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EDITORIAL

Type 2 diabetes among Asian Americans: Prevalence and prevention

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Abstract

Type 2 diabetes mellitus (T2DM) is a growing problem among Asian Americans. Based on the Centers for Disease Control, the age-adjusted prevalence of T2DM for Asian Americans is 9%, placing them at "moderate risk". However differential patterns of disease burden emerge when examining disaggregated data across Asian American ethnic groups; with Filipino, Pacific Islander, Japanese, and South Asian groups consistently described as having the highest prevalence of T2DM. Disentangling and strengthening prevalence data is vital for on-going prevention efforts. The strongest evidence currently available to guide the prevention of T2DM in the United States comes from a large multicenter randomized clinical control trial called the Diabetes Prevention Program, which targets individual lifestyle behavior changes. It has been translated and adopted for some Asian American groups, and shows promise. However stronger study designs and attention to several key methodological considerations will improve the science. Increased attention has also been directed toward population level downstream prevention efforts. Building an infrastructure that includes both individual and population approaches is needed to prevent T2DM among Asian American populations, and is essential for reducing health disparities.

Key words: Type 2 diabetes mellitus; Asian American; Prevalence; Prevention; Health disparity

Core tip: Current estimates suggest that type 2 diabetes affects approximately 9% of Asian Americans overall. However, when examining disaggregated data across different ethnic groups Filipino, Pacific Islander, Japanese, and South Asian groups consistently have the highest prevalence of type 2 diabetes mellitus. This highlights how aggregating Asian Americans into one category can potentially mask the disease burden in high risk groups, while inflating the burden in low risk groups. Prevention efforts therefore need be culturally tailored to meet the unique needs of the various Asian American ethnic groups. In addition, prevention efforts should address both individual and population level strategies.

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a growing epidemic in the United States. In 2012, over 21 million people were diagnosed with the disease as compared to 1.6 million in 1958[1,2]. Moreover, the current cost associated with DM care in the United States is estimated at $113 billion, and is expected to escalate to $336 billion by 2034[3]. The high prevalence of T2DM has been well documented for Native Americans, non-Hispanic Blacks, and Hispanic Americans[4]. However, increasing attention has been drawn to the problem of T2DM among Asian Americans[5,6], one of the fastest growing racial/ethnic minority groups in the United States[7]. Disentangling and strengthening prevalence data will provide more support for activities and resources to address this important health problem among Asian Americans. In addition, understanding best practices and building an infrastructure to prevent T2DM among Asian American populations is essential for reducing health disparities, and more broadly for curbing the growing epidemic of T2DM in the United States.

PREVALENCE OF T2DM

For many years, health services for Asian American populations have been hampered by the model minority myth; the notion that Asian Americans are self-sufficient, well-educated, and have lower burdens of disease[8,9]. In large part, the myth was perpetuated by the lack of reliable data that often lumped Asian Americans into one large category, when in fact they represent a heterogeneous group. This issue is particularly relevant when examining the prevalence of T2DM among Asian Americans. For example, the 2014 Centers for Disease Control (CDC) report estimated that the age-adjusted prevalence of T2DM for Asian Americans as a whole was 9%. This rate is lower than that of Native Americans (15.9%), non-Hispanic Blacks (13.2%), and Hispanic Americans (12.8%), but higher than that of non-Hispanic Whites (7.6%); placing Asian Americans at "moderate risk" for T2DM[2]. When examining disaggregated data across various Asian American ethnic groups though, differential patterns of disease burden emerge.

For example, a study by Choi *et al*[10] using population based data from the 2009 California Health Information Survey (CHIS) found that Native Americans, non-Hispanic Blacks, and Hispanic Americans had higher overall age-adjusted prevalence of T2DM than Asian Americans (as a whole); supporting findings from the CDC report. However, a more complex story is revealed when the data for Asian Americans are disaggregated across six different ethnic groups. Based on disaggregated data, Filipinos had the highest age-adjusted prevalence (15.8% men, 9.4% women), followed by Japanese (11.8% men, 7.6% women), Korean (6.7% men, 5.1% women), South Asian (6.3% men, 2.7% women), Chinese (5.0% men, 3.6% women), and Vietnamese (2.5% men, 2.1% women). These results demonstrated that among some Asian American groups the age-adjusted prevalence of T2DM was even higher than in non-Hispanic Black (8.8% men, 13.3% women), and Hispanics American (6.7% men, 10.7% women) groups. In particular, Filipino (15.8%) and Japanese (11.8%) American men were found to have among the highest rates of T2DM. Other population based prevalence studies have reported similar findings, with Filipino, Pacific Islander, Japanese, and South Asian groups consistently described as having the highest prevalence of T2DM across all Asian American ethnic subgroups[11-14]. These examples highlight how aggregating Asian Americans into one category can potentially mask the disease burden in high risk groups, while inflating the burden in low risk groups.

While disaggregated data has clear advantages, much of the data available on the prevalence of T2DM among Asian Americans is at the aggregate level. When exploring aggregate data, there are some important patterns that can be used to underscore the urgency of addressing T2DM in Asian American populations. Specifically, when examining trends over time, several methodologically rigorous studies suggest that the prevalence of T2DM is increasing faster among Asian Americans than non-Hispanic Whites, non-Hispanic Blacks, and Hispanic Americans[15-17]. Moreover, in a study that used fasting plasma glucose test to estimate the prevalence of T2DM, Asian Americans had higher levels of "pre-diabetes" than non-Hispanic Whites, non-Hispanic Blacks, and Hispanic Americans[18]; foreshadowing further potential increases in the burden of T2DM among Asian Americans over the next few decades. Additionally, across DM prevalence studies that measure Body Mass Index (BMI), the relationship between T2DM and BMI appears to be different among Asians Americans than non-Hispanic Whites, with Asian Americans reporting T2DM at significantly lower BMI levels[12,13,16,19]. These data provide strong evidence of disparities in T2DM among and across various Asian American ethnic groups, and underscores the urgency of addressing T2DM in Asian American populations.

To strengthen future prevalence data, providing disaggregated rates across ethnic subgroups is important. In addition, to simulate demographic data collected at the state and national level, it is helpful to report prevalence rates based on aggregated categories of "Asian" and "Asian Pacific Islander"

groups. When the goal of measuring prevalence is to compare rates across groups and time, it is also important to make sure that: (1) data are collected using a random population-base sampling strategy rather than with convenient samples; (2) adjustments are made to account for age given that T2DM significantly increases with age; and (3) rates are reported with their associated 95% confidence interval, which highlight the fact that prevalence rates are estimates of the population burden and allow readers to assess the precision of the estimate. These strategies will ensure that high quality data are collected and reflective of the realities and needs of the diverse Asian American population. Continued work in this area is essential to garnering ongoing support and resources to address the growing problem of T2DM among Asian Americans.

PREVENTION OF T2DM

The strongest evidence currently available to guide the prevention of T2DM in the United States comes from a large multicenter randomized clinical control trial (n = 3234) called the Diabetes Prevention Program[20]. The program targets individuals with "pre-diabetes," and includes a 16-session "lifestyle" curriculum covering diet, exercise, and behavior change. Each session is taught on a one-to-one basis by a case manager trained in motivational interviewing techniques. In addition, six follow-up sessions are provided on a monthly basis to reinforce behavior changes. The main goals of this intervention include a 5%-7% weight reduction and $\geqslant$ 150 min of moderate physical activity per week. Study results demonstrated that this intervention reduced the development of diabetes by 58%[20], and that the protective benefits persisted over 10 years[21].

While the aims of the Diabetes Prevention Program are straight forward, the resource burden and lack of cultural relevance associated with the program have resulted in calls to test new models of program delivery, as well as to translate the program for use in minority populations[22]. Several studies have demonstrated success in adopting the program in a variety of different minority populations; however they have largely been done with non-Hispanic Black and Hispanic American groups[23,24]. Efforts to translate these findings with Asian American populations are limited, with most published studies designed at the pilot or quasi-experimental level, and focused on select Asian American ethnic subgroups including Chinese, Korean, Filipino, Pacific Islanders, and South Asians[14,25-28]. Continued work in this area is critical because the diets and cultural norms among Asian Americans are vastly different from the general population, and vastly differ across Asian American ethnic groups[29].

This heterogeneity likely explains most of the variance in prevalence rates described earlier, and highlights the importance of culturally tailoring prevention interventions for a given ethnic subgroup. In 2008, the National Institutes of Health held a workshop to discuss strategic options to further investigate cardio-metabolic diseases among Asian American populations in the United States[30]. Among their recommendations, the need to further understand dietary and physical fitness habits of Asian American subgroups was highlighted as a critical component to successfully tailoring interventions. Specifically, they suggests that more research is needed to understand the social context of eating, shopping, cooking, household dynamics on food choices (particularly related to influences on traditionally high carbohydrate diets and unhealthy eating patterns), as well as feasibility, perceptions, barriers (*i.e.,* social role strain), outliers, and motivators to exercising (perhaps by contrasting Asians who exercise and those who do not). To examine these topics, in-depth qualitative and mixed-methods studies will be vital. Of special note- given that the Asian American population represents individuals from over 60 countries with varying languages, cultures, and immigration status, a working group associated with the National Heart Lung and Blood Institute suggests clustering Asians into manageable groups that have similar risk profiles as a way to save cost and ensure broad generalization[31]. These groups include: East Asians, South Asians, Southeast Asians, and Hawaiian/Pacific Islanders.

In addition to exploring these topics, future work aimed at adopting and/or translating the Diabetes Prevention Program should also consider some of the following opportunities and challenges. First, given that Asian Americans tend to have T2DM at lower BMI levels, expanding the inclusion criteria to include individual with BMI values between 23-25 kg/m^2 may be warranted. Related to this, it is advisable to also measure waist circumference, a potentially stronger predictor of DM risk given that Asian Americans tend to gain weight around the abdomen (*i.e.,* central adiposity). Second, many of the existing studies highlighted earlier use community health workers (CHWs) as the "interventionist". Future studies should describe in more detail how standardized training was provided for CHWs; this will enhance intervention fidelity, and provide stronger evidence for the value and use of CHWs. Other "burning" questions that will help move the field forward include evaluating whether or not outcomes differ when the interventionist is a "Certified Diabetes Educator", a designation that requires advanced training and increased cost. Addressing these questions will further promote the benefits of interventions like the Diabetes Prevention Program, and make them more generalizable to Asian American populations.

While there are clear benefits to interventions that target individual lifestyle changes, our current efforts underestimate how hard it is to change behavior not just once or twice, but every day of our

lives. As such, efforts directed toward population level downstream prevention efforts have gained increased attention. Primordial prevention refers to activities (*i.e.,* interventions and policies) that are put in place to prevent the development of risk factors (*i.e.,* obesity, inactivity, poor diet, and chronic stress) in the first place[32]. Creating optimal defaults is the prevailing strategies for addressing primordial prevention. This involves creating environments that enables healthy choices and behaviors for all people across all age groups. This approach necessarily requires a population perspective that engages members of the community not traditionally involved with healthcare (*e.g.,* Boards of Education, Parks and Recreation, Department of Housing, Transportation, and Social Services). Engaging with communities and working with various stakeholders to change the environment (*i.e.,* improve social determinants of health) is especially essential in Asian American communities where coordinated advocacy work may not be as strong.

Whether prevention approaches are targeted at the individual or population level, some final thoughts that are important to consider include upfront planning to (1) estimate the cost and quality of these interventions, and (2) build in continuous longitudinal evaluation. This information will become increasingly relevant as the United States healthcare system enters an era of accountability. Together with improved reporting of prevalence data, these cumulative efforts will ensure that a strong infrastructure is built to prevent T2DM among and across Asian American populations.

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EDITORIAL

Adipose tissue fibrosis

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Abstract

The increasing prevalence of obesity causes a major interest in white adipose tissue biology. Adipose tissue cells are surrounded by extracellular matrix proteins whose composition and remodeling is of crucial importance for cell function. The expansion of adipose tissue in obesity is linked to an inappropriate supply with oxygen and hypoxia development. Subsequent activation of hypoxia inducible factor 1 (HIF-1) inhibits preadipocyte differentiation and initiates adipose tissue fibrosis. Thereby adipose tissue growth is limited and excess triglycerides are stored in ectopic tissues. Stressed adipocytes and hypoxia contribute to immune cell immigration and activation which further aggravates adipose tissue fibrosis. There is substantial evidence that adipose tissue fibrosis is linked to metabolic dysfunction, both in rodent models and in the clinical setting. Peroxisome proliferator activated receptor gamma agonists and adiponectin both reduce adipose tissue fibrosis, inflammation and insulin resistance. Current knowledge suggests that antifibrotic drugs, increasing adipose tissue oxygen supply or HIF-1 antagonists will improve adipose tissue function and thereby ameliorate metabolic diseases.

Key words: Collagen; Hypoxia; Insulin resistance; Immune cells; Adipocyte

Core tip: The close association of adipose tissue fibrosis and metabolic complications in obesity has been corroborated in rodent and human studies. Adipose tissue hypoxia initiates fibrosis which is further aggravated by inflammation. In adipose tissue preadipocytes, adipocytes and resident macrophages produce collagen showing that the fibrotic process differs from the extensively studied scar formation in the liver. Strategies to resolve fibrosis in fat tissues thereby promoting healthy adipose tissue growth are suggested to improve metabolic situation in obese patients.

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INTRODUCTION

Obesity related diseases including type 2 diabetes and non-alcoholic fatty liver disease have become a major health problem. Inappropriate insulin production, insulin

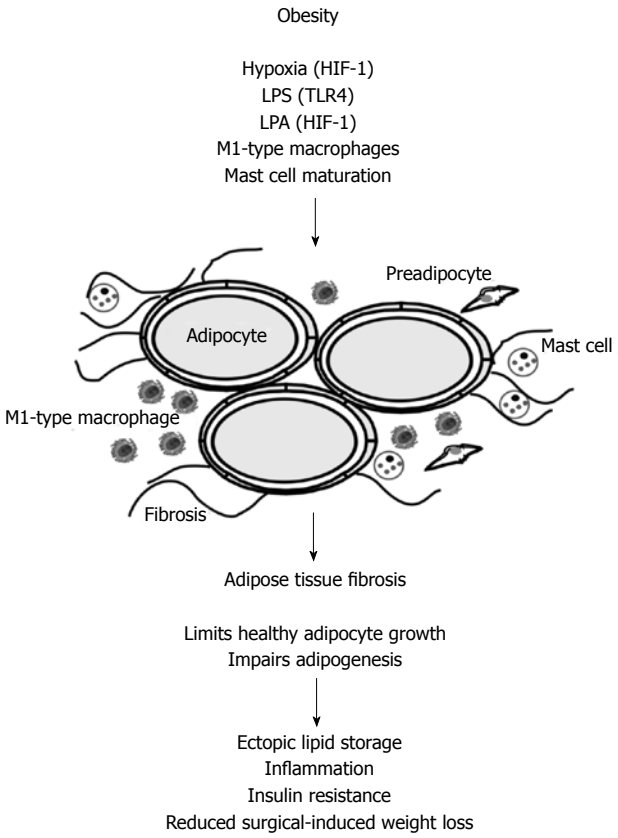

Figure 1 In obese adipose tissue hypoxia, lipopolysaccharide, lysophosphatidic acid, M1-type macrophages and maturated mast cells contribute to adipose tissue fibrogenesis. Hypoxia and LPA mediated effects involve activation of HIF-1 and LPS activates TLR4. Fibrosis impairs adipogenesis and healthy adipocyte growth. Lipids are therefore stored in peripheral tissues like the liver. This is associated with impaired insulin sensitivity and inflammation. Adipose tissue fibrosis negatively affects surgery-induced weight loss. LPS: Lipopolysaccharide; LPA: Lysophosphatidic acid; HIF-1: Hypoxia inducible factor 1.

resistance and dyslipidemia are commonly associated with obesity. It is widely accepted that adipose tissue dysfunction is the major underlying reason for metabolic diseases in obesity[1-3].

White adipose tissue is a highly dynamic organ which rapidly responds to nutrient excess and shortage. In obesity adipose tissue expands by hypertrophy and hyperplasia[2,4]. In epididymal fat of rodents fed a high fat diet adipogenesis is detected after four weeks feeding while subcutaneous fat expands by hypertrophy for up to twelve weeks[5]. Distinct adipose tissue depots also differ in gene expression, adipokine release and function[4,6]. Accumulation of visceral adipose tissue is an independent risk factor for metabolic diseases while gain of subcutaneous fat may even be protective[2,4]. The mechanisms regulating fat pad weight and distribution of body fat are, however, not well understood.

Macrophages are localized in adipose tissues and their number is strongly increased in obesity[2,4]. Macrophages are classified as M1 and M2 types which is a very simplified approach in view of the high diversity of these cells[7]. M1 cells express proinflammatory factors and M2 cells anti-inflammatory proteins. Adipose tissue resident macrophages in the lean state are polarized to the M2 type and in the obese state to the M1 type (Figure 1). Various studies demonstrate a close association between adipose tissue resident macrophages and insulin resistance[8].

Adipocyte inflammatory pathways are, however, essential for adipose tissue growth. Fat tissue expression of: (1) a dominant-negative tumor necrosis factor (TNF); (2) RIDα/β, an adenoviral protein complex that inhibits proinflammatory signaling pathways like toll-like receptor 4 (TLR4)-, TNF- and IL-1 beta-mediated signaling; and (3) a mutated human IκBalpha which inhibits NFκB pathway, impairs adipogenesis and intestinal barrier function and favors ectopic lipid storage, systemic inflammation and insulin resistance. Therefore, adipocyte inflammation may be an adaption to an increased fat storage demand[9].

Not all of the obese suffer from metabolic diseases. Obese people protected from metabolic complications display reduced adipocyte stress, lower inflammation and less accumulation of central fat than obese insulin-resistant individuals. Serum adiponectin is similar to levels in normal-weight controls. Adipose tissue growth of these individuals does not provoke adipocyte dysfunction, inflammation and fibrosis[10]. Evaluation of the mechanisms underlying healthy and unhealthy obesity will help to identify the pathways associated with metabolic disturbances.

Research during the last 20 years revealed that rapid adipose tissue expansion is linked to adipocyte dysfunction[2,3,11]. Adipose tissue inflammation, adipocyte death, low adiponectin, systemic inflammation, increased lipolysis and more recently adipose tissue fibrosis have been identified in obesity and are clearly associated with metabolic disturbances[2,3,11].

ADIPOSE TISSUE EXTRACELLULAR MATRIX

Extracellular matrix proteins in adipose tissues regulate mechanical properties, adipogenesis and lipid droplet growth[12,13]. Disruption of collagens impairs triglyceride storage during adipocyte differentiation and collagen 5 (COL5) and COL6 are essential for proper adipogenesis[13]. High flexibility of the extracellular matrix guarantees healthy adipose tissue expansion. Inappropriately increased and rigid extracellular matrix hinders adipose tissue growth and promotes local and systemic pathologies associated with obesity[3,14].

Fibrosis has been extensively studied in the liver. Liver injury activates "quiescent" hepatic stellate cells and these cells start to proliferate, synthesize connective tissue growth factor (CTGF) and extracellular matrix proteins. Transforming growth factor beta (TGF-β) is the main profibrotic factor in liver fibrosis and upregulates CTGF. CTGF stimulates binding of TGF-β to its receptor and thereby enhances TGF-β activity. CTGF is induced by TGF-β indicating an autocrine or paracrine loop that mutually enhances synthesis of both proteins[1]. TGF-β also upregulates

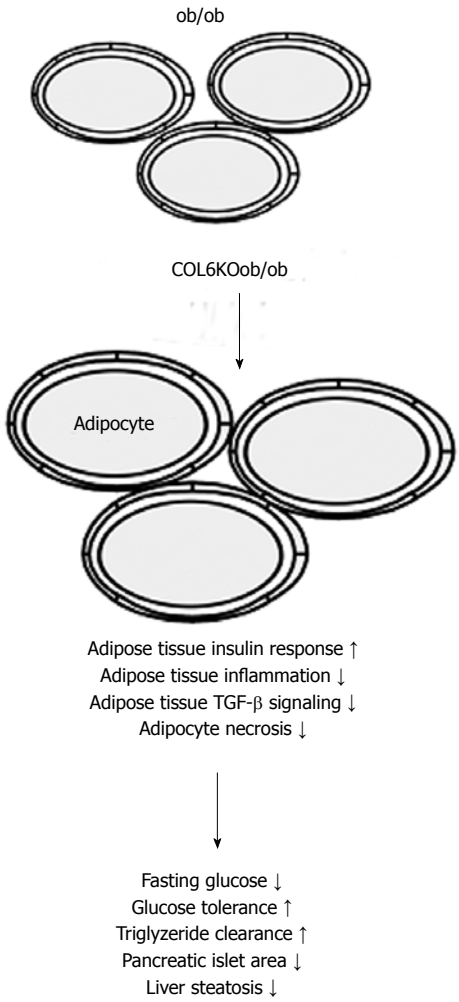

Figure 2 Mice with leptin deficiency (ob/ob mice) have large adipocytes and their size further increases when collagen 6 (COL6KOob/ob) is knocked-out in these animals. Weakening of the extracellular matrix is associated with improved adipose tissue insulin response, reduced inflammation and TGF-beta signaling, and diminished adipocyte necrosis. Subsequently metabolic situation is improved.

CTGF in adipocytes which has been shown to inhibit adipogenesis[15]. TGF-β correlates with adiposity in humans and rodents. Blockage of TGF-β signaling protects from obesity, insulin resistance and fatty liver. Beneficial effects are partly explained by browning of white adipose tissue[16]. In fat tissue, preadipocytes, adipocytes and macrophages produce collagens demonstrating differences in adipose tissue and liver fibrosis where alpha-smooth muscle actin and collagens are mainly synthesized by activated hepatic stellate cells[1,2,17,18].

HYPOXIA IN ADIPOSE TISSUE FIBROSIS

Adipose tissue grows by hyperplasia and hypertrophy which leads to a hypoxic state. Oxygen levels are markedly reduced in white fat of obese rodents and are also lower in white adipose tissues of humans. In obese adipose tissue capillary density is reduced and more large vessels are detected[19].

Hypoxia-inducible factor 1 (HIF-1) is activated when oxygen is low. Hypoxia is supposed to induce tissue fibrosis, and collagen, type I, alpha 1 (COL1A1), COL3A1 and the enzyme lysyl oxidase with a central role in collagen cross-linking are increased when mice are exposed to low oxygen. The HIF-1α inhibitor PX-478 and expression of dominant negative HIF-1α block high fat diet induced HIF-1α activation, lower body weight gain and antagonize the development of metabolic diseases. Adipose tissue fibrosis and inflammation are improved[20]. Therefore, hypoxia mediated activation of HIF-1 seems to be critically involved in limiting healthy adipose tissue growth (Figure 1).

Hypoxia and HIF-1 activation is also believed to significantly contribute to fibrogenic progression of chronic liver diseases and HIF-mediated processes independent of hypoxia have also been described to be involved herein[21].

Hypoxia stimulates cytokine and chemokine release from adipose tissue resident macrophages and inhibits preadipocyte differentiation by lowering peroxisome proliferator-activated receptor gamma (PPARγ) in these cells. Therefore, hypoxia is suggested to link adipose tissue growth, inflammation and adipose tissue fibrosis. Improving adipose tissue angiogenesis lowers hypoxia, HIF-1α, TGF-β pathway and fibrogenesis[22].

COL6 IN ADIPOSE TISSUE FIBROSIS

Inflammation and adipocyte death are reduced by the absence of COL6 which is a highly abundant extracellular matrix component of rodent fat tissues. The increased flexibility of the extracellular matrix enables healthy adipocyte growth, lowers local and systemic inflammation and improves metabolic disease (Figure 2). PPARγ agonist and adiponectin reduce adipose tissue collagens in mice demonstrating that improvement of adipose tissue extracellular matrix composition is one of their beneficial features[23].

The carboxy-terminal domain cleaved from COL6A3 promotes adipose tissue fibrosis, angiogenesis and inflammation. Increased production of this so called endotrophin in obesity contributes to metabolic disturbances. Blockage of endotrophin by a neutralizing antibody protects from adverse metabolic effects of high fat feeding[24]. Higher BMI is a risk factor for cancer and cancer-related deaths. Endotrophin enhances tumor growth and metastasis and thus may be one of the factors connecting obesity and malignant diseases[25,26].

IMMUNE CELLS IN ADIPOSE TISSUE FIBROSIS

Increased cell death of adipocytes in obesity may be partly caused by the rigid extracellular matrix. Crown-like structures representing macrophages surrounding dead adipocytes are characteristic for obese adipose tissue and correlate with the extent of interstitial

fibrosis. Macrophage-inducible C-type lectin is expressed in these macrophages and enhances the formation of crown-like structures, activates myofibroblasts and expression of profibrotic genes[27]. Macrophage depletion and blockage of TLR4 signaling improve while infusion of lipopolysaccharide aggravates adipose tissue fibrosis further confirming a central role of immune cells in fat tissue dysfunction[28].

Mast cells are well known mediators of allergic reactions and it is known for a long time that their number is increased in obese adipose tissue[29]. Mast cells release inflammatory mediators and promote immune cell recruitment. Obese animals lose body weight after mast cell inactivation[30]. Progression of obesity is associated with mast cell maturation which induces COL5 shown to inhibit adipogenesis[31] (Figure 1). COL5 has also been found to be important for adipocyte maturation demonstrating that different experimental designs reveal discordant results[13]. Mast cells in human fat are activated and are mainly localized in fibrotic regions. Mast cell number is positively associated with fibrosis and macrophage accumulation[32].

LYSOPHOSPHATIDIC ACID AND FIBROSIS

Autotaxin is a secreted lysophospholipase D and hydrolyzes lysophosphatidylcholine to produce lysophosphatidic acid. Autotaxin is increased in obesity and liver fibrosis[33]. Lysophosphatidic acid inhibits adipogenesis and mice with an adipocyte-specific knockout of the lysophosphatidic acid receptor 1 (LPAR1) or treated with the receptor antagonist Ki16425 gain more weight and accumulate more adipose tissue. Despite being more obese animals show improved glucose tolerance[34]. Treatment of db/db mice with the LPAR antagonist Ki16425 reduces COL1 and COL4 mRNAs and collagen protein in inguinal and perigonadal adipose tissues. Human adipose tissue explants release autotaxin spontaneously and its levels increase over time. Lysophosphatidic acid in supernatants increases in parallel along with elevated expression of COL1 and COL3, TGF-β and alpha smooth muscle actin and higher level of collagen protein. *In vitro* fibrosis is blocked by the LPAR antagonist and interestingly by the HIF-1α inhibitor YC-1 while it is further increased by oleoyl-lysophosphatidic acid[35] (Figure 1). Upregulation of HIF-1α by lysophosphatidic acid has been shown in colon cancer cells[36]. Current data suggest that HIF-1 is involved in fibrotic processes even in the absence of hypoxia.

COLLAGEN EXPRESSION IN HUMAN OBESITY

In humans COL6A3 is mainly expressed by stromal vascular cells and is higher in subcutaneous than omental fat depot. In both adipose tissues its expression is reduced in obesity and increases upon weight loss in subcutaneous fat. Leptin dose dependently decreases COL6A3 demonstrating a role of this adipokine in adipose tissue extracellular matrix organization[37]. Animal studies have proven that leptin directly promotes liver fibrogenesis. Leptin induces COL1, TGF-β and CTGF in hepatic stellate cells and this effect is mediated *via* enhancing TGF-β release from Kupffer cells[38]. Whether leptin exerts opposing effects in the liver and adipose tissue or whether its activity may be affected by adipose tissue macrophages needs further studies.

In humans with a BMI between 35 and 55 expression of COL3A1, COL5A2 and COL6A3 is lower in omental and subcutaneous adipose tissue of those suffering from the metabolic syndrome compared to the healthy obese[39].

In contrast, positive correlations of COL6A3 expression in abdominal subcutaneous fat with body mass index (BMI) and fat mass have been described in a further study while an association with type 2 diabetes has not been identified. Elevated COL6A3 mRNA levels are found in patients with greater visceral fat mass and higher inflammation. Eight weeks overfeeding increases and pioglitazone reduces COL6A3 expression. Further, in abdominal subcutaneous adipose tissue COL5 is higher expressed in the obese than the lean[40].

In subcutaneous abdominal adipose tissue of patients with a wide range of BMI (19-40 kg/m^2) there is a strong positive correlation of COL6 and CD68 mRNA expression. COL6 and CD68 expression are associated with BMI and inversely with insulin sensitivity. Fibrotic areas are increased in the obese fat tissue and are associated with macrophage number and negatively correlate with insulin sensitivity. Alternatively activated macrophages localize to fibrotic regions and express TGF-β[18]. COL5 is increased and elastin is reduced in obesity[19]. Collagens quantified by picrosirius red staining are found increased in subcutaneous and visceral adipose tissues of obese *vs* lean patients[32].

Picrosirius red has also been used to quantify subcutaneous and omental fat fibrosis in a further investigation. Positive associations with liver fibrosis and systemic IL-6 but not lipid and glucose parameters of the patients have been identified. The subcutaneous white adipose tissue stiffness measured by shear-wave velocity using a prototype vibration-controlled transient elastography method positively correlates with fasting glycemia and insulin, HbA1C and fat-free mass, and negatively with body fat and HDL cholesterol. Diabetes status is also significantly associated with increased shear-wave velocity. These data suggest that subcutaneous white adipose tissue stiffness is not solely defined by collagen content. Cross-linking of collagens and other extracellular matrix proteins such as elastin, laminin, and fibronectin most likely contribute to tissue rigidity[41].

In summary most data in humans find increased adipose tissue fibrosis in obesity. There is, however, no simple explanation for the discrepant results on

the expression of single collagen species which are found reduced and induced in human obesity. Whether this is somehow related to differential composition of the extracellular matrix of the patients analyzed or to regional variations in adipose tissues needs further analysis. Techniques to directly measure adipose tissue stiffness may be more appropriate than determining mRNA expression of individual genes for the analysis of adipose tissue fibrosis.

ADIPOSE TISSUE FIBROSIS AND WEIGHT LOSS

Mild to modest liver fibrosis is reversible[42] and previous studies have shown that adipose tissue collagen levels are reduced by adiponectin and treatment with PPARgamma agonists in mice[23]. In patients, transcriptional and histological analysis of subcutaneous adipose tissue revealed persistence of fibrosis two years after bariatric surgery. As expected, adipocyte hypertrophy and inflammatory infiltration are improved[43]. Adipose tissue fibrosis is even found negatively associated with surgery-induced weight loss[44]. These findings have been confirmed in a second cohort and the association between collagen expression in white adipose tissue and gastric bypass induced weight loss persists even when age, diabetes and IL-6 have been considered[41]. It has also been shown that diet and surgery-induced weight loss increase COL6A3 expression in subcutaneous adipose tissue in accordance with low COL6A3 expression in the fat tissues of obese patients described in this study[37].

CONCLUSION

Adipose tissue fibrosis limits healthy growth of adipose tissue and is associated with metabolic complications in obesity. Hypoxia and subsequent activation of HIF-1 initiate profibrotic mechanisms in fat tissues. Fibrosis in obese fat tissues is not resolved upon weight loss and is even negatively associated with surgical-induced body weight reduction. More detailed analysis of the composition of extracellular matrix, biologic function of the individual constituents and non-invasive techniques to determine adipose tissue fibrosis will give further insights into the complex association of extracellular matrix proteins and metabolic health.

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REVIEW

Toxic stress, inflammation and symptomatology of chronic complications in diabetes

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Abstract

Diabetes affects at least 382 million people worldwide and the incidence is expected to reach 592 million by 2035. The incidence of diabetes in youth is skyrocketing as evidenced by a 21% increase in type 1 diabetes and a 30.5% increase in type 2 diabetes in the United States between 2001 and 2009. The effects of toxic stress, the culmination of biological and environmental interactions, on the development of diabetes complications is gaining attention. Stress impacts the hypothalamus-pituitary-adrenal axis and contributes to inflammation, a key biological contributor to the pathogenesis of diabetes and its associated complications. This review provides an overview of common diabetic complications such as neuropathy, cognitive decline, depression, nephropathy and cardiovascular disease. The review also provides a discussion of the role of inflammation and stress in the development and progression of chronic complications of diabetes, associated symptomatology and importance of early identification of symptoms of depression, fatigue, exercise intolerance and pain.

Key words: Toxic stress; Type 1 diabetes; Inflammation; Type 2 diabetes; Chronic complications; Symptomatology

Core tip: The incidence of diabetes and associated complications are increasing. Toxic stress and inflammation may be contributors to the development and progression of diabetes complications. Current evidence supports early identification of symptoms of toxic stress for preventative strategies of associated risks for diabetes complications as well as assessment of the exacerbation of symptoms related to neuropathy, cardiovascular disease and nephropathy.

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INTRODUCTION

The notion that exposure to chronic stressors predisposes individuals to developing diabetes or succumbing to worsening diabetes complications has gained

attention in recent years[1-4]. The global epidemic of both type 1 and type 2 diabetes[5-7] is occurring in an era of worldwide threats to personal, organizational and societal security due to psychosocial and economic burdens. According to the International Diabetes Federation, diabetes affects at least 382 million people worldwide, and that number is expected to reach 592 million by the year 2035[8]. Although it is well-known that type 2 diabetes comprises the largest proportion of affected individuals, the number of individuals with type 1 diabetes around the world is increasing as well. Worldwide estimates for type 1 diabetes are unknown, but are estimated to be up to 3 million in the United States[9]. A recent report on the prevalence of type 1 diabetes in youth in the United States indicated a 21 percent increase between 2001 and 2009. At the same time, rates of type 2 diabetes in youth rose 30.5%[10].

In the midst of this public health crisis, there is tremendous need to embrace the impact of "toxic stress" from biological and environmental interactions on the development of chronic complications in persons living with diabetes. Toxic stress can result from strong, frequent, or prolonged activation of the body's stress response systems, particularly in the absence of protective mechanisms through daily coping strategies and healthy interpersonal relationships[11]. The impact of toxic stress is apparent in current society and is garnering a paradigm shift regarding a more comprehensive understanding of health and disease across the lifespan[11,12]. Toxic stress can be viewed as the catalyst of a physiological memory that confers life-long risk for disease, especially due to inflammatory processes, well beyond its time of origin[13]. How individuals, institutions, and governments respond to these stressors can have an enormous effect on the collective health of a nation. Health care clinicians serve on the front line of care delivery for identifying the most vulnerable individuals for the ravages of diabetes complications through an understanding of underlying etiologies associated with toxic stress and recognition of resultant symptomatology.

With the growing numbers of individuals diagnosed with diabetes, particularly in younger cohorts, the disease burden is ever apparent, as is the importance of minimizing the role of toxic stress on associated diabetes complications. According to Shonkoff[14], the future consequences of significant adversity and chronic stress in early childhood extend beyond socioemotional and cognitive development. They also have significant implications for the pathogenesis of adult disease[15], including biological manifestations of alterations in immune function[16] and measurable increases in inflammatory markers[17,18] that are known to be associated with poor health outcomes such as cardiovascular disease[19-21], liver cancer[22], asthma[23], chronic obstructive pulmonary disease[24], autoimmune diseases[25], poor dental health[26], and depression[27-29]. Although there is no absolute evidence that chronic stress has a direct effect on the development of diabetes in adults or children, stress can influence the onset of type 2 diabetes secondary to obesity and metabolic syndrome[2].

With regard to the effects of stress on the neuroendocrine system, the hypothalamus-pituitary-adrenal (HPA) axis exerts considerable importance[30]. Upon experiencing a stressor, the hypothalamus secretes corticotropin-releasing factor, which causes the release of adrenocorticotropin (*i.e.,* ACTH). This in turn stimulates the adrenal cortex, which leads to the secretion of glucocorticoid hormones, in particular cortisol. Under normal circumstances, cortisol is secreted according to a circadian rhythm, with cortisol levels highest in the morning and lowest in the evening. However, exposures to stress stimulate the HPA axis to release additional amounts of cortisol to maintain homeostasis and reduce the effects of stress. Cortisol influences a wide range of processes, including the breakdown of carbohydrates, lipids, and proteins to provide the body with energy. Cortisol has an immunosuppressive effect and therefore plays a role in the regulation of immune and inflammatory processes.

The relationship between inflammation and the HPA axis is a complex one since pro-inflammatory cytokines also stimulate the HPA axis and contribute to stress-induced elevation in cortisol[31]. Cortisol in turn, normally plays a fundamental role in limiting the further production of pro-inflammatory cytokines *via* the important cytokine-glucocorticoid feedback cycle. This occurs through cortisol binding to glucocorticoid receptors in the white blood cells (WBCs), which once activated, leads the activated receptor [*e.g.,* Nuclear factor-κB (NF-κB)], to block intracellular cytokine signaling pathways, ultimately stopping the further production of pro-inflammatory cytokines[32] and promotion of anti-inflammatory cytokines[33]. NF-κB consists of a family of transcription factors that play critical roles in inflammatory processes, immune regulation, cell proliferation, differentiation, and survival[34].

With toxic stress, chronic exposure of the WBCs to high cortisol leads to down regulation of the glucocorticoid receptors, resulting in their resistance to cortisol. This stops the cytokine-glucocorticoid feedback cycle, leading to dysregulated cytokine production and chronically elevated cortisol; two states known to worsen disease outcomes. Thus, toxic stress has been associated with inflammation due to glucocorticoid receptor resistance, a mechanism of dysfunctional inflammation regulation that allows proinflammatory mediators to be uncontrolled, adding to stress-related morbidity[35].

ROLE OF INFLAMMATION IN THE PHYSIOLOGY OF DIABETIC COMPLICATIONS

Chronic inflammation contributes to diabetes and its

complications. Features of chronic inflammation include an up-regulation of proinflammatory cytokines, such as TNF-α, interleukin (IL)-1, IL-6, IL-8, monocyte chemo attractant protein-1, and C-reactive protein that are produced by activated immune cells, resident macrophages and adipocytes[36]. Production of these proinflammatory cytokines functions to amplify the immune response. It is recognized that a chronic, low-grade inflammatory response that occurs with an activated immune system is involved in the pathogenesis of obesity-related insulin resistance and type 2 diabetes[37].

Markers of systemic inflammation correlate with risk for the development of diabetes related macrovascular complications[38]. For example, in obesity-related type 2 diabetes, adipose tissue, liver, muscle and pancreatic tissues are sites of inflammation. There is an infiltration of macrophages and other immune cells coupled with a shift in cell population from anti-inflammatory to a pro-inflammatory profile. The shift in the inflammatory profile promotes insulin dysfunction leading to hyperglycemia[39].

One complication of hyperglycemia is the formation and accumulation of advanced glycation endproducts (AGEs), ubiquitous irreversible end products of protein glycation which are formed from Amadori protein products[40]. AGEs crosslink proteins to form stable complexes that are resistant to enzymatic degradation. In addition to hyperglycemia, oxidative stress appears to increase AGE formation. AGEs ligate with their receptor, RAGE, to amplify and perpetuate the inflammatory response through nuclear factor κβ (NF-κβ), cAMP regulated element binding protein (CREB), and activator protein-1 (AP-1) signaling pathways. RAGE is a promiscuous receptor and has multiple ligands including lipopolysaccharide, S100/calcium binding proteins, High Mobility Group Box Protein 1 (HMGB1) and Amyloid-βpeptide (Aβ), as well as many others[40,41]. Data from multiple studies demonstrate that AGEs and their receptor, RAGE, are important contributors to the development of diabetes related complications[40,42].

Oxidative stress, an alteration in redox regulation and control, occurs in response to excessive reactive species production that overwhelms antioxidant defenses[43]. Reactive species may modify glucose, free fatty acids, oxysterols or lipids through oxidation-reduction reactions. For example, oxidize glucose is involved in the formation of AGEs. AGEs ligate with their receptor RAGE to promote an inflammatory response; modification of lipids has been shown to affect mitochondrial metabolic pathways leading to mitochondrial damage[44,45]. Inflammation and mitochondrial damage result in oxidative stress thereby producing an autocrine feedback pathway to perpetuate inflammation and oxidative stress[46]. This pathway has been described in the macrovasculature as well as in peripheral neurons and is recognized as a contributor to the complications of diabetes[47,48].

Vascular dysfunction characterized by an activated endothelium that is primed to facilitate immune cell migration into tissue also occurs in diabetes. Indeed vascular dysfunction is a key contributor of neuropathy, impaired cognition, nephropathy and cardiovascular diseases (*e.g.,* atherosclerosis, cardiomyopathy, *etc.*) that underlie complications of diabetes.

DIABETIC NEUROPATHY

Peripheral neuropathy (PN) affects up to 50% of people with diabetes and the diffuse peripheral neuropathies (distal sensori-motor polyneuropathy and autonomic neuropathy) are major risk factors for foot ulceration and amputation[49]. The etiology of PN is complex; however, studies show that altered blood flow, hyperglycemia and alterations in metabolics (oxidative/nitrative stress, advanced glycation end products and a pro-inflammatory response) are involved.

In animal models of diabetes, evidence of reduced blood flow to the nerve is seen within the first few days of the induction of diabetes with a chemical agent such as streptozosin (STZ). These changes often precede changes in nerve conduction velocity[50-52]. However, the loss of blood flow results in neuronal hypoxia sufficient to compromise nerve function and initiate neurodegeneration[53]. This effect has also been described in autonomic ganglia, dorsal root ganglia and in the hippocampus[54-56].

Hypoxia also induces the expression of numerous pro-angiogenic and pro-inflammatory genes in macrophages[57]. Alterations in the microvasculature effect associated peripheral nerves[58]. Indeed capillary occlusion induces ischemia to the nerve producing ischemic nerve fiber damage and perineural capillary luminal occlusion (due to endothelial cell hypertrophy and hyperplasia)[59]. In rats, hypoxic conditions reduced nerve velocity conduction, and within the context of hyperglycemic hypoxia, blockade of potassium channels leads to intra-axonal acidification by anaerobic glycolysis. This suggests that hypoxia induced neuronal changes may play a role in the development of neuropathy[60,61]. However, reversal of hypoxia in the ischemic limbs of individuals with diabetes does not improve nerve function[62].

Hyperglycemia appears to contribute to the pathogenesis of diabetic neuropathy. Within the first month of inducing diabetes in rats, hyperglycemia resulted in slowing of sensory[63-65] and motor[66,67] nerve conduction velocity coupled with hyperalgesia[68,69] and allodynia[70]. Over time prolonged hyperglycemia produces axonpathy, demyelination and nerve degeneration in diabetic animals[71,72].

Metabolic alterations are thought to play a central role in the development of neuropathy in diabetes. Elevation in polyol pathway activity, oxidative stress, the formation of advanced glycation end products and a persistent pro-inflammatory response through activation of the NF-κβ and p38 mitogen activated protein kinase signaling have been consistently shown

to contribute to diabetic neuropathy[73-75].

There is considerable evidence that pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6 are involved in the pathogenesis of diabetic neuropathy. TNF-α is a potent proinflammatory cytokine that appears to play a role in the pathogenesis of diabetic neuropathy and have a central role in central and peripheral sensitization of neuropathic pain[76]. Pharmacologically inhibiting TNF-α in mice ameliorates the electrophysiological and biochemical effects of the cytokine[77].

IL-1β is an important cytokine that induces the production of a wide variety of cytokines thorough NF-κβ activation. Studies show an increase in the mRNA expression of TNF-α and Il-1β in the spinal cords of STZ-diabetic rats[78]. Activated astrocytes in the spine increase IL-1β expression, which may induce *N*-methyl-*D*-aspartic acid receptor phosphorylation in spinal dorsal horn neurons to enhance pain transmission[79]. Hyperglycemia induces the production of IL-1β through the NOD-leucine-rich repeats-and pyrin domain containing inflammasome[80]. In the spinal dorsal horns of db/db mice, increased IL-1β, TNF-α and IL-6 levels are inhibited by anti-high-mobility group box protein-1, a known RAGE ligand[81].

IL-6 is a member of the neuropoietic cytokine family that participates in neuronal development and has neurotrophic activity. IL-6 is a sensitive marker of diabetic neuropathy and predicts progression and severity of type 1 diabetes[82]. Increased levels of IL-6, IL-1 and TNF-α correlated with the progression of nerve degeneration in diabetic neuropathy[83]. It is believed that these proinflammatory cytokines affect glial cells and neurons to set the pathological process of diabetic neuropathy in motion. However, the role of these cytokines in diabetic peripheral neuropathic pain is unclear[84]. It is clear that inflammation is a complex scenario. To that end other signaling molecules such as interferon-γ, IL-10, C-reactive protein, adhesion molecules, chemokines and adipokines may also play a role in the inflammatory process associated with diabetic neuropathy and neuropathic pain.

NEUROPATHIC PAIN

Pain is the body's perception of actual or potential damage to the nerve or tissue by noxious stimuli. Large A-delta myelinated fibers and small C unmyelinated fibers are sensory afferent nerves that are mainly responsible for carrying nociceptive sensation from the skin, joints, and viscera. Tissue damage results in the release of inflammatory mediators such as prostaglandins, bradykinins, and histamines at the site of injury, which triggers the depolarization of nocioceptors, thereby generating an action potential. The action potential transmits the nociceptive sensation, *via* the dorsal root ganglion (DRG) to the dorsal horn of the spinal cord. The release of glutamate and substance *P* results in the relay of nocioceptive sensations to the spinothalamic tract, thalamus, and subsequently, the cortex where pain is interpreted and perceived.

Nociceptive pain is the normal response to noxious stimuli and nociceptive pain usually subsides upon removal of the stimulus (*e.g.,* healing of injured tissue). Neuropathic pain occurs in the absence of noxious stimuli and represents a pathological change affecting the somatosensory system. Neuropathic pain is characterized by the activation of abnormal pathways of pain at the peripheral nerve and posterior nerve roots. Neuropathic pain is a critical feature in diabetic neuropathy.

The development of painful diabetic neuropathy is complex and not completely understood. However, evidence suggests that glycemic shifts, inflammation and oxidative stress are important contributors. Hyperglycemia affects glial cells leading to demyelination and impaired neurotrophism that culminates in impaired regeneration and decreases nerve conduction velocity; ultimately this results in pain. Hyperglycemia also activated the microvascular endothelium causing endothelial hypertrophy affecting downstream endoneurial circulation to promote hypoxia and ischemia of the nerve. Hyperglycemia and hypoxia affects neurons by promoting axonopathy and neuronal degeneration. Hyperglycemia may also contribute to painful diabetic neuropathy through the polyol pathway[85], advanced glycation end-products[86], hexosamin flux[87], mitogen-activate protein kinases[73], altered activity of the Na^+/K^+-ATPase[88], poly-ADP ribose polymerase (PARP) over activation[89], and cyclooxygenase-2 activation[90]. Nerve cells are prone to hyperglycemic injury as the neuronal glucose uptake is based on glucose concentration.

The expression of voltage-gated sodium and calcium channels and voltage-independent potassium channels in the DRG has a significant role in the generation of nociceptive sensation and peripheral sensitization. Indeed voltage gated sodium channels are active following nerve injury and demonstrate continued generation of ectopic impulses; similar findings have been observed from some voltage-gated calcium channels suggesting that voltage-gated calcium channels play a role in neuropathic pain. Calcium entry through voltage-gated calcium channels causes the release of substance P and glutamate, which results in the modulation of pain at the dorsal horn. The transient receptor potential vanilloid 1 (TRPV1) channel has been found to be associated with neuropathic pain as well. Methylglyoxal, a reactive intracellular by-productive of glycolysis and hyperglycemia, depolarizes the sensory neuron by activating the TRPV1 channel[91] in the DRG and also induces posttranslational modification of the voltage-gated sodium channel Nav1.8[92]. In addition, these changes increase electrical excitability and facilitate firing of nociceptive neurons.

Neuroplasticity is the brain's response to changes within the body or the external environment. In response to chronic neuropathic pain, neuroplasticity

is associated with somatosensory cortex remodeling, reorganization, and hyperexcitibility in the absence of external stimuli. Provoked pain and spontaneous stimuli may reverse the remodeling and reorganization at the somatosensory cortex[93]. In a study of patients with chronic neuropathic pain and nonneuropathic pain Gustin *et al*[93] found using functional and anatomical resonance imaging cortical reorganization and changes in somatosensory activity in patients with neuropathic pain.

