular complications were expressed as frequencies.

RESULTS

Study population

The Pan-European data presenting the current level of glycaemic control and its associated factors in T2DM patients, as well as the data for the Spanish subgroup were published by Depablos-Velasco *et al*[24,25]. The PANORAMA study in Greece enrolled 375 patients. Their mean age was 63.5 ± 10.0 years with males and females proportionally represented (48.9% men *vs* 51.1 % women). Obesity was observed in 42.8% of the patients, while 21.9% were current smokers and 79.7% reported no or very light (less than once a week) physical activity. Demographic and other basic patients' characteristics are presented in Table 1.

Disease characteristics and comorbidities

Mean duration of T2DM in the Greek PANORAMA study population was 9.7 ± 8.8 years with 63.5% of patients presenting the disease for more than 5 years (Table 2). In total, 26.9% of patients suffered microvascular complications, with the most frequent being diabetic nephropathy and chronic diabetic polyneuropathy. In parallel, 24.0% of patients presented macrovascular disease. Coronary heart disease was the most prevalent complication (Table 3).

Diabetes management

Exercise and dietary advice only, was the treatment of 5.3% of the study population. Hence, the majority of the patients (94.7%) were under pharmacological treatment consisting of OADs only (65.1%), 2.7% received GLP-1 agonists, and 24.3% insulin with or without OADs. Regarding OADs, metformin was used by 73.3% patients, while fixed-dose combinations were administered to 12.3% of the patients. The most frequently administered oral hypoglycaemic agents are presented in Table 4.

Glycaemic control

The patients' mean HbA1c, recorded in the index visit of the PANORAMA study, was 6.7% ± 1.0% (50 mmol/mol), while the 32.9% of the patients failed

Table 1 Demographic and anthropometric data in the Greek PANORAMA study population

	n (%)
Age (yr, mean ± SD)	63.5 (± 10.0)
Gender (males)	183 (48.9)
Physical activity	
None	84 (22.5)
Light (less than 1 time/wk)	214 (57.2)
Intense (1 to 2 times/wk)	48 (12.8)
Intense (3 or more times/wk)	28 (7.5)
Body massindex	
Normal (18.5-25 kg/m^2)	66 (17.6)
Overweight (25-30 kg/m^2)	148 (39.6)
Obese (≥ 30 kg/m^2)	160 (42.8)
Smoking status	
Never smoker	205 (54.8)
Former smoker	87 (23.3)
Current smoker	82 (21.9)
Alcohol consumption (units per week)	
Males	2.1 (3.3)
Females	0.6 (1.6)

Table 2 Disease characteristics in Greek PANORAMA study population

	mean ± SD
Average duration of type 2 diabetes in years (*n* = 375)	9.7 (± 8.8)
Duration of type 2 diabetes	*n* (%)
< 5 yr	137 (36.5)
≥ 5 yr	238 (63.5)
Years on insulin treatment, (*n* = 82, yr, mean ± SD)	4.8 (± 7.2)

Table 3 Microvascular and macrovascular complications in Greek PANORAMA study population

	n (%)
Microvascular complications	
Any complication	101 (26.9)
Chronic diabetic polyneuropathy-Asymptomatic	33 (8.8)
Chronic diabetic polyneuropathy-Symptomatic	29 (7.7)
Autonomic neuropathy	6 (1.6)
Diabetic retinopathy	27 (7.2)
Diabetic nephropathy	49 (13.1)
Diabetic nephropathy-Microalbuminuria	32 (8.5)
Diabetic nephropathy-Proteinuria	12 (3.2)
Diabetic nephropathy-Renal insufficiency	9 (2.4)
Diabetic nephropathy-Dialysis	0 (0)
Macrovascular complications	
Any complication	91 (24.0)
Coronary heart disease	70 (18.7)
Cerebrovascular disease	10 (2.7)
Peripheral artery disease	21 (6.6)

Table 4 Diabetes treatment regimens in the PANORAMA study population

Treatment regimen	*n* (%)
No diet, no orals, no injectables (no available data)	10 (2.7)
Only diet and/or exercise	20 (5.3)
Only OADs	244 (65.1)
On oral plus insulin	63 (16.8)
Only on insulin	28 (7.5)
On GLP-1 analogues ± insulin[1]	10 (2.7)
Oral hypoglycaemic agents	316 (84.3)
Sylphonylureas	121 (32.3)
Meglitinides/Glinides	12 (3.2)
Biguanides	275 (73.3)
Thiazolidinediones	41 (10.9)
DPP-4 inhibitors	94 (25.1)
Alpha glucosidase inhibitors	13 (3.5)
Fixed-dose combinations	46 (12.3)
Thiazolidinediones + metformin	3 (6.5)
DPP4 inhibitors + metformin	43 (93.5)

