Insulin Resistance, Type 1 and Type 2 Diabetes, and Related Complications 2015

Journal of Diabetes Research

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Guest Editors: Joseph Fomusi Ndisang, Sharad Rastogi, and Alfredo Vannacci

Editorial Board

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Hindawi Publishing Corporation
Journal of Diabetes Research
Volume 2015, Article ID 234135, 2 pages
http://dx.doi.org/10.1155/2015/234135

Editorial

Insulin Resistance, Type 1 and Type 2 Diabetes, and Related Complications 2015

Joseph Fomusi Ndisang,[1] Sharad Rastogi,[2] and Alfredo Vannacci[3]

[1]*Department of Physiology, University of Saskatchewan College of Medicine, 107 Wiggins Road, Saskatoon, SK, Canada S7N 5E5*
[2]*The Medical Affairs Company, Cardiovascular Division, 43229 Dequindre Road, Troy, MI 48085, USA*
[3]*Department of Neurosciences, Psychology, Drug Research and Child Health, Section of Pharmacology and Toxicology, University of Florence, Viale Pieraccini 6, 50139 Florence, Italy*

Correspondence should be addressed to Joseph Fomusi Ndisang; joseph.ndisang@usask.ca

Received 9 June 2015; Accepted 9 June 2015

The dramatic increase in obesity and diabetes worldwide poses a huge socioeconomic burden to healthcare systems. In type 1 diabetes, autoimmune-mediated destruction of pancreatic beta-cell results in insulin deficiency [1]. Obesity is one of the major causes of type 2 diabetes [1–3]. In type 2 diabetes, a combination of peripheral insulin resistance and aberrant production of insulin are amongst the paradox commonly encountered in the pathogenesis of the disease [1–3]. However, both forms of diabetes are characterized by elevated inflammation/oxidative stress, glucotoxicity, lipotoxicity, endoplasmic reticulum-induced stress with increased apoptosis and necrosis that ultimately leads to destruction loss of beta cells, and related complications including cardiomyopathy, nephropathy, neuropathy, and hepatopathy [1, 4–7]. Although insulin resistance has traditionally been associated with type 2 diabetes, recent evidence suggests that insulin resistance in type 1 diabetes is increasing [8–10]; therefore, novel mechanistic approaches deciphering insulin resistance are needed.

The etiology of insulin resistance is complicated and several factors are implicated, so deciphering this multifaceted disease remains challenging, although a wide body of evidence suggests that oxidative stress, inflammation, genetic, habitual, environmental, and epigenetic factors may be involved [1, 11]. Thus, further research is needed for more in-depth and comprehensive understanding of the pathophysiology of insulin resistance in both type 1 and type 2 diabetes, and especially in situations where diabetes is comorbid with other chronic conditions such as obesity and hypertension.

This special issue will showcase a broad spectrum research and review papers addressing thematic problems associated with insulin resistance, type 1 diabetes, type 2 diabetes, and related complications. To underscore the role of insulin resistance in children, M. P. van der Aa et al. wrote a research article on the prevalence and incidence of childhood insulin resistance, while S.-H. Nam and coworkers investigated the effects of cardioankle vascular index on metabolic syndrome, a multifactorial condition characterized by insulin resistance, dyslipidemia, hyperglycemia, hypertension, and other factors. Similarly, R. Burrows et al. gave further insights for diagnosing metabolic syndrome in adolescents in a research article. In another related research article, M. Fabregat et al. investigated the genetic profile of human leukocyte antigen (HLA) alleles and non-HLA in type 2 diabetes, while P. Tiwari wrote a systematic review about the current therapeutic strategies for the management of diabetes. Given that diabetes and hypertension are characterized by elevated inflammation/oxidative stress [4, 12–14] and these two pathophysiological driving forces are implicated in many cardiac complications [4, 12–14], J. Klen et al. investigated the role of NLRP3 inflammasome polymorphism in type 2 diabetes, shedding novel insights on the role of NLRP3 polymorphism on myocardial infarction, a macrovascular complication of diabetes. Similarly, H. Al-Safar et al. investigated the role of genetic polymorphisms on transcription-factor-7-like 2 and peroxisome proliferator-activated receptors-γ2 in type 2 diabetes and obesity, while M. Guclu et al. wrote a research