IMPAIRED COGNITION AND DEPRESSION

Diabetes can lead to a number of secondary complications, and the most common brain complications include cognitive decline and depression. The incidence of cognitive decline, measured by behavioral testing may be as high as 40% in people with diabetes[94]. Subjective feelings of cognitive decline have also been reported from persons with diabetes[95], which illustrates the impact of diabetes on the individuals perception of how well their brain functions. Indeed multiple studies have reported that diabetic patients have a 2-5 fold increased risk for Alzheimer's Disease compared to non-diabetic subjects[96,97]. Furthermore, alterations in cognitive functioning in type 1 diabetic children (less than 5 years old) has been reported[98,99], as well as evidence of changes in white matter structure[100].

The mechanisms responsible for the development of high rates of cognitive decline in diabetics are not well understood, although evidence suggests that neuroplasticity may play an important role. The dentate gyrus of the hippocampus and the subventricular zone are two important areas in neurogenesis[101], the process of proliferation of progenitor cells or their differentiation into astrocytes, oligodendrocytes or neurons and survival and incorporation of the newborn cells into target regions. Hippocampal neurogenesis is diminished by exposure to environmental stress, HPA axis hyperactivity and increased inflammation[102,103]. Changes in neurogenesis alter a number of key functions of the hippocampus, such as learning and memory, affective expression and regulation of the HPA axis[104,105].

Wide variations in glucose levels and oxidative stress may also play an important role in the development of cognitive decline in diabetics. In animal models, studies show that repeated bouts of hypoglycemia inhibits hippocampal neurogenesis, presumably through oxidative injury to hippocampal CA1 dendrites[106]. Hyperglycemia also promotes oxidative stress and neurodegeneration[107]. Prolonged hyperglycemia promotes the development of AGEs which bind to their receptor, RAGE, to promote and sustain an inflammatory response through NF-κβ, AP-1 and CREB signaling pathways. RAGE ligation also promotes increases expression through an autocrine feedback mechanism[108]. RAGE is also responsible for the transport of amyloid-β (Aβ) across the blood-brain barrier. Aβ contributes to the development of Alzheimer's Disease[109,110] by participating in the formation and accumulation of amyloid plaques and fibrils that facilitate neurodegeneration and impair cognition[107]. Also, Aβ and hyperglycemia have been shown to activate microglia to induce oxidative injury[111].

The relationship between diabetes and depression is reciprocal as either is known to be a risk factor for the other[112]. The importance of depression is diabetes is highlight by studies consistently report a higher prevalence rate for depression among type 1 and type 2 diabetics compared to the general population[113]. Comorbid depression and diabetes is associated with poor self-care, lack of exercise, and nonadherence to dietary or medication routines, leading to inadequate glycemic control.

The mechanisms responsible for the development of depression in diabetics is unclear, although there is likely overlap between physiological and non-physiological factors to account for the pathogenesis of their comorbidity. Non-physiological factors such as sedentary life style, lack of self-care, and diet, as well as the emotion burden of managing diabetes, contribute to the development and progression of diabetes. Insulin resistance is gaining attention as a potential link between diabetes and depression and cognitive decline[114,115]. Neuroendocrine signaling, through hyperactivity of the HPA axis, is thought to cause or exacerbate depression in diabetics[116]. Indeed antidepressant treatment has been shown to abrogate abnormal HPA responses while facilitating recovery from depression[117].

Stress has been shown to decrease brain derived neurotrophic factor (BDNF) in the hippocampus. Stress also appears to decrease the expression of other types of neurotrophic and growth factors such as nerve growth factor and neurotrophin-3[118], which could lead to the alteration in the structure and function of hippocampal neurons. Stress also decreases the expression of vascular endothelial cell growth factor, a growth factor that influences vascular permeability and the proliferation of endothelial cells, in the hippocampus[119]. The significance is that antidepressant treatment increases expression of BDNF and other growth factors in individuals recovering from depression[120-122].

There is also considerable evidence that inflammation plays an important role in the pathogenesis of depression and diabetes[123]. Many studies describe an increase in peripheral cytokine of individuals with depression that is often comorbid with other chronic diseases such as coronary artery disease and chronic obstructive pulmonary disease[124]. Interestingly, cytokines have been shown to be associated with suicidality and depression[125]. Diabetes and inflammation have been associated with alterations of dopamine, serotonin, brain derived neurotrophic factor and insulin growth factor-1 which have been implicated

in depression[126].

CARDIOVASCULAR DISEASE, NEPHROPATHY AND ASSOCIATED SYMPTOMS

Given the worldwide increase in the incidence of diabetes, the dual complications associated with cardiovascular disease and nephropathy heighten the importance of preventive therapies through early identification of biomarkers of inflammation and causative etiologies for stress responses regardless of age or type of diabetes[127]. In 2010, high blood pressure was the leading risk factor for deaths due to cardiovascular diseases, chronic kidney disease, and diabetes in every region of the world, causing more than 40% of worldwide deaths from these diseases[128]. The National Diabetes Statistics Report: Estimates of Diabetes and Its Burden in the United States, 2014[129], indicated that from 2003-2006 after adjusting for population age differences, cardiovascular disease (CVD) death rates were approximately 1.7 times higher among adults (≥ 18 years) with diabetes than among adults without diabetes. Regardless of the type of diabetes, the risk of CVD is evident and likely begins at an earlier age for those diagnosed with type 1 diabetes. Endothelial dysfunction is an integral part of the pathogenesis underlying the increased cardiovascular complications seen in individuals with T1D but it is unclear how early it appears[130].

Results from the Epidemiology of Diabetes Interventions and Complications study, a long term follow up study of the Diabetes Control and Complications Trial (DCCT), showed that adults with T1D had increased carotid intima medial thickness (CIMT) compared to a healthy non-diabetic population 6 years into the study. Individuals receiving intensive insulin treatment during the DCCT had much less progression in their CIMT compared to those who had received conventional treatment. However there was not a significant difference in their percent HbA_{1c} at that time, suggesting the effect of "metabolic memory"[131]. These data suggest that glycemic control may have long lasting effects on cardiovascular morphology and function[130]. Hence, there exists a caveat to minimize exposure to toxic stressors in early life and at the onset of T1D that may aggravate optimal glycemic targets.

Cardiovascular morbidity related to diabetes is associated with vascular changes due to inflammation, resulting in both macrovascular (*i.e.,* atherosclerosis)[132] and microvascular (*i.e.,* cardiovascular autonomic neuropathy)[133] alterations. In type 1 diabetes, several causative factors are implicated in these inflammatory vascular changes[134]. The oxidative modification of LDL and associated immune responses[135] may be one of these key factors, resulting in damage to the endothelium[136], activation of macrophages, adherence of monocytes[137] and impairment of nitric oxide action with resulting vascular cell cytotoxicity[138]. Although markers of inflammation have not been extensively studied in the development of CAD in T1D, the Eurodiab study group, using a standard score based on combined levels of C-reactive protein, IL-6, and TNF-α, reported a significant difference between those with and without CAD ($P < 0.001$) after adjusting for age, gender, HbA_{1c}, diabetes duration, and systolic blood pressure[139]. Research has also indicated that in subjects with known coronary atherosclerosis, low-degree inflammatory activity (*i.e.,* C-reactive protein, fibrinogen, erythrocyte sedimentation rate and white blood cell count) is not only increased in patients with T1D and T2D diabetes, but also increased with increasing HbA_{1c} in non-diabetic individuals. This later finding indicates an early association between degree of glycaemia, inflammation and atherosclerosis prior to the development of diabetes[140].

Cardiovascular autonomic neuropathy is a common form of autonomic neuropathy and one of the most overlooked of all serious complications of diabetes, resulting from microvascular damage to parasympathetic and sympathetic fibers and increased risks for cardiovascular arrhythmias, sudden death, and myocardial infarction in adults with diabetes[141]. There are multiple etiologies of diabetic neuropathy, including hyperglycemic activation of the polyol pathway leading to accumulation of sorbitol causing direct neuronal damage and/or decreased nerve blood flow[142], oxidative stress with increased free radical production leading to vascular endothelium damage and reduced nitric oxide bioavailability[143,144], and the formation of advanced glycosylated end products with reduced blood flow, activation of inflammatory cytokines (*e.g.,* IL-6, TNF-α), nerve hypoxia and altered nerve function[141].

Cardiovascular autonomic neuropathy has been linked to postural hypotension, exercise intolerance, enhanced intraoperative cardiovascular lability, increased incidence of asymptomatic (*i.e.,* painless) ischemia, myocardial infarction, and decreased likelihood of survival after myocardial infarction[145]. The presence of palpitations and tachycardia at rest due to loss of parasympathetic modulation can be present early in the development of this complication prior the onset of other associated symptoms. Cardiovascular autonomic neuropathy occurs in approximately 17% of patients with T1D and 22% of those with T2D. An additional 9% of T1D and 12% of T2D have borderline dysfunction[133]. Since the 1970s, the seminal work by Ewing *et al*[146] unveiled the predictive relationship between cardiovascular autonomic neuropathy and mortality in adults with T1D. The Hoorn Study also found increased mortality in adults with T2D who had decreased cardiovascular autonomic function[147]. Within the pediatric literature, heart rate variability (a measure of cardiovascular autonomic function) was lower in adolescents with T1D compared with healthy control

subjects[148,149] and lower in youth with T2D *vs* T1D[150].

New pathways in the development of diabetic nephropathy also implicate inflammatory processes due to hyperglycemia, renin-angiotensin system and oxidative stress, involving infiltration of the kidneys with monocytes and lymphocytes that increase pro-inflammatory cytokine production, reactive oxygen species and tissue damage[151,152]. This leukocyte activity amplifies the inflammatory response and promotes cell injury and organ tissue fibrosis. Improved future understanding of the inflammatory response in diabetic kidneys is expected to identify novel anti-inflammatory strategies for the potential treatment of diabetic nephropathy. Familial predisposition to disease, including risks for toxic stress, race and other environmental factors interact with hemodynamic changes producing advanced glycation end products, glucose reduction and sorbitol accumulation into the cell, and overproduction of reactive oxygen species[151]. For individuals exposed to toxic stress that may further exacerbate dysglycemia, glycemic control is of upmost importance for preventing the onset and progression of nephropathy by influencing both hyperglycemia itself and hyperglycemia induced metabolic abnormalities. Evidence for this premise is supported by randomized controlled clinical trials in both type 1 and type 2 diabetes[153,154].

CLINICAL IMPLICATIONS FOR SYMPTOM RECOGNITION

The complications of diabetes related to neuropathy, nephropathy and cardiovascular disease are the major contributors to morbidity and mortality in this population. Given the projected increase in the worldwide numbers of individuals to develop diabetes in the coming years, the potential additional burden of toxic stress on the development of disease related complications is of tremendous concern. Key symptoms that warrant clinician recognition during routine assessment in persons with diabetes include signs of cognitive decline, depression, fatigue (including disturbed sleep patterns), exercise intolerance and pain associated with peripheral neuropathy. Although the emphasis in diabetes management is achievement of glycemic targets, weight, lipid and blood pressure control, the environmental and physiological effects of daily stress may be "ticking away" at the emergence of subtle inflammatory changes leading to devastating complications. Therefore, diabetes care management should emphasize symptom palliation as well as cardiometabolic control[155].

Chronic low-grade inflammation in metabolic disorders such as diabetes contributes to behavioral symptoms, including depression, cognitive impairment, fatigue, sleep disturbance and pain[156]. The quality and quantity of sleep may play a key role in the inflammatory processes associated with diabetes and related cardiovascular disease[157]. Additionally, several biomarkers of inflammation, specifically IL-6 and CRP, have been found to be associated with fatigue, poor concentration and sleep quality in a healthy adult cohort[158], which has implication for the stress-induced inflammatory effect on individuals prior to the development of diabetes. There is increasing evidence that hypercytokinemia and activated innate immunity affect the pathogenesis of T2D and related symptoms of fatigue, sleep disturbance and depression[159].

CONCLUSION

Toxic stress exposes individuals at all ages to chronic, low-grade inflammation that is a risk for the development of diabetes and may increase the physiological alterations leading to neuropathy, nephropathy and cardiovascular disease that are so prevalent in diabetes. Evidence supports the importance of minimizing toxic stress to promote glycemic control and lessening immune and inflammatory responses in an attempt to prevent the emergence or worsening of diabetes complications. At a time when the evaluation of immune and inflammatory biomarkers is not standard clinical practice, routine examination strategies are essential for the assessment of stressful life experiences and the effects of these experiences that contribute to the symptoms related to neuropathy, nephropathy and cardiovascular disease and overall quality of life.

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REVIEW

Adapting chronic care models for diabetes care delivery in low-and-middle-income countries: A review

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Abstract

A contextual review of models for chronic care was done to develop a context-adapted chronic care model-based service delivery model for chronic conditions including diabetes. The Philippines was used as the setting of a low-to-middle-income country. A context-based narrative review of existing models for chronic care was conducted. A situational analysis was done at the grassroots level, involving the leaders and members of the community, the patients, the local health system and the healthcare providers. A second analysis making use of certain organizational theories was done to explore on improving feasibility and acceptability of organizing care for chronic conditions. The analyses indicated that care for chronic conditions may be introduced, considering the needs of people with diabetes in particular and the community in general as recipients of care, and the issues and factors that may affect the healthcare workers and the health system as providers of this care. The context-adapted chronic care model-based service delivery model was constructed accordingly. Key features are: incorporation of chronic care in the health system's services; assimilation of chronic care delivery with the other responsibilities of the healthcare workers but with redistribution of certain tasks; and ensuring that the recipients of care experience the whole spectrum of basic chronic care that includes education and promotion in the general population, risk identification, screening, counseling including self-care development, and clinical management of the chronic condition and any co-morbidities, regardless of level of control of the condition. This way, low-to-middle income countries can introduce and improve care for chronic conditions without entailing much additional demand on their limited resources.

Key words: Chronic care models; Context adaptation; Diabetes mellitus type 2; Low-to-middle income countries; Service delivery model

Core tip: This paper introduces strategies that low-to-middle-income countries can employ to introduce feasible care and prevention for diabetes amidst problems of the double burden of disease and scarcity of resources, and presents a context-adapted service delivery model that integrates care for diabetes and similar chronic conditions in the current health services

and assimilates the delivery of diabetes care with other responsibilities of the health system so that people under the care of health services and the health system can experience the whole spectrum of diabetes prevention and care.

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INTRODUCTION

Chronic conditions are said to be a global crisis that threatens human development, especially in low-to-middle income countries (LMIC)[1,2]. A large number of people from LMICs live with chronic conditions. This includes around 291 million with diabetes[3].

The macroeconomic effects of chronic conditions including diabetes in LMICs are substantial. These disproportionately affect the poor[4] and the care for these conditions leads to continued and, as the condition worsens and complications develop, escalating expenditures while decreasing productivity. The costs of care and the disability or death of a household income earner may cause (further) poverty[5]. For every 10% rise in mortality from chronic conditions, the yearly economic growth of a country is estimated to be reduced by 0.5 percentage points[6]. Abegunde *et al*[7] computed projections of foregone national income due to heart disease, stroke and diabetes in 23 LMICs and showed that these countries combined are at risk of losing US$ 84 billion in economic output over the ten-year period 2006-2015.

Thus, LMICs should move towards strategies to deal with chronic conditions including the provision of good quality chronic care in order to address the evolution that is threatening their people. However, the acute disease-oriented health systems of LMICs may face a number of difficulties in adjusting health care delivery to accommodate the growing burden of chronic conditions in general and diabetes in particular. This could be attributed to various reasons including resource constraints, absence of programs directed towards chronic conditions, and difficulties in introducing and/or integrating care for chronic conditions.

An adequate approach to care for chronic conditions such as diabetes is very different from the acute disease-oriented approach practiced in most LMICs: in addition to the disease prevention and drug prescription activities usually done in acute disease care, chronic care also needs to focus on disability limitation and rehabilitation[8]; should give attention to the psychosocial aspects of the patient[9]; and should involve and enable the patient in caring for the condition[10]. Other features that make chronic care different from acute disease care include: (1) case finding for assessment of risk factors, detection of early disease, and identification of high risk status; and (2) long term follow-up with regular monitoring and promotion of adherence to pharmacological and psychological interventions[1].

Analyses have demonstrated that in spite of increased funding in LMICs, progress towards agreed Millennium Development Goals, including the health-related ones, remains slow[11]. This could be attributed to weak health systems, human resource constraints, and over-concentration of resources to specific programs. Introducing chronic disease care *in toto* as practiced in high income countries (HIC) or separately structured and resourced vertical programs to address specific chronic disease problems to LMIC health systems may prove detrimental if not fatal. A better approach could be to strengthen the first line and progressively integrate care for chronic conditions into primary care activities, taking into consideration the capabilities of the health system.

LMICs can take the initiative to undertake the first steps towards the provision of good quality chronic care. Adapting models for chronic care to fit the context of a country and selecting specific elements for implementation is likely to stand a better chance of improving chronic care. This way, specific problems such as resource constraints may be addressed; certain characteristics of the people, the health system and the country that could be capitalized on may be identified; and particular context-adapted strategies may be employed.

For this research, the investigators reviewed existing models for chronic care considering the results of a situational analysis of a low middle-income country, the Philippines, to come up with a model for diabetes care delivery that could be adapted in low-to-middle-income countries with similar characteristics.

BACKGROUND ON THE PHILIPPINES: HEALTH SYSTEMS, CHRONIC CONDITIONS AND DM TYPE 2

Public health care in the Philippines was devolved in 1992 and the responsibility of providing basic health care services for the people was handed down to the local government units, specifically municipalities and cities, through their respective local government health units (LGHU)[12]. A decade before this health care devolution, the country implemented a primary health care policy which led to the creation of a large cadre of community-based health care workers locally called barangay health workers (BHW)[13]. The barangay (village) is the smallest unit of government; a city or a municipality would be composed of a number of barangays. Organizationally, the BHW fall under the governance of the barangay and are selected to work

in their respective areas of residence; functionally, they are under the LGHU. A BHW is assigned approximately 10-20 families and is responsible for dissemination of health information and health promotion activities, and conducts other health-related undertakings to any member of the families being attended to.

The Philippines is among the 23 low-and-middle-income countries where 80% of the LMIC mortality due to chronic conditions is accounted for[7]. It is likewise predicted to be among the 10 countries worldwide with the highest numbers of people with DM type 2 by 2030[14]. For the past decade, eight of the 10 leading causes of mortality in the Philippines are chronic conditions and DM type 2 has been consistently among these[15]. Furthermore, the complications and consequences of DM type 2 in the Philippines are on the rise and have become alarming. For renal complications alone, it is seen that 55% of Filipino diabetics will eventually develop kidney disease; in 2007 there was an increase of more than 2800 diabetic nephropathy patients requiring dialysis[16]. Aside from these, the International Diabetes Federation estimated undetected type 2 diabetes (UDD) in the Philippines at 58.8% in 2011[3]. It seems that the current screening strategies in routine conditions cannot adequately identify previously undetected cases of DM type 2 in the Philippines. The high rate of UDD, the rapidly increasing prevalence of DM type 2, and the poor control of disease progression and emergence of complications only show that current case management of diabetes mellitus in the Philippines is below optimum, and the burden will only escalate if no measures are employed to address these problems. On the macroeconomic level, the Philippines lost US$60 million in 2006 from coronary heart disease, stroke, and diabetes alone[7].

METHODS OF REVIEW AND CONTEXTUAL ANALYSIS

Narratives on models for chronic care and their elements, records of implementation and outcomes of implementation, if any, were analyzed and adapted to the context of the Philippines, an LMIC where the health system is still acute disease-oriented, there is limited organized care for chronic conditions, if at all, and healthcare expenditures are mostly out-of-pocket. The investigators focused on the two main models for chronic care, which have been used by HIC health systems as bases for the organization of chronic care. For the situational analyses, they considered a number of organizational theories: on how an organization may respond to pressure to change and what factors could influence an organization's response; and the factors that may facilitate or hinder adoption of innovations introduced to an organization. Key factors affecting the adaptation of chronic care models and the development of a context-adapted chronic care model-derived service delivery model were explored making use of theories presented by Oliver[17] in her analysis of organizational responses to pressures towards conformity and Greenhalgh *et al*[18]'s theories on diffusion of innovation, taking into consideration the background of the country and its health system and the profiles of the healthcare workers, the people with chronic conditions, and the community. The Philippine context was used and informal interviews with representatives of the community (the government and the people), the healthcare system/service and people with diabetes were conducted.

MODELS FOR CHRONIC CARE

In HICs, models and frameworks for chronic care and its delivery have been implemented, most of which were derived from Wagner's Chronic Care Model (CCM)[19]. The CCM was conceptualized from a primary care perspective and advocates improvements in six essential elements: self-management support, clinical information systems, delivery system redesign, decision support, health care organization, and community resources[20]. The basic idea of the CCM is quite sound: to optimize "productive interactions" between "informed, activated patients" on one hand, and "prepared, pro-active practice teams" on the other, resulting in "functional and clinical outcomes" (Figure 1). However, the CCM appears to be grounded in a preponderantly clinic-based perspective with a background of abundant resources and a highly technological environment such as can be found in HICs. Its focus seems to be on optimizing clinical interaction for more effect in dealing with chronic conditions.

To adapt the basic principles and elements of the CCM to something actionable in developing countries, the World Health Organization[21] introduced the Innovative Care for Chronic Conditions framework (ICCCF) (Figure 2). The same essential chronic care elements specified in Wagner's model were retained. The guiding principles of the ICCCF are evidence-based decision making, population focus, prevention focus, quality focus, integration, and adaptability. It has the following essential elements for taking action: supporting a paradigm shift, managing the political environment, building integrated health care, aligning sectoral policies for health, using health care personnel more effectively, centering care on the patient and family, supporting patients in their communities, and emphasizing prevention. The ICCCF seeks to improve health care at the macro, meso, and micro levels. However, essential components for the policy environment (macro level) are needed (leadership and advocacy, integrated policies that span different disease types and prevention strategies, consistent financing, developing human resources, legislative frameworks and partnership working), requirements that many low-to-middle-income countries, especially those encountering scarcity in human resources for health,

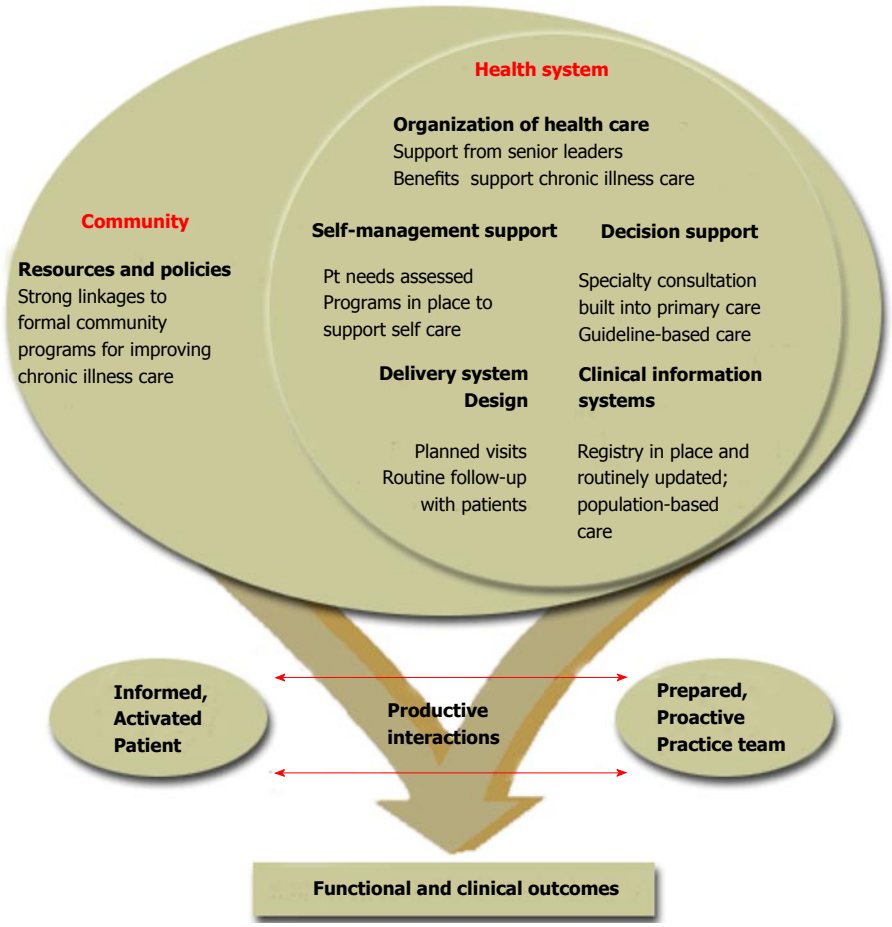

Figure 1 The chronic care model.

economic crisis, and instabilities in leadership, may be unable to fulfill.

In Canada, the Province of British Columbia formulated the Expanded Chronic Care Model (Figure 3) integrating population health promotion and prevention with the existing elements of the CCM to address the social, environmental and cultural factors that affect health[22]. This way, the role of the community has become well delineated and added to the clinically focused initial CCM.

Some countries chose to implement selected elements of these models. In Scotland, key principles in chronic care have been established, namely: pathways of care focused on individuals with chronic conditions; partnership between health care professionals and people with chronic conditions; partnership between primary care, social care, and other agencies; integrated solutions that respond to the needs of people with chronic conditions; focus on providing care in primary care and community settings; and focus on self-care[23].

Certain LMICs have made use of the CCM or the ICCCF to design systems of care for chronic conditions. The CCM-based Vera-Cruz Initiative for Diabetes Awareness in Mexico reports improved glycemia among its study participants 18 mo after implementation[24] while Rwanda made use of ICCCF elements to strengthen its health system and design a system of care for HIV/AIDS, with impressive results[25,26]. However, such implementations of elements of the CCM/ICCCF in LMICs are exceptions rather than the rule. The health systems response in many LMICs is still characterized by a public health system focused on prevention programs; little consideration for the organization, coordination and regulation of health care services; routine medical practice without attention to the opportunities and resources for the specific aspects of chronic care; and large out-of-pocket expenses for patients[27].

Studies conducted on the implementation of the CCM in HICs demonstrated significant correlations between specific elements of the CCM and better health outcomes[28,29]. The number of elements of the CCM and the type and intensity of implementation may vary, depending on many contextual and organizational factors[30].

THE ORGANIZATIONAL THEORIES USED

Oliver's typology of strategic responses to institutional processes[17] lists five behaviors that organizations may enact in response to pressures toward conformity with the institutional environment: acquiescence and its alternative forms of habit, imitation, and compliance; compromise including pacifying tactics and bargaining; avoidance, concealment, buffering and

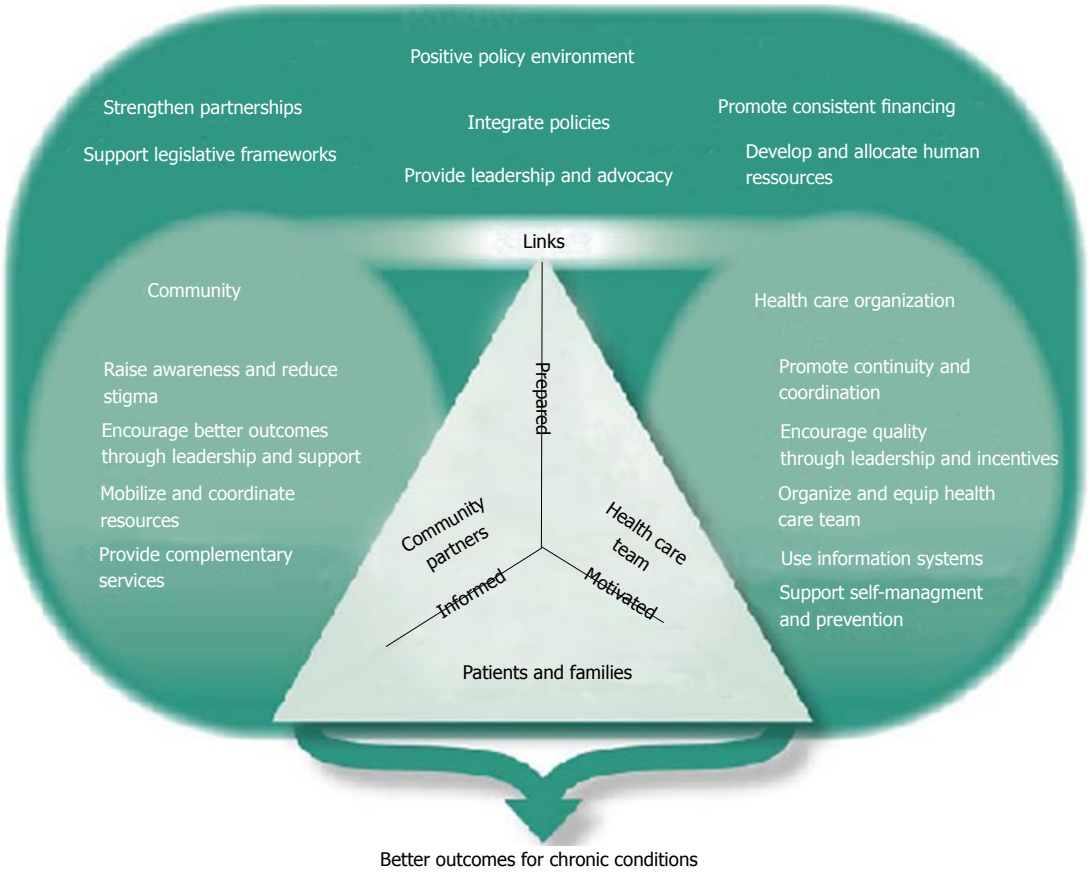

Figure 2 The innovative care for chronic conditions framework.

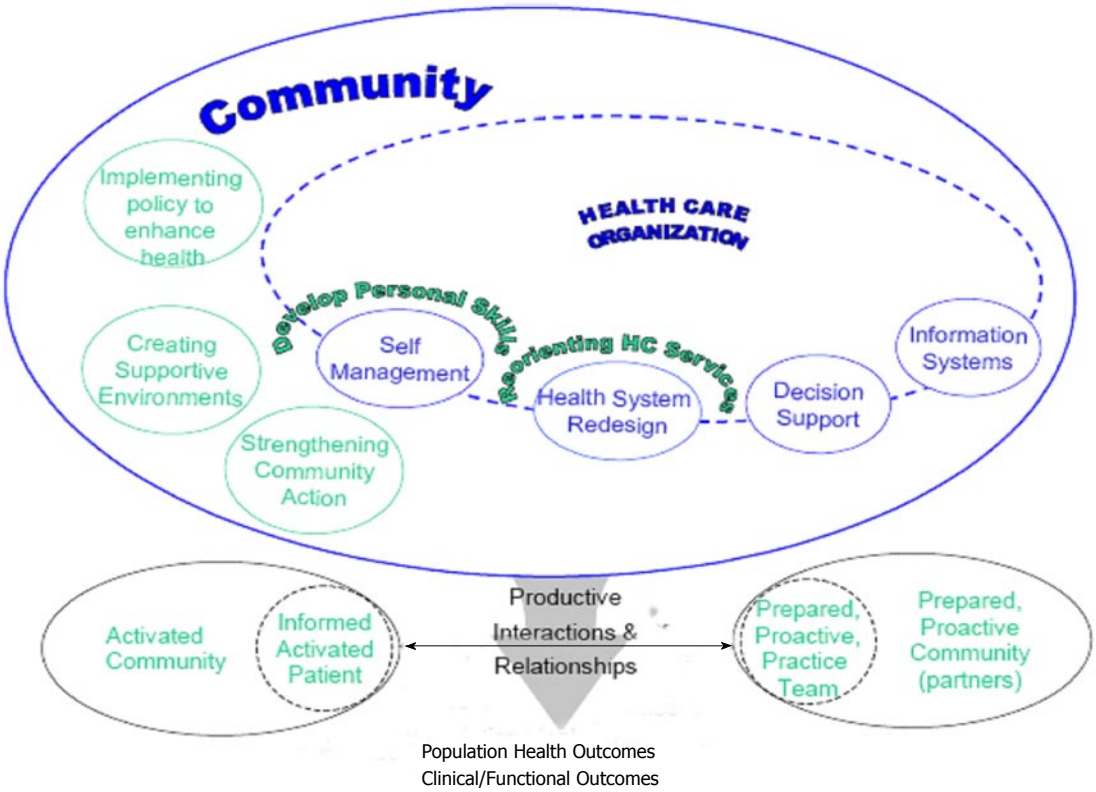

Figure 3 The expanded chronic care model.

escape; defiance, dismissal, challenge and attack; and manipulation including use of co-opting, influencing and controlling tactics. Oliver further states the following antecedents of strategic response: (1) the cause - why the organization is being pressured to conform to institutional norms or expectations; (2) the constituents - who are exerting institutional pressures on the organization; (3) the content - to what norms or requirements is the organization being pressured to conform; (4) the means of control - how or by what means are the institutional pressures being exerted; and (5) the context - what is the environmental context within which institutional pressures are being exerted. Ten predictive dimensions on which the response of the organization will depend are theorized from these antecedents. The investigators considered seven of these theoretical dimensions: social legitimacy; economic efficiency; external dependence on institutional constituents; consistency with organizational goals; constraints on decision-making imposed on the organization; voluntary diffusion of norms; and environmental interconnectedness. The other three dimensions namely multiplicity of constituents demands, legal coercion and environmental uncertainty, although necessary for implementation of top-down policies, were deemed to be less relevant for the present purpose, the perspective of which is rather how institutions would react to bottom-up innovations.

Greenhalgh *et al*[18] theorized that adoption of any intervention by an organization is variable - some interventions may be fully adopted, partially or not at all, while some may be eventually abandoned in time - and presented a unifying conceptual model for considering the different aspects of a complex situation and their many interactions. Certain attributes influence the (non)adoption of interventions based on the characteristics of the innovation, the individuals who will adopt the intervention and the system where the innovation will be assimilated. For this research, the investigators considered the following characteristics of an innovation in designing the context-adapted chronic care model-based service delivery model: relative advantage; compatibility; simplicity; trialability; observability; reinvention; risk; consideration of task issues; knowledge required; and augmentation/support. In constructing the model, the investigators envisioned a care model that could be applied in the context of an LMIC such as the Philippines, and that would engage the two main groups of stakeholders: those who are involved in the provision of care and prevention activities (the care providers, the health service/health system, and the policy makers); and the intended recipients of these activities (the person with the condition and the community members).

A 2-step situational analysis was conducted to help determine what specific interventions for chronic care could be feasibly applied to successfully organize care for chronic conditions, particularly type 2 diabetes mellitus (DM type 2), in the Philippines.

RESULTS OF THE CONTEXTUAL ANALYSIS

Results of analysis of key factors on the proposed organization of care for chronic conditions considering the current situation including awareness and level of knowledge on chronic conditions in general and on DM type 2 in particular, and skills for its care among the leaders and members of the community, the patients, the health system and the healthcare providers are listed in Table 1.

The community, the patients, the health system and the healthcare providers were taken into consideration as these would be the key potential players in implementing any of Wagner's six essential chronic care elements (self-management support, clinical information systems, delivery system redesign, decision support, health care organization, and community resources). The patient is central to all of these chronic care elements; the community, separately as an organized group and as a unit of government, would be responsible for the community resources; and the healthcare provider and the health service would be involved in at least five if not all of the elements.

This first analysis would indicate that there are no insurmountable barriers to the introduction of at least basic interventions for the care of chronic conditions.

The analysis of these factors was taken a step further by applying Oliver's typology and the theories of Greenhalgh *et al*[18] (Table 2), which explored aspects that could affect adaptation of chronic care models towards the development of a chronic care service delivery model.

Based on these, a feasible service delivery model for DM type 2 and similar chronic conditions was constructed, taking into consideration the existing healthcare organization and design, the current duties and responsibilities of individual cadres of healthcare workers, and the chronic care activities that need to be and can be provided.

THE CONTEXT-ADAPTED SERVICE DELIVERY MODEL FOR DM TYPE 2 AND SIMILAR CHRONIC CONDITIONS

Health care reorganization to concentrate primarily on chronic care is neither feasible nor desirable for developing countries still dealing with the problems of acute diseases concurrently with the rising prevalence of chronic conditions. Gradual accommodation of care to include chronic conditions is a better choice as LMICs continue their battle against malaria, pneumonia, diarrheal diseases and other acute illnesses. With this double burden of disease, the health care system should address the care for both chronic and acute conditions in terms of a more inclusive priority setting.

As mentioned previously, creating more vertical programs with specialized structures, dedicated

Table 1 Key contextual factors that is expected to affect chronic care model adaptations for the development of a service delivery model for chronic conditions including type 2 diabetes mellitus

Key factors	Analysis based on context
Community-related	
Policy	No specific policies on chronic care delivery exist at both national and local levels
Politics	Informal interviews with government officials suggested some awareness of chronic conditions such as DM type 2 and the needs that must be addressed for the care of chronic conditions in general and DM type 2 in particular in the political environment
Support	National support is limited mostly to prevention and one-day health promotion campaigns on specific chronic conditions Support from private organizations and civil societies is currently untapped
Awareness	Informal interviews with local government officials and community members suggested a low level of awareness of DM type 2, the care for DM type 2 and other associated factors, and the prevalence and burden of DM type 2 in the locality
Patient-related	
Support	Informal interview with healthcare staff and people with diabetes gave an impression of low level of support given to people with diabetes by the community and health services
Awareness	Informal interview with healthcare staff and people with diabetes gave an impression of low level of knowledge on the condition and care for the condition
Perceived need	Informal interview with people with diabetes revealed a moderate level of perceived need to improve care delivery for their condition
Perceived benefits	Informal interview with people with diabetes revealed a moderate level of perceived benefits of improving care delivery for their condition
Self-efficacy	Informal interview with healthcare staff and people with diabetes suggested a low level of self-efficacy in managing the condition
Provider-related	
Perceived need	Informal interview with healthcare staff revealed a high level of perceived need to improve primary care for chronic conditions
Perceived benefits	Informal interview with healthcare staff revealed a high level of perceived benefits of delivering good quality chronic care
Self-efficacy	Informal interview with healthcare staff suggested an impression of low level of self-efficacy in the provision of good quality chronic/diabetes care
Skill proficiency	Informal interview with healthcare staff suggested an impression of a need for skills and knowledge development regarding delivery of good quality chronic/diabetes care
Health service-related	
Leadership	The (local) government leaders and health officers are supportive of project implementation
Shared vision	The health system has a shared vision in improving the quality of care for chronic conditions
Organizational norms regarding change	The healthcare workers may be open to small, incremental changes as long as these do not lead to a drastic increase in demands on resources and workload
Administrative support	Administrative support for the project is limited

DM: Diabetes mellitus.

personnel and earmarked budget may prove more detrimental to an already-weak health system, and may inadvertently lead to inattention and cause neglect to other health issues that also need to be addressed. Primary care strengthening and capacity building of an existing health service may pave the way towards health care delivery to the people rather than prevention and care of a specific disease, moving health care towards a person-centered, comprehensive approach and veering away from being disease-centered.

LMICs can deliver prevention and care for chronic conditions such as DM type 2 by applying carefully thought-through implementation principles.

Although the care for acute and chronic diseases may seem contrasting, the people involved in health care delivery are basically not. The usual personnel complement of a health service can also be used for chronic care. Chronic care activities may range from simple, standardizable procedures that require low expertise to complex ones that require more expertise and more extensive training and education. These activities may be distributed to different types of health care personnel. A model for the delivery of chronic care services where the activities were stratified according to the level of expertise of health care personnel in LMIC was conceptualized (Figure 4). In this model, healthcare personnel may range from volunteers/community-based health workers and expert patients, constituting the health care personnel with lesser formal expertise, to paramedical personnel (midwives, nurses) to physicians (general practitioners, specialists) constituting health care personnel with more formal expertise, although the highest tier may only involve up to the nurses in certain settings. Chronic care activities may involve health promotion and prevention in the general population, among a population at risk, and a subpopulation with high risk of developing certain chronic conditions; clinical management, counseling and health education of those with good control of their chronic conditions and with stable co-morbidities; and clinical management, counseling and health education of those with poor control of their chronic conditions and/or with unstable co-morbidities.

For DM type 2 (and similar chronic conditions, *i.e.*, hypertension, coronary artery disease, cerebrovascular accidents, some cancers, chronic obstructive pulmonary disease), health care workers with the least formal expertise may perform population-based health promotion and prevention activities, carry out pre-screening activities

Table 2 Contextual analysis of key factors affecting adaptation of chronic care models and subsequent selection of CACCM elements for implementation making use of Oliver's typology and characteristics enumerated by Greenhalgh *et al*[18]

CACCM- and project-related	
Oliver's dimensions	
Social legitimacy	Improving care for chronic conditions and protection and promotion of the health and wellbeing of the LGU population enhances the social fitness of the LGHU and the local government
Economic efficiency	The introduction of additional activities in any organization entails additional expenses. Cost-effective or cost-saving innovations would be preferred
External dependence on institutional constituents	The LGHU are dependent on the LGU for funding; the LGU officials who decide on the allocation of these resources are dependent on the populace for their seats in office
Consistency with organizational goals	The primary goal of the LGHU is to provide good quality healthcare to the people
Discretionary constraints imposed on the organization	The LGHU expects full autonomy especially in substantive decision-making such as resource-allocation, resource acquisition, organizational administration, *etc.*
Voluntary diffusion of norms	A moderate to high degree of voluntary diffusion with some degree of pressure from the LGU officials to diffuse said norms may be most effective in promoting adoption of the intervention
Environmental interconnectedness	A certain degree of predictability of the environment is seen: the general population, especially the people with diabetes and their families will most likely appreciate the intervention. Such appreciation may be reflected on goodwill towards the LGU officials and consequently to the LGHU (for example additional budget allocated to health)
Greenhalgh's characteristics	
Relative advantage	Implementing a diabetes-care project gives the advantage of improving the care for this condition and a number of its comorbidities, but without reduction of other health benefits
Compatibility	Compatibility of the intervention with current/pre-existing activities in the LGHU and with the current duties, responsibilities and workload of the LGHU staff is sought
Simplicity	Simplicity and ease of use of the intervention favors adoption of the intervention
Trialability	Flexibility in accomplishing a number of tasks, *i.e.*, giving leeway to the healthcare staff regarding performance of activities related to the intervention will increase acceptability of the intervention
Observability	Providing information to the intended adopters of the benefits of the intervention, *e.g.*, improvements of glycemia, favors adoption of the intervention
Reinvention	Flexibility of the intervention allowing adaptation and refinement to suit the context, the needs of the individual person with diabetes and the capabilities of the healthcare provider favors its adoption
Risk	Based on outcomes of previous studies conducted on implementation of chronic care models and provision of self-management education, it is certain that the benefits far outweigh the risks
Task issues	Workable and easy to use interventions favor adoption Relevance of the intervention to the work of the staff and tasks that may contribute to the relevance of the work of the individual health care worker is preferred However, the intervention may also be interpreted as an added workload to the LGHU staff
Knowledge required	Knowledge and skills required for full implementation of the intervention need to be supplied/supplemented
Augmentation/support	Provision of a training workshop prior to implementation increases the probability of adoption of the intervention

LGHU: Local government health units; LGU: Local government units.

to identify a subpopulation at risk for developing DM type 2, and may follow-up on people noted to be at high risk or already identified to have prediabetes. These activities are standardizable; operating procedures and work flow diagrams/decision trees may be constructed to instruct the health care worker, and checklists may be prepared to serve as guides. On the next tier of health care workers, activities that may be assigned include specific screening or confirmatory testing for dysglycemia (DM type 2 and prediabetes), identification of co-morbidities, and counseling, health education and clinical management of patients in good glycemic control and with stable co-morbidities. Although certain guidelines and diagrams still make these activities standardizable, a higher level of expertise is expected to clinically manage optimally controlled DM type 2 and co-morbidities and to recognize and know when to refer impending instabilities; special skills also need to be developed to initiate effective communication and counseling/health education. At the higher end of the spectrum, activities are focused on those with poor glycemic control and/or unstable co-morbidities and complications, which would require clinical expertise and judgment for appropriate clinical management and counseling.

CONCLUSION

The context-adapted service delivery model for DM type 2 and similar chronic conditions may be far-removed from that in HICs, but it is designed to deliver prevention and care that encompasses the spectrum of diabetes from those at risk to those with poor glycemic control and/or unstable co-morbidities and includes counseling for self-management education and support. It is likewise designed for the general population to experience this service through general health (diabetes) education and healthy lifestyle promotion. The model incorporates care for diabetes into a current package health care activities making use of pre-existing human resources for health. It taps the potential of a workforce that may assume simple and standardizable diabetes prevention and care activities. In so doing, the additional burden on

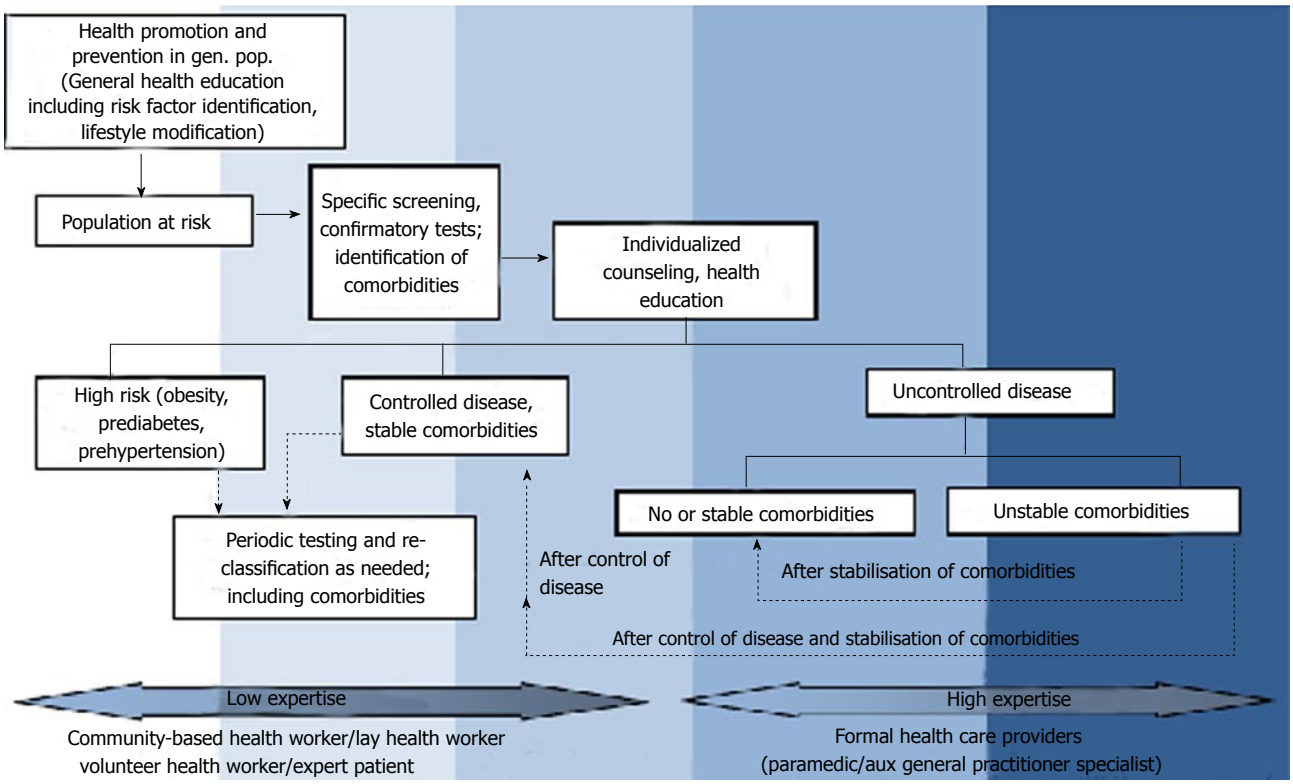

Figure 4 The context-adapted service delivery model for type 2 diabetes mellitus and similar chronic conditions.

professional healthcare workers who are now required to focus attention on both acute and chronic conditions is decreased. This service delivery model, adapted to the Philippine context, may be applicable to other LMICs having a similar situation as the Philippines. The model, however, requires additional support in terms of preparing all cadres for the delivery of diabetes care. These include sustained decision support, and materials such as the flowcharts, decision trees and checklists.