[1]One patient receiving a GLP-1 analogue and insulin was classified in the latter category making the total number of patients 91 when the number should have been 92. OAD: Oral anti-diabetes drugs.

to meet HbA1c target levels presenting with HbA1c ≥ 7.0% (53 mmol/mol) (Table 5). When physicians were asked about the reasons for not reaching HbA1c target, the most frequent answer was poor patient adherence to dietary and exercise recommendations (39.5%), while other common reasons were failure of current drug regimen, resistance or reluctance of the patient to intensify the medication regimen, poor patient adherence to self-monitoring of blood glucose levels, and reluctance of physician to intensify the regimen due to fear of hypoglycaemia. In order to achieve HbA1c target, reported actions taken by the physician included retraining of patients in diet/lifestyle recommendations that need to be adopted (educational approach) (42.7%) and intensification of dose of the current anti-hyperglycaemic medication (27.5%). The addition of another OAD agent was chosen as corrective action in 11.2% of the cases. Initiation of insulin treatment, with or without changing OAD medication, was recorded in a small percentage of cases (Tables 6 and 7).

Cardiovascular risk factors

More than half of the population did not attain LDL-cholesterol (LDL-C) target < 100 mg/dL (2.586 mmol/L) with 55.8% of the patients appearing with LDL-C ≥ 100 mg/dL (2.586 mmol/L). Similarly, 40.4% of the population appeared with triglyceride (TG) levels ≥ 150 mg/dL (1.6935 mmol/L), while 24.3% of the population was off-target at ≤ 40mg/dL (1.0344 mmol/L) for HDL-cholesterol (HDL-C). Additionally, the majority of patients did not also achieve blood pressure targets since 69.8% of the study's patients reported systolic/diastolic blood pressure ≥ 130/80 mmHg (target ≤ 130/80 mmHg) (Table 8).

Patient reported outcomes

DTSQ questionnaire: In the PANORAMA study, the Greek population's mean DTSQ score reported by the patients was 29.1 ± 5.6. Patients reported high satisfaction grades in all domains of the questionnaire; satisfaction with treatment, convenience, flexibility and understanding of diabetes, willingness to recommend treatment to

Table 5 Glycaemic control in the Greek PANORAMA study population

Glycaemic control	*n* (%)
HbA1c value at index visit (mean ± SD)	6.7 (± 1.0) (50 mmol/mol)
HbA1c value at index visit	
< 6.5% (47 mmol/mol)	179 (47.9)
⩾ 6.5% (47 mmol/mol)	195 (52.1)
< 7.0% (53 mmol/mol)	251 (67.1)
⩾ 7.0% (53 mmol/mol)	123 (32.9)

Table 6 Physicians' perceptions on reasons for not reaching HbA1c target

Reasons	*n* (%)
Therapeutic failure of current drug regimen	52 (13.9)
Poor patient adherence to diet and exercise	148 (39.5)
Poor patient adherence to self-monitoring of blood glucose levels	44 (11.7)
Poor patient adherence to recommendations	26 (6.9)
Resistance/reluctance of the patient to intensify his/her medication regimen	46 (12.3)
Reluctance of physician to intensify medication regimen	3 (0.8)
Reluctance of physician to intensify medication regimen-Fear of hypoglycaemia	44 (11.7)
Reluctance of physician to intensify medication regimen-Fear of unwanted side effects	14 (3.7)
Reluctance of physician to intensify medication regimen-Fear of interaction with other medications	6 (1.6)
Reluctance of physician to intensify medication regimen-Cost of treatment	9 (2.4)
Reluctance of physician to intensify medication regimen-Fear of additional weight gain	13 (3.5)

someone else and satisfaction to continue with current treatment. Unacceptably high or unacceptably low glucose levels were rarely reported. DTSQ scores were presented in Figure 1.

ADDQoL questionnaire: The mean ADDQoL questionnaire score reported by patients was -2.0 ± 1.9. Overall, 79.5% of patients reported that their QoL would be better if they did not have diabetes. Following analysis of the individual questionnaire components, the most affected parameters of ADDQoL were freedom to eat and drink (Figure 2).

HFS questionnaire: The mean HFS questionnaire total score for the Greek PANORAMA population was 14.2 ± 14.7. Taking into consideration that the score for the greatest fear equals to 72, the reported HFS score in the study represents an overall mild fear of hypoglycaemia. In particular, 15.3% of the patients were frequently afraid of having a hypoglycaemic episode while alone or during sleep where no one would be present to help (Figure 3).