article about the effects of combination therapy with rosiglitazone and insulin on inflammatory insults in patients with type 1 diabetes. In another related research article, Z. Yida et al. reported that cotreatment with the cholesterol lowering drug simvastatin and edible bird's nest (EBN), a traditional product commonly consumed in Asia for its nutritional value, improved insulin signaling in a rat model of high-fat diet-induced insulin resistance. Consistently, in another related study, A. Ferreira-Hermosillo et al. gave further insights on the role of inflammatory cytokines in patients with metabolic syndrome. Within the same theme of insulin resistance and metabolic syndrome, R. Adela and S. K. Banerjee wrote a review article to underscore the role of growth-differentiation-factor-15 in diabetes and related cardiovascular diseases, whereas J. Zhang et al. gave further insights on obesity and type 2 diabetes in a research article. By the same token, T.-Y. Chuang et al. reported the effects of microRNA-223 on insulin resistance by studying the adipose tissue. Finally, S. Riaz wrote an article giving novel insights on the role of Vitamin B1 on biomarkers of diabetes type 2 diabetes.

Collectively, the articles featured in this special issue cover a wide spectrum of thematic issues of great interest and would benefit a wide audience.

Joseph Fomusi Ndisang
Sharad Rastogi
Alfredo Vannacci

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Hindawi Publishing Corporation
Journal of Diabetes Research
Volume 2015, Article ID 783296, 8 pages
http://dx.doi.org/10.1155/2015/783296

Research Article

Healthy Chilean Adolescents with HOMA-IR ≥ 2.6 Have Increased Cardiometabolic Risk: Association with Genetic, Biological, and Environmental Factors

R. Burrows,[1] P. Correa-Burrows,[1] M. Reyes,[1] E. Blanco,[2] C. Albala,[1] and S. Gahagan[2]

[1]*Institute of Nutrition and Food Technology, University of Chile, Avenida El Líbano 5524, Macul, 7840390 Santiago, Chile*
[2]*Division of Child Development and Community Health, University of California, San Diego, 9500 Gilman Drive, MC 0927, La Jolla, San Diego, CA 92093-0927, USA*

Correspondence should be addressed to R. Burrows; rburrows@inta.uchile.cl

Received 24 November 2014; Revised 6 January 2015; Accepted 7 January 2015

Academic Editor: Joseph Fomusi Ndisang

Objective. To determine the optimal cutoff of the homeostasis model assessment-insulin resistance (HOMA-IR) for diagnosis of the metabolic syndrome (MetS) in adolescents and examine whether insulin resistance (IR), determined by this method, was related to genetic, biological, and environmental factors. *Methods.* In 667 adolescents (16.8 ± 0.3 y), BMI, waist circumference, glucose, insulin, adiponectin, diet, and physical activity were measured. Fat and fat-free mass were assessed by dual-energy X-ray absorptiometry. Family history of type 2 diabetes (FHDM) was reported. We determined the optimal cutoff of HOMA-IR to diagnose MetS (IDF criteria) using ROC analysis. IR was defined as HOMA-IR values above the cutoff. We tested the influence of genetic, biological, and environmental factors on IR using logistic regression analyses. *Results.* Of the participants, 16% were obese and 9.4 % met criteria for MetS. The optimal cutoff for MetS diagnosis was a HOMA-IR value of 2.6. Based on this value, 16.3% of participants had IR. Adolescents with IR had a significantly higher prevalence of obesity, abdominal obesity, fasting hyperglycemia, and MetS compared to those who were not IR. FHDM, sarcopenia, obesity, and low adiponectin significantly increased the risk of IR. *Conclusions.* In adolescents, HOMA-IR ≥ 2.6 was associated with greater cardiometabolic risk.

1. Introduction

Insulin resistance (IR) is the most common metabolic alteration related to obesity and represents an important link between obesity and other metabolic and cardiovascular complications related to oxidative stress and inflammation [1]. IR is acknowledged to be critical in the development of type 2 diabetes (T2D) and has been associated with obesity, metabolic syndrome (MetS), hypertension, and ischemic cardiovascular disease [1, 2]. Although impaired beta-cell function is ultimately responsible for T2D, IR precedes beta-cell dysfunction and, thus, plays a major role in the pathogenesis of this chronic disease [2]. Insulin resistance has become a serious health issue in the pediatric and adolescent age group [3]. In children and adolescents, IR is significantly related to obesity, cardiometabolic risk, and inflammation [3–6]. Family history of type 2 diabetes (FHDM), ethnicity, pre- and postnatal nutritional environment, obesity, puberty, diet, and sedentary lifestyle can all influence insulin sensitivity in the pediatric population [4].