Taking inspiration from models of chronic care and carefully selecting essential elements according to effectiveness potential and local feasibility can result in basic but efficient care strategies[31].

A low resource healthcare system with no specific attention at all for chronicity can be induced to include chronic/lifelong conditions among its priorities, even with minimal means. Well applied, however, such minimal means can make a lot of difference for increasing numbers of people.

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REVIEW

How the kidney hyperfiltrates in diabetes: From molecules to hemodynamics

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Abstract

In this review, we focused on two molecules, connexin and sodium-glucose cotransporter, which can link to diabetic hyperfiltration. In diabetic kidney, the activation of renin-angiotensin system occurs simultaneously with glomerular hyperfiltration. The latter largely depends on pathophysiological afferent arteriolar dilation in the presence of high angiotensin Ⅱ. As a mechanistic basis for the above, tubular hypothesis has been proposed for type 1 diabetic patients as well as experimental models. Although tubular hypothesis has not been well evaluated in type 2 diabetes, clinical observations support that tubular hypothesis is true also in type 2 diabetes. Recent results on tubular hypothesis along with connexin abnormality in type 2 diabetes were revisited. In addition, the importance of sodium-glucose cotransporter in diabetic hyperfiltration is discussed. The link between salt paradox and the activation of renin-angiotensin system will be also reviewed.

Key words: Tubuloglomerular feedback; Salt paradox; Connexin; Glomerular hyperfiltration; Sodium-glucose co-transporter

Core tip: A diminished tubuloglomerular feedback (TGF) in diabetes can explain both glomerular hyperfiltration and the activation of renin-angiotensin system. An enhanced absorption through sodium-glucose co-transporter in proximal tubule decreases the delivery to macula densa, reducing TGF signal generation in diabetes. Connexin phosphorylation and subsequent ubiquitination by oxidative stress in type 2 diabetes reduces its expression in juxtaglomerular apparatus, disabling TGF signal transduction. Clinical as well as experimental evidences support that this tubular hypothesis is working, and suggest that drugs targeting the above to normalize TGF, an intrinsic physiological system, would be effective to ameliorate diabetic nephropathy.

Takenaka T, Inoue T, Watanabe Y. How the kidney hyperfiltrates in diabetes: From molecules to hemodynamics. *World J Diabetes* 2015; 6(4): 576-582 Available from: URL: http://www.wjgnet.com/1948-9358/full/v6/i4/576.htm DOI: http://dx.doi.

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OPENING REMARKS

As reported recently[1], many clinical trials have demonstrated that the inhibitors of renin-angiotensin system (RAS) are effective to prevent the development and progression of diabetic nephropathy (DMN). There is an emerging agreement that the activation of RAS give deleterious influences on DMN. Why is RAS activated even in the early course of diabetes? Many experimental hypotheses for an overproduction of angiotensinogen by hyperglycemia and pathological activation of pro-renin in diabetes have been proposed experimentally[2,3]. They appear very true at least in some aspects of DMN. From the renal hemodynamic point of view, DMN is characterized as glomerular hypertension and hyperfiltration from its early stage. In non-diabetic chronic kidney disease, single nephron glomerular hypertension and hyperfiltration occur in remnant nephrons to suffice the function of lost glomeruli due to its underlying renal disease. Thus, glomerular hyperfiltration starts when renal injury has progressed to some extent. However, all nephrons in diabetes show glomerular hypertension and hyperfiltration before microalbuminuria is developed[4]. The main character of DMN is abnormal afferent arteriolar dilation[5]. Tubular hypothesis is proposed more than 2 decades ago, which explain both glomerular hyperfiltration and RAS activation[6]. Is tubular hypothesis true for type 2 diabetes, which now provides medical as well as socio-economical problems over the world? This has not been well examined. Let us start from basic experiments.

TYPE 1 DIABETES

Tubular hypothesis

Tubular hypothesis is based on physiological responses to hyperglycemia and its mechanisms are following[6]. In type 1 diabetes, insulin deficiency causes marked hyperglycemia, resulting in the ultrafiltrate with high glucose concentration in Bowman capsule. Although proximal tubules reuptake most amounts of filtered glucose, glucose exceeding the capacity of tubular reuptake excretes into urine (glycosuria). Since proximal tubule possesses sodium glucose co-transporter 1 (SGLT1) and SGLT2, glucose is up-taken together with sodium. Then, the reuptake of sodium chloride through SGLT is increased in hyperglycemic condition, which is considered as a cause of salt-sensitive hypertension in diabetes. Furthermore, the delivery of sodium and chloride to macula densa is decreased by the enhanced reuptake through SGLT by proximal tubules (Figure 1). A reduced delivery to macula densa dilates the afferent arteriole by removing constrictor signals from tubuloglomerular feedback (TGF), to induce glomerular hypertension and hyperfiltration. Moreover, TGF signal from macula densa inhibits renin release. Again, a reduced delivery to macula densa during hyperglycemia (due to increased proximal tubular absorption through SGLT) removes TGF signals, to activate RAS which constricts efferent arterioles, worsening glomerular hypertension[7].

TGF mechanisms (adenosine triphosphate + adenosine) and salt paradox

There are still debates how macula densa cell transduces TGF signal to afferent arterioles. Although two hypotheses have been raised for "second messenger" for TGF, our data support the notion that both ATP and adenosine are required for full expression of TGF responses[8]. Macula densa cell releases adenosine triphosphate (ATP) into the interstitium when it reabsorbs sodium chloride delivered by tubular flow (Figure 2). On the one hand, ATP released from macula densa binds to ATP receptor located on extraglomerular mesangial cells to induce membrane depolarization and/or an increase in cytosolic calcium[9]. These signals travel to neighboring mesangial cells through gap junctions, and finally the signals are transduced to afferent arteriolar myocytes through gap junction[10]. Gap junction constitutes an important intercellular communication tool. Indeed, the inhibiting the function of connexin (Cx37 or Cx40), which compose of gap junction, elicits both suppression of TGF-dependent autoregulation and RAS activation. There is a possibility that ATP secreted from macula densa diffuses to afferent arteriolar myocytes and directly interacts with ATP receptors to induce afferent arteriolar constriction. On the other hand, ATP is degraded to adenosine by nucleotidase on extraglomerular mesangial cells, and subsequently adenosine binds to its specific receptor on afferent arteriolar myocytes to induce constriction[11].

Many paracrine factors modulate TGF. Angiotensin and endothelin enhance TGF responsiveness, whereas nitric oxide and prostaglandin diminish it[9]. Under physiological condition, salt load that enhances the renal production of nitric oxide and prostaglandin weakens TGF. However, experimental data indicate that salt intake enhances TGF in type 1 diabetes. Salt load reduces proximal tubular reabsorption, which is enhanced in diabetes. As a result, the delivery to macula densa is increased, thereby restoring TGF that constrict afferent arteriole, thereby ameliorating glomerular hypertension, hyperfiltration and albuminuria in type 1 diabetic model[12]. This is called salt paradox. An inverse relationship between salt intake and glomerular filtration rate is seen in type 1 diabetic patients as well as the animal models. Collectively, both tubular hypothesis and salt paradox are truly working in human[13].

Insulin deficiency and resistance

In contrast to type 1 diabetes, which is characterized by absolute insulin deficiency due to beta-cell damage, type 2 diabetes shows normal or excessive insulin

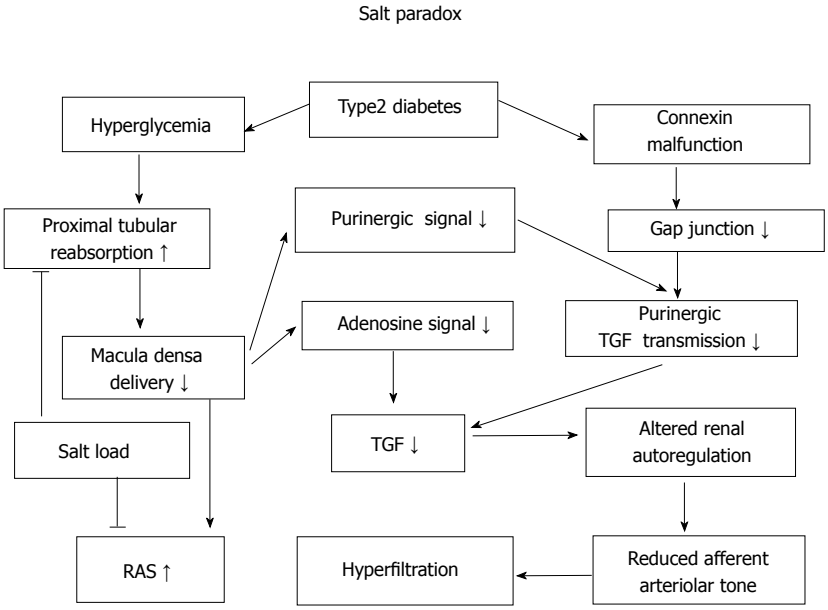

Figure 1 Working hypothesis for glomerular hyperfiltration in diabetes. On the one hand, hyperglycemia enhances sodium reabsorption in type1 and type 2 diabetes, thereby decreasing the delivery to macula densa with resultant weakening of tubuloglomerular feedback (TGF). The latter impairs renal autoregulation that dilats afferent arterioles, and activates renin-angiotensin system (RAS). On the other hand, TGF signal by adenosine triphosphate (P2) is damaged in type 2 diabetes due to connexin phosphorylation and gap junction malfunction, worsening glomerular hyperifiltration. High salt intake inhibits proximal tubular reabsorption, thereby increasing the delivery of sodium chloride to macula densa. This ameliorates pathological afferent arteriolar dilation by the restoration of TGF through adenosine (A1) signal[18].

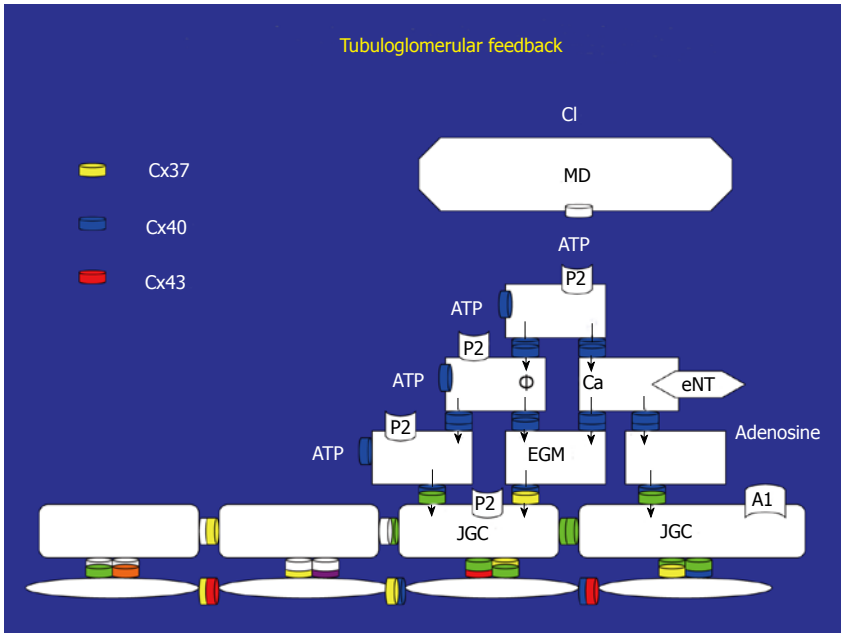

Figure 2 Sodium chloride is delivered to macula densa, macula densa releases adenosine triphosphate. Adenosine triphosphate (ATP) binds to P2 receptor on extraglomerular mesangial cell (EGM), and induces membrane depolarization and/or elevations of cytosolic calcium. These signals are transduced to juxtaglomerular cells (JGC) by intercellular communication through gap junctions consisted of connexins (Cx). In addition, ATP is degraded to adenosine by ecto-nucleotidase (eNT) on EGM. Adenosine binds to A1 receptor on JGC, increasing cytosolic calcium. Calcium waves generated in JGC transduce through gap junctions between afferent arteriolar myocytes to its upstream, eliciting ascending vasoconsitiction (refs 8 and 10). MD: Macula densa; Cl: Chloride.

secretion, especially during its early clinical course. The patients with type 2 diabetes are usually obese and manifest insulin resistance, underlying hyperglycemia in type 2 diabetes. The mechanisms mediating insulin resistance are out of focus of this review, but involve oxidative stress that inhibit insulin signaling by facilitating serine phosphorylation of insulin receptor substrate[14].

HOW ABOUT TYPE 2 DIABETES?

Connexin

As mentioned above, gap junctions are required for the transmission of TGF signal. Juxtaglomerular apparatus

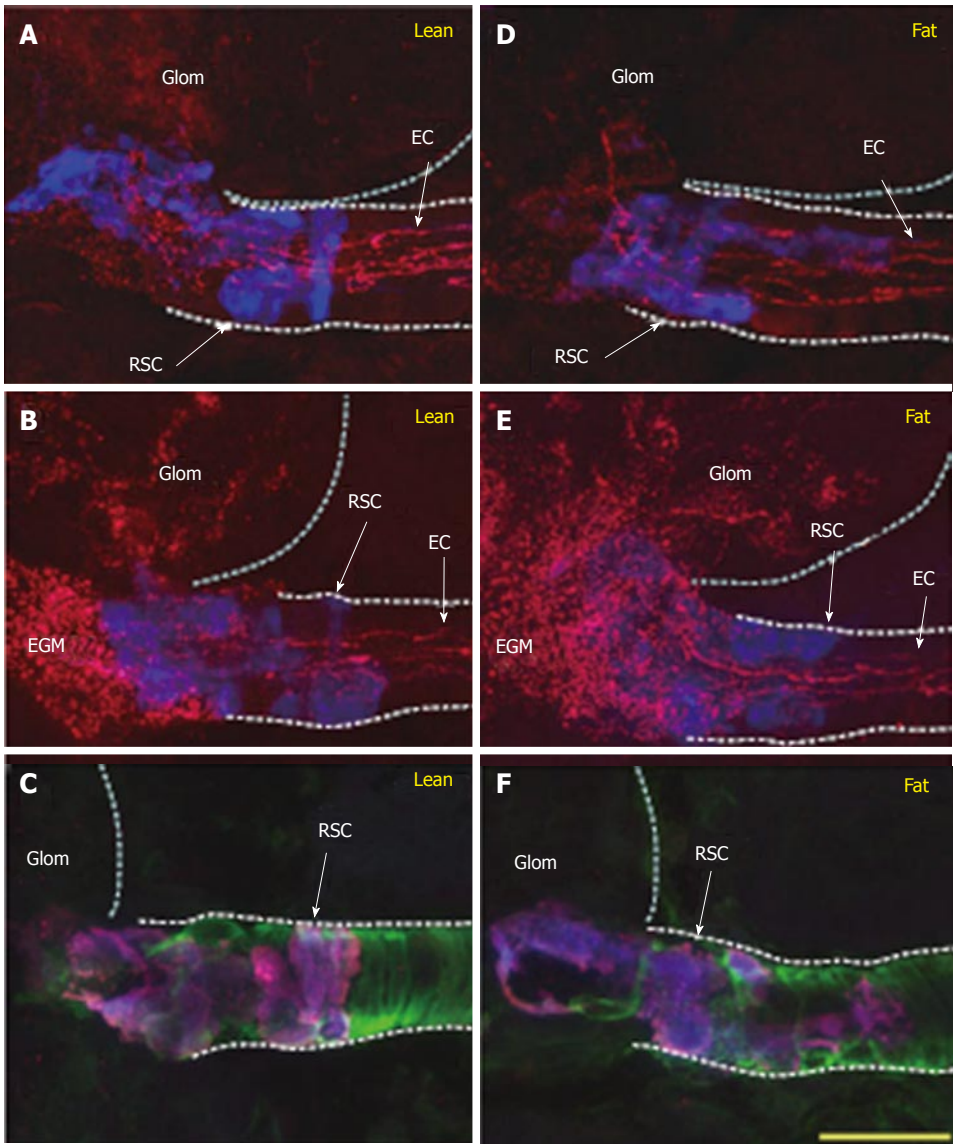

Figure 3 Expression of cennexins (Cx37, 40, 43) in juxtaglomerular apparatus of type 2 diabetic (Zucker diabetic fatty) and control (Zucker lean) rats. A, B, D, E: Renin secreting cells (RSC, blue) and endothelial cells (EC) express Cx37 (A: Lean; D: Fat) and Cx40 (B: Lean; E: Fat). Cx40 is expressed on glomerular and extraglomerular mesangial cells (EGM) in control (B) and diabetes (E). Cx43 is expressed in cytosol of RSC in both groups (C, F). Quantification reveals that the expression of Cx37 in RSC is reduced in diabetic model[15].

shows the expression of Cx37, Cx40 and Cx43. Type 2 diabetic model animals exhibit Cx abnormality[15]. Six Cxs form one hemichannel on cell membrane[16]. The binding of a hemichannel in a cell with the other one in an adjunct cell forms a gap junction. Gap junctions pass through small molecules such as inositol triphosphate and/or calcium, and transduce membrane depolarization, enabling intercellular communication. Post-transcriptional alterations of Cx induce conformational changes and prevent hemichannels to bind each other, especially when their extracellular loops are modified. Serine residue of Cx can be phosphorylated by protein kinase C and/or MAP. In type 2 diabetes, insulin resistance and associated oxidative stress activate these kinase activities. Phosphorylated Cx diminishes its ability to form gap junction, impairing intercellular signal transduction. Abnormal function of gap junction/connexin is considered to be one of the causes of arrhythmia in diabetes. Indeed, the abundance of phosphorylated Cx43 is elevated in type 2 diabetic animal model. Furthermore, functional analyses demonstrated abnormal gap junction function in juxtaglomerular apparatus that inhibiting Cx37 or Cx40 failed to stimulate renin release. Phosphorylated Cx is prone to be ubiquitinated and broken down. Expression of Cx37 on renin-secreting cells is reduced in type 2 diabetic animals (Figure 3). In type 2 diabetes, functional derangements of Cx induce the removal of TGF signal, which dilates afferent arterioles and activates RAS (Figure 1). As discussed, functional impairments of Cx in DMN cause glomerular hyperfiltration through reductions of TGF signal transmission. The latter may allow direct transmission of systemic blood pressure to glomeruli, facilitating glomerular sclerosis together with

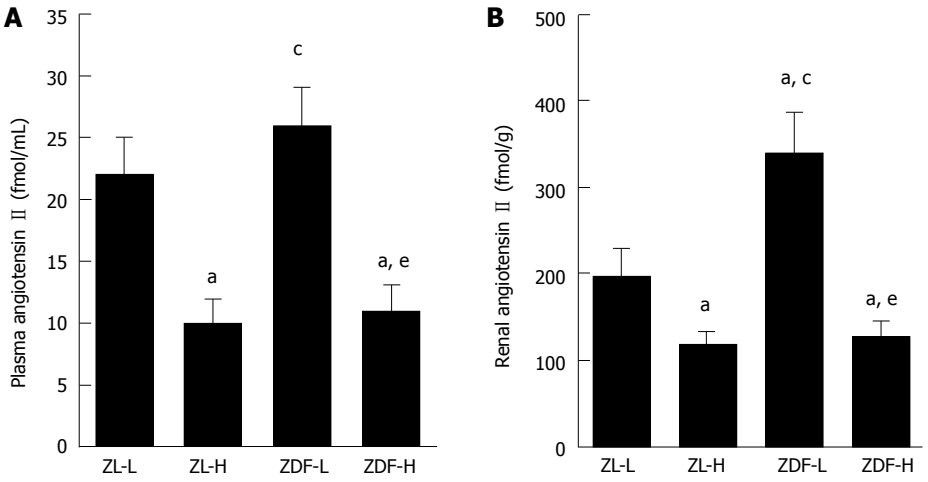

Figure 4 Plasma and kidney concentration of angiotensin II in type 2 diabetic model (Zucker diabetic fatty rat) and control rat (Zucker lean rat). ZL-L and ZL-H indicate ZL fed normal and high salt diet, respectively. Alike, ZDF-L, ZDF-H describe ZDF fed normal and high salt diet, respectively. $^{a}P < 0.05$ *vs* ZL-L, $^{c}P < 0.05$ *vs* ZL-H, $^{e}P < 0.05$ *vs* ZDF-L[18]. ZDF: Zucker diabetic fatty rat; ZL: Zucker lean rat.

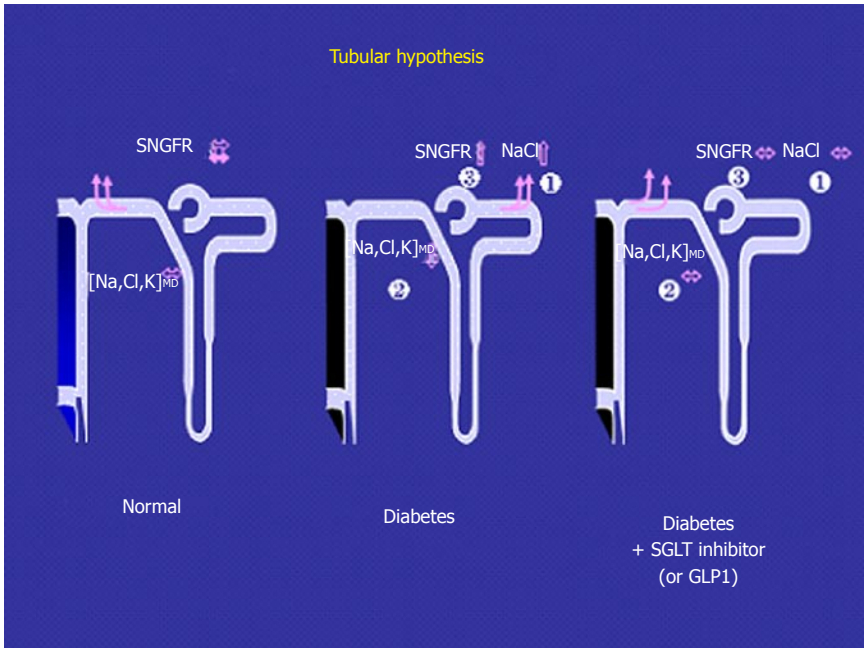

Figure 5 Compared to normal condition (left), ultrafiltrate in Bowman capsule contains significant amount of glucose in diabetes (middle). Because proximal tubules reabsorb more sodium with glucose through SGLT in diabetes (1), the delivery to macula densa is decreased (2). This weakens TGF signals to increase glomerular filtration rate (3), accounting for hyperfiltration in early stage of diabetes. Either GLP or SGLT inhibition (right) inhibits proximal tubular reabsorption (1), restoring sodium chloride delivery to macula densa even under hyperglycemic condition (2). This would have TGF work and normalize glomerular filtration rate (3), ameliorating glomerular hyperfiltration. SGLT: Sodium glucose co-transporter; GLP: Glucagon-like peptide; TGF: Tubuloglomerular feedback.

systemic hypertension induced by RAS activation[4]. Of interest, there is a report that abnormal Cx is related to the prognosis of DMN in type 2 diabetic patients[17].

Hyperglycemia and salt

Is tubular hypothesis true for type 2 diabetes? The answer appears to be YES. Our recent data indicate that enhanced proximal tubular reabsorption and glomerular hyperfiltration exist in type 2 diabetic animal model[18]. In addition, renal RAS is activated in this model, as evident that renal angiotensin concentration is elevated. Furthermore, in this model, acute salt load induces the suppression of proximal tubular reabsorption and the amelioration of glomerular hyperfiltration, together with the decrease in renal angiotensin concentration (Figure 4). The observations that salt load reduces albuminuria in this diabetic model suggest that glomerular hypertension is also controlled by high salt intake. These findings provide compelling evidence that salt paradox exists in type 2 diabetes. It is proved that adenosine is a mediator of salt paradox in type 1 diabetes. When salt consumption is increased in type 1 diabetes, the delivery of sodium chloride is increased, restoring TGF signals to produce adenosine that constricts afferent

arterioles and ameliorates glomerular hyperfiltration. Our experimental results demonstrated that under adenosine receptor blockade, the amelioration of glomerular hyperfiltration by salt load was not happened. Because TGF signal transmission pathway for ATP has been already diminished due to Cx abnormality in type 2 diabetes, residing adenosine pathway works for salt paradox in type 2 diabetes. Is salt paradox truly functioning in type 2 diabetic patients? No answer was given for this question until recently. However, there is a report that proximal tubular reabsorption positively relates to glomerular hyperfiltration in type 2 diabetic black patients[19]. Furthermore, an inverse relation between salt intake and albuminuria is demonstrated in type 2 diabetic Japanese patients[20]. Taken together, salt paradox is working in type 2 diabetic patients regardless of difference in race.

HOPE FOR NEW ANTI-DIABETIC DRUGS

We would not recommend for diabetes to take high salt diet to prevent the development and progression of DMN. Salt load could induce hypertension and facilitate the development of cardiovascular diseases. How can we prevent DMN? Although it should be important to strongly inhibit RAS, new anti-diabetic drugs have some hope. The inhibition of DDP-4 elevates GLP1, which binds to its specific receptor on proximal tubules to induce natriuresis through suppressing proximal reabsorption of sodium chloride (Figure 5). Emerging evidences indicate that DDP-4 inhibitors show blood pressure lowering effects[21]. Thus, according to tubular hypothesis, GLP1 ameliorates RAS activation, glomerular hypertension and hyperfiltration in DMN independently of its blood glucose lowering actions (Figure 1). Indeed, it was recently reported that DDP-4 inhibitors exhibit antiproteinuric effects in DMN. Many SGLT inhibitors are becoming available for clinical use. SGLT inhibitors may share similar renal actions with DDP-4 inhibitors. According to tubular hypothesis, SGLT inhibitors suppress proximal tubular reabsorption, which is enhanced in diabetes, suggesting that SGLT inhibitors possess blood pressure lowering and renal protective actions beyond its blood glucose lowering effects[22]. Indeed, experimentally SGLT inhibitor showed protective effects on diabetic nephropathy. However, the influences on RAS of SGLT and DDP-4 inhibitors have not been examined. Further studies are required to clarify this issue.

CONCLUDING REMARKS

Recently, the number of patients with DMN is progressively increased, showing medical-economic problem. Research on DMN is promising from many aspects, and numerous new findings have been obtained. This in a good news, and we hope that new results could be applied for clinical care as soon as possible. However, we are sorry that they do not intend to integrate the findings, so that they are isolated to each other. We have tried to sum up several findings in this review, although it might be inadequate. Discussion was focused on the mechanisms underlying DMN common in diabetic patients as well as animal model. The treatment with RAS inhibitors on DMN is effective, but we have to admit that they are not enough. In near future, we wish that new therapy that eventually halts DMN will be developed along with complete understanding of underlying mechanisms for DMN.

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REVIEW

Effects of exercise on brain functions in diabetic animal models

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Abstract

Human life span has dramatically increased over several decades, and the quality of life has been considered to be equally important. However, diabetes mellitus (DM) characterized by problems related to insulin secretion and recognition has become a serious health problem in recent years that threatens human health by causing decline in brain functions and finally leading to neurodegenerative diseases. Exercise is recognized as an effective therapy for DM without medication administration. Exercise studies using experimental animals are a suitable option to overcome this drawback, and animal studies have improved continuously according to the needs of the experimenters. Since brain health is the most significant factor in human life, it is very important to assess brain functions according to the different exercise conditions using experimental animal models. Generally, there are two types of DM; insulin-dependent type 1 DM and an insulin-independent type 2 DM (T2DM); however, the author will mostly discuss brain functions in T2DM animal models in this review. Additionally, many physio-pathologic alterations are caused in the brain by DM such as increased adiposity, inflammation, hormonal dysregulation, uncontrolled hyperphagia, insulin and leptin resistance, and dysregulation of neurotransmitters and declined neurogenesis in the hippocampus and we describe how exercise corrects these alterations in animal models. The results of changes in the brain environment differ according to voluntary, involuntary running exercises and resistance exercise, and gender in the animal studies. These factors have been mentioned in this review, and this review will be a good reference for studying how exercise can be used with therapy for treating DM.

Key words: Diabetes mellitus; Involuntary and voluntary exercise; Resistance exercise; Brain function; Animal models

Core tip: Brain is a highly sensitive and vulnerable tissue easily influenced by diabetes mellitus (DM). Physical exercise has been known to be one of the best non-pharmacologic ways to prevent and treat DM. Animal exercise experiments are very useful for research on DM because experiments cannot be performed in humans. Exercise has various benefits that help to improve brain function by reducing chronic inflammatory responses, accumulation of adipose tissue, appetite, insulin resistance, and dysfunction of the negative

feedback mechanism. In this review, the author reports a battery of animal models of exercise, and presents the beneficial effects of exercise on the brain.

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INTRODUCTION

Diabetes mellitus (DM) is one of the most common endocrine disorders and is mainly divided into two types according to the activity of β-cells in the pancreas: type 1 DM (T1DM) is characterized by degeneration of β-cells, while the main cause of type 2 DM (T2DM) is a progressive decline in insulin sensitivity resulting in sustained hyperglycemia[1-3]. Particularly, DM is known as the main factor that can cause various pathologic brain complications and can promote cognitive impairment and vascular dementia in humans[4-8]. A number of studies have reported that DM can cause hormonal dysregulation, systemic vascular changes, dysregulation of the plasma glucose level, changes in blood chemistry, and other organ dysfunctions such as kidney and heart failure[9-19]. Various medical treatments are available to regulate glucose dysregulation, correct hormonal changes and vascular conditions in DM patients; however, these medical treatments cannot always cure the metabolic disorder completely, and physicians also cannot predict the progression of the complications with uncontrolled patient's life styles[20-24].

Particularly T2DM is significantly related to the incidence of obesity and its associated disorders[25-27]. Obesity is defined as a surplus of body fat accumulation, with the excess of adipose tissue really being a well-established metabolic risk factor for the development of obesity-related comorbidities such as insulin resistance, T2DM, cardiovascular diseases, and some common cancers[2,28-32]. The mechanisms linking excess adiposity and cancer are unclear, but the obesity-related low-grade chronic inflammation is widely accepted as a critical factor in the pathogenesis of various diseases such as T2DM, cardiovascular disorders, dementia, cancers, dietary control failure[26,28,33-42]. Currently, particular attention has been placed on the pro-inflammatory microenvironment in the body associated with obesity, specifically underlining the involvement of obesity-associated hormones/growth factors in the cross-talk between macrophages, lymphocytes, adipocytes, and epithelial cells involved in the development of T2DM[28,43]. In addition, accumulated peripheral white adipose tissue (WAT) is an endocrine tissue that secretes hundreds of cell-signaling molecules known as cytokines, chemokines, and adipokines[29,32,33,44,45]. The endocrine function of adipose tissue might be a key factor in the mechanisms linking adipose tissue to insulin resistance, leptin resistance, dietary control failure, T2DM-associated dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis, neurodegenerative diseases, vascular diseases related to aging, cognitive impairment, and dementia[27,35,45-50]. Hence, uncontrolled chronic obese condition can be a critical factor in the development of T2DM, and it also acts as an agent that affects normal brain functions.

Recently many studies have shown the positive effects of regular physical activity on improving complications caused by DM, and hence regular physical activity intervention is regarded as a promising adjuvant therapy[7,37,51-62]. Exercise can affect various physical environments and has decisive effects on improving brain functions for a better quality of life[7,59-61,63-72]. However, the precise mechanisms responsible for the positive effects of exercise on brain functions under obesity and T2DM conditions have not yet been well understood, and many studies have been performed using animal models of different diabetic stages regardless of the DM type and under various kinematic conditions to assess the related mechanisms for changing the microenvironment of the brain. Thus, experiments with animal exercise models mimicking the etiology and progression of human DM have been actively performed and developed to assess the preventive and therapeutic effects of exercise on brain functions[1,73-81].

Therefore, we review recent evidences on the role of exercise in promoting brain functions mainly under T2DM conditions in animal models and provide practical applications for the management of T2DM.

PHYSIOPATHOLOGICAL CHANGES CAUSED BY DM

Most of the DM conditions gradually impair normal brain functions by causing excessive production of pro-inflammatory cytokines, insulin resistance, and reactive oxygen species due to certain causes such as prolonged obese condition or hormonal dysfunction[15,27,28,31,32,35,82-88]. Excessive and/or compulsive overeating disturbs the normal blood composition, deteriorates cardiovascular circulation, induces insulin resistance, and increases the visceral fat[29,45,82,89-91]. Particularly, the infiltrated inflammatory immune cells such as macrophages and lymphocytes in adipose tissues secrete a variety of cytokines into the blood stream, and negatively influence the systemic cardiovascular system and the brain[32,44,82,92-94]. A number of studies have shown that elevations in levels of systemic inflammatory mediators such as adipokines, tumor necrosis factor-α, resistin, interleukin-6, plasminogen activator inhibitor-1, C-reactive protein, monocyte chemoattractant protein (MCP)-1 play a pivotal role in changing the physiology of the brain[3,45,56,82,95-98]. Particularly, results of animal

and human studies have showed that insulin passes *via* the systemic circulation to the brain and it may have some physiologic actions which are different than its peripheral metabolic effects. Insulin resistance in peripheral tissues leads to the elevation of pro-inflammatory cytokines, neurotoxic ceramides, obesity-induced NADPH oxidase-associated oxidative stress in the brain, and insulin action on the brain is thought to be a regulator of peripheral glucose homeostasis in rodent studies *via* melatonin related mechanisms, increased unfolded protein response activation, mitochondrial and ER stress related overeating, leptin and insulin resistance, corticotropin-releasing factor-related islet cell control[47,67,99-102]. Recent studies of the mouse brain have demonstrated that degenerative plaque formation observed in AD (AD is the most prevalent form of dementia) is associated with insulin resistance[47,103]. Insulin regulates food intake and cognitive functions in the brain; however, deranged insulin signaling in the brain has also been implicated in neurodegenerative disorders[104-107].

Insulin action in the brain is regarded as the main factor for maintaining DM patients in a healthy condition due to the interrelationship between peripheral and central insulin resistance.

ANIMAL MODELS IN DM RESEARCH

DM is a chronic disease that is characterized by a relative or absolute lack of insulin release, resulting in hyperglycemia. Since T1DM and T2DM, as endocrine disorders, represent quite complex diseases in which different organ systems are involved, animal models should be chosen carefully depending on what aspect of the disease is being investigated. On the other hand, for developing specific models of T1DM and T2DM, investigators should be aware of the different pathogenic mechanisms of DM that involve different inducible factors.

T1DM animal models

The main characteristic of T1DM is autoimmune destruction of the pancreatic β cells, leading to lack of insulin release. In animal models, investigators can induce this deficiency by chemical ablation of the beta cells in breeding animals that spontaneously develop the autoimmune diabetic condition. The representative chemicals that induce T1DM are streptozotocin synthesized by *Streptomycetes achromogenes*[108-110], and alloxan[1,73,111,112] which causes poor β cell defense mechanisms against free radicals. Thus, these chemicals can be used for developing new insulin, transplantation models for testing treatments that may prevent beta cell death. However, the researchers should be aware that a number of studies using STZ did not consider the time period between chemical injection in animals and sacrifice. Thus, it is true that many researches on T1DM using STZ injection have ignored this factor. Shin *et al*[110] and Yi *et al*[113] demonstrated chronological hippocampal changes in the brain at different time points of animal sacrifice after STZ injection. Therefore, researchers should remember that the results of T1DM *via* the chemical might be different based on how many days or weeks have passed following chemical administration in animals.

The non-obese diabetic mice, Biobreeding rats, and LEW. 1AR1/-iddm rats are the most commonly used animal models of spontaneous autoimmune diabetes showing beta cell destruction due to an autoimmune process[1,73,75]. Akita mice, a genetically induced insulin dependent T1DM diabetic animal model, are characterized by beta cell destruction *via* ER stress. Lastly, T1DM can be induced by viruses such as Coxsackie B virus[114], Encephalomyocarditis virus[115,116], and Kilham rat virus[116,117]. The virus-induced model can be complicated as the outcome is dependent on replication of the virus as well as timing of the infection[118].

Several other large animal models except for rodent animals have been developed to study T1DM extensively. Since it is relatively difficult to expect the development of spontaneous diabetes in large animal models, induced models of T1DM are required. The most commonly used method of inducing T1DM in large animal models is by performing pancreatectomy and chemical ablation of beta cells (STZ)[119-122]. The T1DM rodent models are summarized in Table 1.

T2DM animal models

The main characteristics of T2DM are insulin resistance and β cell dysfunction, and defective insulin secretion from β cells. Therefore, animal models of T2DM tend to include models of insulin resistance and/or β cell dysfunction. Most of the T2DM animal models are characterized by the obese phenotype, which reflects the human condition where obesity is closely related to T2DM development[1]. The T2DM animal models are categorized according to the type of induction mechanism as follows: spontaneously obese models[1], diet/nutrition induced obesity models[123,124], non-obese models[125], genetically induced models of β cell dysfunction[126], and surgically induced diabetic animal models[127]. The T2DM rodent models are summarized in Table 2.

DM AND THE NEURAL SYSTEM

DM and central nervous system

Diabetes is significantly related with brain microenvironments and functions. Diabetes is known to largely affect the intensely vascular organs such as kidneys, liver, and brain[16,18-20,49,50,87,88,95,98,99,128-130]. Brain is the key organ that is involved in hormonal, sensory, and motor regulations so that living organisms can maintain homeostasis *via* the negative feedback system[46,131]. However, diabetic condition can be a serious chronic stress factor, and its secondary negative effects can exert a bad influence on the

Table 1 Summary of animal models of type 1 diabetes mellitus

Induction	Models	Dose(s) (mg/kg)	Main characteristics	Model uses
Chemicals	Streptozotocin	Rat 35-65 (*iv* or *ip*) Mice 100-200 (*iv* or *ip*) Hamster 50 (*ip*) Dog 20-30 (*iv*) Pig 100-150 (*iv*) Primates 50-150 (*iv*)		New formulations of insulin transplantation models
	Alloxan	Rat 40-200 (*iv* or *ip*) Mice 50-200 (*iv* or *ip*) Rabbit 100-150 (*iv* or *ip*) Dog 50-75 (*iv* or *ip*)	Hyperglycemia	
	Multiple low dose Streptozotocin			Treatments prevent beta cell destructions
Spontaneous autoimmune	NOD mice BB rats LEW.1AR1/-iddm rats		Beta cell destruction due to an autoimmune process	Understanding genetics of T1DM Understanding mechanism of T1DM Treatments prevent beta cell destruction Treatments manipulate autoimmune process
Genetically induced	AKITA		Beta cell destruction due to ER stress Insulin dependent	New formulations of insulin Transplantation models Treatments to prevent ER stress
Virally-induced	Coxsakie B virus Encephalomyocarditis virus Kilham rat virus		Beta cell destruction induced by viral infection of beta cells	Establish potential role of viruses in the development of T1DM

iv: Intravenous injection; *ip*: Intraperitoneal injection; T1DM: Type 1 diabetes mellitus.

Table 2 Summary of animal model of Type 2 diabetes mellitus

Induction	Model	Main characteristics	Model uses
Obese models	ob/ob mice db/db mice KK mice KK/Ay mice NZO mice TSOD mice Zucker fatty rat Zucker diabetic fatty rat OLETE rat	Obesity-induced hyperglycemia	Identifying factors involved in obesity-induced diabetes Some models show diabetic complications Treatments to improve beta cell function
Non-obese models	GK rat Cohen diabetic rat	Hyperglycemia induced by insufficient beta cell function	Treatments to improve beta cell function and beta cell survival
Diet/nutrition induced obesity	High fat feeding (mice and rat) Desert gerbil Nile grass rat	Obesity-induced hyperglycemia	Treatments to improve insulin resistance Treatments to improve beta cell function Treatments to prevent diet-induced obesity
Surgical diabetic animals	VMH lesioned dietary Obese diabetic rat Partially pancreatectomized animals (dog, primate, pig and rats)	Avoid cytotoxic effects of chemical diabetogens on other body organs Resembles human T2DM due to reduced pancreatic islet beta cell mass	Occurrence of hyperphagia Pancreatitis
Transgenic/knock-out diabetic animals	Uncoupling protein (UCP1) Knock out mice HiAPP mice	Poor activation of thermogenesis Amyloid deposition in islets	Treatments of obese conditions Increase obesity (energy storage) Treatments to prevent amyloid deposition

body[10,46,113]. What is more important is that, since the brain is a very vulnerable and sensitive organ, the duration and severity of DM might result in serious neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease[10,70,102]. The HPA axis regulates responses to various stress factors, digestion, immune response, mood and emotions, sexuality and energy expenditure/storage[11,59,104,132-134]. In addition, since the HPA axis is also connected with the autonomic nervous system[135,136], it is very important when the brain orders right responses to diverse physiological conditions. If DM persists and/or is increased without any modification, the brain cannot maintain the normal HPA axis regulation, and the HPA axis based on the negative feedback system tends to be highly activated due to uncontrolled DM. However, sometimes exercise-induced stress might influence the beneficial effects of exercise are not observed in certain behavioral test. It is recognized that the amount of psychological stress that an animal encounters determines the degree of

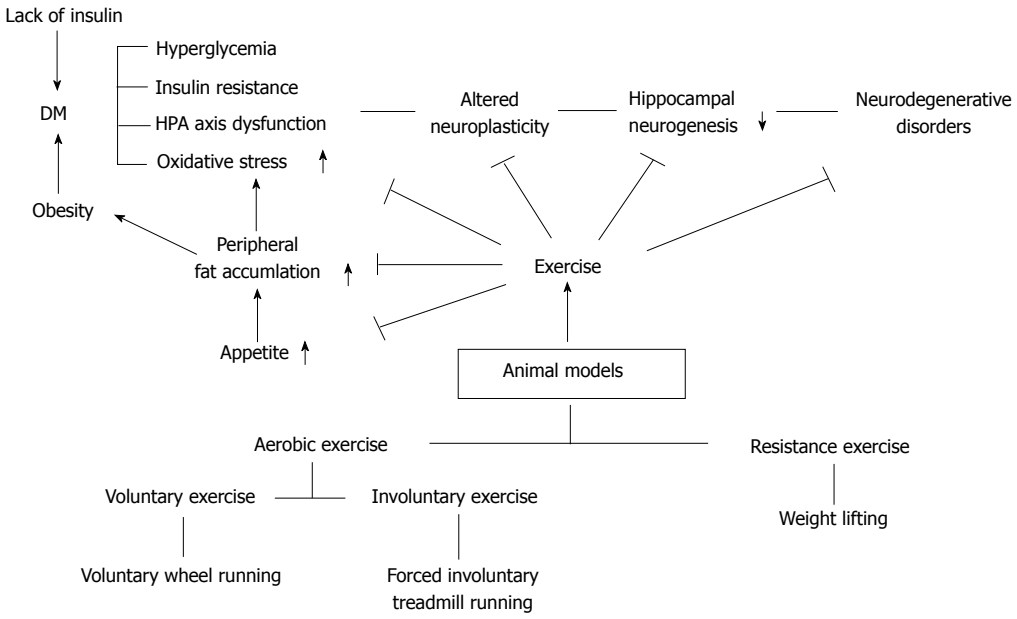

Figure 1 Exercise animal models can separate aerobic voluntary/involuntary exercises and non-aerobic resistance exercise. The exercise can attenuated many neuro-related disease followed DM and obesity. DM can develop many kinds of dysregulation such as hyperglycemia, increasing insulin resistance and HPA axis dysfunction and oxidative stress. Exercise reduces peripheral fat accumulation and appetite in animal models, and it has preventive and therapeutic effects for the many risks to develop obesity and DM. A number of studies about diabetes have been revealed the related mechanisms through exercise animal models. Non-aerobic resistance exercise described in Table 3. DM: Diabetes mellitus; HPA: Hypothalamo-pituitary-adrenal.

response of the HPA axis regulation[137]. Moreover, it has been reported that animals performing an exercise at the stress-induced physiological and environmental factors can be strongly affected[59,137-139]. Therefore, there would be enough possibilities to show different behavioral effects under various kinds of stress factors in exercise animal models such as metabolic DM and psychological depression/anxiety disorders. Cayado *et al*[137] reported that different training showed different exercise effects at the horse exercise training. Martínez-Mota *et al*[138] indicated that the HPA axis response can be different according to sex and age at the exercise animal model. Furthermore, since DM is defined as a chronic systemic inflammatory condition, the disease can contribute to the development of different metabolic disorders. The most significantly affected organs by the chronic inflammatory condition are the vascular converged areas, and thus cardiovascular system is mostly vulnerable and its vascular microenvironment is changed leading to profound damage. Particularly, the occurrence of T2DM is generally characterized by the development of chronic obese condition *via* overnutrition and/or increased hyperphagia[123,124,133,140,141]. In addition, neuroinflammation and neurodegeneration have been known to be closely related with overnutrition-induced disease and diabetic animal models[2,4,9,99] (Figure 1).

T2DM is commonly known to be the consequence of chronic obesity and it is usually accompanied by uncontrollable hyperphagia[142-144]. Many factors contribute to pathologic overeating and mediate feeding behavior in humans and animals, and the most important factor is leptin[104,145,146]. Leptin, which is a cytokine originating mainly from white adipose tissue, plays an important role in regulating energy expenditure, food intake, and obesity[45,71,91,98,104,145,146]. The mechanism by which leptin modulates these hypothalamic neurons involves the binding of leptin to the long form of leptin receptor (Ob-Rb) and subsequent intracellular signaling, initiated by autophosphorylation of Janus kinase 2 (JAK2) and activation of signal transducer and activator of transcription (STAT3). Following the translocation of STAT3 to the nucleus, suppressor of cytokine signaling-3 is activated, exerting feedback inhibition on JAK2. Leptin activation of insulin receptor substrates and the protein kinase B pathway inhibits food intake and modulation of extracellular regulated kinases has been demonstrated to play a role in the control of energy homeostasis[147]. Obese patients and animals cannot regulate their hedonic appetite except for acceptable daily intake of calories. They have excessive WAT in the body and it secretes leptin in the blood; however, the appetite center does not recognize leptin and shows resistance to leptin. Therefore, leptin administration to obese rats and humans has elicited small effects on fat mass and appetite due to leptin resistance[2,53,148]. Likewise, many neuropeptides located in hypothalamic nuclei transmit related anorexigenic or orexigenic signals[104,146]. Furthermore, many kinds of neurotransmitters such as serotonin, dopamine, and norepinephrine participate in regulation of mood, emotions, and appetite[149]. Particularly, specific serotonin reuptake inhibitors (SSRIs) and serotonin-norepinephrine reuptake inhibitors (SNRIs) have been introduced and used as medical treatments to reduce food intake of overweight patients[150-153]. These

drugs have been modified continuously to overcome their side effects or toxicities[154]; however, chronic administration of SSRIs or SNRIs causes rebound body weight gain in the patients. The phenomenon has been observed for a while; however, the cause was not the patients' psychological drug dependence, and same results have been obtained in many studies performing animal experiments[155,156]. Since these agents not only influence the appetite center but also the other areas in the brain, the environment of the brain gets affected[157-159]. Therefore, more specific target regions in the brain and promising regulatory drugs are required. Finally, failure of appetite control can easily lead to T2DM, and then the continuous progression of this metabolic disorder harms the neuroenvironment of the brain and affects learning/memory and cognitive functions[47,49,85,87,160]. According to the severity of DM, hippocampal neurogenesis in the dentate gyrus is significantly reduced and neuronal plasticity is also negatively influenced through reduction in neurotrophic factors[31,130,160]. Uncontrolled DM with hyperglycemia can cause serious brain damage; therefore, appropriate therapies that can slow the progression of this disease are needed.