DISCUSSION

The European PANORAMA study investigated the level of glycaemic control in Europe in addition to patients' treatment satisfaction and QoL[17]. Here, the Greek

Table 7 Actions taken by the physicians to reach HbA1c target

Actions taken	*n* (%)
No specific actions	45 (12.0)
Educational approach	160 (42.7)
Increase dose of current medication	103 (27.5)
Addition of new oral antihyperglycaemic medication	
Sylphonylureas	8 (2.1)
Meglitinides/Glinides	4 (1.1)
Biguanides	5 (1.3)
Thiazolidinediones	3 (0.8)
DPP-4 inhibitors	13 (3.5)
Combination treatment	9 (2.4)
Start insulin therapy without changing oral diabetes medication	9 (2.4)
Start insulin therapy changing oral diabetes medication	14 (3.7)
Other action	9 (2.4)

Table 8 Blood pressure and lipid profile: Percentage of patients who meet AACE criteria for target blood pressure and lipid levels

	n (%)
Hypertension	
SBP/DBP < 130/80 mmHg	113 (30.2)
Triglycerides	
< 150 mg/dL (1.6935 mmol/L)	221 (59.6)
LDL-C	
< 100 mg/dL (2.586 mmol/L)	160 (44.2)
HDL-C	
> 40 mg/dL (1.0344 mmol/L)	278 (75.7)

AACE: American Association of Clinical Endocrinologists, AACE guidelines 2010[35]; SBP/DBP: Systolic Blood Pressure/ Diastolic Blood Pressure; LDL-C: Low density lipoprotein-cholesterol; HDL-C: High density lipoprotein-cholesterol.

PANORAMA study results from primary care T2DM patients in Greece are discussed next. The study was performed in 2009-2010 and enrolled 375 subjects from 25 participating study centres.

Previously, other large, multinational European studies have attempted to assess the level of glycaemic control across Europe. The RECAP-DM study for example, that included Finland, France, Germany, Norway, Poland, Spain and United Kingdom, provided data on glycaemic control on T2DM patients who intensified their treatment by adding either a sulphonylurea or a thiazolidinedione to their standard metformin treatment. Approximately, 26% of European out-patients had adequate glycaemic control, *i.e.,* HbA1c < 6.5% (47 mmol/mol), after a mean of 2.6 years of combined oral antihyperglycaemic therapy. It was observed that glycaemic control modestly declined over time, even though more patients were being treated with insulin[26]. Similarly, another earlier European, epidemiological survey on T2DM that provided data on glycaemic control was the CODE-2 study, of which many participating countries were also included in the PANORAMA study. In the CODE-2 study 69% of the patients did not attain the HbA1c target of less than 7%

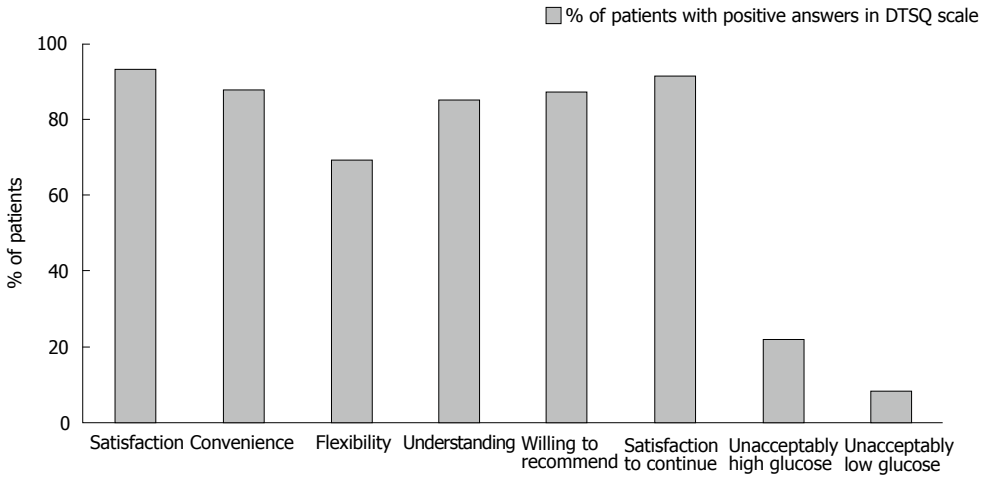

Figure 1 DTSQ questionnaire results (Diabetes Treatment Satisfaction Questionnaire - as assessed by patients) from the Greek PANORAMA study population. Graph presents the percentage of patients that provided positive answers following a series of questions of DTSQ's specific domains related to their treatment satisfaction (grades 4-6 in DTSQ scale).