In many developing countries, the nutritional transition and particularly the "westernization" of life-styles have caused a significant rise in obesity and comorbidities associated with IR, including T2D and ischemic cardiovascular disease [7–9]. Chile is a middle income country that underwent a profound shift from under- to overnutrition in less than two decades. Western dietary patterns and inactive lifestyles are widely spread in all age groups, especially among people from middle-low and low to middle socioeconomic status (SES) [10, 11]. The prevalence of obesity in Chilean children and adolescents more than tripled (5% to 17%), since the early 1990s [12, 13]. In a study of obese children and adolescents, 53% and 30% had IR and MetS, respectively [14]. According to evidence from another sample of obese Chilean adolescents,

insulin resistance was associated with higher risk of Mets [15].

Although hyperinsulinemic–euglycemic clamp is the gold standard method for assessment of insulin sensitivity, it is expensive and invasive. Alternative methods based on surrogate markers derived from fasting insulin and glucose, such as the homeostasis model assessment-insulin resistance (HOMA-IR), have been validated and proposed [16, 17]. HOMA-IR values ≥ 2.5 indicate IR in adults [17], but the corresponding cutoff value for children and adolescents has not been determined [4]. In many studies, IR diagnosis is based on HOMA-IR distribution in a reference population [15, 18]. MetS in pediatric population has been considered for defining the HOMA-IR cutoff point for IR diagnosis, in several contexts around the world [19, 20].

This research aims to determine the optimal cutoff value of HOMA-IR for MetS diagnosis in healthy adolescents, to examine whether IR assessed by using this cutoff value is related to anthropometric, metabolic, and cardiovascular risk profile, and to evaluate the association of IR with genetic, biological, and environmental factors.

2. Methods and Procedures

2.1. Study Design and Population. We studied 667 16- to 17-year-old adolescents living in urban Santiago, from low to middle SES neighborhoods, who were part of an iron deficiency anemia preventive trial and follow-up study beginning in infancy [21]. The participants were assessed in adolescence to understand biological and psychosocial determinants of adolescent obesity and cardiovascular risk. The study was approved by the institutional review boards of the University of Michigan, Institute of Nutrition and Food Technology (University of Chile), and the University of California San Diego. Participants and their primary caregiver provided informed and written consent, which was obtained according to the norms for Human Experimentation, Code of Ethics of the World Medical Association (Declaration of Helsinki, 1995).

2.2. Measurements

2.2.1. Anthropometry and Body Composition. A research physician used standardized procedures to measure the adolescent's height (cm) to the nearest 0.1 cm, using a Holtain stadiometer and weight (kg) to the nearest 0.1 kg using a Seca scale. Body mass index (BMI) (Kg/m^2) was calculated and obesity status was calculated according to WHO references. Measurements were taken twice, with a third measurement, if the difference between the first two exceeded 0.3 Kg for weight and 0.5 cm for height. Waist circumference was measured with nonelastic flexible tape at the high point of the iliac crest around the abdomen and recorded to 0.1 cm. Measurements were taken twice, with a third measurement, if the difference between the first two exceeded 1.0 cms. Dual-energy X-ray absorptiometry (DEXA) was used to measure fat mass (%) and fat-free mass (%). Fat-Free Mass Index (FFMI) was estimated according to Wells and Fewtrell [22]. FFMI values were expressed as percentage of BMI; values ≤ 25th percentile in our sample were considered sarcopenia, after adjusting for sex.

2.2.2. Additional Cardiovascular Risk Markers. After 15 minutes at rest and prior to the physical examination, systolic and diastolic blood pressures (SBP and DBP) were measured, three times on the nondominant arm using a standard mercury sphygmomanometer; the average value was used for analyses. Fasting serum total glucose (Gli), cholesterol, triglycerides (TG), high-density lipoprotein (HDL), insulin, adiponectin, and high sensitivity C-reactive protein (hs-CRP) levels were performed after a 12-hour overnight fast. Radioimmunoassay (RIA DCP Diagnostic Products Corporation, LA, USA) was used for insulin and adiponectin determinations. High sensitivity C-reactive protein (hs-CRP) was measured with a sensitive latex-based immunoassay (latex-enhanced nephelometry method). Glucose was measured with enzymatic-colorimetric test (QCA S.A. Amposta, Spain) and cholesterol profile (Col-HDL and TG mg/dL) was determined by analytical methodology dry (Vitros, Johnson & Johnson, Clinical Diagnostics Inc.). Values of hs-CRP ≥ 1.1 mg/L (75th percentile in our sample) were considered low-grade systemic inflammation and adiponectin ≤ 7.9 μg/mL (25th percentile in our sample) was considered to be low. HOMA-IR was calculated and the optimal cutoff value of HOMA-IR to diagnose MetS was determined with receiver operating characteristic (ROC) regression methodology [23] and used for the diagnosis of IR. CVRF and MetS were diagnosed based on the 2007 International Diabetes Federation (IDF) consensus statement on the clinical definition of the MetS in the pediatric age range. These criteria include central obesity plus 2 of the 4 following factors: abdominal obesity (WC ≥ 80 and 90 cm in females and males, resp.), high blood pressure (SBP ≥ 130, DBP ≥ 85), hypertriglyceridemia (TG ≥ 150 mg/dL), low HDL (≤50 and ≤40 mg/dL in female and male adolescents, resp.) and fasting hyperglycemia (Gli ≥ 100 mg/dL) [24].