DM and peripheral nervous system

Diabetic neuropathy affecting the peripheral nervous and autonomic nervous systems is the most frequent complication of diabetes. The most common neuropathies are chronic sensorimotor distal polyneuropathy (DPN) and autonomic neuropathies[161]. Morphologically, DPN is characterized by alterations in peripheral nerve fibers as well as degeneration and regeneration of both myelinated and unmyelinated fibers in humans, decreased axonal diameter of the sciatic nerve and myelin sheath thickness of the sural nerve, and alteration of the cytoskeletal component in dorsal root ganglia of rats[162-164].

Currently, human life span has dramatically increased due to advances in medical science, and moreover, improving the quality of life has also received attention. Therefore, people are feeling the need to maintain their brain health throughout their life. However, DM poses a threat to the health of people and therefore it has become a problem that needs to be conquered.

EFFECTS OF EXERCISE ON THE BRAIN OF DIABETIC ANIMALS

It is already well known that regular physical activity has a tremendous impact on health and has protective effects against chronic diseases, including heart disease, stroke, hypertension, and DM. Over several decades, many evidences have demonstrated that exercise in human and animal models helps to maintain brain health such as cognitive performance, and it can even protect the central nervous system and improve learning/memory functions following chronic exercise, both in animal models and humans[59,61,104,165-171]. In recent years, many exercise and cognition studies have been carried out in adult rodents. These researches have provided insights into the underlying cellular mechanisms[169,172]. Both voluntary and forced exercise enhanced spatial memory in Morris water maze, Y-maze, T-maze, and radial arm maze test[65,169]. Particularly, running exercise improved performance in hippocampus-dependent tasks that require limited movement, and there were non-hippocampal dependent benefits from voluntary and forced exercise. Chronic involuntary treadmill exercise in an T2DM animal model (ZDF rats) reduced blood glucose levels, caused cell proliferation and an increase in neuroblasts in the hippocampal dentate gyrus; however, the onset of treadmill exercise in the severe chronic diabetic condition has a limitation in increasing neuroblast differentiation although it increases neural plasticity[77,80]. Therefore, for achieving effectiveness of treadmill exercise in increasing neuronal differentiation in the hippocampus and for counteracting the negative effect of DM in the brain, the initiation time of exercise during the early stage of DM may be a very critical point to achieve the positive effects of exercise[77]. Furthermore, Hwang *et al*[77,173] reported that Cox-2 is very important factor for hippocampal neurogenesis in the T2DM animal exercise models. Griffin *et al*[167] reported that voluntary exercise also increased volume of the hippocampus resulting in improved search strategies and decreased perseveration once the platform had been moved to a new location. Voluntary exercise results in elevation of levels of factors such as brain-derived neurotrophic factor (BDNF), whose levels increases with aerobic exercise, and enhances hippocampal function[167].

Interestingly, Burghardt *et al*[174] studied the behavioral effects of voluntary and involuntary running exercise with a battery of behavioral tests; they investigated the effects of 8 wk of forced treadmill running and voluntary wheel running on behavior measures in the elevated plus maze, open field, social interaction and conditioned freezing paradigms. They found that chronic voluntary running produces behavioral changes in the elevated plus maze and open field; however, chronic treadmill running failed to produce behavioral changes with their running protocol. Changes in opioidergic[175], serotonergic[176], GABAergic[177], and catecholaminergic[178] systems have also been observed after wheel running. Regular running exercise is closely associated with food preference and appetite depending on the volitional wheel running and involuntary treadmill exercise. Recently, attention has been paid to various causes of food preference and consumption according to a wide range of conditions for overcoming the obese and DM conditions[179]. Diet composition may lead to changes in neuropeptides within brain nuclei regulating energy metabolism. Dietary manipulation has been thought to influence energy expenditure *via* changes in

central neuropeptide activity. Many studies report that medicines such as morphine, fenfluramine influence the neuro-regulatory systems and exercise can modify palatability in animals[175]. Blundell *et al*[37] asserted that changes in dietary preferences could be due to alterations in the hedonic properties of the food as a result of exercise in rodent models. Shin *et al*[144] also indicated a possibility that treadmill exercise in animals inhibits diabetes-induced increment of the desire for food. Hormonal (leptin and insulin) and nutrient signals from the periphery are mainly integrated in the hypothalamus, and multiple factors regulate food intake. AMP-activated protein kinase (AMPK) is the downstream component of a kinase cascade that acts as a sensor of cellular energy charge, being activated by rising AMP coupled with falling ATP[180]. Although the effects of AMPK on desire for food are still controversial, exercise may contribute to appetite suppressive actions in the hypothalamus due to the effects of leptin and in different causes in the rodent model[147,179-182]. As mentioned above, alterations in opioid or inhibitory neurotransmission systems in both limbic and brainstem areas could be implicated, including the nucleus accumbens[183]. Multiple mechanisms of action in the brain could be responsible for this behavioral difference and lack of gross metabolic difference[171]. In humans, texture, temperature, color, and appearance all play a role in food acceptance[184,185]; however, animals exhibit a wide range of food preferences and animal studies can eliminate the points of dispute in human studies. In addition, an important element in the study of effects of exercise on food preference is sex differences[171,175,179]. Sex differences exist such that female rats tend to prefer carbohydrates over other macronutrients following exercise[134,175]. Unfortunately, there is still no clear evidence on the effect of exercise on macronutrient or carbohydrate selection in different sexes in animal or human studies. Therefore, further research for assessing the sex differences in food preference after exercise is needed.

Chronic inflammation and increased oxidative stress are observed in the animals showing insulin resistance following diet-induced obesity[36,44,45,62,131,186,187]. Indeed, since the brain tissue is highly sensitive to chronic inflammation and oxidative stress due to its high oxygen consumption, iron and lipid contents, and low activity of antioxidant defenses[102,188], energy metabolism impairment and oxidative stress are important events that have been related to the pathogenesis of diseases affecting the central nervous system[47,180]. Exercise has been known to decrease chronic systemic inflammatory response, show antioxidant effects and positive effects on synaptic plasticity in the obese and/or diabetic rodents[55,60]. In the T1DM animal model, significant inflammatory responses are found and they showed different action in a time-dependent manner[113]. These responses induced by DM lead to mitochondrial dysfunction, which can progress to various pathologies such as neurodegenerative diseases (dementia, Alzheimer's disease, Parkinson's disease)[33,34,47,61]. Both T2DM and neurodegenerative diseases are associated with impaired glucose tolerance and cognitive decline in the human and animal studies, and insulin resistance and subsequent hyperinsulinaemia have been found to increase the risk of Alzheimer's disease and promote decline in memory and cognitive dysfunction[3,34,61,133,189]. Regular exercise and dietary restriction can attenuate the progression of metabolic and neurodegenerative disorders[4,5,67,190]. Exercise (particularly vigorous aerobic exercise)[111,167,191-193] and energy restriction (caloric restriction and intermittent fasting)[143,194,195] can result in striking improvements in glucose and lipid metabolism, and can eliminate the need for medications. Exercise and dietary energy restriction activate a wide range of adaptive cellular responses in the peripheral organs (muscle, liver) and the brain, resulting in improved bioenergetics and brain function, and resistance to neurodegenerative disorders.

As mentioned previously, the causes of DM belong to different metabolic conditions and can show diverse pathologic phenotypes in a time-dependent manner[113]. This review mainly focused on the changes in the brain caused by DM and exercise; however, changes in peripheral neuropeptides and organs are also significant. Adiposity, chronic inflammatory response, activation of oxidative stress, dysfunction of pancreatic islets, insulin and leptin resistance, dysfunction of the negative feedback mechanisms, and appetite disturbance constantly affect brain homeostasis. Indeed, exercise has been thought to attenuate brain damage caused by these risk factors; however, exercise during the early stage of diabetes is considered to be a critical factor for preserving brain function[10,80]. The risk factors listed above can be therapeutic targets to treat and ameliorate DM; thus, refinements using various animal exercise models can give new insights into the treatment of DM.

It is well accepted that physical activity by contracting skeletal muscles (resistance exercise) secretes enhanced levels of myokines which have a beneficial endocrine effect on other organs, presenting novel targets for the treatment of metabolic diseases and T2DM[70-72,94]. Pedersen hypothesized that physical inactivity leads to T2DM, depression, dementia, cancers, cardiovascular diseases, and asserted that skeletal muscle should be considered as an endocrine organ[70]. Cytokines and other peptides that are produced, expressed, and released by muscle fibers and exert paracrine or endocrine effects should be classified as myokines. Actually, since skeletal muscle is the largest organ in the human body, skeletal muscle should receive attention for identifying its new multiple functions in metabolic disorders and T2DM. Skeletal muscle has the capacity to express several myokines including IL-6, IL-8, IL-15, BDNF, FGF21, MCP-1, vascular endothelial growth factor, leukemia inhibitory factor, Irisin, and ANGPTL4[71]. IL-6 was discovered as a myokine because of the observation that it increases

Table 3 Exercise animal models on brain function

Exercise type	Method	Measurement	Note[1]	Note[2]
Aerobic exercise	Voluntary running wheel exercise	Freely access to running wheel Exercise strength can be measured *via* digital counter. The running wheel was rotated by animal effort		Cognitive performance Neurogenesis in subgranular zone or subventricular zone Improvements of learning and memory
	Involuntary treadmill exercise	Enforced running exercise Regularly enforced running exercise is enforced with constant speed on a motorized treadmill		Neurophysiological development Relationship between Brain and Stress axis Feeding behavior
	Forced swimming	Animals are forced to swim in an acrylic glass cylinder filled with water	This test is used to see a rodent's response to the threat of drowning whose result has been interpreted as measuring susceptibility to negative mood. It is commonly used to measure the effectiveness of antidepressants	
Non-aerobic resistance exercise	Weight lifting	Kondziela's inverted screen test	The inverted screen is a 43 cm square of wire mesh consisting of 12 mm squares of 1 mm diameter wire	Cognition Neuronal plasticity changes
		Weights test	Seven weights constitute the apparatus Ranging from 20 to 98 g	Anti-inflammatory response in brain
		Grip strength test	Forelimb grip strength is accessed using a digital Grip Strength Meter	Neurogenesis in subgranular zone and subventricular zone

[1]Principal; [2]Uses.

up to 100-fold in the circulation during exercise. In particular, the identification of IL-6 production by skeletal muscle during physical exercise generated renewed interest in the metabolic role of IL-6 since it created a paradox[70]. IL-6 can also alter brain function after peripheral administration, moreover, some myokines might be able to cross the blood-brain barrier[196,197]. IL-6 is significantly produced and released in the post exercise period when insulin action is increased; on the other hand, IL-6 has also been associated with obesity and reduced insulin action. However, many researches during the past decade have reported that in response to muscle contraction, both type 1 and type 2 muscle fibers express the myokine IL-6, which subsequently exerts its effects locally and systemically in several organs[70-72]. Within skeletal muscle, IL-6 acts to signal *via* AMPK and/or PI3-kinase to enhance glucose uptake and fat oxidation. In addition, muscular derived IL-6 mediates anti-inflammatory responses[70].

A few researches on the relationship between myokines and the brain in animal models have just been published, and the effects of skeletal muscle derived myokines on brain function must be plausible enough directly and/or indirectly. Recently, Dun *et al*[198] reported that myokine Irisin was detected in three types of cells; skeletal muscle cells, cardiomyocytes, and Purkinje cells of the cerebellum. Moreover, they reported that Irisin not only mediates the animal's movements but also regulates adipose tissue thermogenesis by neurons in the caudal ventrolateral medulla and rostral ventrolateral medulla that are an integral component of the medullary sympathetic circuitry and these neurons project their axons to spinal sympathetic premotor neurons[198]. Similarly, it is known that resistance exercise improves body and brain bioenergetics for PD risk reduction[4], insulin and leptin signaling in obese rats[2,45,82,104,199], and exerts antidepressant-like effects *via* improving the impaired neuroplasticity[101,200]. Aerobic exercise and non-aerobic resistance exercise described in the Table 3.

Evidences of positive effects of resistance exercise on brain health in T2DM for therapeutic purposes with other aerobic exercises and pharmacologic treatments have been reported recently, and further studies on the mechanisms of treatment according to the severity of DM are needed.

CONCLUSION

It is confirmed that exercise is an incredible therapeutic option for treating DM patients. Animal exercise models are significant methods to study the network between central and peripheral organs. Brain is an extremely sensitive and soft tissue that can be damaged due to chronic insulin resistance, hyperglycemia, and chronic inflammation; however, various kinds of exercise can attenuate the brain damage and delay neurodegeneration caused by the risk factors. Many diabetic experimental animals with a genetic background and nutrition induced diabetic animals can be used in various DM studies; however, many physiopathologic conditions should be considered, and researchers should choose the animal models after giving careful consideration. Many aerobic running exercises and resistance skeletal muscle exercises have been performed recently in various animal models to study their therapeutic effect on brain function; however, more careful considerations reflecting the clinical conditions should be added in the animal models. Furthermore, it is important to study the therapeutic effects of exercise on brain health during

the stages of DM in animal models; however, dramatic effects of more prospective methods for maintaining brain health during DM seem to be achieved through development of various combinations of animal models in the pre-diabetic condition. A number of target signals from the exercise studies can also be the candidates for development of pharmacologic medicines.

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REVIEW

Type 2 diabetes mellitus: From a metabolic disorder to an inflammatory condition

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Abstract

Diabetes mellitus is increasing at an alarming rate and has become a global challenge. Insulin resistance in target tissues and a relative deficiency of insulin secretion from pancreatic β-cells are the major features of type 2 diabetes (T2D). Chronic low-grade inflammation in T2D has given an impetus to the field of immuno-metabolism linking inflammation to insulin resistance and β-cell dysfunction. Many factors advocate a causal link between metabolic stress and inflammation. Numerous cellular factors trigger inflammatory signalling cascades, and as a result T2D is at the moment considered an inflammatory disorder triggered by disordered metabolism. Cellular mechanisms like activation of Toll-like receptors, Endoplasmic Reticulum stress, and inflammasome activation are related to the nutrient excess linking pathogenesis and progression of T2D with inflammation. This paper aims to systematically review the metabolic profile and role of various inflammatory pathways in T2D by capturing relevant evidence from various sources. The perspectives include suggestions for the development of therapies involving the shift from metabolic stress to homeostasis that would favour insulin sensitivity and survival of pancreatic β-cells in T2D.

Key words: Diabetes mellitus; Inflammation; Insulin resistance; β-cell dysfunction; Adipose tissue

Core tip: Immuno-metabolism, the confluence of metabolism and immune system has emerged as a chief breakthrough especially in the field of diabetes mellitus; a metabolic disorder of great magnitude. Activation of immune system by metabolic stress has opened new insights in the pathogenesis and progression of type 2 diabetes (T2D). The link between metabolic overload and activation of the immune system form the core tip of this review. Metabolic stress can cause pathologic activation of the immune system, thus metabolic disorders like T2D manifest and progress as an inflammatory disorder with severe consequences thereof.

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INTRODUCTION

Diabetes mellitus, a life style disease affecting 8.3% of the adult population of the world and increasing at an alarming rate, is one of the most common non-communicable diseases of current era[1]. The burden of this disease is immense owing to transition in lifestyle and dietary habits, ageing of the population and urbanization in the setting of a genetically predisposed environment[2]. The fact that the number of subjects with diabetes mellitus has doubled over the past three decades has made this disease a global challenge[3]. The number of diabetes mellitus patients is projected to increase from 382 million in 2013 to 592 million by 2035, denoting a net increase of 55%[1]. The predominant form is type 2 diabetes (T2D) which accounts for nearly 90% of all diabetes cases.

Diabetes mellitus-not so sweet

T2D is a metabolic disorder characterized by insulin resistance and pancreatic β-cell dysfunction as a consequence of unsettled hyperglycemia[4,5]. In response to nutrient spill over in the setting of insulin resistance and eventual β-cell dysfunction, the general fuel homoeostasis of body is altered[2]. Insulin resistance in target tissues and a relative deficiency of insulin secretion from pancreatic β-cells are the major features of T2D. β-cell hyperplasia and hyperinsulinaemia in response to insulin resistance occur in the preclinical period of disease. Relative insulin deficiency as a consequence of failure of β-cells to compensate for insulin resistance, progresses into overt T2D[6].

Metabolic alterations associated with T2D are well characterised by epidemiological and research based studies. The pathogenesis and progression of T2D is ascribed to four mechanisms; increased advanced glycation end product (AGE) formation, increased polyol pathway flux, activation of protein kinase C (PKC) isoforms, and increased hexosamine pathway flux[7]. Till recently no common linking element was apparent for these mechanisms: however, recently production of superoxide emerged as a unifying mechanism for these four pathways. Downstream to oxidative stress, activation of inflammatory pathways has emerged as an imperative link between T2D and inflammation. Since, abundant data have elucidated the role of oxidative stress in T2D pathogenesis. In this review, we will evaluate the inflammatory component of T2D and underscore the link between metabolic alterations in T2D and inflammation.

T2D AS AN INFLAMMATORY CONDITION

Studies investigating the relation between inflammation and T2D have coalesced sufficient data implicating the role of inflammation towards the development of insulin resistance and pathogenesis of T2D[8,9]. Metabolism and immune system were conventionally regarded as two distinctive mechanisms governing nutrient disposal and body defense, respectively. Typically, little was known about the coordination and interplay between these two systems. However, present research has led to combining these distinct entities as studies perceive pathological activation of the immune system as a regulatory mechanism associated with multiple disorders underlying the metabolic syndrome[10]. Potency of steroid hormones as immune suppressors and hyperglycemic inductors, metabolic alterations associated with pyrexia, wasting syndrome initiated by chronic infections and of late, markers of acute-phase response have been associated with insulin resistance, insulin secretion defects, T2D and vascular complications of T2D[8,11-15].

T2D encompasses colossal cellular factors characteristic of triggering inflammatory signalling cascades. A detailed analysis of these molecules cannot be underscored in this review, however their particular roles in T2D has been outlined in Table 1. Consequently, T2D at the moment is considered an inflammatory disorder triggered by disordered metabolism[16]. The probable history of diabetes involves a more or less latent prodromal period followed by progressive deterioration of glucose tolerance culminating into explicit disease. Progression of islet β-cell failure results in hypertrophy of pancreatic islets and proliferation of β-cells. This phase is associated with an inflammatory response precipitating into reduction of cells by apoptosis and fibrosis of islets. In fact, an analogy of sequence of events involving an incipient inflammatory phase is associated with other T2D complications also[17]. Hyperglycemia is regarded as the major upstream mechanism, and micro-inflammation is regarded as the subsequent downstream driving force of diabetes related complications[17]. Epidemiological data advocate that markers of inflammation are predictive of T2D[18]. The role of inflammation in insulin resistance is traced by the integration of metabolism and innate immunity *via* nutrient-sensing pathways mutual to pathogen-sensing pathways. Components of nutrition (free fatty acids, glucose, and amino acids) signal through collective receptors and pathways in a similar way as pathogens and/or cytokines. Cells of the immune system (macrophages) and metabolism (adipocytes) also share many functions like secretion of cytokines, and trans-differentiation into macrophages. Nutrients can activate macrophages and adipocytes through common receptors, such as toll-like receptors (TLRs) that sense broad classes of molecular structures common to pathogen groups, and are central to innate

Table 1 Role of various inflammatory molecules in type 2 diabetes

Category	Molecule	Role
Pro-inflammatory cytokines and signaling molecules	TNF-α	Increased levels related to IR and T2D
		Reduces insulin sensitivity by influencing the phosphorylation state of the insulin receptor
	IL-6	Major pro-inflammatory cytokine that induces inflammation and IR leading to T2D
	CRP	Elevated serum CRP associated with the incidence of T2D
	IL-1	Associated with obesity and IR
		Affects insulin signaling directly through the induction of SOCS-3
	IL-8	Leads to IR *via* the inhibition of insulin-induced Akt phosphorylation in adipocytes
	IL-1β	Mediates auto-inflammatory process resulting in β-cell death
Transcription factors	NF-κB	Increase the expression of genes encoding cytokines, chemokines, transcription factors and various receptors involved in IR and pathogenesis of T2D
	JNK	Promotes IR through phosphorylation of serine residues in IRS-1
	IKKβ	Leads to IR through transcriptional activation of NF-κB
Adipokines	Leptin	High leptin levels, reflecting leptin resistance predict increased risk of T2D
	Adiponectin	Low levels of this protective adipokine correlate with T2D. Adiponectin is downregulated by TNF-α
	Resistin	Promotes IR and decreases insulin-stimulated glucose transporters in adipose tissue
	Adipsin	Role in maintaining β cell function
		Lower levels of adipsin found in T2D patient
	Visfatin	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
Chemokines	MCP-1	MCP-1 expression in adipose tissue contributes to the macrophage infiltration into this tissue, IR and T2D
	IP-10/CXCL10	Downstream effector of pro-inflammatory cytokines involved in T2D-related complications
	CCR2	Imitates tissue inflammation and IR
Toll like receptor	TLR2 and TLR4	TLR2 and TLR4 play a critical role in the pathogenesis of IR and T2D
Adhesion molecules	E-slectin/P-slectin	Lead to leukocyte recruitment in local tissue and contributes to inflammation, IR and T2D
	ICAM-1/VCAM-1	Alters endothelial and sub-endothelial structure leading to reduced vascular permeability, reduced insulin delivery to peripheral insulin sensitive tissues and ultimately T2D
Nuclear receptors	PPARα, PPARγ, and PPARβ/δ	Mutations in *PPAR* genes associated with IR and T2D
	VDR	Regulates expression of insulin receptor preferentially by binding as a heterodimer with the RXR to VDREs in the promoter regions of insulin receptor gene

IR: Insulin resistance; CRP: C-reactive protein; T2D: Type 2 diabetes; SOCS-3: Suppressor of cytokine-3 signalling; NF-κB: Nuclear factor κB; JNK: c-Jun NH2-terminal kinase; IRS-1: Insulin receptor substrate; IKKβ: Inhibitor of nuclear factor κB kinase subunit β; MCP: Monocyte chemoattractant protein-1; IP-10: Interferon gamma-induced protein 10; CXCL10: Chemokine (C-X-C motif) ligand 10; CCR2: Chemokine (C-C) motif receptor 2; ICAM-1: Intracellular adhesion molecule 1; VCAM-1: Vascular cell adhesion molecule 1; PPAR: Peroxisome proliferator activated receptor; VDR: Vitamin D receptor; RXR: Retinoid X receptor; VDREs: Vitamin D response elements.

immunity and inflammation.

ADIPOSE TISSUE AS A SITE OF INFLAMMATION

Clinical and experimental studies show that adipose tissue acts as a site of inflammation. The first insight came from the study on adipose tissue of obese mice exhibiting elevated production of TNF-α[11]. Consequently, increase in adiposity is associated with upregulation of genes encoding pro-inflammatory molecules and associated with accumulation of immune cells[19-21]. Adipocytes hoard excessive nutrient load and become hypertrophic gradually. Events initiating a pro-inflammatory response involve synergistic contributions of various mechanisms like an increase in nuclear factor κB (NF-κB) and c-Jun NH2-terminal kinase (JNK) activity by hypertrophied adipocytes, endoplasmic reticulum (ER) stress causing altered unfolded protein response (UPR), hypoxic stress in adipose tissue, activation of TLR by excess free fatty acids (FFAs), or increased chylomicron-mediated transport from the gut lumen into the circulation in a lipid-rich diet[16,22,23]. Stressed adipocytes produce various cytokines and chemokines promoting immune-cell activation and accumulation in adipose tissue[24]. A pro-inflammatory loop is formed by several macrophages by clustering around adipocytes, particularly with dead adipocytes forming crown-like structures[19,21,25]. Sustained accumulation of lipids in adipose tissues results in switching of macrophages from an anti-inflammatory "M2" (alternatively activated) to a pro-inflammatory "M1" (classically activated) phenotype[19,21,26,27]. The skew in balance results in an increased secretion of inflammatory molecules that subsequently stimulate the hypertrophied adipocytes resulting into a pro-inflammatory response[28]. The inflammatory response in macrophages is induced by adipocyte-derived FFAs *via* TLR or NOD-like receptor family, the pyrin domain containing 3 (NLRP3) dependent pathways[29,30]. Local hypoxia as a result of vasculature insufficiency in hypertrophied adipocytes has been proposed to stimulate expression of inflammatory genes in adipocytes as well as immune cells[31]. However, the hypothesis lacks confirmation in the situation of human obesity[32]. Instead, mechanisms like ER stress and

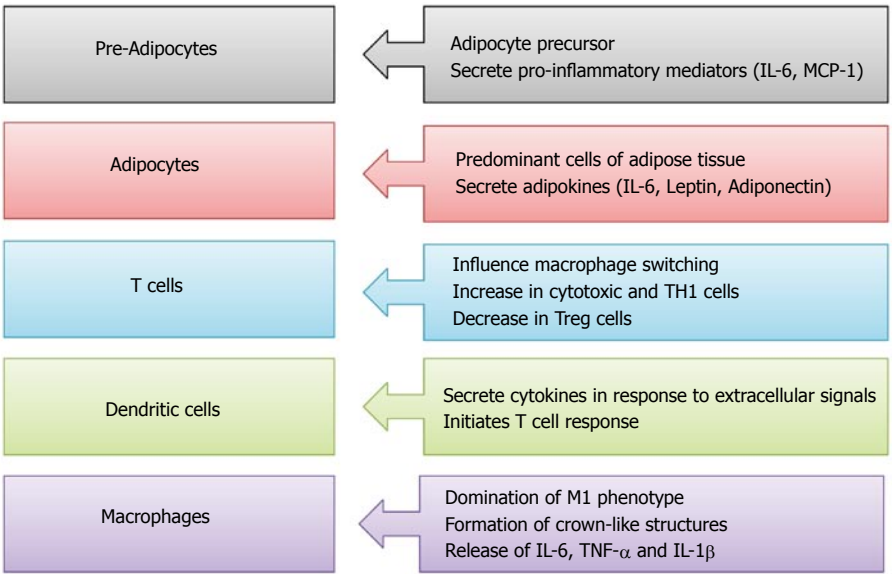

Figure 1 Functions of various immune cell types in pathogenesis of type 2 diabetes. IL: Interleukin; MCP-1: Monocyte chemoattractant protein-1; TNF-α: Tumor necrosis factor α.

autophagocytosis have been proposed as origin of local inflammatory signalling pathways in adipose tissue[22,33]. Recently, the role of the incretin hormone glucose-dependent insulinotropic peptide has also been implicated[34,35]. In addition to adipose tissue, a pro-inflammatory state in liver and skeletal muscle result in disruption of systemic insulin sensitivity and glucose homeostasis that are characteristic of T2D[36-38].

Metabolic inflammation is regulated by critical orchestration of innate and adaptive immune cell interactions[39,40]. Studies investigating immuno-metabolism have recognised that the inflammatory status of immune cells is dictated by their metabolic programming, mitigating the progression of T2D. T2D is preceded by an extensive period of disease development, and inflammation has been shown to be a precipitating factor underpinning insulin resistance, preceding T2D[41,42]. The progression of T2D involves an intricate interplay between metabolism and immunity. The progression of T2D has been causally linked to various types of immune cells but the primary sources of inflammatory effectors contributing to insulin resistance are macrophages[43-45]. Among various cell types, pre-adipocytes, adipocytes, T cells, dendritic cells and macrophages are major cell types involved in obesity-induced inflammation and insulin resistance[46]. Their prime functions are shown in Figure 1. The key inducers of cytokine release in metabolic organs leading to impaired insulin action are tissue-resident macrophages[47].

Nutrient overload corresponds to increased infiltration of macrophages in metabolic tissues promoting a pro-inflammatory environment characterised by augmented TNF-α, IL-1β and inducible nitric oxide synthase (iNOS) levels. The accrual of these pro-inflammatory macrophages in metabolic organs like liver, adipose tissue and muscle directly supresses insulin action, thereby promoting hyperglycemia[48].

ROLE OF INFLAMMATION IN INSULIN RESISTANCE

Insulin is a key endocrine hormone produced by β-cells of pancreatic islets. Insulin is regarded as "hormone of abundance" owing to the array of functions it performs, the effects of which extend from metabolic to mitogenic activity (Figure 2). It is likely that disruption of insulin-mediated pathways will have pleiotropic effects that are not confined to carbohydrate metabolism only. Various mechanism working separately or in synergy have been linked to the development of insulin resistance among which chronic inflammation represents as a triggering point[8].

Inflammation is an important component linking insulin resistance with nutrient overload and increased visceral adipocyte mass[42]. During an insulin-sensitive state, the signalling cascade of insulin upon binding to its receptor results in phosphorylation of tyrosine residues of the insulin receptor substrate 1 (IRS-1) ensuing in downstream insulin signalling[49]. However, in an insulin-resistance state, pro-inflammatory molecules activate various other serine kinases like JNK, inhibitor of NFκB kinase subunit β (IKK-β), extracellular-signal regulated kinase (ERK), ribosomal protein S6 kinase (S6K), mammalian target of rapamycin (mTOR), PKC and glycogen synthase kinase 3β[50]. The activation of these kinases inhibits insulin action by phosphorylating serine residues instead of tyrosine residues in the insulin signalling pathway[49].

The development of insulin resistance is linked to two prime transcription factor-sinalling pathways: JNK and IKKβ/NF-κB[51]. Activation of these two pathways involves a series of proinflammatory stimuli, many of which comprise of both activators and upregulators of

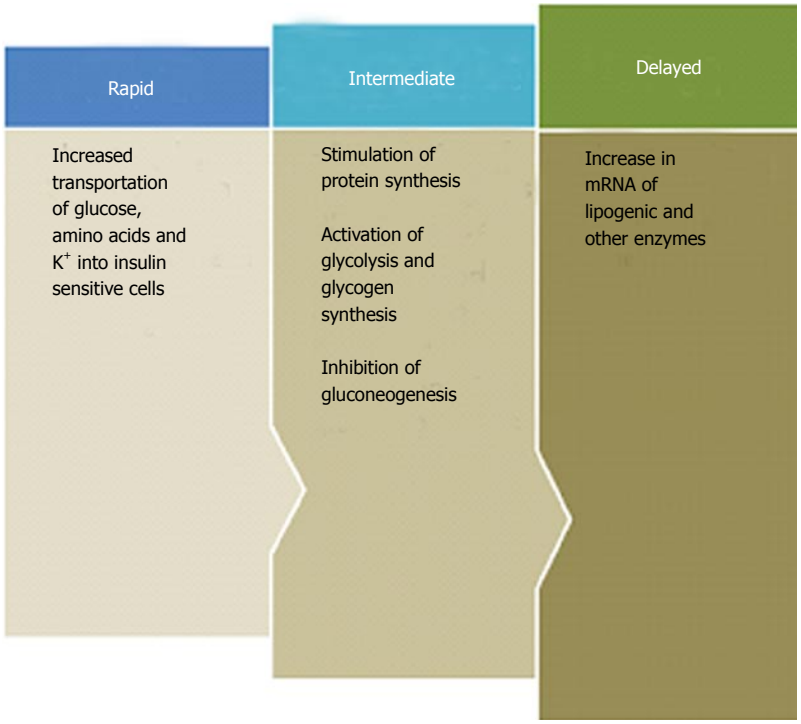

Figure 2 Various hormone functions of insulin.

NF-κB. In addition, these pathways are also activated by pattern recognition receptors like TLRs and receptors for advanced glycation end products (RAGE). Elevated levels of FFAs result in an increase in diacylglycerol (DAG) that activates PKC isoforms leading to concomitant activation of JNK and NF-κB pathways[52]. Further stimuli involve production of reactive oxygen species (ROS), ER stress and changes in adiposity[53-55].

The mechanisms in development of inflammation-induced insulin resistance are different for JNK and IKKβ. Unlike JNK that phosphorylates the serine residues of IRS-1, IKKB induces insulin resistance by transcriptional activation of NF-κB[56-59]. The physiological substrates of IKKβ are IκB protein inhibitors of NF-κB. IKKβ phosphorylation promotes proteosomal degradation of IκBα liberating NF-κB for nuclear translocation where it stimulates the expression of several target genes (Figure 3)[9]. The products of these target genes of NF-κB induce insulin resistance. The production of inflammatory molecules further activates JNK and NF-κB pathways promoting a vicious loop of insulin resistance by feed-forward mechanism.

PANCREATIC ISLET INFLAMMATION IN T2D

Increasing evidence suggests the presence of an inflammatory milieu in pancreatic islets in T2D, such as increased cytokine levels, chemokine levels and immune cell infiltration. Evidence of islet inflammation was initially observed in hyperglycemia induced β-cell apoptosis[60]. Recent studies on human islets and monocytes have shown that the combination of hyperglycemia and elevated FFAs induces a more efficient pro-inflammatory phenotype[61,62]. Various T2D experimental animal models like db/db mice and Goto-Kakisaki rats showed increased infiltration by immune cells in the pancreatic islets[63]. Studies on experimental animal models elucidated islet inflammation and macrophage infiltration as an event occurring as early as eight weeks before the onset of frank diabetes[63]. Recruitment of macrophages is a consequence of phagocytic clearance owing to the death of islet β-cells[64]. Alternately, in a diabetic milieu endocrine cell-derived inflammatory molecules like IL-6 and IL-8 produced in islets are also attributed to increased macrophage infiltration[63]. Production of pro-inflammatory cytokines and secretion of chemokines by β-cells results in a vicious cycle speeding up islet inflammation. In humans, IL-1β secreted by infiltrating immune cells is related to the pathogenic process of T2D, as blockade of IL-1 has been associated with reduced hyperglycemia, improved β-cell function and reduced expression of inflammatory markers[65]. However, recent studies involving human islets have shown that induction of IL-1β plays a role in precipitating the clinical features of diabetes and is unlikely involved in initial pathogenesis[66-68]. The first study demonstrating the hyperglycemia-induced IL-1β secretion documented a pro-inflammatory response induced by a non-autoimmune mechanism in β-cells[12]. *Ex vivo* experiments on isolated human islets exposed to high glucose levels showed increased IL-1β production preceding activation of NF-κB, upregulation of Fas, fragmentation of DNA, and reduction

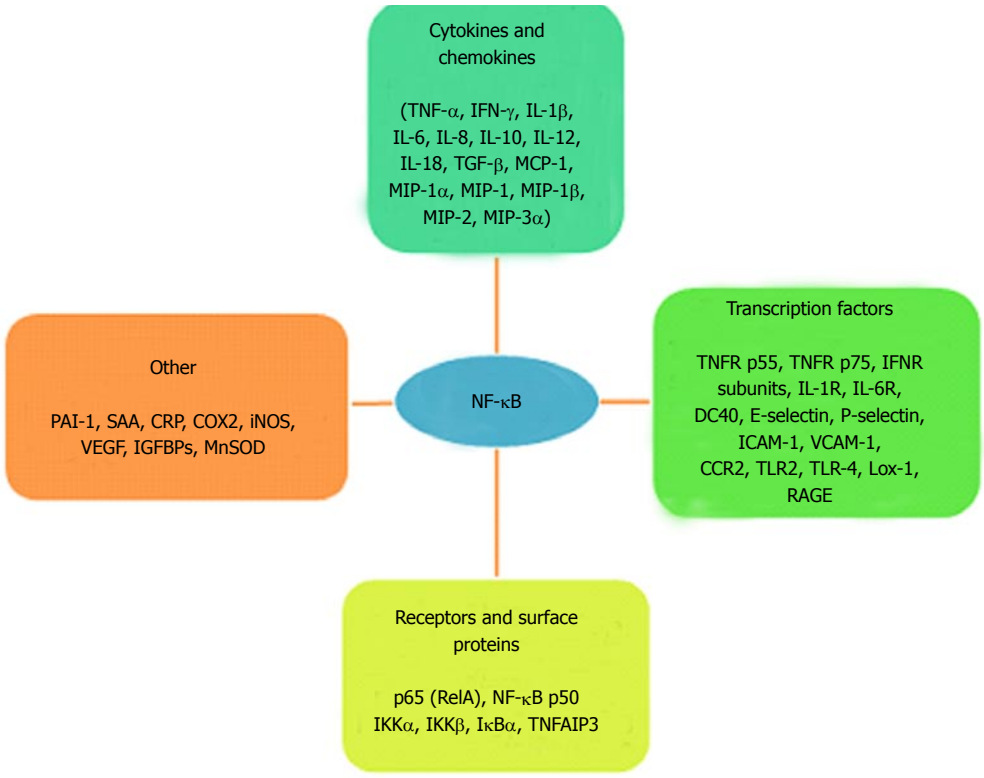

Figure 3 Target genes activated by NF-κB. TNF-α: Tumor necrosis factor-alpha; IFN-γ: Interferon-gamma; IL: Interleukin; TGF-β: Tumor growth factor-beta; MCP-1: Monocyte chemoattractant protein-1; MIP: Major intrinsic protein; TNFR: Tumor necrosis factor receptor; INFR: Interferon receptor; IL-R: Interleukin receptor; CD: Cluster of differentiation; ICAM: Intracellular cell adhesion molecule; VCAM: Vascular cell adhesion molecule; CCR: Chemokine CC receptor; TLR: Toll-like receptor; Lox: Lysyl oxidase; RAGE: Receptor advanced glycation end product; PAI: Plasminogen inhibitor activator; SAA: Serum amyloid; CRP: C-reactive protein; COX: Cyclo-oxygenase; iNOS: Inducible nitric oxide synthase; VEGF: Vascular endothelial growth factor; IGFBPs: Insulin-like growth factor binding protein; MnSOD: Manganese superoxide dismutase; RelA: Reticuloendotheliosis viral oncogene homolog A; NF-κB: Nuclear factor-kappa B; IKK: Inhibitor Kappa B kinase; IκBα: Inhibitor of NF-κB; TNFAIP3: TNF-α induced protein 3.

of insulin secretion[69]. Upregulation of IL-1β plays a predominant role as a major cytokine regulating other chemokines and cytokines in islets of T2D patients[12,66,70,71]. This master cytokine elicits a broader response by recruitment of various immune cells and also by induction of IL-1β in β-cells, provoking a vicious inflammatory cycle[66]. The critical role of IL-1β in islet inflammation was recently confirmed by analysing global gene expression in pancreatic islets of humans that showed an association of a group of co-expressed modules enriched for IL-1 related genes with T2D and insulin resistance[72]. *SFRP4* gene encoding the secreted frizzled-related protein 4 was one of the interesting genes that were overexpressed, likely mediating the effect of IL-1β on islets[72]. In islets of both T2D subjects as well as in animal models, an eminent number of immune cells along with cytokines and chemokines has been observed[63,66,73]. In fact, T2D animal models invariably exhibit islet immune cell infiltration[63,71].

ISLET INFLAMMATION AND β-CELL DEATH

Islet tissue sections of T2D subjects show well-defined fibrosis which is a hallmark of the late stage of a chronic inflammatory process. In clinically overt T2D subjects a decreased β-cell mass has been reported indicating a probable role in its pathogenesis[4,74]. Decreased β-cell mass in T2D has been attributed to pancreatic β-cell apoptosis and to β-cell dedifferentiation[75]. In slowly progressing T2D, the probability of detecting β-cell damage in pancreatic sections is low, thus very few studies on this aspect have been reported[4,76]. Several mechanisms like amyloid deposition in islets, presence of long-chain FFAs[77], and chronic hyperglycemia[60] has been implicated in β-cell apoptosis. Sustained gluco-lipotoxic conditions amplifies the β-cell stress responses by potentiating effects of elevated levels of FFAs, glucose causing ER stress and mTORC1 activation[78,79,54]. The underlying mechanism for hyperglycemia-induced β-cell apoptosis is attributed to the glucose-induced IL-1β production that upregulates the Fas receptor[80,81,12]. FFAs act as important effector molecules causing β-cell dysfunction by lipoapoptosis (a metabolic cause of programmed cell death). The most abundant saturated FFA in blood is palmitate that has direct lipotoxic effects on β-cells by inducing ER stress and ROS[82-85]. Ceramide, an effector molecule responsible for inducing lipoapoptosis of β-cells, is a metabolic product of FFAs that activates JNK[86-88]. Likewise, incomplete β-cell oxidation of fatty acids resulting in metabolites like DAG and triglycerides (TGs) also elicits final effector molecules contributing to FFA-

induced lipotoxicity as well as insulin resistance[89-91]. In addition to this, FFA-induced activation of JNK by Src has also been reported in a recent study[92]. These studies show that islet inflammation contributes to β-cell dysfunction.

TRIGGERING OF THE INNATE IMMUNE SYSTEM IN T2D

Nutrient excess in metabolic tissues resulting in metabolic inflammation, *i.e.*, a low-level pro-inflammatory milieu, has emerged as an important factor underlying the development of T2D[11-15,93,94]. Activation of innate immunity in T2D is linked to the activation of TLRs. These receptors have been implicated in diabetes-induced inflammation and vascular complications[95]. TLRs comprise the pattern-recognition receptors characteristic of the innate immune system. Various pathogen-associated molecular patterns (PAMPs) encompassing carbohydrates, proteins, nucleic acids and lipids, are recognised by TLRs followed by initiation of an immune response. TLR2, a receptor for pathogen lipoproteins and TLR4, a receptor of lipopolysaccharides, are activated by FFAs[96,29]. Binding of FFAs to TLRs has been postulated to directly induce a pro-inflammatory response[97,98]. Also, various indirect ways of TLR activation by FFAs has been postulated recently[99]. *In vitro* studies have demonstrated that, unlike the short chain FFAs, the long chain palmitate and oleate that comprise 80% of circulating FFAs are pro-inflammatory in various cell types[29,96,98,100,101]. Contemporary studies report the activation of TLR signalling by FFA-induced formation of lipid rafts that favour TLR dimerization in cell membranes[92,102]. Recently, fatty acid transporter CD36 binding to TLR2 and liver-derived glycoprotein fetuin-A binding to TLR4 were identified as endogenous ligands linking FFAs to TLRs, eliciting inflammation and prompting insulin resistance[103,104]. In addition, damage-associated molecular patterns (DAMPs) like high-mobility group box 1 (HMGB1) and AGEs also act as endogenous ligands which are recognised by TLRs, thereby activating pro-inflammatory pathways[105]. TLR2 is responsible for upregulation of inflammatory molecules like NF-κB, myeloid differentiating factor 88 (MyD88) and chemokine (C-C motif) ligand 2 (CCL2)[106]. TLR4 knockout mice have been shown to be protected from insulin resistance as well as from fat-induced inflammation[106]. TLR4 silencing by siRNA technology has been shown to attenuate the hyperglycemia-induced activation of IκB/NF-κB[107]. TLR5 is a receptor for bacterial flagellin that controls metabolic pathways through sensing gut microbiota. TLR5 knockout mice have been reported to exhibit increased adiposity along with hyperphagia, hypertension, hyperlipidemia and insulin resistance[108]. Activation of inflammatory pathways in a TLR-independent mechanism by metabolic stress involves generation of ROS that induce stress kinases and NLRP3 inflammasome (multiprotein complexes responsible for production of bioactive IL-1β) formation[109].

Both TLR-dependent and TLR-independent mechanisms function in concert. This finding is demonstrated by animal models of diabetes in which there is partly protection of pro-inflammatory cytokine production in case of deficiency of TLR2 or TLR4, whereas deficiency of a universal intracellular docking protein MyD88 required for TLR signalling, exerted total protection[61]. Apart from FFAs, systemic inflammatory responses are also elicited by elevated glucose levels[110]. Sustained hyperglycemia results in non-enzymatic glycation of lipids and proteins resulting in the formation of AGEs. AGEs stimulate the pattern recognition receptor RAGE. Numerous cell types, like macrophages, T cells, smooth muscle cells, neuronal cells, podocytes and cardiomyocytes, express RAGE[111]. RAGE activates the pleiotropic pro-inflammatory transcription factor NF-κB along with stress kinases ERK1 and ERK2[112]. Excessive glucose metabolized by oxidative phosphorylation to ATP results in ROS generation that tends to activate the NLRP3 inflammasome concomitantly with FFAs[67]. This results in release of active IL-1β along with IL-1-dependent cytokine and chemokine production[61].

FROM INNATE TO ADAPTIVE IMMUNITY IN T2DM

The role of specific or adaptive immunity comes from the recent clinical overlap between type 1 diabetes (T1D) and T2D such as younger age of onset in T2D and increasing body mass index (BMI) coinciding with increased incidence in T1D. Moreover, progressive decrease in β-cell mass observed in T2D and evidence of insulin resistance in T1D has blurred the etiology[113]. The argument supporting the involvement of autoimmunity in islets of T2D patients is evident from the presence of β-cell specific antibodies in nearly 10% of T2D patients and presence T cells reactive to β cell antigens in some patients[114]. The number of autoantigen-responsive T lymphocytes in islets from T2D patients has been reported to correlate with disease progression[114], however the exact role of islet autoimmunity in T2D requires further studies. A monogenic form of diabetes characterised by typical features of T1D like lean body mass, young age of onset, autoantibodies to β-cells, rapid disappearance of C-peptide and insulin requirement concomitantly with T2D-associated insulin resistance provides genetic support for the overlap between T1D and T2D[115]. The genetic alteration is attributed to an autosomal-dominant mutation in the *SIRT1* gene, and the pathogenesis involves β-cell impairment and death, paralleling a state of activation of immune system[115]. As a consequence of insulin resistance, stress induced β-cell death results in the release of autoantigens along with alarmins (endogenous molecules released by necrotic cells causing activation of immune system). Alarmins have potentiating effects of promoting pathologic self-antigen presentation, resulting in enhanced adaptive

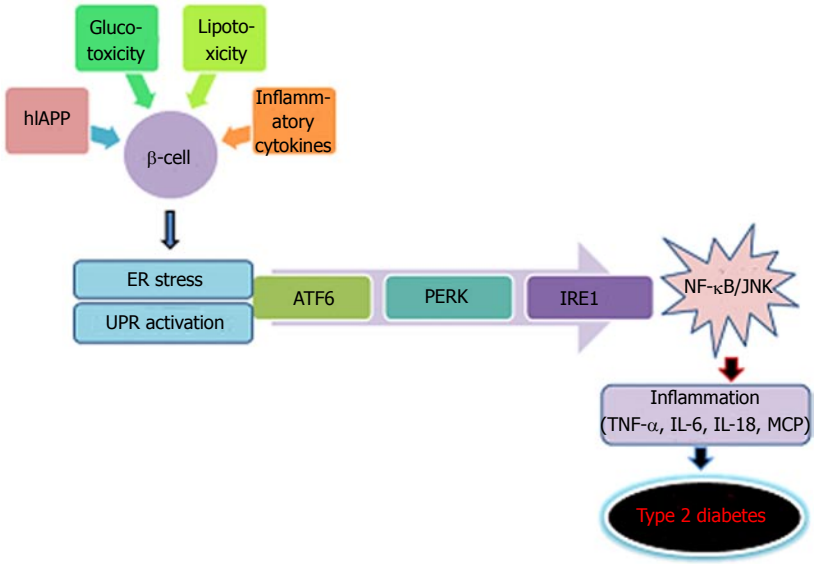

Figure 4 Mechanism of endoplasmic reticulum stress. ER: Endoplasmic-reticulum; ATF6: Activating transcription factor 6; PERK: Double-stranded RNA-activated protein kinase (PKR)-like ER kinase; IRE1: Inositol-requiring kinase 1; IL: Interleukin; MCP: Monocyte chemoattractant protein; TNF-α: Tumor necrosis factor α; JNK: c-Jun NH2-terminal kinase; NF-κB: Nuclear factor κB.

immune response[116]. In light of these observations, sirtuins are recognised as novel regulators of immuno-metabolism in humans. Apart from SIRT1, SIRT2 has been recently linked to cytoskeleton remodeling and activation of NLRP3 in intracellular pathways[117]. Apart from the activation of innate immunity, the contribution of adaptive immune cells in inducing inflammation is now established in T2D at the cellular level.