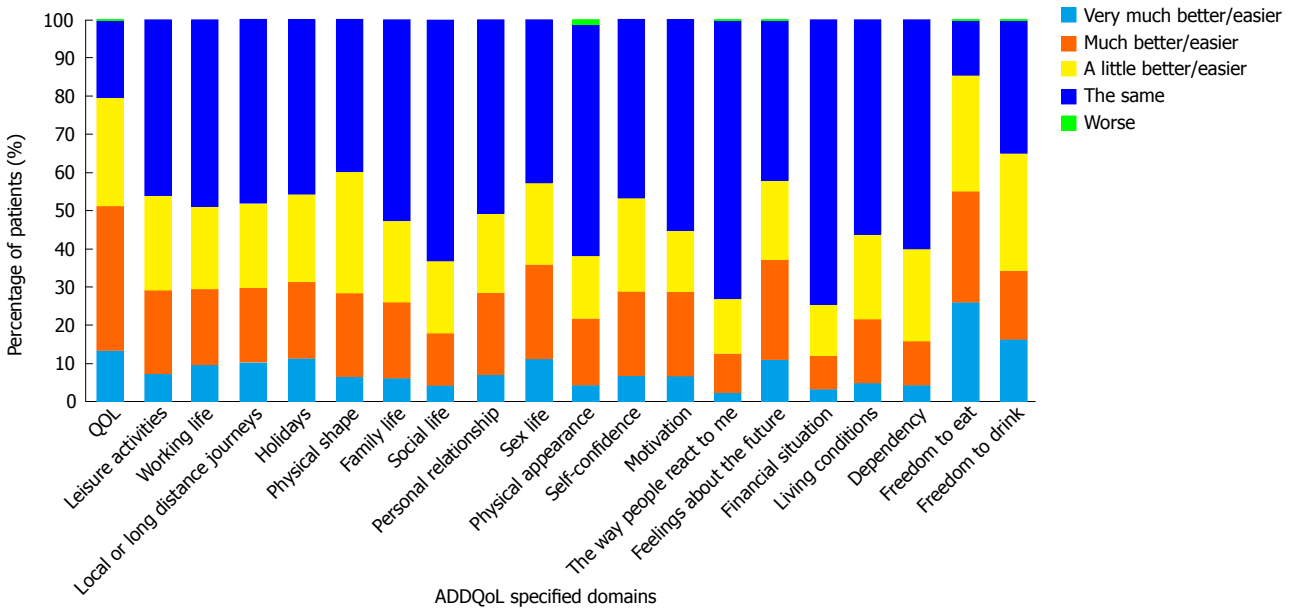

Figure 2 ADDQoL questionnaire results (Audit of Diabetes Dependent QoL) from the Greek PANORAMA study population. ADDQoL is an individualized measure of the impact of diabetes on QoL. Graph presents the distribution of answers reported by patients following the question: "If I did not have diabetes my QoL would be…".

(53 mmol/mol), as opposed to the 37.4% of the patients from the PANORAMA study and 32.9% of the Greek population from the PANORAMA study[24,27]. The much better glycaemic control observed in PANORAMA and its Greek population in comparison to other studies can be attributed to the fact that patients were enrolled in the study only if medical records for at least the past 1 year existed in the study site. This could suggest that the study population was more closely followed.

Regarding CV risk factor control in T2DM patients from the Greek PANORAMA study, data showed that a large percentage of patients failed to meet the recommended target levels for LDL-C, triglycerides and especially blood pressure. This is in line with previous studies conducted in Greece, showing that a considerable percentage of patients do not meet treatment goals for better CV risk control[28,29]. CV risk factors continue to be the most critical determinants of mortality and morbidity in T2DM patients, and account for more than half of the observed mortality and morbidity in this population.

The issue of CV risk factor control emerges as a great challenge in the management and treatment of T2DM patients, especially when joint standards of medical care for patients with diabetes are considered. For example, the present data indicate inadequate control for LDL-C with 55.8% of patients not achieving LDL-C target < 100 mg/dL (2.586 mmol/L). This observation highlights the difficulty in regulating LDL-C and the status of