2.2.3. Diet and Physical Activity. Food intake and physical activity (PA) habits were evaluated using validated and standardized self-report questionnaires, scored from 0 to 10, with higher scores denoting more nutritious food intake or more physical activity [25, 26]. The questionnaires were administered by a researcher during the half-day assessment. The quality of food intake was measured by the amount of saturated fat, fiber, sugars, and salt in the food items. We applied cutoffs established by Burrows et al. [25] to classify the eating habits of participants into three groups: unhealthy (scores ≤ 25th percentile), intermediate (scores 26th to 74th percentile), and healthy (scores ≥ 75th percentile). PA was measured by the total amount of time devoted to sedentary activities, recreational games, active commuting, and weekly scheduled exercise either school or nonschool organized. Participants with scores ≤ 25th percentile were considered as physically inactive, those with scores ≥ 75th percentile were physically active, and those in between were moderately active.

2.2.4. Family History of Type 2 Diabetes. A standardized questionnaire of FHDM, including first- and second-degree relatives, was answered by the participant's primary caregiver (father, mother, or grandparents). Reporting FHDM in at least one parent or grandparent was required to fit our definition of positive FHDM.

2.3. Statistical Analysis. Data were analyzed using Stata for Windows version 12.0 (Lakeway Drive College Station, TX, US). A P value of <0.05 denoted statistical significance. Statistical analysis included chi-square tests for categorical variables and Student's t-test for comparison of mean values of anthropometric and cardiometabolic variables. ROC analysis was used to find the optimal cutoff of HOMA-IR for diagnosis of IR. Bivariate analyses were used to test the association between obesity, sarcopenia, unhealthy food intake, physical inactivity, and FHDM and the outcome, IR. We used multiple logistic regressions to assess the influence of the variables significantly associated with IR. We excluded data on participants who reported unknown information in at least one parent or grandparent ($n = 125$). Four models were estimated. The first one included FHDM and low PA as independent variables. In the second model, obesity was added. The third model included sarcopenia. Finally, a full adjusted model contained all mentioned covariates with the addition of low adiponectin. The likelihood ratio test was performed to test the overall significance of all coefficients in the multiple adjusted models, whereas the Hosmer-Lemeshow goodness-of-fit test was used to evaluate their effectiveness in predicting the outcome of IR.

3. Results and Discussion

3.1. Results

3.1.1. Patients' Characteristics. Our sample was made up of 52.2% male and 47.8% female adolescents who were on average 16.8 (SD 0.3) years old. The prevalence of obesity was 16.2% whereas 9.4% of participants met criteria for MetS.

3.1.2. Optimal Cutoff Point for the Diagnosis of Insulin Resistance. A HOMA-IR value of 2.6 showed the best sensitivity (59%) and specificity (87%) for diagnosing MetS (AUC: 0.821; correctly classified: 87%; LR+: 4.5) (Figure 1). According to this optimal cutoff point, 16.3% of adolescents in the sample had IR.

As shown in Table 1, adolescents with IR (HOMA-IR ≥ 2.6) had significantly higher ($P < 0.001$) BMI z score, WC, fat mass (%), systolic and diastolic BAP, total cholesterol, LDL-chol, triglyceride, glucose, and insulin, as well as significantly lower mean values of lean mass (%), HDL-chol, and adiponectin compared to adolescents with HOMA-IR < 2.6. Obesity (40.4% versus 9.3%; $P < 0.001$) and physical inactivity (51.4% versus 37.4; $P = 0.006$) were significantly higher among participants with IR compared to the insulin sensitive group. Similarly, adolescents with IR (Figure 2) show a significantly higher prevalence of abdominal obesity (61.5% versus 27.8%; $P < 0.001$), high blood pressure (18.4% versus 4.8%; $P < 0.001$), fasting hyperglycemia (19.3% versus 6.6%; $P < 0.001$), hypertriglyceridemia (20.4% versus 5.6%; $P < 0.001$), and MetS (33.0% versus 4.8%; $P < 0.001$).