Experimental animal models of insulin resistance have demonstrated a Th2/Th1 shift in favour of Th1, shifting the T_{reg}/Th17 shift towards Th17 and shifting the CD8/CD4 ratio in favour of CD8 and finally reduction of T-cell receptor (TCR) diversity[118-121]. These studies have recently been extrapolated to human subjects[122] and confirm the observation that an increase pro-inflammatory stimuli (IFN-γ) causing M1 phenotype switching of adipose tissue macrophages result in the activation of a Th1 type response[121]. IFN-γ and IL-17 produced by these T cell populations interact directly with adipocytes in addition to contributing to a pro-inflammatory loop in cells of innate immunity. IFN-γ inhibits the JAK-STAT pathway, and IL-17 induces the secretion of IL-6 from adipocytes[123]. β-cells isolated from T2D patients exhibit increased IL-8 and decreased IL-10 secretion[124]. Recent studies regard the contribution of B-cell humoral immunity in adipose tissue inflammation. A study on experimental mice models involving B-cell knockout mice and anti-CD20 therapy showed a significantly improved metabolic phenotype and adipose tissue inflammation[120].

LINK BETWEEN ER STRESS AND INFLAMMATION IN T2D

Activation of ER stress and the UPR forms a convincing hypothesis for the induction of inflammatory pathways in T2D. ER stress in T2D occurs by virtue of nutrient overload, hypoxia and accumulation of unfolded proteins in metabolic organs[22]. Under normal conditions, the flux of proteins through ER is high, and in the setting of insulin resistance or glucotoxicity, a prolonged state of insulin need generates ER stress[125].

Three ER localized sensors control the activation of ER stress and UPR (Figure 4): (1) the double-stranded RNA-activated protein kinase (PKR)-like ER kinase (PERK); (2) inositol-requiring kinase 1 (IRE1); and (3) activating transcription factor 6 (ATF6). ER stress by protein overload or accumulation of unfolded proteins causes dissociation of GRP78, and the subsequent binding to unfolded proteins in ER prevents their transport to cis Golgi.

Prominently, UPR activation stimulates inflammatory stress kinases like JNK and IKK and their critical downstream transcriptional targets; activator protein 1 (AP-1) and NF-κB, respectively[126,127].

These transcription factors control the induction of inflammatory cytokines and chemoattractants that are known to have a direct link with the development of insulin resistance[128,129]. ER stress can also impair insulin signalling by activation of stress kinases (JNK, IKK) that can inhibit insulin receptor substrates by direct phosphorylation. Recently, death protein 5 (DP5) and p53-upregulated modulator of apoptosis (PUMA) have been reported as inducers of β-cell apoptosis by mediating ER stress[130]. ER stress can also cause induction of lipogenic genes that promote lipid accumulation and thereby contributes to the development of lipid-induced insulin resistance[131].

ER stress and UPR pathways

Triggering of inflammatory signals by three pathways of UPR is initiated by activation of JNKs and NF-κB in B

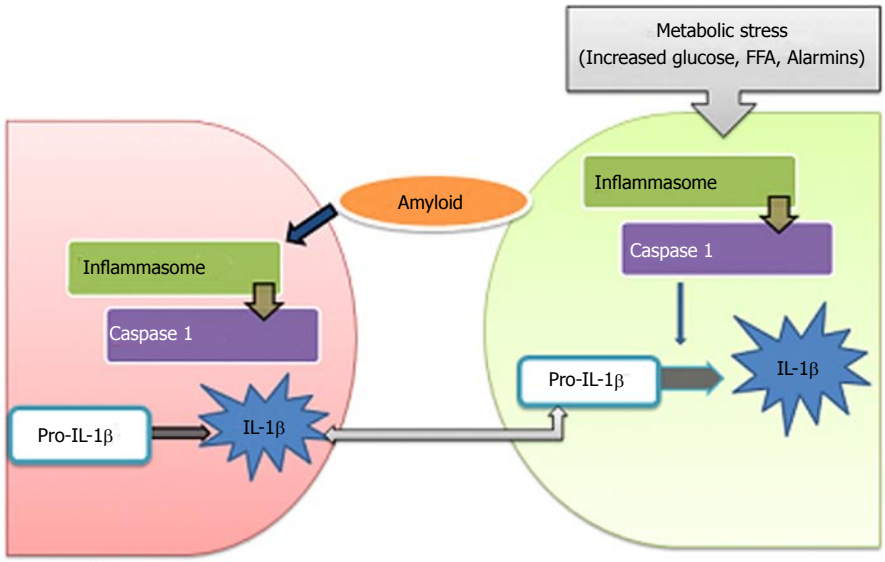

Figure 5 Activation of inflammasomes in type 2 diabetes (metabolic stress activates multiprotein complex, inflammasome in β-cells that induce caspase-1 to cleave pro-interleukin-1β (pro-IL-1β) into active IL-1β. β-cell-derived IL-1β promote the release of chemokines and recruitment of macrophages that are activated by human islet amyloid polypeptide, leading to deleterious concentrations of IL-1β. FFA: Free fatty acid; IL: Interleukin.

cells. This activation acts as the linkage point between metabolic and immune pathways since the activation of these very kinases is analogous to that elicited by an immune response[94,132]. JNKs play an important role in T2D, as increased activity has been shown to promote insulin resistance [56,133].

The first responses for opposing ER stress involve decreasing the translation of proteins. This involves phosphorylation of α subunit of eIF2 by PERK. In humans and mice, loss of PERK expression is linked to dysregulation of the UPR response which is fundamental to ER stress, resulting in increased cell death and T2D[134]. A permanent form of neonatal diabetes in humans is related to elevated ER stress markers as a result of a mutation in PERK, confirming the pivotal role of PERK in regulating ER stress during fetal development[135-137].

A factor in the second pathway of UPR, IRE1, is a prime regulator of ER stress and is highly expressed in the pancreas. An *in vitro* knockdown study on IRE1 signalling showed a decreased synthesis of insulin[138,139]. Upon activation, IRE1 initiates activation of X-box binding protein 1 (XBP1) that leads to upregulation of ER expansion and biogenesis[140]. The critical role of XBP1 in achieving an optimal insulin secretion and glucose control was demonstrated in β-cell-specific XBP1-deficient mice that exhibited impaired pro-insulin processing and secretion, reduced β-cell proliferation and hyperactivation of IRE1[141].

The third pathway of UPR involves the activation of ATF6, the basic leucine zipper domain protein, that upregulates PERK1 and IRE1 pathways by suppressing the apoptotic UPR signalling cascade under chronic ER stress. The role of ATF6 activation in β-cell dysfunction has been concluded in studies that showed decreased expression of insulin gene by ER stress-induced ATF6 activation and a decrease in ER chaperones along with induction of apoptosis in ATF6 knockdown insulinoma cells[142,143].

ACTIVATION OF INFLAMMASOME IN T2D

Inflammasomes are multiprotein complexes in the intracellular machinery responsible for production of bioactive IL-1β in response to multiple stimuli[144]. NLRP is a subfamily of Nod-like receptors containing a central nucleotide binding and oligomerization (NACHT) domain with flanking C-terminal leucine-rich repeats (LRRs) and N-terminal caspase recruitment (CARD) or pyrin (PYD) domains[145]. The NOD-like receptor family, the pyrin domain containing 3 (NLRP3) inflammasome is in a pathway that controls the production of IL-1β and IL-18[146-148]. Unlike TLR, a potential role of NLR in metabolic abnormalities has not been extensively investigated. NLRP forms a constituent of the inflammasomes responsible for maturation and release of IL-1β, and thus is a relevant candidate for metabolic disorders and T2D[149]. NLRP3-dependent activation of inflammaosomes in diabetes was proposed by studies implicating the release of IL-1β as a consequence of elevated levels of glucose, FFAs and human islet amylopeptide (hIAPP)[16,150,151]. However, the effective metabolites involved in activation of inflammasomes are not clearly elucidated yet (Figure 5).

The NLRP3 inflammasome is a general metabolic alarmin stimulated by different endogenous and exogenous stimuli[152]. NLRP3 inflammasome activation is augmented in T2D patients[153]. Dysregulation of lipid metabolism, paving the way to aberrant lipid accumulation, as well as formation of oxidized LDL and cholesterol, triggers NLRP3 activation[30,153,154] similar

to ER stress that acts as one of the important factors triggering NLRP3 activation[155,156]. In T2D subjects, increased oxidative stress also contributes to NLRP3 inflammasome activation[157,158].

Studies on obesity-induced inflammation and insulin resistance are also indicative of the role of NLRP3. In experimental models of calorie-restricted mice, a positive correlation has been observed between IL-1β/NLRP3 mRNA and body weight[30] whereas disruption of *NLRP3* gene in obese mice has revealed changes in metabolic profiles. Insulin resistance as a consequence of inflammasome activation is directly related to FFAs and LPS[109]. Apart from Insulin resistance, activation of inflammasomes is related to β-cell dysfunction, as NLRP3-knockout mice exhibit improved glycemic profiles after consumption of a high-fat diet, likely due to attenuation of IL-1β[67]. In response to hyperglycemia-induced increased production of ROS, NLRP3 activation occurs as a result of dissociation of thioredoxin interacting protein (TXNIP) from thioredoxin and its subsequent binding to NLRP3[67]. Nevertheless, shortage of TXNIP has shown effects on glucose metabolism in addition to the NLRP3 activation[159]. A substantial role of inflammasome activation in β-cell dysfunction was recently reported by ablation of NLRP3 that conferred protection to β-cell function and structure from injury inflicted by metabolic stress[160].

Secretion of IL-1β requires two induction stimuli; the first stimulus induces pro-IL-1β expression and the second inflammasome activation. Inflammasome activation triggers caspase-1 resulting in cleavage of pro-IL-1β and release of mature IL-1β. In T2D, the first stimulus comes from minimally-modified LDL in islets which prime the macrophages for processing of IL-1β by activation of TLR4 signalling. Recently, the second stimulus was recognized to regard islet hIAPP, secreted by β-cells in response to high glucose levels[151]. hIAPP was shown to direct NLRP3 activation by inducing β-cell injury. In islets, interaction of macrophages and β-cells is essential for the activation of inflammasomes. hIAPP, a soluble oligomer induces activation of NLRP3 and subsequent release of IL-1β from macrophages and dendritic cells which are primed with TLR4 agonists like LPS or modified LDL molecules[151]. The macrophages are attracted to islets by hIAPP-induced synthesis of chemokines (CCL2 and CXCL1). It has been reported that overexpression of hIAPP in islet grafts increases the recruitment of macrophages by 50%[161]. Recently the activation of inflammasomes in myeloid cells in T2D patients was elucidated. A study on untreated T2D subjects showed upregulation of IL-1β production and maturation in macrophages[153]. Treatment of macrophages with various alarmins like FFA, hIAPP, HMGB1and ATP resulted in release of inflammasome products. Studies have shown that T2D subjects exhibit elevated levels of circulating alarmin molecules thereby advocating a possible role of these molecules in NLRP3 inflammasome activation in myeloid cells[162].

PERSPECTIVES

The concept of chronic low-level inflammation in T2D has given an impetus to the field of immune-metabolism. Elucidation of various cellular mechanisms linking inflammation to insulin resistance and β-cell dysfunction has revolutionized insights in the molecular pathogenesis of diabetes. Insights into intricate pathways provide a platform to tackle the distinct pathway without compromising immuno-surveillance. Nutritional and therapeutic interventions aimed at controlling/inhibiting the escalating pro-inflammatory response can help in attenuating the pathogenesis and progression of T2D. Well-designed studies should offer the development of novel targeted therapeutics to deal with the disease burden of T2D and its associated complications.

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MINIREVIEWS

Lean diabetes mellitus: An emerging entity in the era of obesity

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Abstract

Much has been published on the characteristics of type 2 diabetes mellitus and its association with the epidemic of obesity. But relatively little is known about the incidence of lean diabetes, progression of disease and fate of the patients with low-normal body mass index (< 25). Studies in developing countries have shown that the clinical characteristics of these patients include history of childhood malnutrition, poor socioeconomic status, relatively early age of onset and absence of ketosis on withdrawal of insulin. In the United States, recent studies showed that the lean, normal weight diabetes is not rare especially among minority populations. They showed that these patients are mainly males, have higher prevalence of insulin use indicating rapid beta cell failure. They might have increased total, cardiovascular and non cardiovascular mortality when compared to obese diabetic patients. In this review, the epidemiologic and clinical features of lean diabetes are presented. The potential causal mechanisms of this emerging diabetes type that may include genetic, autoimmune, acquired and behavioral factors are discussed. The need for studies to further elucidate the causation as well as specific prevention and treatment of lean diabetes is emphasized.

Key words: Lean diabetes; Beta cell failure; Ketosis resistant diabetes of young; Obesity paradox; Sarcopenic obesity

Core tip: Little is known about lean diabetes (patients with low-normal body mass index). Studies in developing countries have shown that these patients have history of childhood malnutrition, poor socioeconomic status and early age of onset with absence of ketosis. In the United States, recent studies showed that the lean, normal weight diabetes is not rare especially among minorities. These patients are mainly males and have higher prevalence of insulin use indicating rapid beta cell failure. They might have increased total, cardiovascular and non cardiovascular mortality when compared to obese diabetic patients. The potential causal mechanisms of this diabetes type may include genetic, acquired and behavioral factors.

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INTRODUCTION

The prevalence of diabetes mellitus (DM) has increased exponentially over the past decade globally. In the United States, nearly 26 million adults have diabetes with over 9 million being prediabetic. The numbers are expected to double by 2050. Nearly 35% of American adults are obese putting them at an increased risk for the development of Diabetes. Healthy people 2020 initiative has stressed the importance of reduction in obesity to lower the incidence of this morbid condition among the United States population[1].

Since early times obesity has been a well known risk factor for diabetes. However, beyond the classical obesity related type 2 diabetes and other well defined types of diabetes like type 1, Maturity onset Diabetes of the Young, Gestational diabetes, *etc.*, there is a renewed interest in the underweight or normal weight lean diabetes that is emerging also in the developed countries like United States. This review will focus on the low-normal weight diabetes that is so far not well characterized and defined. More importantly, this review will highlight the differences in the clinical features of diabetes mellitus in normal body weight patients in the United States in comparison to the classical obese diabetes. The evidence related to the profile, peculiarities, morbidity, mortality and pathogenesis associated with lean type diabetes will be described.

DIABETES IN LOW BODY WEIGHT GROUP (BODY MASS INDEX < 18 KG/M^2)

Though recognized as a distinct entity early on, very little attention has been paid to the qualitative changes associated with it. Any "atypical diabetes" syndrome which did not meet the classical American Diabetes Association or World Health Organization Classification suffered from imprecise definitions which led to a group of complex phenotypes[2]. Further studies have been undertaken only recently shedding much insight into their disease burden, progression and natural history. Adult onset diabetes with body mass index (BMI) < 25 was initially placed under the category of "malnutrition related diabetes mellitus" in a subcategory termed "protein deficient pancreatic diabetes"[3]. Later this syndrome was noted to be similar to that originally described as "Jamaica type Diabetes", a term used to represent around 5% of Caribbean diabetics[4]. Similar clinical syndromes were subsequently described in regions of south Asia and Africa and has acquired various names; "Tropical Diabetes, Mixed onset type Diabetes, Phasic insulin dependent Diabetes, J type Diabetes, Z type Diabetes, M type or type 3 Diabetes, Ketosis resistant growth onset type Diabetes"[5].

Many of the overlapping categories described above could be lumped together under the term coined by Ahuja[6] as "Ketosis Resistant Diabetes of the Young (KRDY)". This category includes a broad subset of patients mostly of Asian and African ethnicity. The following criteria were suggested for the diagnosis of KRDY[6]: (1) blood glucose > 200; (2) onset < 30 years of age; (3) BMI < 18 kg/m^2; (4) absence of ketosis on insulin withdrawal; (5) poor socio-economic status or history of childhood malnutrition; and (6) insulin requirement > 60 units/d or 1.5 units/kg.

Although early age of onset and low BMI may raise suspicion for type 1 diabetes, the presence of islet cell specific antibodies has consistently been lower than those with type 1 diabetes across multiple population groups. It can be argued that the wasting or leanness noted in around 25%-50% of patients could represent the effects of long standing glycosuria[7]. Though BMI mostly improved with weight gain following optimal glycemic control, the mean value remained within the definition of low body weight among both genders[8]. Phenotypic similarities have been described from various other regions of the world with nearly all of them demonstrating a male preponderance with the most extensive data being described from Ethiopia[9,10].

A study from India on around 10000 type 2 diabetics revealed that around 3.5% patients were lean with a BMI < 18.5, with the larger share of around 63% patients having ideal body weight at diagnosis. Age of diagnosis (45 ± 13) and smoking patterns were not significantly different among the lean, ideal body weight and obese groups, although a male preponderance was noted only in the former two. This study also highlighted the fact that HbA1c, fasting and postprandial blood glucose levels were higher among those in the lean group. Micro-vascular complications of Diabetes such as retinopathy, nephropathy and neuropathy were more common among the lean male patients presumed to be related to the higher plasma glucose and HbA1c levels[11]. Other studies have also highlighted the higher incidence of peripheral neuropathy among lean diabetic males[12], whereas hypertension and coronary artery disease tend to be more common in the obese group[13]. A limitation of these studies were that auto antibodies were measured only in a very small number, thereby leaving a chance that the lean diabetics could represent type 1 diabetics[14], although patients who had an abrupt onset, ketosis or ketoacidosis at any time or required insulin at time of diagnosis were excluded from the study to avoid bias. In whom C-peptide levels were measured, they were found to be significantly higher compared to the type 1 diabetics being followed at the respective centers. Moreover the results of islet cell antibodies and antibodies against Glutamic acid decarboxylase were not significantly different between the 3 groups[15]. Nearly 48% of the lean NIDDM patients responded to diet or oral hypoglycemic agents after a mean duration of 9.2 ± 8.1 years, which is a clear distinction from type 1 diabetics. Symptomatic ketoacidosis was absent in this group[11].

The pathophysiology and its distinction from classic

type 2 diabetes is still unclear and a subject of much debate. The key feature appears to be a defect in insulin secretory capacity as opposed to peripheral insulin resistance as noted in classical diabetes. Multiple studies have shown an association between lean diabetes, malnutrition in early years of life and poor socioeconomic status. Although prospective human studies are lacking, experiments done on rats and primates have shown that low protein diet in early life leads to decreased beta cell mass and insulinopenia. Insulin mediated glucose disposal appears to be similar in KRDY and type 1 Diabetic patients[16]. These patients have fasting C-peptide levels intermediate between type 1 and type 2 diabetics[17,18]. Despite the "decent" C-peptide levels suggesting a good beta cell reserve, the circulating insulin levels at baseline and post stimulation with insulin secretagogues (glucose, tolbutamide and amino acids) have been consistently lower in lean diabetics when compared to their obese counterparts[8,19-24]. The 2 mechanisms that have been postulated to cause this are excessive extraction of insulin in the porto-hepatic circulation from raised glucokinase activity and hyperactive futile cycles of carbohydrate metabolism[8]. Resistance to ketosis noted in these cases is due to a small but sufficient insulin secretory reserve which is absent in type 1 diabetics[2]. They have also been noted to have lower fasting plasma free fatty acid and ketone levels and a blunted response to catecholamines further delaying the development of ketoacidosis[19-21]. The occurrence of fat malabsorption in a small subset of patients contributes evidence to an exocrine defect in KRDY patients[25].

Auto-antibodies can be present in anywhere between 2%-25% of KRDY patients. Glutamic acid decarboxylase (GAD), tyrosine phosphatase like protein (IA-2) or high mobility group box transcription factor SOX- 13 (ICA-12) antibodies are commonly found, though co-occurrence of GAD and IA-2 was observed in only 4.7% of lean patients compared to 22% type 1 diabetics[26]. Any single antibody by itself is neither sensitive nor specific to distinguish between the phenotypes of type 1 or type 2 diabetes as GAD or ICA positivity has been reported even in 4%-13% of obese type 2 diabetics[8]. In summary the primary etiology appears to be depressed beta cell function most likely due to malnutrition in utero and early infancy in addition to autoimmune modulations. However more studies are needed on this front to unearth the metabolic, hormonal and immunological characteristics of lean type 2 diabetics.

DIABETES IN NORMAL BODY WEIGHT GROUP (BMI 18-24.9)

The term "lean" has been described variously in different studies. The major distinction seems to originate from the geographic region where the study was conducted. Those from developing countries use a BMI < 18 to describe leanness whereas studies performed in the United States describe lean patients to have a BMI ranging from 18-24.9.

A study cohort of 18000 patients with type 2 diabetes in Chicago, United States showed that around 13% belonged to this group, with ideal body weight defined as a BMI ranging from 17-25. The study failed to demonstrate a significant difference between the age of diagnosis (43 ± 13) between the lean and obese diabetics, and corroborated the previous finding of male preponderance among the lean group (62%). Asians were shown to have a five-fold higher prevalence in the lean group (17% *vs* 4%). Environmental insults such as use of alcohol and cigarette smoking were more common among lean diabetics. As confirmed by various other studies, glycemic control was worse among lean diabetics and coronary complications more prevalent among the obese with no significant difference noted among micro-vascular complications[27].

The major pathophysiology in this group appears to be rapid beta cell failure as opposed to insulin resistance. This was highlighted by the fact that lean individuals had both a higher prevalence and early initiation of insulin use[27]. They were also noted to have lower TG/HDL ratios, which is an indirect marker of lower insulin resistance[28-30]. In diabetics, central obesity (waist circumference > 102 cm in males and 88 cm in females by NCEP) correlates with the degree of insulin resistance[31]. In the above cohort, 96.9% of the lean males did not have central obesity which points away from insulin resistance causing hyperglycemia[27].

The key defect responsible for hyperglycemia in the lean diabetics is impaired pancreatic insulin secretion[32-34] which is partly due to a reduced beta cell mass as demonstrated *via* autopsies[35,36]. The more severe beta cell dysfunction in these patients may be functional rather than structural as beta cell mass was noted to be equally reduced in both lean and obese patients[34]. Lean healthy Caucasian subjects born with a low birth weight have been demonstrated to develop several physiological defects of type 2 diabetes such as decline of insulin secretion, reduced muscle glucose uptake, reduced insulin stimulated glycolysis, lower fasting plasma glycerol levels and increased fat accumulation more prematurely than expected[37-40]. Dutch Famine Study has also proven that a brief period of malnutrition during postnatal period or early childhood increases the risk of diabetes[41]. These findings have been further validated in Asian populations too.

In addition, there was a higher prevalence of smoking, alcoholism and pancreatitis in the lean group in the Chicago study[27]. Chronic alcohol consumption induces pancreatic beta cell dysfunction and apoptosis[42]. Exposure to passive and active smoking are positively and independently associated with the risk of diabetes[43]. Whether the pronounced male prevalence among the lean is due to inherent genetic differences or unhealthy life style that can promote beta cell failure in comparison to females is still a matter of debate.

Genetic modulators might also predispose to reduced beta cell function in the lean body weight group. Polymorphisms of transcription factor FL2 gene (TCFFL2) and a genetic defect of ATP sensitive potassium channel Kir6.2 (or KCN JII) are associated with defective insulin secretion. Carriers of TCFFL2 gene polymorphism were also shown to be leaner and more insulin sensitive as compared with other type 2 diabetics[44]. Genetic scores for insulin resistance have shown association of lower subcutaneous fat mass and ectopic fat deposition highlighting the role of impaired adipose expandability and body fat distribution even among lean type 2 diabetic individuals[45].

Higher prevalence of Asians in the lean group could be from greater intrauterine insults and under-representation of overweight and obesity amongst them based on standard Body Mass Index definitions available. GAD antibodies though less frequently noted[46,47] may play a role in the autoimmune destruction of beta cells. It remains to be proven whether occurrence of these antibodies is merely secondary to the loss of beta cell function as compared to an etiological agent in itself[48]. The role of genetics and autoimmunity in lean diabetics needs to be further elucidated prior to drawing more concrete conclusions in this group.

THE OBESITY PARADOX AND SARCOPENIC OBESITY

Another interesting aspect is the occurrence of complications and increased rate of mortality in certain normal weight diabetes patients in comparison to their obese counterparts. This phenomenon is called the "obesity paradox". A pooled analysis done on 2625 participants from 5 longitudinal cohorts showed that normal weight adults at the time of incident diabetes had higher mortality than adults who were overweight or obese[49]. This "obesity paradox" has also been previously noted in other studies on diabetics and in various other chronic conditions such as hypertension, end stage renal disease and heart failure. It is likely that lower body weight in the presence of obesity related metabolic disorders may just be a reflection of preexisting illness that may predispose to mortality[49]. It is also known that despite having a leaner body mass, cigarette smokers are more insulin resistant, more likely to develop diabetes, and have a higher mortality from chronic lung disease and malignancies as compared with non-smokers. Carnethon *et al*[49] concluded that the elevated mortality in normal weight participants could not be entirely attributed to smoking, though a subgroup analysis demonstrated that there is no statistically significant difference between mortality in non-smoker adults of either cohort. A main limitation of this analysis was inability to assess the smoking burden and relatively low statistical power that limited the body mass index classification to 2 broad and heterogeneous groups (BMI 18.5-24.9 and BMI $\geqslant$ 25).

Further research into this obesity paradox by Tobias *et al*[50] in over 11000 participants, showed a J-shaped association between body mass index among all participants and in current or previous smokers. A direct linear relationship was observed in those who had never smoked. The lower mortality was observed among participants with a BMI of 22.4-24.9. The obesity paradox was therefore not observed in this study[50]. The increased mortality among lean diabetic smokers has been observed in the general population as well[51-54] and further studies are needed to address whether they represent an effect modification or is secondary to bias[55]. In participants 65 years or older, a null or weaker linear association was observed, which was probably due to increased prevalence of co-existing chronic diseases and decreased validity of BMI as a measure of adiposity due to age related decline in muscle mass and wasting[50,56].

A possible explanation for the observed obesity paradox could be sarcopenic obesity, defined as the presence of high body fat with reduced or normal lean body mass[1]. The localization of adipose tissue particularly abdominal obesity, independent of total obesity is associated with increased risk of cardiovascular disease. Sarcopenic obesity reduces the cardiopulmonary fitness and physical functioning possibly leading to premature death and could account for the higher mortality eventually seen in individuals who are normal weight at the time of diabetes onset[23]. The sarcopenic obesity may also indicate a catabolic underlying illness that leads to increased mortality. This was further supported by a systematic review, which showed that all cause mortality was lower among those with a high body mass index and good aerobic fitness as compared with individuals of a normal body mass index and poor fitness[57]. A study on veterans has also supported this view[58]. Hence questions can be raised regarding the sufficiency of such a simplistic classification of diabetes into obese or non-obese groups solely based on BMI.

IS FURTHER WEIGHT LOSS RECOMMENDED IN LEAN DIABETICS?

Weight loss is recommended for all overweight or obese diabetics. Studies such as the diabetes prevention program outcome study (DPP) and Look AHEAD[59] have primarily focused on weight management in overweight and obese individuals[1]. Could such recommendation be also effective in lean diabetics? Some concerns may be raised that additional weight loss could worsen both bone loss and decrease further the lean body mass contributing further to sarcopenic obesity. In the Chicago study, it appeared that leaner individuals had worse beta cell failure. One can then postulate that lean diabetics is a special variant of type Ⅱ diabetes whereby the failing beta cell cannot even

Table 1 Clinical differences between type 1, type 2, ketosis resistant diabetes of the young and lean type 2 variant diabetes

Clinical features	Type 1 diabetes	Type 2 diabetes	KRDY	Lean type 2 variant
Age of diagnosis	Can occur at any age, usually < 25	Usually > 25 yr, but increasing prevalence in adolescents	< 30 yr	Average age around 40 with male preponderance
Weight	Usually lean	Overweight and obesity	BMI < 18	BMI 18-25
Autoantibodies	Present	Absent	Variable	Absent
Family history of diabetes	5% to 10%	75% to 90%	Unknown	Around 50%
Insulin sensitivity	Normal	Decreased	Normal	Normal, in females might be decreased
Insulin dependent at diagnosis	Yes	No	40% at diagnosis	35% at diagnosis
Risk of ketoacidosis	High	Low	Low	Low

KRDY: Ketosis resistant diabetes of the young; BMI: Body mass index.

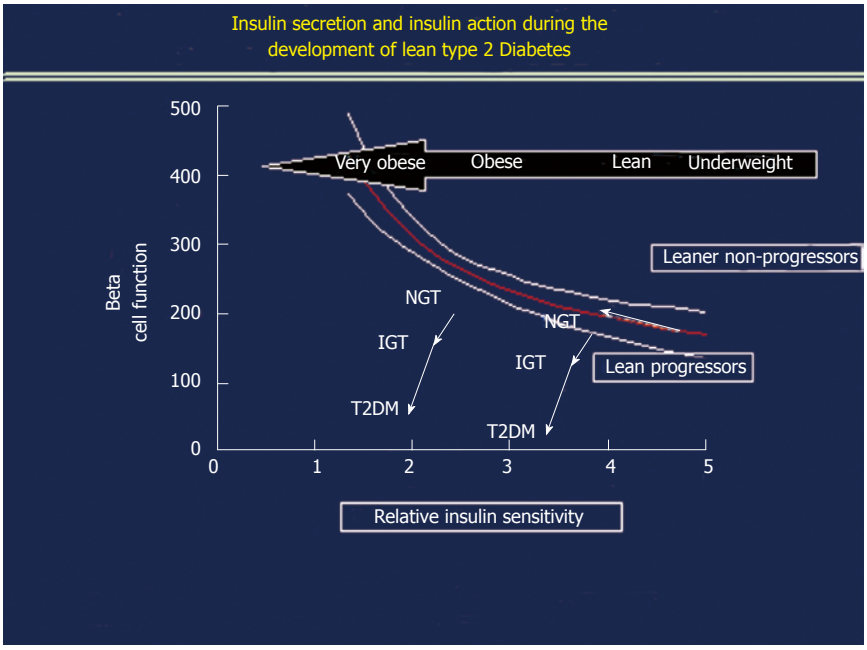

Figure 1 Pathogenetic model of development of lean type 2 diabetes. In the obese individuals the diabetes develops once the beta cell cannot cope with the insulin resistance conferred by the growing obesity. In the lean diabetes the early failure of the beta cells results in development of diabetes at much lower insulin resistance. It might be speculated that in individual with similar beta cell dysfunction but lower insulin resistance (lower weight) diabetes might not develop. NGT: Normal glucose tolerance; IGT: Impaired glucose tolerance; T2DM: Type 2 diabetes; Modified from Weyer *et al*. *J Clin Invest* 1999; 104: 787–794.

cope with the small amount of insulin resistance that lean body weight confers. Could then achievement of a lower body weight or lower adiposity in these patients prevent diabetes? Currently there is no answer to this intriguing hypothesis (Figure 1).

CONCLUSION

The patients with lean diabetes in comparison to classical obese type 2 diabetes (Table 1) are characterized by younger age at onset, earlier and more prevalent use of insulin, higher prevalence in males and higher rates of cigarette smoking and alcohol abuse. It was initially shown that patients with diabetes who are leaner have higher mortality rate in comparison to the obese (the obesity paradox). This was explained by the concept of sarcopenic obesity whereby these patients are metabolically obese with excess of adiposity but have decreased muscle mass (sarcopenia) which is the predisposing factor for the increased mortality. The obesity paradox however could not be confirmed in a most recent study in a larger cohort.

The underlying pathogenetic mechanism of lean diabetes has not yet been clarified and more studies are needed for its elucidation. It could be a completely new pathogenic entity, however there is a possibility that it may just be a variant of type 2 diabetes. In type 2 diabetes, the beta cells that are genetically destined to fail gradually over the years cannot cope with the increasing insulin resistance that is conferred by obesity. Lean diabetes might be a variant of these main operating pathogenic mechanisms. The difference is the much more pronounced beta cell failure that occurs earlier and results in more rapid exhaustion. Several potential mechanisms could be involved in the beta cell failure. The initial predisposing factors may be an adverse intrauterine or early postnatal environment with insufficient nutrients that result in a smaller beta cell mass. Then genetic predisposition of a more fragile beta cell mass may cause early destruction and apoptosis. Studies have showed that genetic markers of such fragility are more common in the lean than in obese diabetics. In addition, in developed countries like the United States, acquired insults like cigarette

smoking and alcoholism might be newly defined and significant pathogenetic contributors that could further weaken the beta cells. In these circumstances, even the little insulin resistance associated with lean body weight could precipitate diabetes (Figure 1). Recent study has also shown that genetically determined insulin resistance may also play a role in the pathogenesis of lean diabetes.

Considering the inadequacy of BMI in distinguishing leanness, future studies should investigate the complex interaction between body composition, amount and distribution of adipose tissue and physical functioning in determining the development of lean diabetes. In the meantime emphasis on modifiable risk factors like smoking and alcohol abuse that may further accelerate beta cell failure in lean patients may prevent further progression of the disease.

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MINIREVIEWS

Psychological themes that influence self-management of type 1 diabetes

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Abstract

It has long been accepted that psychological factors adversely influence efforts to optimise glycaemic control. These are often unrecognised in terms of clinical assessment and therefore under reported. This essay presents an introduction to psychological issues that interact with psychiatric co-morbidities and diabetes-specific distress, and a case scenario illustrating the interconnectedness of presenting problems and themes. In the way that we cannot separate carbohydrate counting, blood glucose monitoring and insulin dose adjustment in the understanding of a presenting problem such as poor control, so we cannot separate the concurrent thoughts, feelings, and behaviours. Each of these emotional aspects are self-managed either through avoidance, or by delayed disclosure and are frequently associated with poor health outcomes. There is a requirement for the healthcare team to be sensitised to these issues and to develop styles of communication that are empathic, reflective and non judgemental. A brief outline of evidence-based psychotherapy treatments is given.

Key words: Psychological factors; Glycaemic control; Anxiety; Depression; Eating disorder; Diabetes distress; Maladaptive coping; Psychotherapy

Core tip: Psychological factors adversely influence efforts to optimise glycemic control. The focus on psychiatric diagnosis has done a disservice to people with diabetes who experience significant levels of sub-clinical distress and it is essential to develop an understanding of the psychological issues that underpin poor self-management of type 1 diabetes. The diabetes healthcare team needs to be sensitive to the underlying issues and to be confident in the use of consultation styles that facilitate recognition and appropriate signposting for specialised support and treatment.

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INTRODUCTION

It has long been accepted that psychological factors

adversely influence efforts to optimise glycemic control and for many years these have been addressed in the context of psychiatric diagnoses anxiety[1], depression[2,3] and eating disorders[4,5]. Until recently the Quality Outcomes Framework[6] has been used to remunerate United Kingdom General Practitioners for recording assessments of anxiety and depression in those living with long-term conditions. This focus on diagnosis has been to the detriment of people with diabetes who experience significant levels of distress, visible in terms of poor control but unrecognised in terms of clinical assessment and therefore under reported. More recently attention has been given to the need to differentiate between clinical diagnoses and diabetes-emotional distress[7-9]. This article, informed by literature and the clinical experience of the author, presents an introduction to psychological issues that interact with psychiatric co-morbidities and diabetes-specific distress, and a case scenario illustrating the interconnectedness of presenting problems and themes. A brief outline of psychotherapeutic models available to treat these difficulties is given.

Management of type 1 diabetes requires optimising a sequence of actions or behaviours that include blood glucose monitoring, carbohydrate counting, insulin administration and physical activity, in the context of cognitions and emotions (thoughts and feelings). On the face of it the most straightforward approach to management is to advise people what to do, and expect it will be done. Living with and managing diabetes in the context of education, employment, recreation, ill health and relationships to name a few aspects of daily living, can be challenging. Diabetes is not an exact science and day to day life, early learning, emotions and motivation can, and do emerge as barriers to the consistent application of the "straight-forward" approach.

EMOTIONS AND COGNITIONS ASSOCIATED WITH DIABETES

Diabetes is the ultimate gatecrasher. There is no satisfactory explanation to answer the question "why me?" and at best glycaemic control can be managed, but unlike the party gatecrasher, diabetes cannot be sent away. Clinicians are familiar with the lexicon offered by people living with diabetes in response to the question "How does diabetes make you feel?" that includes words like anxious, worried, afraid, mood swings, depressed, euphoric, shame, guilty, angry and frustrated. Similarly when asked about thoughts triggered by living with diabetes the responses reflect what it means to live with the condition and its impact on quality of life, and the difficulties of management. This thinking interacts with beliefs about the self, the reaction of others and unconscious motives derived from early experience.

TOWARDS AN UNDERSTANDING OF THE MISMANAGEMENT OF DIABETES

In the way that we cannot separate carbohydrate counting, blood glucose monitoring and insulin dose adjustment in the understanding of a presenting problem such as poor control, so we cannot separate the co-existing thoughts, feelings, and behaviours.

Psychiatric diagnoses of anxiety, depression and eating disorders are frequently listed as psychological aspects of diabetes, and improvements in glycaemic control (HbA1c) reported as the primary outcome measure of treatment. As stated in the introduction, in recent years there has been increasing attention given to the need to differentiate psychiatric comorbidities and diabetes-related emotional distress. Identifying the factors that influence the decisions for particular actions that contribute to poor control is the crux of psychological formulation. Assessment of the presenting problem and the context in which it occurs offers a pathway to a personalised understanding that informs the treatment of choice. This might include further diabetes education, pharmacological treatment, appropriate psychological therapy, or indeed any combination of these.

Assessment of an individual presenting with poor control may reveal distress associated with, for example, a fear of hypoglycaemia. Thoughts might reflect a fear of loss of control, or of drawing unsolicited attention giving rise to emotions such as anxiety, frustration or guilt. Actions to elevate blood glucose such as reducing insulin or eating to maintain a "safe" blood glucose level result in both a reduction of risk of hypoglycaemia and a reduction in anxiety. Alternatively, high blood glucose readings frequently trigger guilt and fears about long term health. Evidence of high blood glucose is denied by avoidance of blood monitoring, and the emotional equilibrium thereby maintained. These are examples of the mismanagement of diabetes being used to manage emotional distress.

Managing diabetes requires multifactorial consideration of food choice and quantity, activity, blood glucose, ill health, ambient temperature, alcohol consumption, and menstrual cycle to name a few of the factors. The psychological responses are equally complex and influence actions with consequences on a continuum ranging from subtle to extreme outcomes on glycaemic control and psychological well-being.

PRESENTING PROBLEM AND UNDERLYING PSYCHOLOGICAL ISSUES

The following case history is used to illustrate psychological issues underlying poor control evidenced by elevated HbA1c and frequent episodes of hypoglycaemia. It invites consideration as to whether the presenting problem requires direct treatment or

is a symptom of underlying difficulties that need to be addressed.

CASE STUDY (REPORTED WITH PERMISSION)

Sam is a 60-year old post menopausal woman approaching retirement. She has been married for 38 years and although she experienced difficulty with conception she has one adult child. Her family of origin practised strict religious beliefs and she was brought up to be sensitive to the needs of others before her own. She had little preparation for independent living and intimate relationships: indeed such topics were taboo and usually associated with duty, guilt, shame and blame. There was an expectation that she would be self-sufficient and undemanding which contributed to considerable reluctance to seek help and resulted in her needs not being met. She suffered a sexual assault in her late teens not disclosed at the time, for which she did not receive emotional support and which subsequently influenced intimate relationships and generated a sense of shame. Her predominant employment has involved looking after others in a variety of roles, consistently prioritising the needs of others above her own. Sam has had type 1 diabetes for 20 years with significant fluctuations in control over that time and frequent hypoglycaemia, severe episodes occurring most frequently during sleep. Weight and body-image concerns are relevant in terms of her self-perception as unloveable and unattractive. She engages in regular exercise and has the not uncommon challenge of balancing energy needs, insulin dose and extremes of blood glucose levels. Whilst there is no indication that Sam uses insulin omission to influence her weight she tends to maintain elevated blood glucose to protect against hypoglycaemia. Pervading themes reflect guilt, shame, blame, a sense of being undeserving and either not good enough or a failure.

On the face of it a diabetes-specific intervention might focus on insulin adjustment, reducing episodes of hypoglycaemia, optimising exercise and blood glucose control or, less likely, weight management. The brief history offers insight into interconnecting psychological factors that contribute to poor glycemic control (Figure 1).

We can map the interlinking of the psychological factors, many of which are derived from early experience, others of which are accentuated by the role of diabetes and its impact on daily living. The experience of hypoglycaemia potentially predisposes fear of further episodes interconnecting with blame from others for "getting it wrong", shame and personal failure for "getting it wrong" and embarrassment and a fear of being out of control. Maladaptive coping behaviours include overeating and/or the reduction of insulin to elevate blood glucose, avoiding any physical exertion that may result in hypoglycaemia and avoiding social situations. The awareness that the coping mechanism results in short term relief with adverse consequences on long-term health perpetuates the cycle with resulting feelings of guilt, shame, and body-image concerns.

The spider's web gives some indication of why managing diabetes can be so challenging. Advice to adjust insulin:carbohydrate ratios, conservative treatment of hypoglycaemia, dietary advice and information about women's sexual function and diabetes may all be appropriate but do not address the psychological themes underpinning the maladaptive behaviours contributing to poor control.

PSYCHOLOGICAL ISSUES AND MALADAPTIVE COPING

The earlier paragraphs describe circumstances and events with psychological consequences, in the context of diabetes, that lead to maladaptive coping and mismanagement of diabetes. The following is a brief account of some of these with reference to the literature for more detailed exploration. Evidence has been provided for different components of type 1 diabetes distress: emotional burden, interpersonal and social distress, regimen related, and health care related[9-12]. The psychological issues are inextricably interconnected and related to specific aspects of diabetes-distress and presenting problems such as hypoglycaemia, fear of complications and body-image concerns.

Fear and anxiety

Fear and anxiety are the cognitive and emotional responses to threat. In order to reduce emotional discomfort individuals either "overdo" in an attempt to prevent the feared event or "under do" (avoid) the action in the misapprehension that by not addressing it, it will go away. The lack of exposure to the feared event (*e.g.*, hypoglycaemia) means that the individual reinforces the avoidance behaviour and does not learn how to cope were the threat to occur.

Blame and shame

Blame and shame indicate perceived negative judgement. Both emotions result from and give rise to the instigation of thoughts of not being good enough, having done wrong, or having failed, and are consistent with the experience of distress. "Shame plays a major role in the eventual consequences of diabetes self-management"[13]. Embarrassment and shame are also associated with specific diabetes symptoms which are both embarrassing to experience and for which to seek help[14].

Stigma

"Health related stigma is a negative social judgement based on a feature of a condition or its management that may lead to perceived or experienced exclusion, rejection, blame, stereotyping and/or status loss"[15].

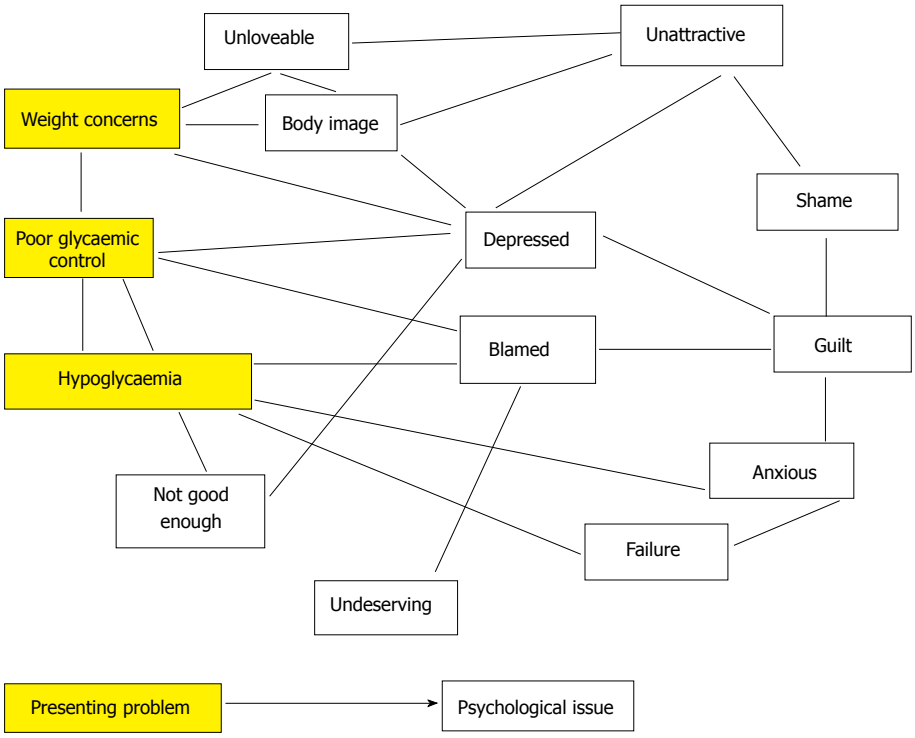

Figure 1 The spider's web: Visual representation of interconnecting psychological issues associated with the presenting problems of a female with type 1 diabetes.

Consequences of stigma span emotional, behavioural and social domains with specific implications of an unwillingness to disclose the condition which may compromise care, and fear of being judged or blamed for suboptimal diabetes management. A model is proposed to understand diabetes-related stigma[15].

Guilt

Guilt is a personal emotion experienced when there is recognition that something has not been done as believed it should have been, or something has been done that should not have been. It is similar to shame in its negative impact on self esteem but tends to relate to a specific action whereas shame is more to do with the perception of self. It evokes efforts to correct or make reparation, however this can result in perpetuating the negative feelings as a consequence of negative thoughts about self worth.

Each of these emotional aspects are self managed either through avoidance, striving to maintain invisibility, with poor outcomes or by delayed disclosure which can intensify the distress, at least in the short term, and frequently are also associated with poor outcomes.

There is a requirement for the healthcare team to be sensitised to these issues and to develop styles of communication that are empathic, reflective and non judgemental.

Models of therapy

Assessment and formulation guide treatment plans and until there is a robust evidence base specific to people with diabetes that suggests otherwise there is a choice of psychological therapies available for accredited practitioners to use. Behaviour therapy[16] focuses on modification of observable behaviours without taking into account "invisible" emotions and cognitions and is rarely an appropriate model to use with people with type 1 diabetes.

Cognitive behavioural therapy

Traditional cognitive behavioural therapy is a goal oriented, problem focussed therapy that combines behavioural and cognitive models. It is a collaborative approach that focuses on current problems rather than past issues. It has evolved as a specific treatment for symptoms associated with specific diagnoses, and is used to challenge cognitive distortions thereby promoting behaviour change[17].

Acceptance and commitment therapy

Acceptance and Commitment Therapy is an example of "Third Wave Therapies". It is particularly relevant in the context of fluctuating and frequently negative thoughts and feelings associated with diabetes. Rather than focus on the influence of emotions and cognitions as drivers for behaviour, the emphasis is on promoting value driven behaviour. The treatment facilitates a willingness to step back, notice and accept thoughts as they occur without becoming ensnared by the emotional response. Individuals commit to value driven actions whilst noticing the thoughts and feelings that invite self-sabotaging behaviour. For a detailed review of the full range of Third Wave therapies the reader is

invited to a recent review article by Kahl *et al*[18].

Cognitive analytic therapy

Cognitive analytic therapy is a time limited therapy which integrates concepts from cognitive and psychodynamic models[19]. The treatment involves the identification of sequences of thoughts and emotions that explain how a problem is established and maintained. The recognition of unhelpful self-sabotaging patterns of interaction derived from early experience, replayed in later life are interpreted in the context of diabetes management.

CONCLUSION

Non-psychiatric psychological aspects of living with type 1 diabetes interconnect with the day to day self-management tasks and related diabetes distress. There is a need for the diabetes healthcare team to be sensitive to the underlying issues and to be confident in the use of consultation styles that facilitate recognition, assessment, and appropriate signposting for specialised support and treatment. There is an unequivocal need for the multi-disciplinary team to include experienced psychological-therapists with considerable knowledge of diabetes management so that difficulties can be addressed as an integral part of the diabetes care available.