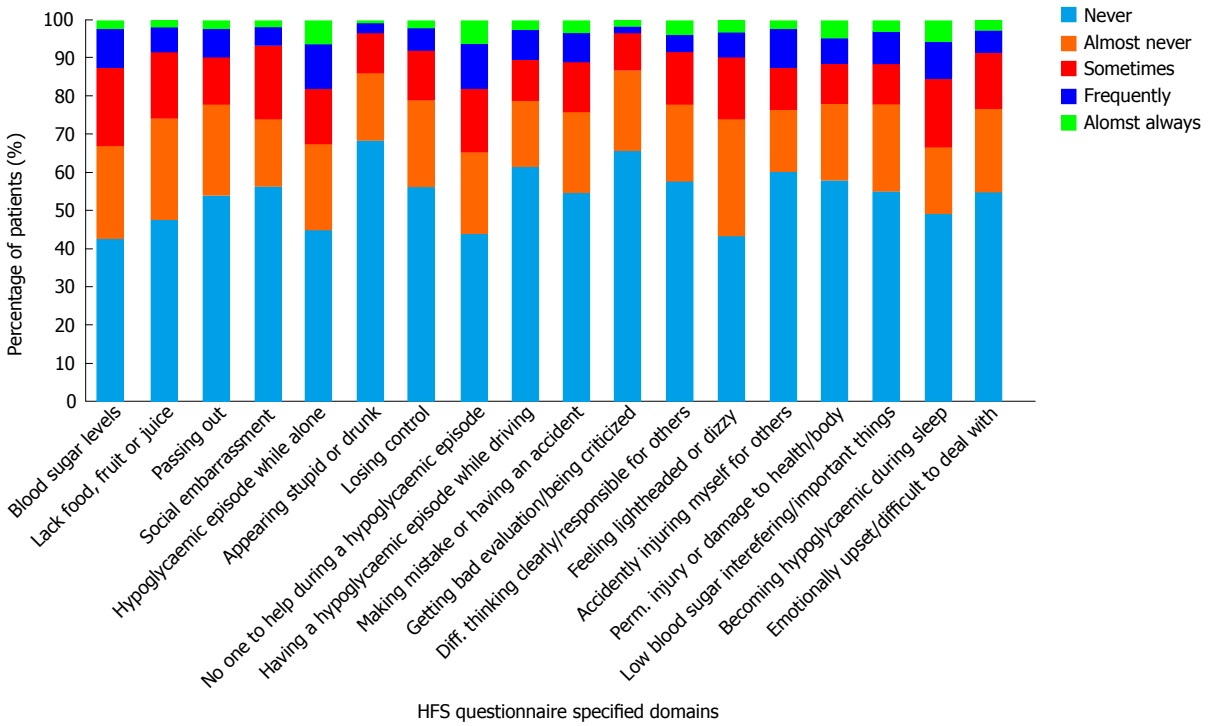

Figure 3 HFS questionnaire results (Hypoglycaemia Fear Survey-II) from the Greek PANORAMA study population. Graph presents the distribution of answers ("never", "almost never", "sometimes", "frequently" and "almost always") following questions in the 18 specific domains of the HFS questionnaire related to patients' fear of hypoglycaemia. Each of the 18 items was preceded by the statement "Because my blood sugar could drop, I worried about …".

current available therapies and drugs.

The difficulty in total cardiovascular risk reduction observed in our results was also clearly shown in the total PANORAMA population recently published by de Pablos-Velasco *et al*[24], that reported that the joint triple target for HbA1c, blood lipids (total cholesterol) and blood pressure was achieved only in the 7.5% of the patients. This observation denotes an unmet medical need and that despite new improvements in pharmacotherapy, still a great deal of work is warranted for better T2DM disease management.

The majority of the Greek PANORAMA study population perceived positively their diabetes treatment. The high scores of the DTSQ questionnaire in the Greek PANORAMA study (29.1 ± 5.6 out of 36), are attributed to the high level of satisfaction reported in the sections concerning satisfaction with treatment, satisfaction to continue with treatment and willingness to recommend treatment to someone else.

DTSQ outcomes have been shown to correlate significantly with the duration of diabetes and the perceived glucose control by the patients, showing that the longer the diabetes duration and the less controlled glucose levels the more patients appear unsatisfied with their treatment[19]. On the other hand, satisfaction also appears to be sensitive to treatment changes[13,30] and differences between treatment groups[31]. Furthermore, in a study about diabetes patients' perception of their disease, a clear correlation was demonstrated between patients' responses to the questionnaire, demographic characteristics, the health status and the type of their anti-diabetic treatment[32].

The overall ADDQoL questionnaire mean score in the present study suggests that diabetes exerts a negative impact on patients' perception of QoL. The QoL parameter identified to be most commonly, negatively affected by diabetes in the study population was freedom to eat as wish, a parameter valued as important or very important from 80.1% of the patients.

Use of the ADDQoL in people with type 1 or type 2 diabetes has shown, on average, almost universally, negative impact of diabetes on all domains[13]. Significantly improved T2DM management has also been shown in non-insulin treated patients without complications in comparison to those insulin-treated with complications[33]. The ADDQoL has also proven useful in detecting the negative impact of diabetes on QoL despite the high levels of treatment satisfaction, measured by the DTSQ[34].

Lastly, the results of the use of the HSF questionnaire as a measure of the impact of hypoglycaemia in the patients' QoL, suggested a presence of a mild fear of hypoglycaemic episodes among the study population. History of hypoglycaemic episodes seems to also play an important role in shaping patients' perceptions on hypoglycaemic events[35].