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Insulin action in muscle and adipose tissue in type 2 diabetes: The significance of blood flow

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Abstract

Under normal metabolic conditions insulin stimulates microvascular perfusion (capillary recruitment) of skeletal muscle and subcutaneous adipose tissue and thus increases blood flow mainly after meal ingestion or physical exercise. This helps the delivery of insulin itself but also that of substrates and of other signalling molecules to multiple tissues beds and facilitates glucose disposal and lipid kinetics. This effect is impaired in insulin resistance and type 2 diabetes early in the development of metabolic dysregulation and reflects early-onset endothelial dysfunction. Failure of insulin to increase muscle and adipose tissue blood flow results in decreased glucose handling. In fat depots, a blunted postprandial blood flow response will result in an insufficient suppression of lipolysis and an increased spill over of fatty acids in the circulation, leading to a more pronounced insulin resistant state in skeletal muscle. This defect in blood flow response is apparent even in the prediabetic state, implying that it is a facet of insulin resistance and exists long before overt hyperglycaemia develops. The following review intends to summarize the contribution of blood flow impairment to the development of the atherogenic dysglycemia and dyslipidaemia.

Key words: Insulin resistance; Muscle blood flow; Glucose uptake; Adipose tissue blood flow; Diabetes

Core tip: Insulin resistance and type 2 diabetes present with diminished glucose transport and disposal in muscles and fat and inadequate inhibition of lipolysis after meal ingestion or during physical exercise. This defect lies mainly in the cellular and subcellular level of insulin action. However, the resistance in the haemodynamic properties of insulin is another facet of type 2 diabetes and the metabolic syndrome. In this review, we intend to summarize the contribution of this impairment to the development of the atherogenic dysglycemia and dyslipidaemia.

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INTRODUCTION

The role of insulin in regulating glucose disposal in peripheral tissues, such as skeletal muscle and adipose tissue is well established. In the 50s, when it was discovered that insulin stimulated glucose uptake and disposal into the muscles, this effect was thought to be the most important way by which insulin regulated glycaemia *in vivo*. When the glucose/fatty acid regulatory cycle was discovered in the 60s a new insight of the way that insulin regulates glucose metabolism was introduced[1]. However, apart from its direct action on cells, insulin is also a vasoactive hormone, and it is now recognized that its vascular and metabolic actions are closely linked. Baron *et al*[2-4] originally introduced the concept that insulin might control its own access and that of other substrates - like glucose, lipids and several signalling molecules- to peripheral tissues, by increasing blood flow, and that this effect is compromised in states of insulin resistance.

In states of metabolic dysregulation, as in diabetes and obesity, there is deterioration in the cellular effects of insulin in peripheral tissues, which leads to a reduced ability of the latter to stimulate glucose uptake from the skeletal muscle and adipose tissue, as well as to inhibit lipolysis in fat depots[5]. Apart from the defects at the cellular level, the metabolic derangement could also be a result of the inability of insulin to cause vasodilatation and delivery of substrates to peripheral tissues especially in the postprandial period. This could contribute to the progression to type 2 diabetes as well as to the development of atherosclerosis, which is often evident even before overt hyperglycaemia develops[6].

In this review we summarize the current understanding of insulin action on peripheral blood flow and its implications on metabolic impairment both under fasting and postprandial conditions in type 2 diabetes.

INSULIN AND THE VASCULATURE IN NORMAL METABOLIC PHYSIOLOGY

Skeletal muscle

In skeletal muscle, insulin promotes the rate of glucose transport and the activities of hexokinase and 6-phosphofructokinase and subsequently the rate of glycolysis. In terms of protein metabolism, insulin increases synthesis and decreases degradation of proteins, in favour of an anabolic process[1]. Insulin also enhances vasodilatation and capillary recruitment, consequently increasing the flow of nutrients in peripheral tissues and especially in skeletal muscle[7]. It acts through traditional insulin receptors on the vascular endothelium to stimulate production of nitric oxide and induce vasodilatation[8]. The endothelial insulin response is mediated through a PI3-kinase pathway, which after several intermediate steps ends up activating endothelial nitric oxide synthase (eNOS)[6].

Blood flow is highly important for the metabolic function of skeletal muscle and under normal conditions increases after meal ingestion and during exercise and a correlation between the rate of insulin stimulated glucose uptake and the extent of vasodilatation seems to exist[9].

Insulin stimulates skeletal muscle glucose disposal and total muscle blood flow in a time- and dose-dependent fashion. *In vivo,* it enhances nitric oxide synthase-dependent vascular actions, in order to increase total skeletal muscle blood flow and to recruit muscle capillaries (by relaxing resistance and terminal arterioles, respectively). It is speculated that enhancing blood flow in this way on resistance vessels may induce the delivery of glucose and insulin to peripheral tissues and thus contribute to overall glucose disposal.

Capillary blood volume increases when precapillary arterioles dilate, thus increasing the flow to previously unperfused or underperfused areas, and total blood flow to skeletal muscle increases when larger resistance vessels relax[10].

Insulin increases tissue perfusion by augmenting microvasculature and, at normal concentrations, the rise in total muscle blood flow follows 60-90 min later[11,12].

Both haemodynamic effects of insulin, muscle blood flow increase and capillary recruitment seem to be independent of each other. Capillary recruitment occurs earlier *in vivo*, and at lower doses of insulin[13].

Insulin resistance may correlate to endothelial dysfunction in many ways, including dysregulation of sub-cellular signalling pathways that influence both insulin action and nitric oxide production[14,15].

Adipose tissue

Subcutaneous adipose tissue represents about 85% of whole body fat stores in subjects with various degrees of adiposity. Its main metabolic role is the storage of triglycerides which derive from energy overflow, and the release of stored lipids when other tissues are in need. Adipose tissue metabolism is under distinct control: usually, when a person consumes a meal, within the first hour postprandially, fat catabolism converts to fat storage, while the opposite happens in the case of physical activity. Adipose tissue interacts with the circulation by providing or drawing triglycerides and non-esterified fatty acids depending on metabolic needs. There are two kinds of triglyceride-rich lipoproteins: (1) chylomicrons, the largest particles, that carry the fat from absorbed nutrients within the intestine; and (2) very-low-density lipoproteins, that carry "endogenous" triglycerides and are released by the liver. Chylomicron- triglycerides are preferably stored within adipose tissue, and the fatty acid composition of adipose tissue (*i.e.*, the kind of fatty acids that form its triglycerides) usually represents the

composition of a person's dietary fat intake, suggesting that adipose tissue triglycerides derive mainly from the ingested fat through diet. However, a proportion of plasma triglycerides are endogenously produced from non-lipid substrates (*de novo* lipogenesis) in adipose tissue[16-22].

In terms of metabolic regulation, adipose tissue can be divided into central (abdominal) and peripheral (lower body) depots. An unfavourable metabolic profile has been related to central fat accumulation (visceral and subcutaneous, each with distinct metabolic, endocrine and paracrine characteristics and blood flow rates)[23,24].

Adipose tissue regulates its metabolism, at least in part, by increasing its blood flow rate mainly in the early postprandial period[16]. Capillary perfusion is essential for that function. In the case of increased energy demands, as in physical activity, blood flow increases to facilitate the delivery of lipolytic products to peripheral tissues. Furthermore, after meal consumption, it helps the delivery of ingested substrates to fat depots for storage[16]. Adipose tissue blood flow responses are subject to adrenergic stimulation or inhibition. Adrenaline administration stimulates postprandial increases whereas beta-blockers inhibit the latter. Genetic studies in subcutaneous adipose tissue biopsies have identified expression of the type A receptor of A natriuretic peptide and of the synthase of nitric oxide, and have found an association of those with post-challenge blood flow responses[16,25-28].

Lipid kinetics and subcutaneous adipose tissue blood flow alterations are closely linked. More specifically, blood flow rises in response to an increased demand for lipolytic products as energy, or that for cleavage of free fatty acids from the circulation. In euglycaemic subjects with normal weight blood flow peaks within the first hour after a glucose load or a mixed meal. This facilitates the postprandial delivery of energy substrates and insulin to the fat depot, leading to adipose tissue lipoprotein lipase stimulation which stores circulating triglycerides and the suppression of hormone-sensitive lipase, which results in the inhibition of endogenous lipolysis[16,29-31].

On the other hand, visceral adiposity exerts even more unfavourable metabolic actions. Increased visceral fat has been associated with atherogenic dyslipidaemia and the development of atherosclerosis, even in non-diabetic individuals[32]. Although increased abdominal fat is in general positively associated with markers of inflammation and atherosclerosis, visceral fat is more strongly correlated with C-reactive protein, monocyte chemoattractant protein-1, interleukin-6 and isoprostanes independently of total adiposity, indicating a major role in systemic inflammation[33]. Furthermore, visceral fat has been more strongly related to hypertension both in men and women, and provides information towards the latter above BMI and waist circumference. However, subcutaneous adipose tissue is also contributing to vascular dysfunction, possibly through the actions of leptin apart from the presence of insulin resistance[34]. Both adipose tissue beds' size has been found correlated to adipose tissue blood flow, independently of BMI, leptin or adiponectin concentrations[35].

INSULIN AND BLOOD FLOW IN TYPE 2 DIABETES

Skeletal muscle

Insulin provokes microvascular recruitment in skeletal muscle[10]. Impaired muscle blood flow as a facet of insulin resistance in subjects with either dysglycaemia or diabetes is well recognized in the literature. In the early 90s Steinberg *et al*[36] have shown that obese insulin resistant subjects present with an endothelial dysfunction and during a euglycaemic hyperinsulinaemic clamp they fail to increase endothelium-dependent vasodilation. In these trials, catheterizations of the femoral artery was used to measure the response to an intra-arterial vasodilator stimulus, comparing control to euglycemic-hyperinsulinemic clamp conditions[36].

Thereafter, it was suggested that since insulin exerts its vasodilatory effects through endothelial nitric oxide release, *in vivo* stopping nitric oxide production could inhibit insulin's vasoactive actions in skeletal muscle and consequently reduce glucose uptake[37]. Moreover in obese insulin resistant subjects, insulin resistance in skeletal muscle was promoted by the increased endogenous endothelin action[38].

A rat model of insulin resistance has shown that endothelial-dependent vasodilation is blunted, in part due to an unresponsive nitric oxide synthase to insulin, leading to decreased nitric oxide levels in the endothelial cells[39,40].

In type 2 diabetes and other insulin-resistant states, impaired suppression of adipose tissue lipolysis and postprandial hyperglycemia favour non-esterified fatty acid utilization and oxidation and increase glucose uptake from insulin independent tissues (like liver). Dyslipidaemia, usually related to lack of insulin sensitivity, enhances atherosclerosis and triggers inflammation in endothelial cells[41].

In insulin-resistant patients basal blood flow is generally not altered[42-44]. Laakso *et al*[45], demonstrated that insulin cannot effectively increase muscle blood flow in type 2 diabetic patients, using the combined euglycemic clamp and leg balance techniques during different insulin infusions. They also concluded that impaired insulin-dependent rise in skeletal muscle blood flow can be attributed to the diabetic milieu and not to obesity, in a study of obese diabetic patients[45].

Lambadiari *et al*[46] studied simultaneously lean subjects with insulin sensitivity varying from normoglycaemic insulin-resistant first-degree relatives of diabetic subjects to prediabetic and diabetic patients with either isolated postprandial hyperglycaemia or overt diabetes[46]. They demonstrated that using a

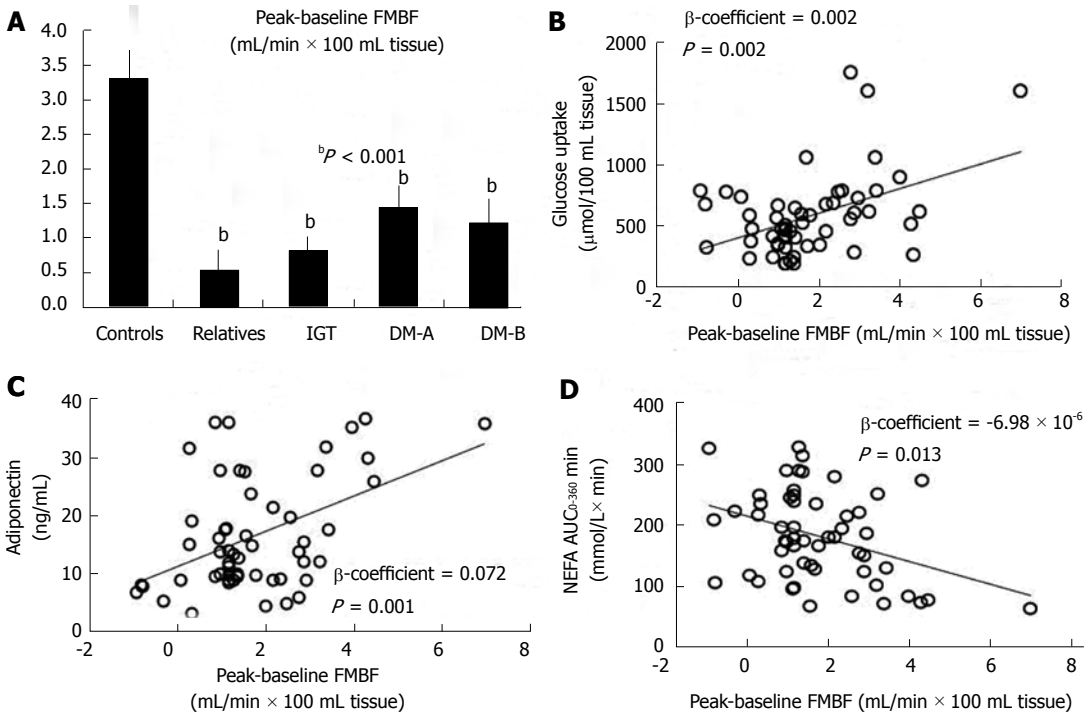

Figure 1 Forearm muscle blood flow peak-baseline values in all groups (A), and associations between peak-baseline forearm muscle blood flow and forearm muscle glucose uptake (B), plasma adiponectin (C) and postprandial non-esterified fatty acids (D), in subjects at all stages of type 2 diabetes. A: ^{b}P overall < 0.001; B: Forearm muscle glucose uptake = 427.9 + 101.4 peak-baseline FMBF, P = 0.001; C: Adiponectin = 12.17 + 3.05 peak-baseline FMBF, P < 0.001; D: Postprandial NEFA ($AUC_{0\text{-}360}$) = 209.5 - 18.52 peak-baseline FMBF, P = 0.005. FMBF: Forearm muscle blood flow; NEFAs: Non-esterified fatty acids. IGT: Impaired glucose tolerance; DM: Diabetes mellitus. Adapted from Lambadiari *et al*[46].

physiological mixed meal as a stimulus, the postprandial augmentation in forearm muscle blood flow is blunted throughout all stages of metabolic impairment compared to controls; this occurs even before overt hyperglycaemia develops. The latter affected glucose disposal in muscle, which was also unresponsive after meal delivery and was also positively correlated to the postload muscle blood flow differences. Lipid substrates affected blood flow peak as well. Triglyceride levels had a negative impact on blood flow responsiveness in the fed as well as in the fasting period. Post-challenge non-esterified fatty acids levels exhibited a negative effect on blood flow responsiveness, suggesting a possible mechanism for the decrease in muscle glucose clearance after the meal. A lower serum adiponectin level was also seen in the diabetic and the prediabetic insulin-resistant subjects, with the latter being positively related to the decreased postload blood flow rise[46] (Figure 1).

In subjects with morbid obesity postprandial muscle blood flow was also blunted in a study by the same group and this contributed to the decrease in muscle glucose uptake postprandially[47]. The same was observed by the same group in another insulin resistant state, such as hypothyroidism, in which a decreased postprandial blood flow response was coupled with an impairment in muscle glucose uptake[48]. In a study by Magalhães *et al*[49] administration of metformin to non-obese type 2 diabetic patients increased post-load forearm muscle blood flow and lowered free fatty acids, thus improving glucose oxidation and insulin sensitivity in the muscle bed.

However, there is not universal agreement with the above mentioned results, since numerous studies have failed to reveal a defect in insulin-mediated blood flow in type 2 diabetic patients[42,50]. There is a certain discrepancy since the literature either confirms or not a substantial[45,51], or an unimportant correlation between insulin kinetics, muscle blood flow and glucose disposal[52,53].

Some of these discrepancies may at least partially explained by the different studies populations and by the experimental protocol used. The commonly used clamp technique is not physiological, because these exceptionally high insulin concentrations are not normally present for long after meal consumption. Hence, one could question the physiological significance of such an increase in blood flow rates. A normal stimulus, such as a mixed meal, can provide evidence of a real life metabolic state[46,54]. However, not only the type of meal but the method for the detection of blood flow is important in this evaluation.

Adipose tissue

In lean insulin-sensitive subjects, abdominal adipose tissue blood flow increases by two- to four-times in response to feeding. The same seems to be true for blood flow in lower body fat depots (thigh) and forearm tissues. Physiologically, adipose tissue blood flow peaks within half to one hour after nutrient ingestion. This rise

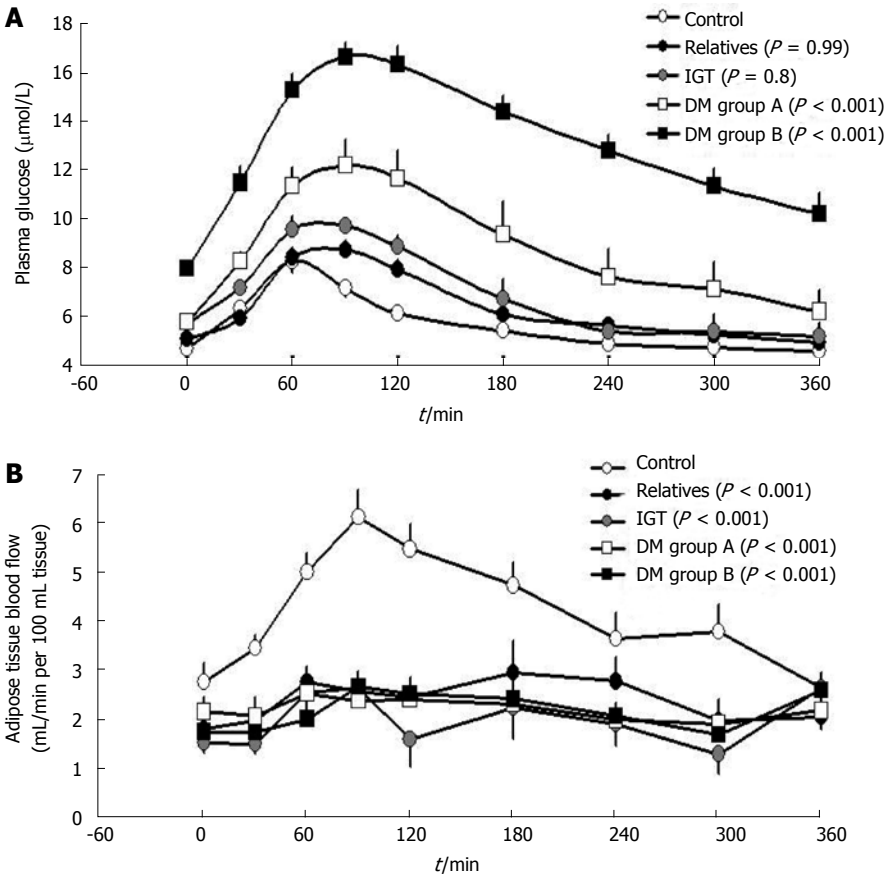

Figure 2 Plasma glucose, and adipose tissue blood flow in healthy subjects (control), first degree relatives of subjects with type 2 diabetes (relatives), subjects with impaired glucose tolerance, subjects with type 2 diabetes with postprandial hyperglycemia and normal fasting plasma glucose levels (diabetes mellitus group A) and subjects with type 2 diabetes with both postprandial and fasting hyperglycemia (diabetes mellitus group B). "*P*" stands for overall comparison (repeated measures ANOVA) between control and patient groups. IGT: Impaired glucose tolerance. Adapted from Dimitriadis *et al*[62].

coincides with plasma insulin peak and the inhibition of lipolysis[23].

By studying obese or diabetic individuals in the 90s, Jansson *et al*[55,56] detected impairment in adipose tissue blood flow response as a facet of insulin resistance coupled with hypertension and elevated lipolysis products.

Since then, numerous studies have shown that in states of decreased insulin sensitivity, as in "diabesity", the postprandial increase in adipose tissue blood flow is reduced[57-60]. Karpe *et al*[61] showed that the postprandial blood flow rise is associated with insulin sensitivity independently of weight. Moreover, they showed that hyperinsulinaemia affects adipose tissue blood flow indirectly by stimulation of sympathetic activity[61].

Previous reports in healthy subjects by the same research group have demonstrated that nitric oxide determines the actual rate of adipose tissue blood flow, whereas postprandial augmentation of it is mainly under adrenergic regulation *in vivo*, and that blood flow regulation and lipolysis are co-regulated[25].

Dimitriadis *et al*[62] showed an altered fasting and postprandial adipose tissue blood flow in all stages of metabolic regulation, from the prediabetic state to clinical diabetes, even in lean first-degree relatives of diabetic patients. This study, using a mixed meal as a stimulus, showed significant association of postprandial adipose tissue blood flow with insulin sensitivity. Basal and post-challenge triglycerides were negatively correlated to the responsiveness of adipose tissue blood flow; the same was true for postprandial non-esterified fatty acids but not for fasting values[62] (Figure 2).

Fatty acid overflow (mainly palmitic acid), a well recognized factor to interfere with insulin sensitivity, causes both cellular and vascular insulin dysfunction[63]. The increased rate of lipolysis in diabetes may result in increased lipid oxidation and a decreased glucose oxidation rate[61,64].

Impairment in blood flow response of adipose tissue has been found in other insulin resistance states. Mitrou *et al*[47] study in morbidly obese subjects, shows a drop in postprandial adipose tissue blood flow response and in glucose disposal per 100 mL fat tissue. However, glucose fractional extraction from subcutaneous fat depot was unaltered and glucose uptake per total fat mass was increased. Thus, it seems that although an expanded adipose tissue causes insulin resistance, total fat mass provides a buffer for glucose overflow and compensates for insulin resistance.

Diabetic subjects fail to increase adipose tissue blood flow during prolonged exercise of moderate

intensity, in combination to the inability to regulate non-esterified fatty acid mobilization and adipose tissue glucose clearance[65]. Exercise augments adipose tissue lipolysis in diabetic patients, but due to an impaired blood flow response, a high proportion of free fatty acids that come from lipolysis cannot be released into the circulation. Visceral glucose release is lower than whole-body glucose utilisation during exercise and post-exercise recovery[66].

The cause of the impairment in postprandial adipose tissue blood flow reactivity in insulin resistance is still obscure. One potential explanation is the downregulation of the adrenergic receptor during chronic sympathetic stimulation in a milieu of long-standing hyperinsulinaemia. Sympathetic nervous system overactivity induces oxidative stress. Increased levels of circulating free oxygen radicals consumes nitric oxide, and inhibits physiological insulin-dependent vasodilatation[23]. Interestingly, the transcription of eNOS and natriuretic peptide receptor-A, which are expressed in adipose tissue and interfere with vasoactive actions, was associated with adipose tissue blood flow responsiveness to feeding. This finding suggests that part of blood flow regulation is at a transcriptional level and it is independent of adiposity[28].

At the bottom line, adipose tissue is an important buffer against the postprandial spill-over of nonesterified fatty acids in the circulation, thus protecting other peripheral tissues. This buffering effect is dysregulated in states of an over-expanded inflammatory, hypoxic adipose tissue, where the postprandial blood flow response is minimized, potentially leading to atherogenic dyslipidaemia[67].

CONCLUSION

Resistance in the haemodynamic actions of insulin is essential for the development of type 2 diabetes and insulin resistant states as well as their complications, namely cardiovascular disease, the development of which often precedes overt hyperglycaemia and which is the primary cause of mortality within the diabetic population.

Insulin normally stimulates microvascular perfusion (capillary recruitment) of skeletal muscle and subcutaneous adipose tissue and thus increases blood flow mainly after meal ingestion or physical exercise. This effect is impaired in insulin resistance and type 2 diabetes early during metabolic dysregulation development and reflects early-onset vascular dysfunction. Failure of insulin to increase muscle blood flow results in the inability to regulate its own delivery and that of other substrates and hormones and consequently to a decrease in glucose disposal. In fat depots blood flow is closely related to triglyceride clearance and non-esterified fatty acid kinetics. Therefore, we may speculate that dysregulation of post-challenge blood flow responsiveness in skeletal muscle and adipose tissue may together underlie some of the detrimental aspects of insulin resistance.

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MINIREVIEWS

Assessment of cardiovascular risk in diabetes: Risk scores and provocative testing

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Abstract

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality among patients with diabetes mellitus, who have a risk of cardiovascular mortality two to four times that of people without diabetes. An individualised approach to cardiovascular risk estimation and management is needed. Over the past decades, many risk scores have been developed to predict CVD. However, few have been externally validated in a diabetic population and limited studies have examined the impact of applying a prediction model in clinical practice. Currently, guidelines are focused on testing for CVD in symptomatic patients. Atypical symptoms or silent ischemia are more common in the diabetic population, and with additional markers of vascular disease such as erectile dysfunction and autonomic neuropathy, these guidelines can be difficult to interpret. We propose an algorithm incorporating cardiovascular risk scores in combination with typical and atypical signs and symptoms to alert clinicians to consider further investigation with provocative testing. The modalities for investigation of CVD are discussed.

Key words: Diabetes; Cardiovascular risk; Risk scores; Provocative testing; Silent ischaemia; Atypical symptoms

Core tip: Current guidelines focus on testing for cardiovascular disease in symptomatic patients. However, patients with diabetes often present with atypical features of underlying vascular disease. An individualised approach to cardiovascular risk estimation and management is needed in patients with diabetes. We propose an algorithm incorporating cardiovascular risk scores in combination with typical and atypical signs and symptoms to alert clinicians to consider further investigation with provocative testing. The modalities for investigation of cardiovascular disease are discussed.

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INTRODUCTION

The incidence of diabetes mellitus is increasing globally. The World Health Organisation estimated there were 30 million people who had diabetes worldwide in 1985. This number increased to 217 million in 2005, and by the year 2030, it is predicted this number will increase to at least 366 million[1].

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality among people with diabetes mellitus, who have a risk of cardiovascular mortality two to four times greater than that of people without diabetes[2]. Diabetes is commonly associated with other cardiovascular risk factors, interacting with these to accelerate atherogenesis[3-6]. Multifactorial interventions, such as those targeting hyperglycaemia, hypertension and hypercholesterolaemia, significantly reduce the risk of both fatal and non-fatal CVD[7]. The National Cholesterol Education Programme Adult Treatment Panel III (NCEP-ATPIII) has listed diabetes as a coronary heart disease (CHD) equivalent, which would obviate the need for risk stratification. However, clearly not all patients with diabetes have the same cardiovascular risk. An individualised approach to cardiovascular risk estimation and management is needed[8]. Furthermore there is a high prevalence of asymptomatic coronary artery disease (CAD), and higher incidences of silent ischaemia and of atypical symptoms[9].

Over the past two decades, there has been a significant reduction in the incidence of diabetes-related complications. The greatest absolute decline was in the number of cases of acute myocardial infarction, likely reflecting a combination of enhanced awareness, detection and early management of risk factors[10]. The development of statistical models, such as the Framingham equations, has allowed the probability of future cardiovascular events to be calculated based on multiple risk factors[11]. This allows targeted preventative therapy for those with highest absolute risk[12]. However, the majority of these risk equations have not been validated enough in the diabetic population, and either overestimate or underestimate cardiovascular risk.

USE OF CARDIOVASCULAR RISK SCORES IN DIABETES MELLITUS: PREDICTORS, VALIDATION AND IMPACT ON CLINICAL OUTCOME

There have been a multitude of risk scores developed over the past decades, but only a few have been specifically developed for use in the diabetic population. In a systematic review of prediction models for CVD risk in type 2 diabetes[13], 12 of 45 prediction models were specifically developed for patients with type 2 diabetes. The majority of these predicted 5-year risk of CHD or total CVD, with the most commonly used predictors being age, sex, duration of diagnosed diabetes, HbA1c (glycosylated haemoglobin A1c) and smoking. Non-traditional risk factors, such as novel biomarkers and low birthweight, have generally not been incorporated into these models, and are of questionable clinical significance[14,15]. Prediction models derived from the general population, in which diabetes was used as a predictor, included other risk factors such as age, sex, systolic blood pressure, smoking and cholesterol. Of the risk scores, only a third had been externally validated in a diabetic population[13].

The International Diabetes Federation recommends calculating cardiovascular risk in patients with type 2 diabetes with prediction models that can be applied to the diabetes population, including the United Kingdom Prospective Diabetes Study (UKPDS) risk engine[16]. This risk engine provides a comprehensive model for predicting CHD risk in patients with type 2 diabetes. The Australian National Vascular Disease Prevention Alliance[17] recommends using both the Framingham prediction model and UKPDS risk engine. However, certain subgroups (Table 1) are at high risk of cardiovascular events because of their comorbidities, and a calculation of absolute CVD is not considered necessary[17].

Kengne *et al*[11] evaluated the performance of the Framingham and UKPDS models in a cohort of patients with established type 2 diabetes, and found both models to overestimate the 4-year risk of CHD; by 146% and 198% respectively. The Action in Diabetes and Vascular Disease: Preterax and Diamicron MR Controlled Evaluation model[18] was developed from a contemporary multinational cohort of diabetic patients, and includes both retinopathy and microalbuminuria as risk predictors. They are both significantly associated with CVD. It has largely outperformed the Framingham models in validation studies, with only a modest risk underestimation[19]. Similarly, the Fremantle prediction model[20], developed from a type 2 diabetic cohort, had good positive and negative predictive values, but requires further validation[13].

Very few studies have examined the impact of applying a prediction model in clinical practice. In a cohort of patients with type 2 diabetes at high risk of CVD, clear documentation of a cardiovascular risk prediction score on patient medical records was associated with more intensive intervention through prescription of lipid-modifying or antihypertensive medications[21]. Furthermore, use of risk scores has resulted in improvements in lipid profiles and significant reductions in risk of CHD[22].

The use of cardiovascular risk scores has been

Table 1 Clinical features suggesting diabetes patients at high risk

Diabetes and age > 60 yr
Diabetes and microalbuminuria (> 20 mcg/min or urine albumin to creatinine ratio > 2.5 mg/mmol for males, > 3.5 mg/mmol for females)
Diabetes and moderate or severe chronic kidney disease (persistent proteinuria or eGFR < 45 mL/min per 1.73 m^2)
Diabetes and a previous diagnosis of familial hypercholesterolaemia in the individual
Diabetes and systolic blood pressure ⩾ 180 mmHg or diastolic blood pressure ⩾ 110 mmHg
Diabetes and serum total cholesterol > 7.5 mmol/L

Albumin to creatinine ratio - confirmed on second test and not due to another cause (*e.g.*, urinary tract infection).

Table 2 American Diabetes Association guidelines on stress testing in diabetic patients[23]

Typical or atypical cardiac symptoms
Resting electrocardiogram suggestive of ischaemia or infarction
Peripheral or carotid occlusive arterial disease

Table 3 Signs and symptoms of concern in an otherwise asymptomatic patient

Symptoms suggestive of cardiovascular autonomic neuropathy
Resting tachycardia
Postural hypotension
Signs/symptoms suggestive of coexisting vascular disease
Erectile dysfunction
Claudication symptoms
Carotid bruit
Diminished/absent peripheral pulses
Inappropriate exercise tolerance
Shortness of breath without clear pathology

incorporated into multiple guidelines, and may be a useful initial step towards CVS risk stratification. However, given the modest performance of most prediction models, and need for more extensive validation studies, further decision-making may be useful before proceeding to provocative testing.

PROVOCATIVE TESTING: FACTORS INFLUENCING DECISION MAKING

The onset of microvascular and macrovascular complications in diabetic patients is frequently insidious, with the absence of typical symptoms often delaying diagnosis. Studies have demonstrated that a significant percentage of patients with diabetes who have no symptoms of CAD have abnormal stress tests, either by stress electrocardiogram (ECG), stress echocardiogram or stress nuclear perfusion imaging[23]. CAD in patients is often silent, more advanced and associated with less favourable prognosis than those in the non-diabetic population[23]. Diabetic cardiovascular autonomic neuropathy (CAN) resulting in damage to the neural fibres responsible for innervation of the heart and cardiac vessels can lead to atypical clinical manifestations, hence the concept of screening an asymptomatic patient is complex[24]. However, the American Heart Association recommends against routine screening in diabetic patients who are asymptomatic, as there is currently no outcome data to support stress testing in this group of patients[25]. In contrast, the American Diabetes Association (ADA) recommends exercise stress testing in both symptomatic and asymptomatic patients with specific criteria (Table 2).

There are further specific guidelines for screening for CVD before beginning moderate to vigorous exercise training program which expand to include the length of disease; 15 years for type 1 diabetes and 10 years for type 2 diabetes, and age ⩾ 35 for type 2 diabetes. Given we encourage all our patients to exercise as part of a general care plan for diabetes, it may be argued that all patients should be screened prior to this recommendation.

Furthermore, given symptoms may be atypical in the diabetic patient, there may be some clues to the presence of CVD to alert the treating clinician to investigate (Table 3). Symptoms of exercise intolerance and erectile dysfunction may suggest underlying coronary artery disease and may prompt further investigation. Peripheral arterial disease and the presence of Q waves and or ST/T wave abnormalities on ECG have also been shown to predict presence of coronary artery disease[26].

Erectile dysfunction

Erectile dysfunction may be the manifestation of endothelial dysfunction in many cases and is recognised to represent the coexistence of vascular disease in other areas[27]. It has been documented that men with

Table 4 Sensitivity and specificity of provocative tests in patients with diabetes

Diagnostic test	Sensitivity (%)	Specificity (%)
Exercise stress test[33]	47	81
Stress echo[35]	82	54
Stress nuclear perfusion study[36]	86	56
CT coronary angiogram[40]	76	90
Coronary calcium score[39]	64-75	75-83

CT: Computed tomography.

Table 5 Sensitivity and specificity of provocative testing in women[44]

Diagnostic test	Sensitivity (%)	Specificity (%)
Exercise electrocardiogram	31-71	66-78
Exercise echocardiogram	80-88	79-86
Pharmacological echocardiogram	76-90	85-94
Nuclear perfusion study	78-88	64-91
Computed tomography coronary angiogram	97	79

no cardiac symptoms and erectile dysfunction have increased risk of cardiac events over the following 3-5 years[28]. Furthermore a large meta-analysis found patients with erectile dysfunction have an increased risk of CVD, cerebrovascular disease, stroke and all-cause mortality independent of traditional risk factors[29]. The suggestion that patients with erectile dysfunction are likely to be vasculopathic validates the investigation of cardiovascular and peripheral vascular disease, even in the absence of typical symptoms. We therefore propose that patients with a history of erectile dysfunction be investigated further for underlying vascular disease.

Exercise tolerance and CAN

Autonomic dysfunction in diabetes leads to exercise intolerance. Suboptimal cardiac output in times of exertion can be a result of CAN as well as vascular disease and silent ischaemia[30]. Signs and symptoms of CAN may include resting tachycardia due to impaired vagal tone or orthostatic hypotension[30]. CAN significantly increases the risk of fatal or non-fatal cardiovascular event[24]. The suspicion of CAN may therefore justify further investigation for coronary vascular disease.

Claudication symptoms

Symptoms of claudication in the diabetic patient justify consideration of investigating other vascular disease including coronary artery disease, even in the absence of symptoms. Patients with peripheral vascular disease have increased mortality from cardiovascular causes[31]. Assessment of peripheral pulses should be performed in all patients, given this is a simple method of screening. If abnormal, further investigation with ankle brachial indices and provocative testing for cardiac ischaemia may be warranted[31].

CHOICE OF INVESTIGATION FOR RISK STRATIFICATION IN DIABETIC PATIENTS WITH SUSPECTED CVD

The choice of investigation will depend on a number of factors including mobility, exercise tolerance, plans for future increases in exercise and potentially gender. As a baseline investigation, the American Heart Association recommends that a resting electrocardiogram (ECG) is a reasonable tool for risk assessment in asymptomatic adults with diabetes[32]. Beyond this, the factors influencing selection of a particular modality for provocative testing are similar between diabetic and non-diabetic patients and include availability, sensitivity and specificity and risk. Each modality has varying performance accuracy in terms of sensitivity and specificity with some specific differences in patients with diabetes (Table 4).

Exercise ECG

Exercise ECG (stress testing) is widely regarded as the first line test in mobile patients with a normal baseline electrocardiogram and it has been found to have similar predictive value between diabetic and non-diabetic populations[33]. However sensitivity is variable, and in some studies is less than 50%[33]. A positive test will identify the majority of patients with left main or significant multi-vessel coronary artery disease[33]. One study found a positive predictive value of 94% in a cohort of asymptomatic older males with poorly controlled diabetes[34].

Stress ECG is less sensitive and specific in asymptomatic populations, *i.e.,* where there is a lower pre-test probability. The test is highly dependent on the patient's capacity to exercise long enough to provide a valid test. Whilst a patient reaching above expected exercise capacity provides useful prognostic and clinical information, many diabetic patients with obesity, peripheral neuropathy, decreased physical conditioning or other co-morbidities are unable to exercise long enough to determine low cardiovascular risk. It can therefore be argued that this form of investigation is suboptimal for patients with diabetes who are unlikely to be able to reach an appropriate workload owing to co-morbidities. In women, the test may also be less useful, with quoted sensitivities of 31%-71% (Table 5).

Stress echocardiography

In the general population addition of imaging modalities such as echocardiography to stress testing provides greater diagnostic accuracy. Addition of echocardiography gives additional information about regional wall motion abnormalities (suggesting prior infarcts) and ventricular dysfunction, both of which are more common in people with diabetes. However data regarding diagnostic accuracy of stress echocardiography specifically in diabetic populations is relatively limited. Hennessy *et al*[35] evaluated

dobutamine stress echo in 52 patients with diabetes, finding a sensitivity of 82% but a specificity of only 54%. The positive predictive value was 84% with a poor negative predictive value of 50%[35]. Availability may be limited by cost and operator expertise.

Nuclear perfusion scans

Stress nuclear imaging has been the most widely investigated modality for the detection of CAD in people with diabetes. The sensitivity of this tool has been quoted as 86% with a specificity of 56% in patients with diabetes[36]. Wackers *et al*[37] examined asymptomatic patients with diabetes using adenosine Single Photon Emission Computed Tomography (SPECT) imaging and found positive test results for CAD in 22%. Interestingly, 41% of these patients with abnormal imaging findings would not have met usual criteria for further investigation of coronary disease according to previous ADA guidelines. Thus, use of stress imaging in selected people with diabetes who have high absolute cardiovascular risk is reasonable even if they are asymptomatic. Nuclear imaging studies can be performed with exercise, or in subjects with limited exercise capacity with other modalities to increase coronary flow such as adenosine. This modality provides information about coronary flow at rest, with exercise or stimulated stress, as well as regional wall motion, although the last is much less precise than the information obtained with echocardiography.

Computed tomography coronary angiogram and coronary calcium score

Computed tomography (CT) coronary angiogram (CTCA) may provide information on the vascular lumen and the arterial wall[38]. In people without diabetes it has been reported to have high sensitivity[39]. However, a study comparing the use of CTCA in diabetic *vs* non-diabetic patients found reduced sensitivity and specificity in people with diabetes, due to differences in artefacts and calcification[40]. While coronary calcium score may be able to predict coronary disease beyond standard risk factors, significant stenosis can occur in the absence of calcification, so this tool should not be used in isolation[31,41,42].

A study by Maffei *et al*[42] showed that coronary plaque burden and coronary calcium scores were higher in diabetic *vs* non diabetic patients. Furthermore it has been shown that asymptomatic patients with diabetes with high coronary artery calcium scores have a high prevalence of inducible ischaemia on stress imaging[43]. The American Heart Association acknowledges that measurement of coronary artery calcium score is reasonable for cardiovascular risk assessment in patients with diabetes who are asymptomatic and age over 40[32]. The efficacy of this test in women with diabetes is less clear, see below.

Gender effects

Both symptoms and pathophysiology of coronary artery disease can differ between males and females. Women, whether diabetic or not, are more likely to have atypical symptoms and are often older at the time of onset of disease or events. Prognosis is poorer in women than men with higher mortality rates from acute myocardial infarction[44]. Detection of disease in women is more difficult given the lower likelihood of obstructive coronary disease and apparently lower levels of clinical suspicion[45].

As well as these issues, currently available provocative tests are both less sensitive and less specific in women[44]. Information regarding the characteristics of coronary artery disease in diabetic women *vs* the general female population is surprisingly sparse. To date, guidelines suggest the use of exercise ECG testing as first line investigation in women with symptoms of coronary disease with a normal baseline ECG[46]. If either the baseline ECG or exercise ECG is abnormal, the addition of stress testing with imaging is recommended[46]. However, these investigations are well known to have limitations in the female population due to interference from breast soft tissue and differences in coronary anatomy in women[45].

Stress SPECT and stress echo are considered superior to exercise ECG in women for both sensitivity and specificity. Adenosine stress nuclear imaging has similar prognostic ability in men and women, though it has been shown that women have worse clinical profiles for the same degree of imaging abnormality[47]. However, the ultimate decision may be limited by cost and local expertise. Table 5 summaries the sensitivity and specificity of the different provocative investigations in women.

Risks associated with different testing modalities

There are risks associated with each of the tests discussed. For non-invasive stress testing such as exercise stress tests and exercise stress echo, 5% of patients may experience mild angina, shortness of breath or musculoskeletal pain. Less commonly (< 5%) chest pain, hypotension or syncope may occur and rarely (< 1%) there is a risk of acute myocardial infarction, stroke or arrhythmia[45]. Investigations requiring contrast such as CTCA carry risks of associated renal toxicity or allergic reaction, and exposure to significant radiation with resultant cancer risk. Nuclear perfusion scans may employ the use of agents such as adenosine, which are known to induce asthma in some individuals and also involve some radiation exposure. Each of these factors must to be considered in the decision to utilise a certain modality. In women who are considering pregnancy, stress ECG or stress echocardiography are radiation-free, which is an important consideration.

SUGGESTED ALGORITHM

The decision to proceed with provocative testing should be based on a combination of cardiovascular risk

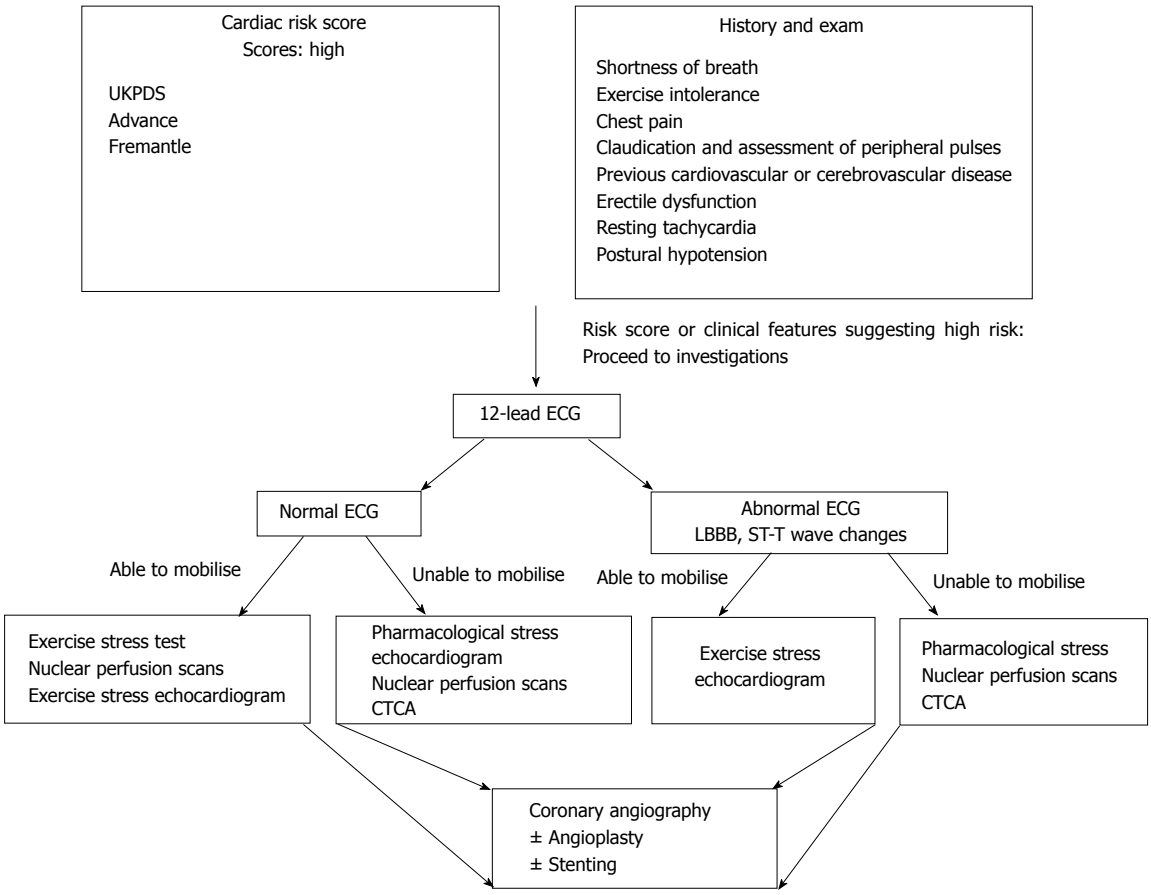

Figure 1 Suggested algorithm for investigation of cardiovascular disease in patients with diabetes. Since a 12-lead ECG is a safe and cheap test, it should be performed in people with diabetes with a low threshold. At each layer of testing, if the test is normal or unchanged from previous testing, consider whether the next level of testing is needed. ECG: Echocardiogram; LBBB: Left bundle branch block; CTCA: CT coronary angiogram; UKPDS: United Kingdom prospective diabetes study.

score and suspicious features on clinical history and or examination. As discussed above, risk calculators do not consider specific features such as erectile dysfunction or cardiac autonomic neuropathy. Therefore, risk calculators may fail to identify potential high risk features when used in isolation.

Firstly, a baseline 12-lead ECG should be performed in all patients considered at risk. Following this, the choice of modality for provocative testing will depend on factors such as abnormal resting ECG (left bundle branch block or ST-T wave changes at baseline), mobility including ability to perform exercise testing, gender, cost and access to local expertise. There is a need to highlight and alert the treating clinician to recognise novel markers of disease that have been previously under-recognised by traditional risk scores. Considering these risk factors, we propose the algorithm in Figure 1.

CONCLUSION

Patients with diabetes are at high risk of mortality from CVD. Given this group of patients often present with atypical symptoms and silent ischaemia, traditional recommendations for screening in symptomatic individuals may not be applicable. National guidelines recommend incorporation of a cardiovascular risk score in risk stratification. Risk scores have arguably suboptimal performance when used in isolation and have not been extensively validated. Additionally, to date such clinical signs as erectile dysfunction or autonomic neuropathy have not been incorporated into cardiovascular risk prediction models, though it is well recognised that these pathologies represent underlying cardiac disease. We propose the use of a combination of a risk score and relevant clinical findings in the overall assessment of cardiovascular risk. The algorithm (Figure 1) presented may provide treating clinicians with various clues to prompt further investigation with provocative testing. There is an ongoing need for re-evaluation of guidelines for screening in this high risk patient group.

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Gene-gene, gene-environment, gene-nutrient interactions and single nucleotide polymorphisms of inflammatory cytokines

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Abstract

Inflammation plays a significant role in the etiology of type 2 diabetes mellitus (T2DM). The rise in the pro-inflammatory cytokines is the essential step in glucotoxicity and lipotoxicity induced mitochondrial injury, oxidative stress and beta cell apoptosis in T2DM. Among the recognized markers are interleukin (IL)-6, IL-1, IL-10, IL-18, tissue necrosis factor-alpha (TNF-α), C-reactive protein, resistin, adiponectin, tissue plasminogen activator, fibrinogen and heptoglobins. Diabetes mellitus has firm genetic and very strong environmental influence; exhibiting a polygenic mode of inheritance. Many single nucleotide polymorphisms (SNPs) in various genes including those of pro and anti-inflammatory cytokines have been reported as a risk for T2DM. Not all the SNPs have been confirmed by unifying results in different studies and wide variations have been reported in various ethnic groups. The inter-ethnic variations can be explained by the fact that gene expression may be regulated by gene-gene, gene-environment and gene-nutrient interactions. This review highlights the impact of these interactions on determining the role of single nucleotide polymorphism of IL-6, TNF-α, resistin and adiponectin in pathogenesis of T2DM.

Key words: Cytokines; Gene-environment interaction; Diabetes mellitus; Single nucleotide polymorphism; Gene-gene interaction

Core tip: Single nucleotide polymorphisms (SNPs) in inflammatory cytokines play role in insulin resistance and type 2 diabetes mellitus (T2DM). These SNPs are found to be correlated with cytokine serum levels, body mass index, insulin resistance and dyslipidemia although these findings are challenged by other studies. Gene-gene, gene-environment and gene-nutrient interactions alter the impact of these SNPs in pathogenesis of T2DM. These interactions may explain the inter-ethnic variations in role of inflammatory cytokines in T2DM reported in international studies. This mini-review highlights these gene-genes, gene-environment and gene-nutrient interactions and their impact on inflammatory cytokine SNPs.

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INTRODUCTION

The prevalence of diabetes mellitus (DM) has increased globally over decades and is projected to continue increasing[1]. The etiology of DM is multi-factorial and inflammation plays a role in its pathogenesis. DM is considered as chronic low grade inflammatory state and markers of sub clinical inflammation increase in type 2 diabetes mellitus (T2DM) years before diagnosis of the disease[2]. They have a role in pathogenesis of the disease, obesity, insulin resistance and apoptosis of beta cells of endocrine pancreas by various mechanisms. Among the recognized markers are interleukin (IL)-6, IL-1, IL-18, tumor necrosis factor-alpha (TNF-α), C-reactive protein (CRP), tissue plasminogen activator (tPA), heptoglobulins, fibrinogen, resistin and adiponectin. The single nucleotide polymorphisms (SNPs) of pro- and anti-inflammatory cytokines have been found to influence the cytokines at translational level and modify serum levels. Inter-ethnic variations have been reported regarding association of SNPs of cytokines with T2DM and serum levels (Table 1). Pro-inflammatory cytokines induce glucotoxicity and lipotoxicity which in turns leads to mitochondrial damage, oxidative stress and beta cell apoptosis[2].

Although the relationship between single nucleotide polymorphisms of cytokines/adipokines and risk of T2DM has been robustly proven, inter-ethnic and intra-ethnic variations in this relationship cannot be explained in generalized terms. A number of factors including demographic features, sample size, gene-gene, gene-environment and gene-nutrients interaction, average age of onset, duration of disease, life style, degree of obesity, glucose tolerance and pathogenesis of disease can confound association studies.

GENE-GENE INTERACTION

Variable results regarding association of cytokines SNPs with T2DM in different ethnic groups have been reported in international studies[1-7]. Even different studies in the same ethnic group have also reported varying results[8,9]. This variation is also found in association of cytokines SNPs with serum levels of respective cytokines, insulin resistance, serum insulin, lipid profile and body mass index (BMI). Gene-gene interaction is an established fact and a few studies have reported this factor as a contributing one in inter-ethnic variations in various parameters. There might be another unidentified functional gene polymorphism in close linkage disequilibrium with cytokine SNPs.

It has been found that presence or absence of a minor allele of inflammatory cytokine genotype may influence the binding of transcription factors to promoter region altering the promoter activity from almost non-existent to augmented by many folds[4]. Similarly gene-gene interactions may also influence either the binding of transcription factors or translational activity. Polymorphism of a stress protein gene; P2/P2 genotype of heat shock protein 70-2 is found to be close to and in linkage disequilibrium with TNF-α promoter area and is statistically associated with obesity in Tunisian subjects and may influence the impact of TNF-α polymorphism[3].

IL-6 -174 G/C SNP is in the promoter region (*-173* to *-145*) that contains multiple response elements. These functional sites respond to many factors including IL-1, TNF-α, NF-κB and glucocorticoids[6]. Presence or absence of a minor allele may influence the binding of transcription factors to DNA response elements. Moreover, *IL-6* gene promoter function is also effected by a variable run of *A* and *T* bases (*-257* to *-276*)[4]. *IL-6* promoter variants; *G* and *C* are also affected by the *IL-6* haplotype, age and sex of the individual and presence of sex steroids[5]. Estrogen regulates protein synthesis at transcriptional levels, particularly those proteins which are involved in glucose and lipid metabolism.

In overweight IGT Finnish subjects, simultaneous polymorphism *C-174G* in *IL-6* and the *G-308A* polymorphism in TNF-α showed a 2.2 fold increased risk of T2DM than any other SNP[6] neither of SNP, although risk was not higher in simultaneous SNP's as compared to the *G-308A* SNP alone showing a gene-gene interaction[6].

Individually, *IL-6-597 G/A, TNF-α -308G/A* and *IL-10 -592C/A* do not show any association between SNP and risk of T2DM in Indian population but combined genotypes of *IL-6 -597 GA* and *TNF-α -308 GG* increased the risk of T2DM up to 21 times, while triple combination of *IL-6 -597 AA, TNF-α -308 GG* and *IL-10 -592 CA* increased the risk to 314 times. Presence of minor allele *A* in all 3 genes increased the risk up to 1.41 times in Indian population, showing strong gene-gene interaction[7].

Table 1 Frequencies of single nucleotide polymorphisms of cytokines in various ethnic groups

S no	Ethnic group	*n*	IL6 -174G/C genotype frequency			Ref.
			GG (%)	GC (%)	CC (%)	
1	Diabetic Turkish	96	83.9		16.1	Karadeniz *et al*[5]
2	Diabetic Indians	40	57	28	15	Mukhopadhyaya *et al*[33]
	Healthy Indians	40	30	33	37	
3	Diabetic Finnish	737	61		39	Razquin *et al*[27]
4	Diabetic Mexicans	90	76.7	20	3.3	Guzmán-Guzmán *et al*[34]
			IL6 -572 G/C allele frequency			
			GG (%)	GC (%)	CC (%)	
5	Healthy Chinese	581	3.74	39.8	56.4	Zhou *et al*[35]
6	Diabetic Mexicans	90	61.1	30	8.9	Guzmán-Guzmán *et al*[34]
7	Healthy Caucasians	677	6.2	39	54.8	Paik *et al*[36]
			TNFα -308G/A allele frequency			
			GG (%)	GA (%)	AA (%)	
8	Diabetic Mexicans	278	76		24	Pérez-Martínez *et al*[31]
9	Diabetic Caucasians	350	87		13	Chan *et al*[37]
10	Diabetic Indians	40	87.5	5	7.5	Mukhopadhyaya *et al*[33]
	Healthy Indians	40	92.5	0	7.5	
			TNFα -238 G/A allele frequency			
			GG (%)	GA (%)	AA (%)	
11	Healthy Iranians	202	79.3	19.2	1.5	Hedayati *et al*[38]
12	Diabetic Canadians	123	85.4	14.6		Fontaine-Bisson *et al*[14]
13	Diabetic Chileans	230	90	10	0	Santos *et al*[30]
			RETN -420 G/C allele frequency			
			CC %	GC%	GG%	
14	Diabetic Japanese	161	47.2	46	6.8	Hishida *et al*[39]
	Healthy Japanese	2491	42.6	43.7	13.7	
15	Diabetic Han Chinese	318	60	40		Chi *et al*[40]
	Healthy Han Chinese	370	61.5	38.5		
16	Diabetic Finnish	258	65	35		Kunnari *et al*[41]
	Healthy Finnish	494	73	27		
			ADIPOQ -11377 G/C allele frequency			
17	Diabetics White	503	79	21		Chiodini *et al*[42]
18	Diabetic Han Chinese	212	68.6	31.4		Yang *et al*[43]
19	Diabetic Germans	365	71	29		Schwarz *et al*[44]

Impact of SNPs in the resistin promoter region is also found to be influenced by gene-gene interaction. Presence of Pro/Pro genotype of PPAR gamma acts synergistically with *RETN -420 G* allele in augmenting serum resistin levels in Japanese population[8]. SNPs in regions outside the coding region may influence transcription or mRNA stability and thus affect the expression of the gene. Nuclear proteins are specifically recognized with a single base difference at SNP-*358* in *RETN* gene but not at SNP *RETN -638*. Therefore, *A* at *RETN -358* is required for *G* at *RETN -420* to confer the highest plasma resistin in the general Japanese population[9]. In Caucasians, the association between SNP *RETN C -420G* and plasma resistin is not strong, and A at *RETN -358* may not exist, suggesting that SNP *RETN -358* could explain this ethnic difference[9].

Similarly, in Filipino population, common SNPs in *ADIPOQ* gene were found to be in linkage disequilibrium with a rare coding variant in *R221S* at *ADIPOQ* locus. This variant was unique to Filipino population and was not found in 12514 European individuals[10].

PPAR-γ agonists are identified transcription factor for *ADIPOQ* expression. In differentiated 3T3L1 mice adipocytes cell line, the haplotype *AC* or *AG* both at *-11426* and *-11377* position results in 2-3 fold increase in rosiglitazone induced promoter activity whereas *GG* haplotype result in almost non-existent promoter activity. The result indicates that inducibility of *ADIPOQ* promoter activity by rosiglitazone (PPAR-γ agonist) depend on SNP variant combination rather than a particular allele[11].

Genome-wide association studies (GWAS) on large sample size and various ethnic groups are required to reach a definitive conclusion about gene-gene interaction.

GENE-ENVIRONMENT INTERACTION

T2DM is a disease in which environmental factors play a very significant role. Physical activity, BMI, physical and mental stress, dietary habits, smoking and lifestyle not only influence the pathogenesis of the disease but also the age of onset of disease, response to treatment, and onset of complications of the disease. These environmental factors are not functioning alone and there is strong gene-environmental interaction.

Adipose tissue is the source of 30% of IL-6. Some studies suggested the role of adiposity in modulating the association between IL-6 genetic variability and T2DM risk[12,13]. Association of *IL-6* SNP with S. IL-6 levels has also been found highly variable in different ethnic groups. *IL-6 C-174G* SNP is suggested to

enhance IL-6 expression, IL-6 mRNA levels and thus serum levels but these effects are cell-type specific[12]. Various IL-6 producing cell types may respond differently to various risk factors like inflammation, obesity, insulin resistance or yet unidentified parameters and thus altering the impact of SNP. This hypothesis is supported by the finding that TNF-α -308A allele was associated with significant elevation of TNF-α expression but when catering for genotypes, *A* allele association with insulin resistance in presence of obesity but not in absence of it, has been reported in diabetic Canadians[14]. Interestingly not many studies were found in literature indicating positive association of *A* allele presence with higher insulin resistance even in absence of obesity. In one study, insulin resistance was found greater in both obese and lean Romans[15] in either allele carriers. It may indicate the possibility that insulin resistance may be secondary to raised BMI, in all obese irrespective of genotype; a fact supported by studies on obese Australian[16], obese Americans[17], overweight IGT Finnish subjects[6] and obese female Polish Caucasians[18], where insulin resistance was present in obese subjects carrying either allele. TNF-α polymorphism may act as a genetic factor enhancing the insulin resistance in presence of obesity, irrespective of serum TNF-α levels as found in many studies. Why in some studies, this association between polymorphism and insulin resistance is not found even in presence of obesity is not known.

Smoking is a known factor inducing oxidative stress and increased levels of inflammatory cytokines are found in smokers. Genotype-smoking interaction has been found statistically significant in Korean healthy subjects. *IL-6 -174C* allele was associated with significant elevation of IL-6 expression after coronary artery bypass surgery, although IL-6 levels were same in either C or G allele carriers before surgery. It indicates that probably stress led to an altered impact of the IL-6 G-174C SNP on IL-6 expression[19]. Similarly, *C* allele was found to be associated with reduced levels of IL-6 after long term exercise although no difference was reported between genotypes before exercise training program[4,20]. Although, the low level of physical activity may increase the risk of T2DM in the absence of the risk allele, presence of the risk allele may not always assure the protection from the disease with exercise.

Association of *TNF-α -308 G/A* polymorphism with T2DM is explained by increased insulin resistance caused by raised serum levels of TNF-α. Despite quite a large number of studies on association of T2DM with this SNP, insulin resistance and BMI; very few studies correlated the TNF-α SNPs with serum levels of TNF, insulin resistance, BMI. Association of *A* allele with higher serum levels of TNF has been reported various ethnic groups such as in overweight Finnish subjects[6], Danish Caucasians[21] and in Chinese[22]. Negative association has been reported in healthy controls, impaired glucose tolerant and diabetic Czech Caucasians[23] and in healthy Chinese[24].

Homozygous *IL6 -572GG* genotype results in higher serum IL-6 in smokers as compared to *GC* and *CC* genotype (*P* value = 0.04)[25]. Evidences indicate that environmental factors not only alone through metabolic derangement but also through gene-environmental interaction influence the impact of cytokine SNPs on pathogenesis of T2DM.

GENE-NUTRIENT INTERACTION

Single nucleotide polymorphisms in cytokines also interact with dietary factors influencing the cytokine expression induced by diet. *IL-6 -174G* allele has been found to be associated with higher energy expenditure as compared to *C* allele which could be one of the possible causes of lower BMI in *G* allele carriers in this cohort[26]. A recent study indicate the higher BMI in *IL-6 -174 CC* genotype in Koreans but greater reduction in weight in *CC* genotype as compared to *GG* genotype by low fat diet/virgin olive oil diet for 3 years. The effect of gene variant on obesity indices was reversed by low fat diet[27].

TNF-α -238 A allele alter the post-prandial suppression of FFA and levels remain high in obese, but not in non-obese A carriers. High TNF-expression in obese due to presence of *A* allele but also due to larger adipocytes may explain the absence of this effect in non-obese despite having *A* allele in Canadians[14].

A recent study highlighted the strong gene-nutrient interaction affecting the serum levels of adiponectin. Marine fish oil contains unsaturated fatty acids which are ligands for transcription factor; PPARγ which enhances the adiponectin expression thus increasing its serum level. The presence of SNPs in *ADIPOQ* fosters the effect of dietary marine fish oil on adiponectin expression[28].

Another example of gene-nutrient interaction is dependence of insulin sensitivity on plasma saturated fatty acid (SFA) levels in the presence of homozygous minor alleles *ADIPOQ -11377 GG.* Insulin resistance was higher in *GG* carriers with high SFA levels. In the presence of homozygous major alleles; *ADIPOQ -11377 CC* and heterozygous *CG* genotypes, the insulin sensitivity was not altered by plasma concentrations of SFA[29]. *ADIPOQ -11377* SNP also influences the extent to which energy is derived from dietary fat in obese women[30]. *ADIPOQ CC* homozygotes men have less insulin resistance after consumption of a monounsaturated fatty acid and carbohydrate diet as compared to saturated fatty acid diet; an effect not seen in females[31]. On the other hand, serum adiponectin was reduced after α-linolenic acid supplementation in obese individuals irrespective of *C* or *G* allele at *-11377 ADIPOQ*[29].

Heterogeneity in serum levels of cytokines in response to lipid lowering drugs in various patients may be due, in part to genotypes of inflammatory cytokines. *IL-6 -572 GG* is associated with higher S.

IL-6 levels as compared to *GC* and *CC* genotypes in those who are not taking lipid-lowering drugs while levels are comparable in *GG, GC* and *CC* genotypes who were taking those drugs. It is possible that statins might reduce the augmented effect of *GG* genotype on inflammation[32].

CONCLUSION

Pathogenesis of T2DM is multi-factorial. Genetic background has a profound effect especially in T2DM. The genetic makeup itself is governed by many factors; some of which are still unidentified. Contribution of T2DM susceptibility genes in insulin resistance and beta cell failure and their interaction with cytokine genes, environment and nutrients need to be explored. There could be additional, still unidentified risk factors which obscure the impact of SNP with specific genetic/environmental/nutrient background in various ethnic groups in population. Single nucleotide polymorphism may be considered as a susceptibility factor in certain population segments based on other risk factors; both genetic and environmental. GWAS with large number of sample sizes may indeed be required to statistically manifest SNP-related risk factors. Most of the studies are done in relatively small population groups in a particular ethnic group. Sampling might include patient selection inherent biases and do not reflect the general risk of population for the disease.

Gene-gene, gene-environment and gene-nutrient interactions strongly influence the impact of cytokine polymorphisms on not only serum levels of cytokines but also on the insulin sensitivity, susceptibility to disease, response to weight reduction and lipid lowering drugs, energy expenditure and energy derivation from dietary fats. Such multi-dimensional regulatory factors can explain the wide variations in the role of single nucleotide polymorphisms in cytokines in pathogenesis of T2DM and MS reported in various ethnic groups.

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Attenuating type 2 diabetes with postpartum interventions following gestational diabetes mellitus

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Abstract

Women with a history of gestational diabetes should be screened during and after the postpartum period because of a high risk for developing type 2 diabetes mellitus. Although differences exist between guidelines practiced throughout various parts of the world, all recommend the use of cutoffs for fasting and/or post-load plasma glucose to diagnose diabetes or pre-diabetes. The use of these glycemic parameters could be optimized when a trend is observed, rather than considering them as isolated values at various time points. As the presence of insulin resistance and beta-cell dysfunction start before glycemic changes are evident, the estimation of insulin sensitivity and beta-cell function by Homeostatic Model Assessment is suggested for women who have additional risk factors for diabetes, such as obesity. Disease-modifying lifestyle intervention should be the first-line strategy to prevent or delay the onset of diabetes in women with a history of gestational diabetes mellitus. Intensive lifestyle interventions are designed to decrease caloric intake and increase physical activity in order to reduce body weight and fat, which will in turn reduce insulin resistance. This article also reviews unique problems of postpartum women, which should be considered when designing and implementing an intervention. Innovative "out of the box" thinking is appreciated, as continued adherence to a program is a challenge to both the women and the health care personnel who deal with them.

Key words: Gestational diabetes mellitus; Glycemic parameters; Lifestyle intervention; Screening; Type 2 diabetes mellitus

Core tip: This article reviews and highlights important areas concerning diabetic risk during and after the postpartum period in women with gestational diabetes mellitus. Optimizing the use of glycemic parameters and assessing beta-cell function, particularly in high-risk women, will facilitate early recognition of those on the path to pre-diabetes and diabetes. Lifestyle interventions designed to attenuate the progression should be carefully planned, taking into consideration the unique set of problems in these women. "Out of the box" thinking is necessary to design lifestyle intervention protocols that will have high acceptance by these women.

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DIABETIC RISK FOR WOMEN WITH GESTATIONAL DIABETES MELLITUS

Gestational diabetes mellitus (GDM), which occurs and is diagnosed during pregnancy[1], is a condition that increases the risk of developing type 2 diabetes mellitus (T2DM)[2,3]. In a large meta-analysis of 20 cohort studies in 2009, Bellamy *et al*[4] showed that women with GDM have a more than seven-fold increased risk of developing T2DM when compared to women with normoglycemic pregnancies. However, the incidence of diabetes in these women varies, with relative risks ranging from 6[5] to 12[6], possibly due to differences in screening and diagnostic criteria, associated risk factors[7], and inclusion of subjects with overt diabetes uncovered by pregnancy[8]. Feig *et al*[6] further demonstrated an increase in the probability of developing diabetes from 3.7% at 9 mo to 18.9% at 9 years after delivery, suggesting the need for long-term follow-up and monitoring of women with a history of GDM.

The development of peripheral insulin resistance during pregnancy is facilitated by the increased maternal adiposity and release of insulin-desensitizing hormones from the placenta[9]. The secretion of insulin is increased to compensate, and women with a deficit in this secretion can develop GDM. The effects of pregnancy on glucose homeostasis are alleviated following delivery of the offspring and removal of the placenta, such that the glycemic profile should return to normal within 6-12 wk postpartum.

POSTPARTUM SCREENING OF PATIENTS WITH A HISTORY OF GDM

Despite the lack of a consensus concerning precise recommendations for postpartum screening of women with a history of GDM[10], the importance of optimal screening is universally accepted. The American Diabetes Association recommends using the oral glucose tolerance test (OGTT) to screen these women for persistent diabetes at 6-12 wk postpartum, and lifelong screening for development of diabetes or pre-diabetes at least every three years[1]. However, the Mexico City Diabetes Study demonstrated that the progression from normoglycemia to diabetes ranges over three years with a probable phase of impaired glucose tolerance[11], which suggests that three years between screens is insufficient for high-risk individuals. In the United Kingdom, the National Institute for Health and Clinical Excellence guidelines recommend glucose estimation prior to discharge, at 6 wk postpartum, and annually thereafter using fasting plasma glucose (FPG)[12]. In 2010, however, Kakad *et al*[13] used retrospective data of 470 women to show that diabetes was missed in 26% of women when only the FPG was used for screening. Furthermore, unlike OGTT, FPG does not allow for detection of impaired glucose tolerance. Hemoglobin A1c, an additional parameter introduced to the diagnostic criteria of pre-diabetes and diabetes in 2009[14], is also considered unsuitable for use in postpartum women due to its low sensitivity on its own[15] or in combination with FPG[16]. Thus, OGTT with 75 g fasting glucose challenge and two-hour glucose measurements is the preferred screening method for women with previous GDM[17]. The interpretations should be based on diagnostic cutoffs for pre-diabetes and diabetes for non-pregnant adults[1].

Tabák *et al*[18] used serial measurements of yearly glucose levels over 13 years to evaluate glycemic parameters in normoglycemics and diabetics. They found that during the transformation from normoglycemia to diabetes, FPG and post-load glucose gradually increased, followed by an abrupt increase approximately two years before a diagnosis of DM. This indicates that continual glycemic measurements during screening can be even more informative and predictive, despite being within the normal range. Therefore, it is suggested that rather than looking solely at isolated values at any given time, changes in glycemic measures should be observed.

With the global increase in the prevalence of DM[19], the current recommendations for screening women with GDM for the development of T2DM should be revised. The present guidelines detect problems only when they reach the end point (diabetes), or a landmark very close to the end point (pre-diabetes). Can we use knowledge of the underlying pathophysiology to identify these cases earlier, before they reach the end point? The transition from normoglycemia to diabetes is a continuous process[11,18,20]. Although the glycemic profile assessed by FPG or post-load glucose should return to normal after delivery in a woman with a diagnosis of GDM, these parameters are not indicators of the ongoing pathophysiologic process. An analysis of the British Whitehall Ⅱ study showed a steep decline in insulin sensitivity, along with a marked increase followed by a steep decrease in insulin secretion, approximately 3-5 years before the onset of diabetes[18]. These parameters can be estimated by the Homeostatic Model Assessment[21]. However, this assessment by itself is inappropriate for evaluation of beta-cell function, and serial measurements are required in order to observe the longitudinal changes in insulin secretion[22]. Repetitive monitoring of insulin sensitivity and secretion may be confined to the initial postpartum years due to increased cost, as Kim *et al*[3] showed that T2DM appears rapidly within the first five years and plateaus after ten years. Furthermore, these measurements can be limited to women with a higher predictive risk of developing diabetes, such

as those who are overweight[23], have a higher pre-pregnancy body mass index[24,25], were diagnosed with GDM before the 24th week of gestation[25], and who needed insulin for glycemic control during pregnancy[23]. Finally, the recent call for developing standardized screening protocols for Indian women with GDM[26] is worth considering for all Asian women, as they show a greater risk than Caucasian women[23].

Nonetheless, the risk of developing T2DM can persist for more than 25 years in women with a history of GDM[8,18,27]. Therefore, continued life-long follow-up of these women is justified, particularly with recognition of the fact that ageing is an independent risk factor for T2DM. In addition, women who are not diagnosed with GDM but have mild glucose abnormalities[28] or a single abnormal value in the OGTT[29] should be screened because of the increased risk for developing T2DM. However, as revised recommendations stipulate that only one abnormal value, not two, is sufficient to for a diagnosis of GDM[1], more women may be recommended for T2DM screening.

LIFESTYLE INTERVENTIONS

Lifestyle interventions are the most appropriate initial approach to mitigate the development of diabetes in high risk individuals, such as those with a history of GDM[30], and can reduce the incidence of DM by at least 50%[27,31]. Such interventions may slow down or arrest the pathophysiologic processes, such as the beta-cell exhaustion that occurs in response to chronic insulin resistance[32,33].

Lifestyle intervention programs designed for high-risk individuals generally propose a low-calorie, low-fat diet with moderate intensity physical activity (*e.g.*, brisk walking) for 150-180 min per week to achieve a weight reduction of 5%-7% of the initial body weight[31,34-36]. The recommended calorie limit varies between 1000-1200 kcal/d[35] and 1200–1800 kcal/d[34]. Although it is advised that no more than 30% of energy should come from fats[36], a recent study found adequate glycemic control with a very low-carbohydrate, high-fat, non-calorie-restricted diet[37]. Other simple measures include increasing the amount of fiber in the diet[36], decreasing the amount of energy-dense foods, such as fast foods, increasing the amount of fruit and vegetable intake[38], and controlling portion size[35]. Although it is important to combine physical activity with dietary support to enhance the efficacy of an intervention program[39], results of a small study showed that women perceived diet as more important for the prevention of T2DM than physical activity[40], emphasizing the importance of effective counseling to reinforce the value of both aspects for weight reduction and maintenance[35].

Although almost all published protocols are based on similar principles of intervention, a thorough investigation of these illustrates minor but important differences between them, especially when it comes to the stage of implementation. To augment dietary and exercise interventions, Gabbe *et al*[35] suggested incorporation of behavioral therapy, which includes stress management, stimulus control, problem solving, and goal setting. The Mothers After Gestational Diabetes in Australia Diabetes Prevention Program offers an intervention program handbook, six face-to-face sessions, and two follow-up telephone calls within the 12-mo follow-up period to ensure that participants achieve the program goals[36]. Substantial decreases in glycemic and anthropometric parameters after one year of intervention[41] is strong evidence for implementation of an effective lifestyle intervention program by community health workers[34]. A randomized control study for high-risk Hispanic women initiated interventions during late pregnancy, and continued for 12 mo postpartum[38]. Further support for prenatal implementation was provided by greater weight loss and improved health behaviors in the postpartum period in mothers who underwent a low glycemic index dietary intervention during pregnancy[42]. It is the responsibility of the researchers and health care personnel planning the interventions to utilize such reported evidence when designing implementation strategies for a particular population.

Although almost all programs aimed at preventing T2DM promote increased physical activity, healthy eating, and weight loss, "out of the box" thinking is necessary in order to increase participant acceptance of, and thus adherence to, a given intervention. A high level of acceptance was reported in a novel intervention in England that used group leisure activities for adults at risk for DM[43], though the recruitment procedure may have contributed to the these results. Another interesting study protocol published in 2013 used motivational interviews to influence lifestyle changes in individuals with impaired fasting glucose[44], a method based on the transtheoretical model of health behavior change[45].

Although pharmacologic interventions are also beneficial in attenuating the onset of T2DM in women with a history of GDM[27,46], a discussion of these is beyond the scope of this review.

Barriers to effective screening and lifestyle interventions and strategies to overcome them

Despite the importance of clear understandings of the nature of the disease, the risk for developing DM, and measures to prevent or delay its onset, the knowledge itself may not be enough. A recent qualitative study exploring factors that influence postnatal health behaviors in women with GDM showed that, although nearly all participants were aware of the increased risk for diabetes, this knowledge did not motivate them for action[47]. However, a low level of awareness remains, even among college-educated affluent women[48], which justifies the need for intensive awareness programs to counsel these women.

The health care team has an enormous respon-

sibility to educate these patients about the diabetes risk and the importance of regular screening, to motivate them to adapt to healthy lifestyles, and to support them to adhere to these changes. Although an OGTT is mandatory for women with prior GDM, a population-based cohort study in Canada found that women who chose an obstetrician for follow-up as opposed to a family physician were more likely to undergo a postpartum OGTT[49], which highlights the importance of educating all levels of health personnel on current recommendations. However, there are conflicting results concerning the efficiency of obstetricians for enforcing postpartum T2DM screening of GDM women[50,51]. It is the responsibility of the health care personnel to maintain records of these women and routinely remind them[52], preferably through some form of written information[53], as postal reminders[54] or laboratory slips[48] greatly increase the screening rates. Text message-reminder systems for screening[55] and internet-based programs for lifestyle intervention[56] are novel approaches worth trying in this era of technological dependence.

Postpartum women are a special group with a unique set of problems. The most common barriers to lifestyle interventions reported by these women were insufficient time[40,57,58], lack of support for child care[40,47,57], and other family commitments[40,47]. As the amount of available social support is associated with adherence to lifestyle interventions[57], educational and counseling sessions should be extended to the spouse and the immediate family of these women.

CONCLUSION

This review highlights important aspects concerning the screening of women with GDM, during the prenatal and postpartum periods, and thereafter. Women with GDM are a unique group for whom diabetes prevention strategies can be applied. In addition to being familiar with the general recommendations for screening and managing these patients, health care personnel should be able to appropriately support their patients to ensure greater acceptance of these valuable screening tests and interventional programs. The real challenge is not the planning of a lifestyle intervention, but implementing it effectively within the target population.

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MINIREVIEWS

Role of peroxisome proliferator-activated receptors gene polymorphisms in type 2 diabetes and metabolic syndrome

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Abstract

Metabolic syndrome (MetS) and type 2 diabetes mellitus (T2DM) are the serious public health problems worldwide. Moreover, it is estimated that MetS patients have about five-fold greater risk of the T2DM development compared with people without the syndrome. Peroxisome proliferator-activated receptors are a subgroup of the nuclear hormone receptor superfamily of ligand-activated transcription factors which play an important role in the pathogenesis of MetS and T2DM. All three members of the peroxisome proliferator-activated receptor (PPAR) nuclear receptor subfamily, PPARα, PPARβ/δ and PPARγ are critical in regulating insulin sensitivity, adipogenesis, lipid metabolism, and blood pressure. Recently, more and more studies indicated that the gene polymorphism of PPARs, such as Leu^{162}Val and Val^{227}Ala of PPARα, +294T > C of PPARβ/δ, Pro^{12}Ala and C1431T of PPARγ, are significantly associated with the onset and progressing of MetS and T2DM in different population worldwide. Furthermore, a large body of evidence demonstrated that the glucose metabolism and lipid metabolism were influenced by gene-gene interaction among PPARs genes. However, given the complexity pathogenesis of metabolic disease, it is unlikely that genetic variation of a single locus would provide an adequate explanation of inter-individual differences which results in diverse clinical syndromes. Thus, gene-gene interactions and gene-environment interactions associated with T2DM and MetS need future comprehensive studies.

Key words: Polymorphisms; Metabolic syndrome; Type 2 diabetes mellitus; Peroxisome proliferator-activated receptors

Core tip: Recently, more and more studies indicated that the gene polymorphism influence of peroxisome proliferator-activated receptors (PPARs), including PPARα, PPARβ/δ and PPARγ, acted as a pivotal role in the onset and progressing of metabolic syndrome (MetS) and type 2 diabetes mellitus (T2DM). We reviewed the recent advances in the relationships between PPARs polymorphisms and MetS and T2DM. Also, we discussed

the effects of gene-gene interaction among *PPARs* genes on the MetS and T2DM herein.

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INTRODUCTION

Globally, about 25% and 5.4% of adult population have been estimated to have metabolic syndrome (MetS) and type 2 diabetes mellitus (T2DM), respectively[1]. MetS is defined as a constellation of metabolic disorders including insulin resistance, central obesity, dyslipidemia and hypertension. The underlying cause of the MetS has been linked to the disorders of glucose metabolism including insulin resistance and glucose intolerance[2,3]. One study in Nigeria reported that the prevalence of the MetS in T2DM patients is up to 86%[4]. The study in Cameroon indicated that 71.7% T2DM patients diagnosed with the MetS[5]. Thus, it is estimated that MetS patients have about five-fold greater risk of the T2DM development compared with people without the syndrome[6].

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors that are part of the superfamily includes receptors for steroid hormones, thyroid hormones, retinoic acid and fat-soluble vitamin A and D. The primary role of PPARs is to regulate glucose, fatty acid and lipoprotein metabolism, energy balance, cell proliferation and differentiation, inflammation and atherosclerosis[7]. PPARα, the first member of the PPAR family identified in 1990, is mainly expressed in tissues in which fatty acid catabolism is important[8,9]. Since that time, two additional members of the family, PPARβ/δ and PPARγ, have been identified[10,11]. Recently, more and more studies on the associations of PPARs polymorphisms and disorders of glucose metabolism and abnormal lipid metabolism have been published, indicating that the gene polymorphism influence of PPARs acted as a pivotal role in the development of MetS and T2DM[12-15]. This review is aimed to summarize the recent advances in the relationships between PPARs polymorphisms and the metabolic disorders that related with MetS and T2DM. Moreover, the effects of gene-gene interaction among *PPARs* genes on the MetS and T2DM also will be discussed.

PPARα

PPARα gene is located on chromosome 22q12.2-13.1, and it is the first member of the *PPAR* isotypes to be cloned and was named based on its ability to be activated by peroxisome proliferator chemicals. PPARα is robustly expressed in tissues with elevated fatty acid catabolism and regulates transcription of multiple genes involved in glucose metabolism, such as the liver, heart and skeletal muscle, where it functions as a major regulator of fatty acid homeostasis[8,9]. Along with regulation of lipid and glucose metabolism, PPARα is as an attractive candidate gene for the risk of MetS and T2DM[7].

Role of PPARα gene polymorphisms in T2DM

Until now, more than 20 different base substitutions have been identified in the *PPARα* gene. Among of them, Leu^{162}Val (rs1800206) has been shown to be significantly related with the risk of T2DM in different population[16-20]. Flavell *et al*[17] reported that the variant of Leu^{162}Val variant was associated with increased plasma levels of total-cholesterol, HDL-cholesterol, and apoA-I, indicating that PPARα gene variation influences the onset and progression of T2DM. Furthermore, the PPARα haplotype significantly influenced age at diagnosis, with the C-L-C and C-V-C haplotypes [rs135539 (intron 1)-Leu^{162}Val (rs1800206)-rs4253778 (intron 7)] accelerating onset of diabetes by 5.9 and 10 years, respectively, as compared with the common A-L-G haplotype, and was associated with an odds ratio for early-onset diabetes (age at diagnosis $\leqslant$ 45 years) of 3.75. Intron 1 C-allele (rs135539) carriers also progressed more rapidly to insulin monotherapy (AA 9.4 ± 1.5 and AC + CC 5.3 ± 1.1 years). In another study, Andrulionyte *et al*[19] reported that the presence of the G (162V) allele of rs1800206 in *PPARα* gene increased the risk of developing diabetes. Moreover, haplotypes C-G-C and A-G-C, based on SNPs rs135539, rs1800206, and rs4253778, increased the risk of developing diabetes by 4.58-fold and 3.18-fold, respectively, compared with the C-C-C haplotype. Additionally, it should be noted that the Leu^{162}Val polymorphism has different effects on gene transcription. Evans *et al*[20] demonstrated that the Leu^{162}Val polymorphism was associated with a lower body mass index (BMI) in two independently recruited groups of patients with T2DM, suggesting that Leu^{162}Val polymorphism in PPARα protects T2DM patients from the overweight which is frequently associated with their condition.

Role of PPARα gene polymorphisms in MetS

Leu^{162}Val polymorphism not only plays a pivotal role in the T2DM development, but also significantly associated with the risk of MetS. In young Caucasians males, Uthurralt *et al*[21] found Leu^{162}Val polymorphism of PPARα to be a strong determinant of serum triglyceride levels, where carriers of the V allele showed 78% increase in triglycerides relative to L homozygotes. Moreover, men with the V allele showed lower HDL, but women did not. Recently, Smalinskiene *et al*[22] reported that males with the G (162V) allele of rs1800206 in *PPARα* gene had higher OR of elevated

triglyceride levels *vs* carriers of PPARα genotype CC, indicating that PPARα Leu^{162}Val polymorphism gene influences the onset and development of MetS.

Val^{227}Ala, a non-synonymous variant at the PPARα locus encoding a substitution of valine for alanine at amino acid residue 227, is another important PPARα polymorphism reported that associated with MetS development[23-28]. In Japanese population, significant interactions between PPARα Val^{227}Ala polymorphism and triglyceride levels and AST/ALT ratios were found in drinkers[23,24]. Chan *et al*[26] reported that the level of weight, BMI, hip circumference, waist circumference, waist-hip ratio, percentage of body fat, abdominal wall fat thickness in Chinese subjects with Val^{227}Ala variant were significantly lower than that in Val227wide type. Additionally, in Chinese females, the presence of the A227 allele was significantly associated with lower serum concentrations of total cholesterol and triglycerides[26,27]. Moreover, Chan's results also showed that the Val^{227}Ala polymorphism modulates the association between dietary polyunsaturated fatty acid intake and serum high density lipoprotein concentration[26].

In addition, the other variants of *PPARα* gene associated with MetS were also demonstrated in previous studies[29-33]. A Rotterdam study observed that the minor alleles of the PPARα rs4253728 and rs4823613 polymorphisms are associated with a better total and LDL-cholesterol-lowering response to simvastatin, possibly through influence on CYP3A4[33]. Therefore, better understanding the associations between PPARα polymorphisms and lipo-protein metabolism would be helpful for the prevention and treatment of MetS.

PPARα

PPARδ, also known as PPARβ, has 441 amino acid residues. Its coding gene is located in 6p 21.1-21.2, which includes 11 exons. PPARδ is widely expressed in the liver, kidneys, cardiac and skeletal muscle, adipose tissue, brain, colon and vasculature[34,35]. Animal studies found that PPARδ knockout mice showed glucose intolerance on normal chow, and were prone to obesity on high-fat diet[36,37]. PPARδ activation in the liver also appears to decrease hepatic glucose output, thereby contributing to improved glucose tolerance and insulin sensitivity[36,37]. Meanwhile, treatment with PPARδ-specific agonist enhanced β-oxidation, decreased free fatty acid, and improved insulin sensitivity in mice and moderately obese men[38,39]. Hence, PPARδ has emerged as a key role for the development of MetS and T2DM in recent years.

Role of PPARδ gene polymorphisms in T2DM

PPARδ is an important candidate gene for T2DM. About ten years ago, Vänttinen *et al*[40] reported that a statistically significant increase in insulin-stimulated whole-body and skeletal muscle glucose uptake in carriers of the alleles of three variants in *PPARδ* (rs6902123, rs2076167 and rs1053049), and the association was strongest for the rs6902123 variant. After that, the results from "The STOP-NIDDM Trial" demonstrated an increased risk of conversion to overt T2DM in carriers of the rs6902123 variant[41]. Similar to these findings, Lu *et al*[42] observed that rs6902123 was significantly associated with risk of T2DM and impaired fasting glucose in Chinese Han population. The minor C allele of rs6902123 was associated with increased levels of fasting glucose and HbA1c. In addition, a previous study revealed that the haplotype, composed of -13454G>T, -87T>C, 2022+12G>A, 2629T>C, and 2806C>G, is closely related to fasting plasma glucose and BMI of normal people in Korea[43]. Also, Hu *et al*[44] and Yu *et al*[45] reported that gene polymorphism of *PPARδ*, -87T>C, is significantly associated with higher fasting plasma glucose concentrations in both normal glucose tolerant and diabetic subjects.

However, with 886 middle age Chinese female T2DM patients, Villegas *et al*[46] did not find a main gene effect of *PPARδ* on T2DM or an interaction between the genes with BMI or exercise participation and the risk of T2DM. The similar result was also observed in another study of 7495 middle age white people that sequenced the *PPARδ* gene and found no association between variants and T2DM[47]. The reason for this disparity is not clear. It should be considered that that both genetic and environmental heterogeneity, including differences in their interaction, could give rise to population-specific discrepancies in the association of allelic variants and insulin resistance and thereby account for the inconsistent findings.

Role of PPARδ gene polymorphisms in MetS

Based on the analysis of a *PPARδ* null mouse model, it was demonstrated that *PPARδ* gene-deficient mice who bypassed the lethal placental defect displayed a lean phenotype, with a significantly smaller amount of fat mass. In addition, the muscle-specific PPARδ transgenic mice displayed increased mitochondrial-rich, oxidative type-1 myofibers with enhanced oxidative enzymatic activities[36,37,48,49]. Skogsberg *et al*[50] screened the 5'-untranslated region of the human *PPARδ* gene and found that a +294T > C (also named -87T > C, rs2016520) polymorphism in nucleotide 15 of exon 4 (located 87 nucleotides upstream of the start codon), was significantly associated with plasma levels of LDL and cholesterol in two cohorts of healthy men. In a Canada study, Robitaille *et al*[51] reported that *PPARδ* +294T > C polymorphism may be associated with a lower risk to exhibit the MetS and this association is influenced by dietary fat intake. Also, Aberle *et al*[52] showed that a highly significant association between the +294T > C and lower HDL- cholesterol levels in dyslipidemic female subjects. Moreover, MetS patients with CC genotype had significantly higher total and LDL-cholesterol

levels than those with TT and TC genotypes. The risk variant of PPARδ +294T >C marker was associated with higher LDL-cholesterol and increased serum total cholesterol[53]. Additionally, several other studies demonstrated that the PPARδ +294T >C polymorphism was associated with modifications of serum lipid concentrations in healthy subjects and the risk of CAD in dyslipidemic women and hypercholesterolemic men and cholesterol metabolites in Alzheimer's disease patients[54,55].

However, previous studies of *PPARδ* +294T >C polymorphism provided conflicting results regarding association with MetS. In another study in Scottish males, Skogsberg *et al*[56] reported that the +294C allele did not influence LDL-cholesterol concentrations. Gouni-Berthold *et al*[57] demonstrated that the presence of the C allele had no effect on triglyceride, HDL-cholesterol, and LDL-cholesterol levels, both in diabetic and non-diabetic German controls, or both in men and in women. In a Chinese study, Wei *et al*[58] showed that serum total cholesterol, HDL-cholesterol, LDL-cholesterol, ApoA1, and ApoB levels were not correlated with +294T >C polymorphism in nondrinkers. In addition, Grarup *et al*[47] also did not replicate the associations of +294T >C polymorphism with metabolic traits in 7495 middle-aged white people. Therefore, more studies focused on the impact of *PPARδ* gene polymorphism on the MetS development should be performed in different populations in future.

PPARγ

The gene of *PPARγ* (isoforms *PPARγ1*, *PPARγ2* and *PPARγ3*) is located on chromosome 3p25 encodes a nuclear transcription factor involved in the expression of hundreds of genes. *PPARγ* gene contains 9 exons, spans more than 100 kb. Since 1997, more and more evidences indicated that both common and rare polymorphisms of the genes of *PPARγ* acted as key roles in the regulation of lipid and glucose metabolism[59-62]. Rare mutations of *PPARγ (*loss-of-function mutations) exhibit a limited impact due to their low frequency but are associated with severe phenotypes such as hypertension, T2DM and MetS[63]. Conversely, common polymorphisms of *PPARγ* significantly associated with the risk of T2DM development, obesity and cardiovascular diseases in the general population due to their relatively high frequency[64].

Role of PPARγ gene polymorphisms in T2DM

PPARγ was the first gene reproducibly associated with T2DM. The association between the substitution of alanine by proline at codon 12 of PPARγ2 (Ala12 allele) and the risk for T2DM has been widely studied since Yen *et al*[65], first reported this polymorphism. In a recent study on the association between Pro^{12}Ala polymorphism with both T2DM and the development of diabetic nephropathy, the results demonstrated that the Pro/Pro genotype was the most common in diabetic patients as well as in controls followed by Pro/Ala genotype and Ala/Ala genotypes was the least common one. The allelic frequency of Pro was significantly higher in diabetic patients than controls and also significantly higher in diabetics with nephropathy than without nephropathy[66]. In South Africa population, Vergotine *et al*[67] reported that the Pro^{12}Ala of *PPARγ2* is significantly associated with insulin resistance and this polymorphism interacts with IRS1 Gly^{972}Arg, to increase the risk of T2DM. In addition, Wang *et al*[68] demonstrated that the presence of the Ala allele may contribute to improved insulin secretory capacity and may confer protection from T2DM and obesity in the Chinese population. Moreover, a meta-analysis confirmed the association between the PPARγ2 Pro12 allele and T2DM, and suggested that patients who carry the Pro12 allele have a 1.27-fold higher risk for developing T2DM than Ala12 carriers. This seemingly modest effect translates into a staggering 25% population-attributable risk because of the higher frequency of the Pro12 allele, especially in Japanese and European populations[69].

Compared to the effects of the common Pro^{12}Ala variant, rare mutations of *PPARγ* gene affecting the ligand-binding domain of PPARγ, such as 185Stop, Arg^{425}Cys, and Pro^{467}Leu, also associated with decreased transcriptional activity, improves glucose homeostasis and insulin sensitivity[70-72]. Additionally, the other PPARγ polymorphisms such as Cys^{114}Arg, Cys^{131}Tyr and Cys^{162}Trp could restrict wild-type PPARγ action *via* a non-DNA binding, transcriptional interference mechanism. Heterozygous carriers of these new mutations are severely insulin resistant also been reported in the previous studies[73,74].

Role of PPARγ gene polymorphisms in MetS

The functional mutation Pro^{12}Ala has also been reported to be associated with MetS in several populations[75,76]. Tellechea *et al*[75] reported that individuals carrying the Ala12 allele of PPARγ have a high risk for MetS and IR, especially among nonsmokers from Buenos Aires, Argentina. Also, The Québec Family Study observed that Ala12 carriers had a higher BMI, WC, fat mass than Pro/Pro homozygotes, suggesting that this polymorphism can modulate the association between dietary fat intake and components of the MetS[76]. However, studies investigating the association between Pro^{12}Ala polymorphisms and the risk of MetS in different populations have been inconsistent. In a large French population-based study, Meirhaeghe *et al*[77] found no association between Pro^{12}Ala polymorphism of PPARγ and MetS. Based on the analysis of 423 subjects with MetS and families without MetS, Yang *et al*[78] reported that Pro^{12}Ala polymorphism was not associated directly with MetS, although MetS patients with Ala allele have higher fasting blood sugar (FBS) and higher left ventricular voltage. Similar to these findings, Ala carriers of middle-aged Swedish individuals did

not show statistically significantly different levels of fasting blood glucose, triglycerides, HDL-cholesterol, waist circumference or BP when compared with Pro^{12}Pro homozygotes, suggesting that Pro12Ala polymorphism in *PPARγ* gene does not have a major role in determining MetS prevalence[79]. More recently, a meta-analysis included 4456 cases and 10343 controls from10 case-control studies, indicated that no significant statistical difference was observed between the variant and metabolic syndrome, even if stratified by ethnicity, definition of metabolic syndrome, source of control groups, and quality score of selected studies[80].

Another polymorphism, the C1431T silent substitution (rs3856806) in the 6th exon of *PPARγ*, has also been shown to be associated with MetS in the different populations[78,81]. In Iranian population, a significant difference in the frequencies of the C1431T genotypes was observed between MetS and control subjects. The T allele carriers had a significantly increased risk of MetS compared to the CC genotype even after correction for multiple-testing and adjustment for age, sex and genotype[81]. In Chinese population, the association of C1431T polymorphism with MetS has also been observed. There were significant differences in terms of gender, FBS, LDL-cholesterol levels, triglyceride between CC genotype and CT +TT genotype groups in patients with MetS[78]. However, not all studies had similar results. In Meirhaeghe's French population study, polymorphisms of C1431T were not individually associated with MetS. However connected with the other three polymorphisms, -681C>G, P2-689C>T, Pro^{12}Ala, haplotypes are significantly associated with the risk for MetS[82].