It was clear from the present data that patients were more often worried about having a hypoglycaemic episode while alone, at a time where no one would be available to help, or during sleep which by itself yields a negative impact on QoL.

The PANORAMA study has some inherent limitations such as the mixing of sampling techniques and

the cross-sectional design of the study that cannot determine the causal nature of the associations[24]. In the present study, the A1CNow® (Bayer) was used to reduce the high variability of blood glucose measurements between centres. In addition, patient recruitment in Greece followed a sequential, rather than randomized manner, which was adopted in other European participating countries. This may raise concerns towards specific variables that could be affected by the lack of randomization at selection, such as duration of diabetes and diabetes-related problems or macro/microvascular complications, since the patients selected solely by their attendance to a participating centre may be more prone to clinic/hospital visits or diabetes related comorbidities and complications than others.

In conclusion, the Greek Panorama study data analysis demonstrated that a considerable part of the T2DM patient population does not achieve glycaemic target levels despite the coincident patient satisfaction by their administered antidiabetic treatment. Despite this high level of satisfaction, a mild fear of hypoglycaemia was detected and a considerable percentage of primary care patients, approximately 1 in 3, did not meet glycaemic goals. In parallel, the majority of the study's population reported that their QoL would be better without diabetes. Finally, since CV risk factors are proven to be inadequately controlled among T2DM patients in Greece, further intensification efforts regarding treatment and management are required to enable better management towards diminishing CV risk and to improve treatment of type 2 diabetes patients.

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COMMENTS

Background

Diabetes mellitus is a common disease with a rising prevalence worldwide. Its complications are divided to macrovascular disease (atherosclerosis) and microvascular disease which includes diabetic neuropathy, retinopathy and nephropathy. Glycaemic control is of paramount importance in patients with diabetes and it can be estimated by glycated haemoglobin (HbA1c) blood levels. According to treatment guidelines, for most patients with diabetes, a level of HbA1c lower than 7% is recommended. There are many antidiabetic medications of different categories that can be administered for blood glucose control as monotherapy or in combination in order to achieve the treatment targets.

Research frontiers

Despite the multiple available antidiabetic treatment options, a large proportion of patients with diabetes do not achieve the glycaemic goals. Furthermore, only a few studies have examined the impact of diabetes on the quality of life as well as the patients' perception of their treatment.

Innovations and breakthroughs

PANORAMA study was a multinational Pan-European study that provided an update on glycaemic control in European patients with type 2 diabetes mellitus. The authors present the results from the Greek population of the study which showed that a large proportion of patients with diabetes do not achieve the glycaemic targets. The other cardiovascular risk factors as LDL cholesterol, triglycerides and blood pressure were also out of control in the majority of Greek patients that were enrolled in our study. Although the majority of the Greek PANORAMA study population perceived positively their diabetes treatment, most of them reported that their life has a negative impact from diabetes, especially because they do not have the freedom to eat and drink.

Applications

The primary care physician must know that most of the Greek patients with diabetes are not under control. More effort is needed to achieve glycaemic targets and cardiovascular risk factors control in these patients. Apart from treatment targets though, it must also be remembered that quality of life in these patients is reduced because of diabetes mellitus. More emphasis must be given on this issue by the physician.

Peer review

The control level of blood glucose seems to be better compared to Steno study.

REFERENCES

1 **Shaw JE**, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Res Clin Pract* 2010; **87**: 4-14 [PMID: 19896746 DOI: 10.1016/j.diabres.2009.10.007]

2 **Panagiotakos DB**, Pitsavos C, Skoumas Y, Lentzas Y, Stefanadis C. Five-year incidence of type 2 diabetes mellitus among cardiovascular disease-free Greek adults: findings from the ATTICA study. *Vasc Health Risk Manag* 2008; **4**: 691-698 [PMID: 18827919]

3 **Panagiotakos DB**, Pitsavos C, Chrysohoou C, Skoumas I, Stefanadis C. Prevalence and five-year incidence (2001-2006) of cardiovascular disease risk factors in a Greek sample: the ATTICA study. *Hellenic J Cardiol* 2009; **50**: 388-395 [PMID: 19767280]

4 **Davies M**. The reality of glycaemic control in insulin treated diabetes: defining the clinical challenges. *Int J Obes Relat Metab Disord* 2004; **28** Suppl 2: S14-S22 [PMID: 15306833 DOI: 10.1038/sj.ijo.0802745]

5 **Fritsche A**, Häring H. At last, a weight neutral insulin? *Int J Obes Relat Metab Disord* 2004; **28** Suppl 2: S41-S46 [PMID: 15306837 DOI: 10.1038/sj.ijo.0802749]