GENE-GENE INTERACTION AMONG PPARα, PPARδ AND PPARγ

Until now, increasing evidences suggested that gene-gene interaction among *PPARα*, *PPARδ* and *PPARγ* influenced the onset and progressing of T2DM and MetS[41,83-88]. Andrulionyte *et al*[41] reported that SNP rs6902123 of *PPARδ* alone and in combination with the Pro^{12}Ala polymorphism of *PPARγ2* predicted the conversion from impaired glucose tolerance (IGT) to T2DM. More recently, our results indicated that there was a significant association between plasma Lp(a) level and gene-gene interaction among the polymorphisms rs1800206, rs135539 in PPARα and rs10865710, rs1805192, and rs4684847 in PPARγ, suggesting that PPARα/γ gene may influence the risk of T2DM and MetS by regulating Lp(a) level[83,84]. In addition, the results from our another study demonstrated that gene-gene interaction among *PPARα/δ/γ* polymorphisms contribute to the risk of hypertriglyceridemia independently or in an interactive manner[86,87]. Thus, gene-gene interactions among SNPs in *PPARα*, *PPARδ* and *PPARγ* should be further investigated in future in order to better understand the small single gene effects that cannot be detected by single-locus studies.

CONCLUSION

Although the molecular mechanisms are still uncovered, more and more studies indicated that the gene polymorphism influence of PPARs acted as a pivotal role in the development of MetS and T2DM. Therefore, identification of polymorphic variants of PPARs in different populations and the genotypic associations between SNPs and gene-gene interactions would be helpful for the prevention and treatment of T2DM and MetS. However, given the complexity pathogenesis of metabolic disease, it is unlikely that genetic variation of a single locus would provide an adequate explanation of inter-individual differences which results in diverse clinical syndromes. To this end, gene-gene interactions and gene-environment interactions associated with T2DM and MetS needs future comprehensive studies.

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ORIGINAL ARTICLE

Basic Study

Advanced glycation end-product expression is upregulated in the gastrointestinal tract of type 2 diabetic rats

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Author contributions: Gregersen H and Zhao JB designed the research; Chen PM and Zhao JB performed the research and analyzed the data; Chen PM and Zhao JB wrote the paper; Gregersen H revised the paper.
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Ethics approval: Ethics of the study was approved by the Danish Committee for Animal Experimentation. The license number is 2008/561-1530.
Institutional animal care and use committee: All procedures involving animals were reviewed and approved by the Danish Committee for Animal Experimentation. The license number is 2008/561-1530. Animals in poor clinical condition were euthanized and excluded from the study. The rats were euthanized with CO_2 inspiration during the anesthesia. The animals were acclimatized to laboratory conditions (22 °C, 12 h/12 h light/dark, 50% humidity, ad libitum access to food and water) for 2 wk prior to experimentation. The animal protocol was designed to minimize pain or discomfort to the animals.
Conflict-of-interest: We declare that we have no proprietary, financial, professional or other personal interest of any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitle "Advanced Glycation End-Product expression is upregulated in the Gastrointestinal Tract of Type 2 Diabetic Rats".
Data sharing: No additional data are available.

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Abstract

AIM: To investigate changes in advanced glycation end products (AGEs) and their receptor (RAGE) expression in the gastrointestinal (GI) tract in type 2 diabetic rats.

METHODS: Eight inherited type 2 diabetic rats Goto-Kakizak (GK) and ten age-matched normal rats were used in the study. From 18 wk of age, the body weight and blood glucose were measured every week and 2 wk respectively. When the rats reached 32 wk, two-centimeter segments of esophagus, duodenum, jejunum, ileum, and colon were excised and the wet weight was measured. The segments were fixed in 10% formalin, embedded in paraffin and five micron sections were cut. The layer thickness was measured in Hematoxylin and Eosin-stained slides. AGE [N epsilon-(carboxymethyl) lysine and N epsilon-(carboxyethyl)lysine] and RAGE were detected by immunohistochemistry staining and image analysis was done using Sigmascan Pro 4.0 image analysis software.

RESULTS: The blood glucose concentration (mmol/L) at 18 wk age was highest in the GK group (8.88 ± 1.87 *vs* 6.90 ± 0.43, $P < 0.001$), a difference that continued to exist until the end of the experiment. The wet weight per unit length (mg/cm) increased in esophagus, jejunum and colon from the normal to the GK group (60.64 ± 9.96 *vs* 68.56 ± 11.69, $P < 0.05$ for esophagus; 87.01 ± 9.35 *vs* 105.29 ± 15.45, $P < 0.01$ for jejunum; 91.37 ± 7.25 *vs* 97.28 ± 10.90, $P < 0.05$ for colon). Histologically, the layer thickness of the GI

tract was higher for esophagus, jejunum and colon in the GK group [full thickness (μm): 575.37 ± 69.22 *vs* 753.20 ± 150.41, $P < 0.01$ for esophagus; 813.51 ± 44.44 *vs* 884.81 ± 45.31, $P < 0.05$ for jejunum; 467.12 ± 65.92 *vs* 572.26 ± 93.60, $P < 0.05$ for colon]. In esophagus, the AGE and RAGE mainly distributed in striated muscle cells and squamous epithelial cells. The AGE distribution was much stronger in the GK group compared to the normal group both in the striated muscle layer and mucosa layer (immuno-positive area/total measuring area %: 4.52 ± 0.89 *vs* 10.96 ± 1.34, $P < 0.01$ for muscle; 8.90 ± 2.62 *vs* 22.45 ± 1.26, $P < 0.01$ for mucosa). No visible difference was found for RAGE distribution between the two groups. In the intestine AGE and RAGE distributed in epithelial cells of villi and crypt. RAGE was also found in neurons in the myenteric and submucosal plexus. The intensity of AGE staining in mucosa of all segments and RAGE staining in neurons in all segments were strongest in the diabetes group. Significant difference for AGE was found in the epithelial cells of villi and crypt in duodenum (immuno-positive area/total measuring area %: 13.37 ± 3.51 *vs* 37.48 ± 8.43, $P < 0.05$ for villi; 0.38 ± 0.12 *vs* 1.87 ± 0.53, $P < 0.05$ for crypt) and for RAGE in neurons of all segments (*e.g.,* for jejunum: no staining neurons% 0 *vs* 0, mild 36.0 ± 5.2 *vs* 28.7 ± 3.5, moderate 53.2 ± 4.8 *vs* 55.8 ± 5.4, strong 10.7 ± 1.1 *vs* 15.4 ± 2.0, $P < 0.05$). In the colon, RAGE was primarily found in neurons in the myenteric and submucosal plexus. It was stronger in the diabetes group than in the normal group (no staining neurons% 6.2 ± 0.2 *vs* 0.3 ± 0.04, mild 14.9 ± 2.1 *vs* 17.6 ± 1.5, moderate 53.1 ± 4.6 *vs* 44.7 ± 4.4, strong 25.6 ± 18 *vs* 43.6 ± 4.0, $P < 0.05$). In the rectum, RAGE was primarily found in the mucosa epithelial cells.

CONCLUSION: The AGE and RAGE expression was up-regulated in the GI tract of GK diabetic rats and may contribute to GI dysfunction in type 2 diabetic patients.

Key words: Diabetes mellitus; Gastrointestinal complications; Advanced glycation end products; Receptor of advanced glycation end products

Core tip: Changes in advanced glycation end products (AGEs) and their receptor (RAGE) expression in the gastrointestinal (GI) tract in type 2 diabetic rats were studied. The AGE and RAGE were widely distributed in epithelial cells of all segments as well as in striated muscle cells in the esophagus. RAGE also distributed in neurons in all segments. Up-regulated AGE and RAGE expression was found in the GI tract of GK diabetic rats. The altered AGE and RAGE may be a contributing factor for GI dysfunction in type 2 diabetic patients.

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INTRODUCTION

Sensory-motor abnormalities are common in the gastrointestinal (GI) tract in diabetes mellitus patients. Symptoms may arise from the entire GI tract. Common complaints are dysphagia, heartburn, abdominal pain, early satiety, nausea, vomiting, constipation, and diarrhea[1-3]. The pathogenesis of such symptoms in diabetes mellitus is complex, multi-factorial with motor dysfunction, glycemic control, autonomic neuropathy, and psychological factors, and is not well understood[4].

Previous studies demonstrated changes in the morphological and biomechanical properties of the GI tract during diabetes, *e.g.,* the wall thickness and stiffness of GI tract increased[5-7]. The structure or deformation changes may alter the relative positions of the mechanosensitive afferents (zero setting of the mechanosensitive afferents). The changes in stress distribution and wall stiffness likely alter the stress in the vicinity of the mechanosensitive afferents. Consequently, the perception and motility of the intestinal tract will change as well. Therefore, the morphological changes and biomechanical remodeling are likely to affect the function of mechanosensitive afferents in the GI wall and further affect the motor and sensory function. Only sparse information, however, is available about the mechanisms for these changes.

Advanced glycation end products (AGEs) are formed in physiological states and gradually increases with age but AGEs formation is accelerated in diabetes[8]. AGEs can lead to changes in structure and function directly in the target protein. They also can bind to their receptor (RAGE), leading to activation of signaling pathways resulting in serial changes[9-11]. AGEs and RAGE play important roles for diabetic complications in the cardiovascular system[12-14] and for retinopathy[15] and nephropathy[16,17]. It was also demonstrated that AGEs and RAGE were associated with diabetic-induced arterial wall stiffening[18-20]. Therefore, they may also play an important role in the diabetic GI tract. In our previous study we demonstrated that AGE [N epsilon-(carboxymethyl)lysine, CML and N epsilon-(carboxyethyl)lysine, CEL] and RAGE were up-regulated in the small intestine and colon of streptozotocin (STZ)-induced diabetic rats[21]. However, to the best of our knowledge, data on the distribution of AGE and RAGE in the GI tract of type-2 diabetes have never been described.

The aims of this study were to investigate the AGE and RAGE distribution in the GI tract in type-2 diabetic rats and to compare those with normal rats. The data obtained may serve as the basis for further studying AGE and RAGE effects on type 2 GI diabetic dysfunctions.

MATERIALS AND METHODS

Reagents

Anti-AGEs mouse monoclonal antibody (6D12), against N(epsilon)-(carboxymethyl)lysine (CML, a major immunological epitope in AGEs) and N epsilon-(carboxyethyl)lysine (CEL) was purchased from COSMO BIO CO.,LTD. Japan. Other substances were rabbit polyclonal antibody against the N-terminal of human RAGE from Cell Applications, INC, United States; LSAB2 System-HRP for rat specimens, proteinase K, citrate buffer (pH = 6.0, 10xconcentrated), bovine serum albumin (BSA) and Mayer haematoxylin from Dako A/S, Denmark; soluble RAGE from Shanghai Yanji Bio; STZ, ethanol, methanol and xylene from Sigma-Aldrich Denmark A/S, Vallensbæk Strand, Denmark. Blood glucose analyzer and test strips were supplied by Hemocue Corporation, Sweden. The slides and cover glasses we used for immunohistochemical staining were Menzel-Glaser products, Germany.

Animal

Approval of the protocol was obtained from the Danish Committee for Animal Experimentation. Eight inherited type 2 diabetic Goto-Kakizak rats (GK group), 12 wk old and weighting about 330 g, were purchased from Taconic Europe DK-8680 Ry, Denmark. Ten age-matched normal rats (same strain as GK rats) served as controls (Normal group). During the breeding, the rats freely drank tap water and ate food except fasting over night before measuring body weight and blood glucose, which were done every week for body weight and every 2 wk for blood glucose from week 18. The rats survived until 32 wk of age.

Sampling

At the termination of the experiments, the rats were anaesthetized with Hypnorm 0.5 mg and Dormicum 0.25 mg per 100 g body weight (Hypnorm: Dormicum: sterile water = 1:1:2; subcutaneous injection). Two-centimeter segments of esophagus, duodenum, jejunum, ileum, and colon were excized. The esophageal segment was taked from the distal end of esophagus; the duodenal segment from 5 cm distal to pylorus sphincter; the jejunal segment from 5 cm distal to the ligament of Treitz; the ileal segment from 5 cm proximal to the ileo-cecal valve; and the colon from the middle part. Residual contents in the lumen were gently cleared using Krebs solution of the following composition (mmol/L): NaCl, 118; KCl, 4.7; $NaHCO_3$, 25; NaH_2PO_4, 1.0; $MgCl_2$, 1.2; $CaCl_2$-H_2O, 2.5; Glucose, 11; ascorbic acid, 0.11 and the wet weight was measured. Thereafter the rats were killed by injecting an overdose anesthetics.

General histological staining

All samples were fixed in 10% phosphate-buffered formalin about 24 h. The specimen were dehydrated in a series of graded ethanol (70%, 96% and 99%) and embedded in paraffin. Five-micron sections were cut perpendicular to the mucosa surface and the paraffin was cleared from the slides with coconut oil (over 15 min, 60 ℃). The sections were rehydrated in 99%, 96% and 70% ethanol followed by a 10 min wash in water and stained with hematoxylin and eosin (HE). The layer thickness was measured by the same pathologist in a blinded review and sixteen determinations were made on each specimen and averaged.

Immunohistochemical staining

Tissue pretreatment: The tissue samples for immunohistochemistry were also fixed in 10% phosphate-buffered formalin about 24 h, embedded in paraffin. Five-micron sections were cut perpendicular to the mucosa surface and placed in a water bath at 40 ℃. Thereafter, sections were transferred onto pretreated microscopic slides, which electrostatically attracted formalin fixed tissue and binding them to the slides. After drying the slides completely at room temperature, they were treated in an oven at 37 ℃ overnight to enhance the attachment of tissue to the slides. The sections were deparaffinized two times in xylene, 15 min per time, and rehydrated in 100%, 95%, 90%, 80%, 70%, 60% and 50% ethanol two times respectively, 3 s per time, followed by rinsing for 10 min and washing in 0.01M PBS (pH 7.4).

AGE: After treatment with H_2O_2 (3% in ethanol, room temperature, 15 min) and proteinase K (100 μg/mL, 100 μL, 37 ℃, 20 min), the sections were incubated with 5% BSA-PBS buffer (room temperature, 30 min) for blocking non-specific staining. Afterwards, the sections were incubated with the primary antibody 6D12 [1:100, diluted in 1% bovine serum albumin-Phosphate buffered saline (BSA-PBS)], which has been thoroughly characterized by Ikedas group[22], or normal mouse IgG (250 μg/mL) pre-treated with excessive CML (1:250, diluted in 1% BSA-PBS, negative control) overnight at 4 ℃. The sections were then washed and incubated with LINK (biotinylated anti-rabbit and anti-mouse immunoglobulins) and afterwards with STREPTAVIDIN PEROXIDASE (streptavidin conjugated with horseradish peroxidase) at room temperature for 10 min (both are part of reagents of LSAB2 System-HRP, products of Dako Company, Denmark). Then the peroxidase activity was visualized by incubating the sections in substrate working solution containing hydrogen peroxide and 3,3'-diaminobenzidine tetrahydrochloride at room temperature for 5 min. The sections were rinsed for 10 min, counterstained with Mayer Haematoxylin for 1 min, treated in HCl-ethanol for 3 s, dehydrated in 80%, 90%, 95%, 100% ethanol for 3 s, respectively. Then the slides were immersed in xylene for 15 min two times and mounted.

RAGE: The primary antibody against RAGE was produced in rabbits immunized with a synthetic peptide corresponding to a sequence at the N-terminal of

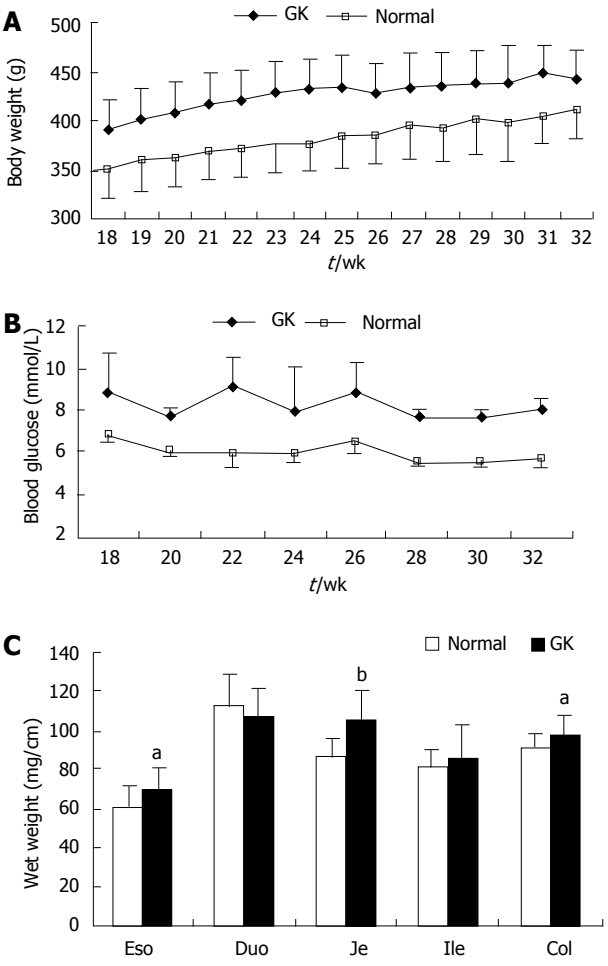

Figure 1 Body weight (A) and the blood glucose level (B) were higher in Goto-Kakizak group than in the normal group ($P < 0.001$ and $P < 0.01$). The wet weight per unit length of intestinal and colon segments is shown in Figure 1C (compared with normal group: $^{a}P < 0.05$, $^{b}P < 0.01$). Values are mean ± SD, $n = 8$ for each group. Eso: Esophagus; Duo: Duodenum; Je: Jejunum; Ile: Ileum; Col: Colon; GK: Inherited type 2 diabetic Goto-Kakizak rats.

human RAGE. Only two amino acids are different from the related rat sequence. For RAGE immunostaining, instead of treating sections with proteinase K, the sections were boiled in 10 mmol/L citrate buffer (pH = 6.0) 18 min for retrieving antigen and using normal rat lung as positive control as the RAGE is highly expressed in the lung[23]. The primary antibody was diluted (1:60) with 1% BSA-PBS and normal rabbit serum (diluted 1:60) pre-treated with excessive soluble RAGE was used as negative control. Other processes were similar to the AGE immunostaining.

Image analysis

To minimize errors, 6 to 10 photographs were randomly taken of different locations of same layer in each slide. After that, images of the different parts such as villus and crypt were saved as individual image files. The region of interest (ROI) was defined using Sigmascan Pro 4.0 image analysis software (Jandel Scientific, Germany). The color due to 3,3'-diaminobenzidene staining was distinguished in the ROI using intensity thresholds. Finally the images were exported as binary images and the area fraction of AGE or RAGE positive staining was calculated by a MATLAB program (MATLAB 6.5, The MathWorks Inc. United States).

Data analysis

According to the image analysis above, the fraction of AGE in mucosa (villi and crypt were analyzed separately in the intestinal segments), muscle layers, and the fraction of RAGE in the mucosa and muscle layer were computed as: Fraction of AGE or RAGE = immuno-positive area/total measuring area. It was difficult to calculate the fraction of RAGE in neurons in the same way. Therefore, the immunoreactivity of RAGE in each neuron was categorized by the stained intensity, *i.e.*, negative, mild, moderate and strong[24].

Statistically analysis

The results were expressed as mean ± SD unless indicated in the text. The differences between the diabetes and normal groups were tested using Student's *t* test and Anova. The results were regarded as significant when $P < 0.05$.

RESULTS

General information

The body weight and blood glucose level of GK group were significantly higher than those of the Normal group during the whole experimental period (Figure 1A and 1B, $P < 0.001$ and $P < 0.01$, respectively).

The wet weights per unit length of esophagus, jejunum and colon segments were highest in the GK group (Figure 1C, $P < 0.05$ and $P < 0.01$, respectively). No significant difference were found for duodenum and ileum between the two groups (Figure 1C, $P > 0.05$).

General histological changes

Compared with the Normal group, the full wall thickness of esophagus, jejunum and colon remarkably increased in the GK group (Figure 2A, $P < 0.05$ and $P < 0.01$, respectively). No significant difference was found in duodenum and ileum between two groups. The smooth muscle thickness of esophagus and colon (both circumferential and longitudinal smooth muscle) increased remarkably in GK group. The villous height of jejunum increased in the GK group (Figure 2B-D, $P < 0.05$ and $P < 0.01$). No significant difference was found for other layers.

Distribution of AGE

The immune-positive area of AGE was yellow-brown (Figure 3A and B). These colors were not found in the negative control slides (without primary antibody), demonstrating that the stained color was specific for AGE.

In the esophagus, AGE distribution was inhomogeneous and mainly distributed in striated muscle

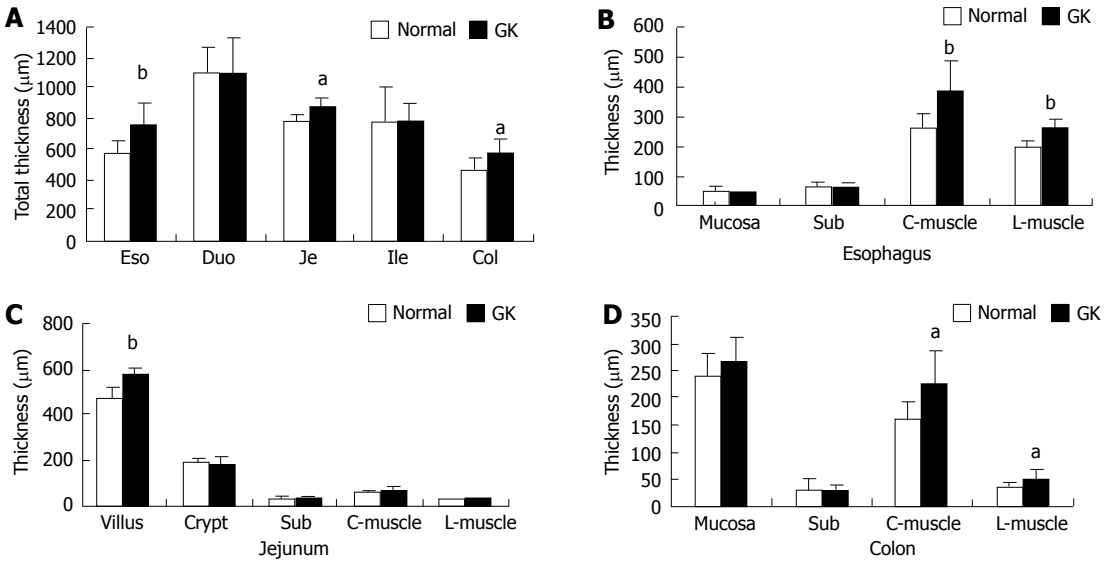

Figure 2 The wall and layer thickness. A: Total wall thickness; B: Layer thickness of esophagus; C: Layer thickness of jejunum; D: Layer thickness of colon. Values are mean ± SD, $n = 8$ for each group (compared with normal group: $^{a}P < 0.05$, $^{b}P < 0.01$). Eso: Esophagus; Duo: Duodenum; Je: Jejunum; Ile: Ileum; Col: Colon; Sub: Submucosa; C-muscle: Circumferential smooth muscle; L-muscle: Longitudinal smooth muscle; GK: Inherited type 2 diabetic Goto-Kakizak rats.

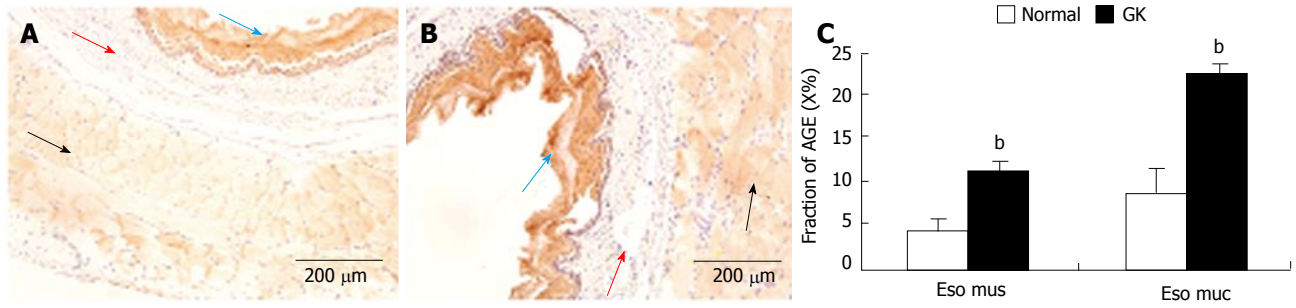

Figure 3 Example of advanced glycation end products immune-staining in esophagus (A, normal; B, diabetic); C: Shows the statistic result of immune-staining intensity in esophagus muscle and mucosa. Values are mean ± SD, $n = 8$ for each group (compared with normal group: $^{b}P < 0.01$). The immune-positive area of AGE showed yellow-brown color. Eso mus: Esophageal muscle; Eso muc: Esophageal mucosa; GK: Inherited type 2 diabetic Goto-Kakizak rats; AGE: Advanced glycation end product. Blue arrow: mucosa; Red arrows: Submucosa; Black arrows: Muscle.

and squamous epithelial cells. Compared with normal group, the intensity of immune-staining for AGE was much stronger in the GK group (Figure 3C, $P < 0.01$). No visible stained color was found in submucosa layer (Figure 3A and B).

In the intestine, AGE was mainly distributed in the mucosa layer, especially in epithelial cells of villi (Figure 4A-4E) and crypt. No visible stained color was found in submucosa, smooth muscle and ganglia. The crypt epithelial cells in ileum and colon were slightly stained. The distribution of AGE in the epithelial cells was inhomogeneous, the surface part was much stronger than the bottom part in villous epithelial cells but it showed an opposite pattern in the crypt epithelial cells. The intensity of AGE staining of the epithelial cells in villi was stronger than that in crypts ($P < 0.01$). In the mucosa, the intensity of AGE staining was similar between duodenal and jejunal segments (Figure 4A-C, F, G) but they were stronger than those in colon (Figure 4A, 4C, 4E and 4F). The mucosa of ileum showed the weakest intensity of AGE staining among different intestinal segments (Figure 4D). Compared with the Normal group, the intensity of AGE staining in mucosa of all segments were stronger in the GK group (Figure 4F and G). Significant difference was found in the epithelial cells of villi and crypt in duodenum (Figure 4F and G, $P < 0.05$).

Distribution of RAGE

The immune-positive area of RAGE also showed yellow-brown color (Figure 5) that was not found in the negative control slides (without primary antibody). Therefore, the stained color was specific for RAGE.

In esophagus, the immune-positive staining for RAGE was mainly observed in the striated muscle cells and mucosa squamous epithelial cells. The RAGE distribution was inhomogeneous in the striated muscle layer and graduated decreased from bottom to surface. No visible stained color was found in the submucosa layer (Figure 5). The intensity of RAGE staining did not differ between Normal and GK group both in the striated muscle cells and mucosa squamous epithelial

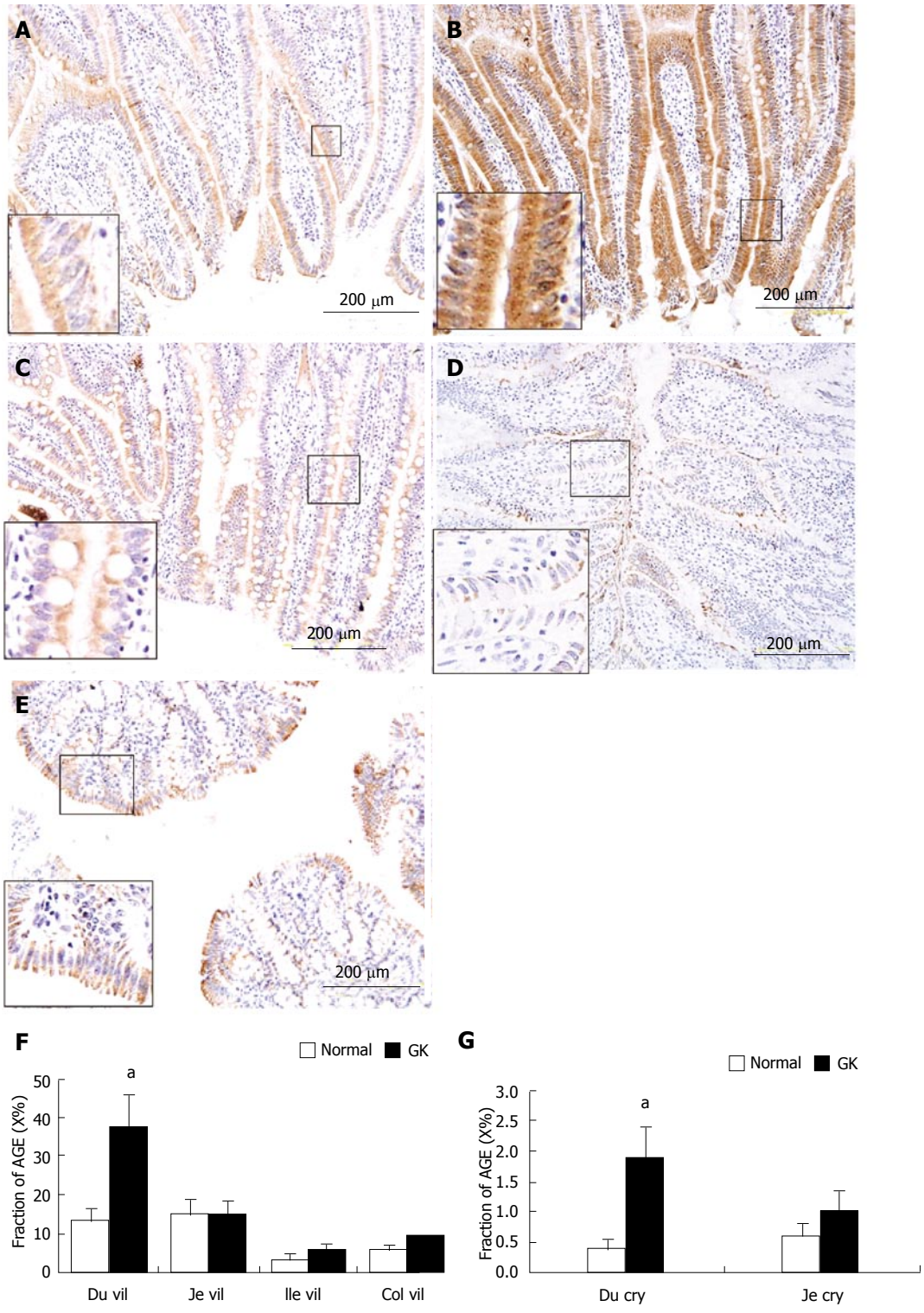

Figure 4 Example of advanced glycation end products immune-staining in villi of duodenum (A, normal; B, diabetic), jejunum (C), ileum (D) and mucosa in colon (E). The immune-positive area of AGE showed yellow-brown color; 4F and 4G: Show the statistical result of immune-staining intensity in villous epithelial cells of duodenum, jejunum, ileum and colon as well as in crypt epithelial cells of duodenum and jejunum. As shown in the magnification area (big frame *vs* small frame), the AGE distribution in epithelial cells was inhomogeneous, the surface part was much stronger than bottom part. Values are mean ± SD, *n* = for each group (compared with normal group: $^{a}P < 0.05$). Du vil: Duodenum villi; Je vil: Jejunum villi; Ile vil: Ileum villi; Col mu: Colon mucosa; Du cry: Duodenum crypt; GK: Inherited type 2 diabetic Goto-Kakizak rats; AGE: Advanced glycation end products.

cells ($P > 0.05$).

In the small intestine, the immune-positive staining for RAGE was observed in the epithelial cells of villi (Figure 6A, C, E) and crypts (Figure 6B, D, F), and in neurons in the myenteric and submucosal plexus (Figure 6G). The RAGE was homogeneously distributed in the cells, as shown in villus and crypt epithelial cells, but the intensity of immune-staining was

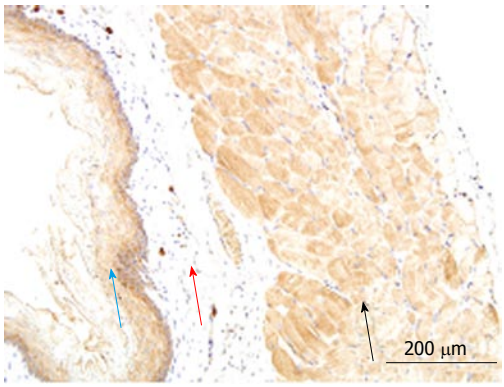

Figure 5 Example of receptor of advanced glycation end products immune-staining in normal esophagus. The immune-positive area of RAGE showed yellow-brown color. RAGE: Receptor of advanced glycation end products. Blue arrow: mucosa; Red arrows: Submucosa; Black arrows: Muscle.

much stronger in villous epithelial cells than in crypt epithelial cells. The strongest staining color occurred in duodenum and the weakest in ileum among the three segments (duodenum > jejunum > ileum, Figure 6A-F). In neurons, RAGE distributed both in cytoplasm and cell membrane (Figure 6G). Compared with the Normal group, the intensity of immune-staining for RAGE increased in the neurons for the three segments (Figure 6G and H) in the GK group ($P < 0.05$), but no significant difference was found in other cells between two groups.

In the colon, the immune-positive staining for RAGE was observed in neurons of the myenteric (Figure 7A) and submucosal plexus. It was stronger in the GK group than in Normal group ($P < 0.05$) (Figure 7A and B). Furthermore, a mild positive staining was also observed in mucosa epithelial cells both in the GK and Normal groups (Figure 7C).

DISCUSSION

Our previous study showed that the expression of AGE and RAGE was up-regulated in the small intestine and colon of STZ-induced type 1 diabetic rats[21]. STZ rats have high blood glucose but formation of free radicals and STZ cytotoxicity plus its direct effects on AGE formation and RAGE expression in GI tract may be questioned. Using the present model, the confounding effect of STZ can be avoided. The major discovery was that the intensity of AGE immune-staining was significantly increased in striated muscle and mucosa layer of esophagus, and in epithelial cells located in intestinal villi and crypts in the GK group compared to normal rats. RAGE was significantly increased in myenteric and submucosa plexus neurons of all intestinal segments in the GK group.

The distribution of AGE and RAGE in normal GI tract

Ling *et al*[25] reported the existence of four kinds of AGEs in stomach and small intestinal epithelial cells in normal rats. Our previous study[21] showed homogenous AGE distribution in the cytoplasm of smooth muscle cells, epithelial cells, and neurons of the myenteric and submucosal plexus in the layers of colon and small intestine. Furthermore, homogeneous distribution of RAGE was found in epithelial cells and neurons. The present study confirmed the distribution of both compounds in the colon and small intestine as reported from our previous study. Furthermore, we also found that the AGE and RAGE distributed in the striated muscle and squamous epithelial cells of esophagus and also in the stomach (unpublished data). The present study together with our previous study[21] is the first reports of the localization of AGE and RAGE in the whole rat GI tract. This provides a basis for further comparison study of the distribution of AGE and RAGE on GI tract with diseases, such as diabetes.

AGE and RAGE changes in GI tract of GK diabetic rats

RAGE and AGE distribution in the GI tract of GK rats was similar to that in normal controls. However, compared with the normal controls, the level of AGE and RAGE at some GI locations was increased in GK rats. However, compared with our previous study[21], the intensity of AGE and RAGE immune-staining were not so strong in the present study. It is well known that the accumulation and production of AGEs and expression of RAGE are associated with blood glucose level[26]. The blood glucose level is much lower in GK diabetic rats compared to STZ-induced diabetic rats. This is one plausible explanation for the weaker increasing AGE and RAGE in the GK type-2 diabetic rat model.

Histomorphological and biomechanical GI remodeling occurred during the development of diabetes[27]. For example the esophagus and colon were morphologically and biomechanically remodeled during the development of diabetes[6,7]. Abnormal levels of AGE and RAGE found in the present study may be associated with the GI remodeling in the GK diabetic rats. In the present study we found that the mucosa of small intestine and muscle layer of all segments proliferated, accordingly the AGE expression is up-regulated in the mucosa in all segments and esophageal muscle layer in the GK diabetic rats. However, the intensity of immune-staining in muscle had no apparent increase in muscle layer of intestine in GK group despite the fact that the muscle layer showed hyperplasia. Therefore it is speculated that in addition to the affection of AGE, other factors may affect the hyperplasia of GI muscle in GK diabetic rats, such as glucagon-like peptide 2[28].

In the present experiment no direct evidence showed how tissue growth was affected by AGEs and RAGE. From studies in other organs, it is known that AGE through AGE-RAGE-mediated ROS generation activating angiotensin Ⅱ-tissue growth factor beta (TGF-β)-Smad signaling can increase renal interstitial fibroblasts mitogenesis and type Ⅰ collagen production[29], mesangial cell hypertrophy and fibronectin synthesis[9], through AGE-RAGE interaction can cause epithelial

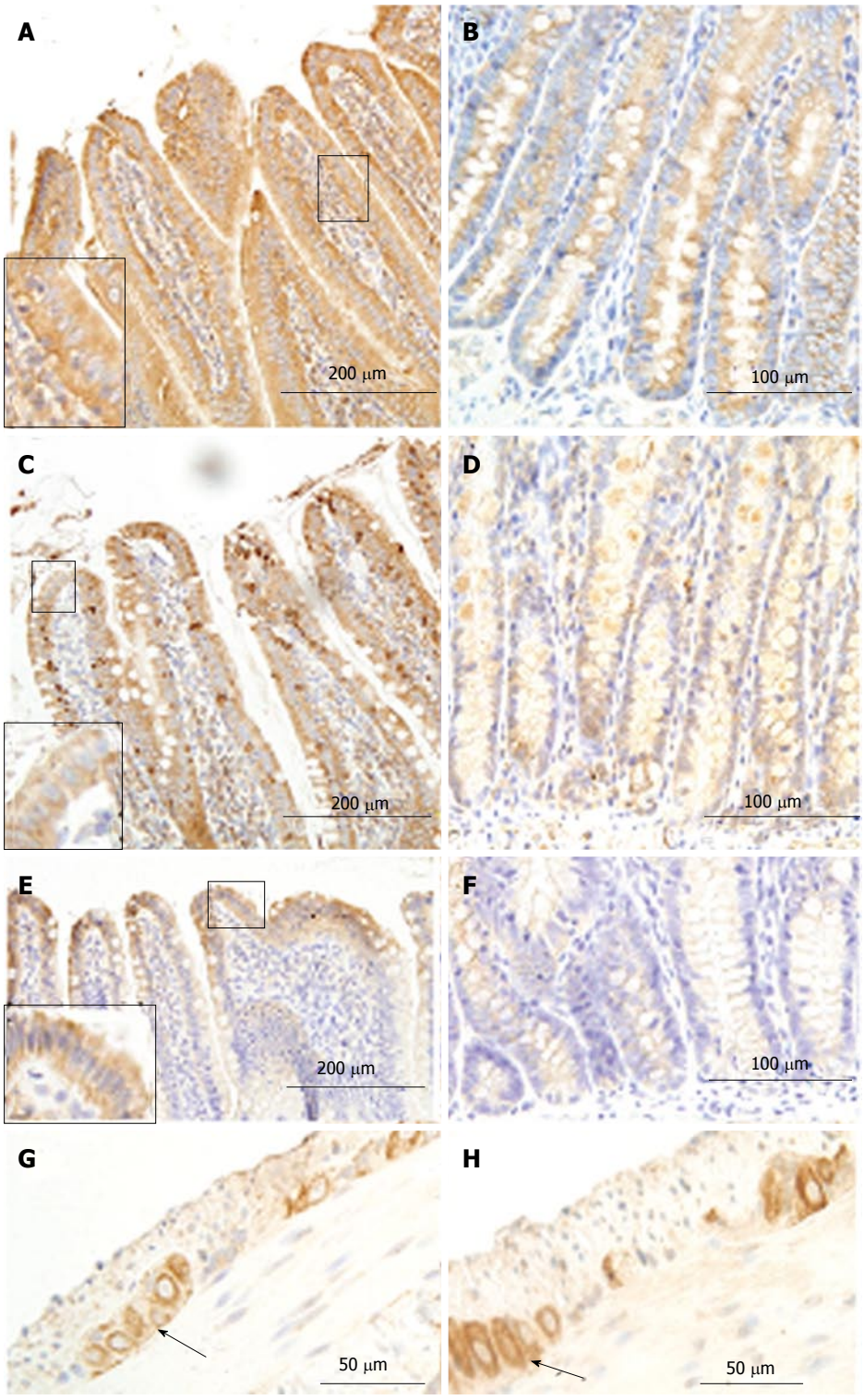

Figure 6 Receptor of advanced glycation end products immune-staining in villi (A, C, E) and crypt (B, D, F) of duodenum (A, B), jejunum (C, D) and ileum(E, F) as well as in ileum ganglia (arrowhead; G: Normal group; H: GK group). As shown in the magnification area (big frame *vs* small frame), the RAGE homogeneously distributed in the epithelia cells. The intensity of immune-staining in ganglia was stronger in the diabetic group (H) than in the normal group (G) (arrowhead). RAGE: Receptor of advanced glycation end products; GK: Inherited type 2 diabetic Goto-Kakizak rats.

myofibroblast transdifferentiation[30] and vascular smooth muscle proliferation[31], and through galectin-3 induce smooth muscle proliferation[32]. Therefore, it is feasible that GI tissue proliferation at least in part may be induced by AGE accumulation through the same pathways. Further studies must explore mechanisms for AGE- and RAGE-induced GI tissue growth and the association with the biomechanical remodelling in diabetes.

AGE and RAGE accumulation impact on GI dysfunction

It is well documented that small intestinal epithelial

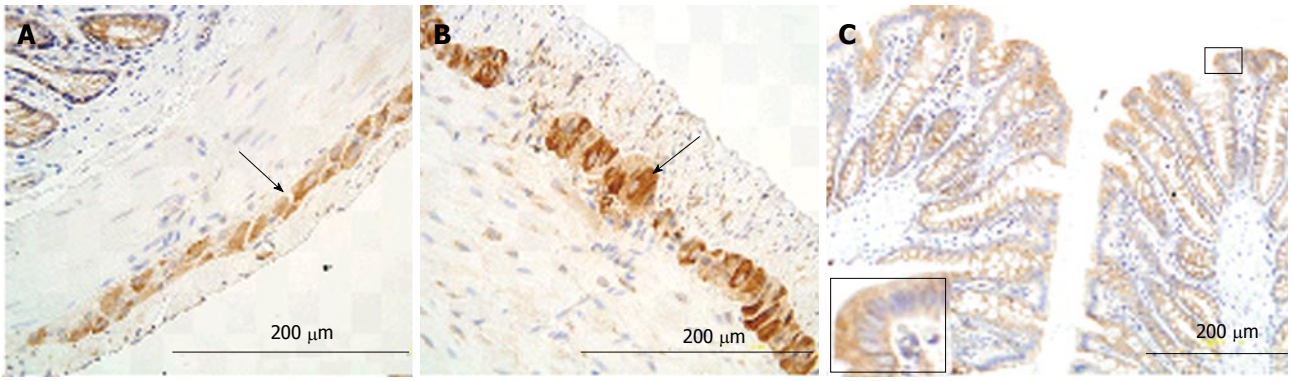

Figure 7 Receptor of advanced glycation end products immune-staining in colon ganglia (arrow; A: Normal; B: Diabetic) and mucosa (C). The intensity of immune-staining in ganglia was stronger in the GK group (B) than in the normal group (A) (arrow). Big frame area *vs* small frame area. RAGE: Receptor of advanced glycation end products; GK: Inherited type 2 diabetic Goto-Kakizak rats.

cells are important for digestion and absorption. Many kinds of enzymes located in the enterocytes lining the intestinal villi brush border are involved in digestion[33]. Furthermore, the small intestinal mucosa is important for absorptive function[33]. We demonstrated a stronger intensity of AGE immune-staining in the epithelial cells of intestinal crypts and villi in the diabetes group compared to the normal group. Digestive enzyme activity and cell membrane properties may potentially be affected by AGE accumulation. It is also known that non-enzymatic glycation and oxidative stress are important for changes of brush border membrane fluidity[34]. Digestion and intestinal transport processes occur at the brush border membrane. Changes in fluidity as well as in the membrane composition can alter the enzyme activity in the villi brush border membrane[35,36]. Multiple cellular signaling cascades can be activated by binding of AGEs to RAGE[37]. The increased AGE linking with RAGE may change epithelial cell function. Mechanisms linking AGEs/RAGE compounds to intestinal mucosa function in diabetics need more work.

Numerous studies demonstrated abnormal GI motility in diabetics [27,38]. Diabetic autonomic neuropathy is considered important in the pathogenesis of sensory-motor disordered function in diabetic patients[39-41]. AGEs and RAGE likely are key players in development of diabetic neuropathy[23]. Synergistic action of AGEs and endogenous nitric oxide can lead to neuronal apoptosis *in vitro*[42]. The neuronal AGE formation and accumulation may account for the development of GI neuropathy, primarily as a direct effect on structural and functional proteins, alternatively by activating RAGE indirectly[43]. GI nerves express nitric oxide synthase (nNOS), which generates a key transmitter nitric oxide in the regulation of GI motility[44]. Korenagas group demonstrated that AGEs inhibit *via* RAGE nNOS expression *in vitro*[45]. The expression and function of neuronal nitric oxide synthase decreased in the stomach of spontaneously diabetic BB-rats[41] and also decreased in duodenum of STZ-induced diabetic rats, which can be prevented by aminoguanidine (a drug that prevents AGE formation) and ALT-711 (AGE cross-link breaker)[8]. In the present study direct evidence was provided that RAGE is localized in myenteric and submucosal plexus neurons in the esophagus and intestine. In addition, DM enhanced RAGE intensity and therefore, AGE-RAGE interaction is likely of importance for GI diabetic autonomic neuropathy. We also demonstrated that the intensity muscle tissue AGE immune-staining was strongest in the diabetic esophagus. The accumulation of AGEs in muscle may alter the architecture and contractile proteins of smooth muscle[46], resulting in the alteration of muscle contraction propertiess.

AGE was mainly distributed in striated muscle and squamous epithelial cells in esophagus; in small intestinal epithelial cells of crypt and villi and in epithelial cells in colon and rectum. RAGE was mainly distributed in striated muscle and squamous epithelial in esophagus; in epithelial cells in intestine mucosa and neurons in ganglia. High AGE density was found in striated muscle and mucosa layers in esophagus, and villus, crypt in the GK rat small intestine, and the expression of RAGE in the intestine increased in ganglia of GK rats. Increased expression of AGE and RAGE likely contributes to GI disorders associated with DM.

ACKNOWLEDGMENTS

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COMMENTS

Background

In this previous study the authors demonstrated that advanced glycation end products (AGEs) and their receptor (RAGE) were up-regulated in the small intestine and colon of streptozotocin-induced diabetic rats. However, to the best of our knowledge, data on the distribution of AGE and RAGE in the gastrointestinal (GI) tract of type 2 diabetes have never been described.

Research frontiers

Previous studies demonstrated the morphological and biomechanical properties of the GI tract were remodeled during diabetes. However the mechanisms for these changes are not well understood. Therefore, investigation on the

distribution of AGE and RAGE in the GI tract of type-2 diabetes is important for understanding the mechanism of GI remodeling in diabetes.

Innovations and breakthroughs

At present study the authors demonstrated that the AGE and RAGE expression was up-regulated in the GI tract of GK diabetic rats. The increased AGE and RAGE levels may contribute to diabetic GI dysfunction in type 2 diabetic patients.

Applications

The most common type diabetes is type 2 diabetes; therefore it is important to understand the expression of AGE and RAGE in the GI tissues in type 2 diabetes. Knowing the over-expression of AGE and RAGE in the diabetic GI tract may in somehow direct the treatment in the patients suffering from type 2 diabetes.

Terminology

AGEs are formed by non-enzymatic attachment of sugars to the amino groups of various proteins through a series of complex intermediary reactions. Diabetic hyperglycemia accelerates the accumulation of AGEs in the tissues. RAGE is a 55kD transmembrane receptor of the immunoglobulin super family, which binds AGEs. AGEs contribute to diabetic complications through receptor-dependent and -independent pathways.

Peer-review

This is an interesting study. In future, more data should provide to further demonstrate the relationship between increasing AGE and morphological or functional changes in diabetes.

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