6 **Voorham J**, Haaijer-Ruskamp FM, Wolffenbuttel BH, de Zeeuw D, Stolk RP, Denig P. Differential effects of comorbidity on antihypertensive and glucose-regulating treatment in diabetes mellitus--a cohort study. *PLoS One* 2012; **7**: e38707 [PMID: 22679516 DOI: 10.1371/journal.pone.0038707]

7 **Rubin RR**, Peyrot M. Quality of life and diabetes. *Diabetes Metab Res Rev* 1999; **15**: 205-218 [PMID: 10441043 DOI: 10.1002/(SICI)1520-7560(199905/06)15:3<205::AID-DMRR29>3.0.CO;2-O]

8 **Pladevall M**, Williams LK, Potts LA, Divine G, Xi H, Lafata JE. Clinical outcomes and adherence to medications measured by claims data in patients with diabetes. *Diabetes Care* 2004; **27**: 2800-2805 [PMID: 15562188 DOI: 10.2337/diacare.27.12.2800]

9 **UK Prospective Diabetes Study Group**. Quality of life in type 2 diabetic patients is affected by complications but not by intensive policies to improve blood glucose or blood pressure control (UKPDS 37). U.K. Prospective Diabetes Study Group. *Diabetes Care* 1999; **22**: 1125-1136 [PMID: 10388978 DOI: 10.2337/diacare.22.7.1125]

10 **Eaton WW**, Armenian H, Gallo J, Pratt L, Ford DE. Depression and risk for onset of type II diabetes. A prospective population-based study. *Diabetes Care* 1996; **19**: 1097-1102 [PMID: 8886555 DOI: 10.2337/diacare.19.10.1097]

11 **Egede LE**. Diabetes, major depression, and functional disability among U.S. adults. *Diabetes Care* 2004; **27**: 421-428 [PMID: 14747223 DOI: 10.2337/diacare.27.2.421]

12 **Kovacs M**, Iyengar S, Goldston D, Stewart J, Obrosky DS, Marsh J. Psychological functioning of children with insulin-dependent diabetes mellitus: a longitudinal study. *J Pediatr Psychol* 1990; **15**: 619-632 [PMID: 2283571 DOI: 10.1093/jpepsy/15.5.619]

13 **DAFNE Study Group**. Training in flexible, intensive insulin

management to enable dietary freedom in people with type 1 diabetes: dose adjustment for normal eating (DAFNE) randomised controlled trial. *BMJ* 2002; **325**: 746 [PMID: 12364302 DOI: 10.1136/bmj.325.7367.746]
14 **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]
15 **Anderson RT**, Narayan KM, Feeney P, Goff D, Ali MK, Simmons DL, Sperl-Hillen JA, Bigger T, Cuddihy R, O' Conner PJ, Sood A, Zhang P, Sullivan MD. Effect of intensive glycemic lowering on health-related quality of life in type 2 diabetes: ACCORD trial. *Diabetes Care* 2011; **34**: 807-812 [PMID: 21346183 DOI: 10.2337/dc10-1926]
16 **Biderman A**, Noff E, Harris SB, Friedman N, Levy A. Treatment satisfaction of diabetic patients: what are the contributing factors? *Fam Pract* 2009; **26**: 102-108 [PMID: 19254969 DOI: 10.1093/fampra/cmp007]
17 **Bradley C**, de Pablos-Velasco P, Parhofer KG, Eschwège E, Gönder-Frederick L, Simon D. PANORAMA: a European study to evaluate quality of life and treatment satisfaction in patients with type-2 diabetes mellitus--study design. *Prim Care Diabetes* 2011; **5**: 231-239 [PMID: 21752743 DOI: 10.1016/j.pcd.2011.04.004]
18 **Bradley C**, Lewis KS. Measures of psychological well-being and treatment satisfaction developed from the responses of people with tablet-treated diabetes. *Diabet Med* 1990; **7**: 445-451 [PMID: 2142043 DOI: 10.1111/j.1464-5491.1990.tb01421.x]
19 **Bradley C.** The Diabetes Treatment Satisfaction Questionnaire: DTSQ. Handbook of Psychology and Diabetes: a guide to psychological measurement in diabetes research and practice. Chur: Harwood Academic Publishers, 1994
20 **Bradley C**, Speight J. Patient perceptions of diabetes and diabetes therapy: assessing quality of life. *Diabetes Metab Res Rev* 2002; **18** Suppl 3: S64-S69 [PMID: 12324988 DOI: 10.1002/dmrr.279]
21 **Bradley C**, Todd C, Gorton T, Symonds E, Martin A, Plowright R. The development of an individualized questionnaire measure of perceived impact of diabetes on quality of life: the ADDQoL. *Qual Life Res* 1999; **8**: 79-91 [PMID: 10457741 DOI: 10.1023/A:1026485130100]
22 **Cox DJ**, Irvine A, Gonder-Frederick L, Nowacek G, Butterfield J. Fear of hypoglycemia: quantification, validation, and utilization. *Diabetes Care* 1987; **10**: 617-621 [PMID: 3677982 DOI: 10.2337/diacare.10.5.617]
23 **Irvine A,** Cox D, Gonder-Frederick L. The Fear of Hypoglycaemia Scale. Handbook of Psychology and Diabetes; a guide to measurement in diabetes research and practice, ed. Bradley C. Chur, Switzerland: Harwood Academic Publishers, 1994: 133-155
24 **de Pablos-Velasco P**, Parhofer KG, Bradley C, Eschwège E, Gönder-Frederick L, Maheux P, Wood I, Simon D. Current level of glycaemic control and its associated factors in patients with type 2 diabetes across Europe: data from the PANORAMA study. *Clin Endocrinol* (Oxf) 2014; **80**: 47-56 [PMID: 23194193 DOI: 10.1111/cen.12119]
25 **Depablos-Velasco P**, Salguero-Chaves E, Mata-Poyo J, Derivas-Otero B, García-Sánchez R, Viguera-Ester P. Quality of life and satisfaction with treatment in subjects with type 2 diabetes: results in Spain of the PANORAMA study. *Endocrinol Nutr* 2014; **61**: 18-26 [PMID: 24055176 DOI: 10.1016/j.endonu.2013.05.005]
26 **Alvarez Guisasola F**, Mavros P, Nocea G, Alemao E, Alexander CM, Yin D. Glycaemic control among patients with type 2 diabetes mellitus in seven European countries: findings from the Real-Life Effectiveness and Care Patterns of Diabetes Management (RECAP-DM) study. *Diabetes Obes Metab* 2008; **10** Suppl 1: 8-15 [PMID: 18435669 DOI: 10.1111/j.1463-1326.2008.00881.x]
27 **Liebl A**, Mata M, Eschwège E. Evaluation of risk factors for development of complications in Type II diabetes in Europe. *Diabetologia* 2002; **45**: S23-S28 [PMID: 12136408 DOI: 10.1007/s00125-002-0863-0]
28 **Guallar E**, Banegas JR, Blasco-Colmenares E, Jiménez FJ, Dallongeville J, Halcox JP, Borghi C, Massó-González EL, Tafalla M, Perk J, De Backer G, Steg PG, Rodríguez-Artalejo F. Excess risk attributable to traditional cardiovascular risk factors in clinical practice settings across Europe - The EURIKA Study. *BMC Public Health* 2011; **11**: 704 [PMID: 21923932 DOI: 10.1186/1471-2458-11-704]
29 **Hermans MP**, Brotons C, Elisaf M, Michel G, Muls E, Nobels F. Optimal type 2 diabetes mellitus management: the randomised controlled OPTIMISE benchmarking study: baseline results from six European countries. *Eur J Prev Cardiol* 2013; **20**: 1095-1105 [PMID: 22605788 DOI: 10.1177/2047487312449414]
30 **Witthaus E**, Stewart J, Bradley C. Treatment satisfaction and psychological well-being with insulin glargine compared with NPH in patients with Type 1 diabetes. *Diabet Med* 2001; **18**: 619-625 [PMID: 11553198 DOI: 10.1046/j.1464-5491.2001.00529.x]
31 **Lewis KS**, Bradley C, Knight G, Boulton AJ, Ward JD. A measure of treatment satisfaction designed specifically for people with insulin-dependent diabetes. *Diabet Med* 1988; **5**: 235-242 [PMID: 2967144 DOI: 10.1111/j.1464-5491.1988.tb00976.x]
32 **Marra G**. The DIAB.& amp; TE.S Project: how patients perceive diabetes and diabetes therapy. *Acta Biomed* 2004; **75**: 164-170 [PMID: 15796090]
33 **Sundaram M**, Kavookjian J, Patrick JH, Miller LA, Madhavan SS, Scott VG. Quality of life, health status and clinical outcomes in Type 2 diabetes patients. *Qual Life Res* 2007; **16**: 165-177 [PMID: 17033903 DOI: 10.1007/s11136-006-9105-0]
34 **Speight J,** Bradley C. ADDQoL indicates negative impact of diabetes on quality of life despite high levels of satisfaction with treatment. *Diabetologia* 2000; **43** Supplement 1: A225-A225
35 **Marrero DG**, Guare JC, Vandagriff JL, Fineberg NS. Fear of hypoglycemia in the parents of children and adolescents with diabetes: maladaptive or healthy response? *Diabetes Educ* 1997; **23**: 281-286 [PMID: 9257618 DOI: 10.1177/014572179702300306]

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