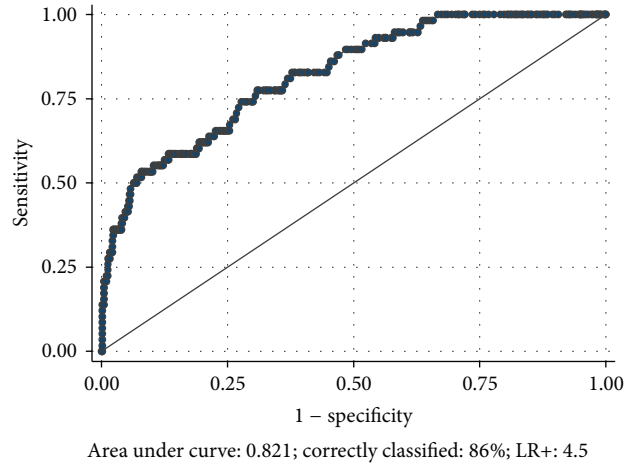

Figure 1: ROC curve to determine the optimal cutoff value of HOMA-IR for MetS diagnosis in healthy adolescents.

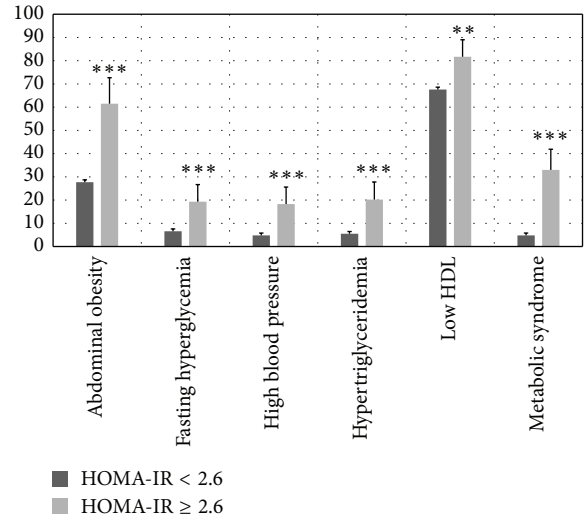

Figure 2: Prevalence rates of cardiovascular and metabolic risk factors by HOMA-IR. Error bars are 95% CI. Statistical significant difference by Pearson's Chi2. Significance level: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

3.1.3. Association of IR with Genetic, Biological, and Environmental Factors. Table 2 contains the results of the bivariate analysis that was used to identify explanatory variables of IR in adolescents. We found a significant association between IR and obesity (OR: 6.6; 95% CI: 4.1–10.6), sarcopenia (OR: 4.9; 95% CI: 3.2–7.5), low adiponectin (OR: 2.5; 95% CI: 1.6–4.0), physical inactivity (OR: 1.8; 95% CI: 1.2–2.7), and FHDM (OR: 1.7; 95% CI: 1.1–2.9).

Table 3 shows the results of the multivariate logistic regression analyses assessing the relationship between genetic, biological, and environmental variables significantly

Table 1: Background characteristics of adolescents in the sample by insulin sensitivity ($n = 667$).

	HOMA-IR < 2.6 ($n = 558$)	HOMA-IR ≥ 2.6 ($n = 109$)	Total sample	P value[c]
Age (years)	16.8 ± 0.3[a]	16.9 ± 0.2	16.8 ± 0.3	n.s.
Anthropometrics				
BMI (z score)	0.48 ± 1.1	1.53 ± 1.2	0.65 ± 1.2	<0.001
WC (cm)	79.4 ± 9.9	90.4 ± 14.3	81.2 ± 11.4	<0.001
Lean Mass (%)	68.7 ± 11.4	62.0 ± 9.4	67.6 ± 11.1	<0.001
Fat mass (%)	27.9 ± 10.7	34.7 ± 9.4	29.0 ± 10.8	<0.001
BMI $z \geq 2$ SD	52 (9.3)[b]	44 (40.4)	96 (14.4)	<0.001[d]
CVM profile				
SBP (mm Hg)	111.1 ± 10.2	118.3 ± 10.7	112.2 ± 10.6	<0.001
DBP (mm Hg)	68.7 ± 7.0	72.1 ± 6.5	69.3 ± 7.0	<0.001
TG (mg/dL)	82.5 ± 45.5	118.2 ± 71.6	88.3 ± 50.1	<0.001
HDL-chol (mg/dL)	40.8 ± 10.6	36.6 ± 10.2	40.1 ± 10.6	<0.001
Glucose (mg/dL)	87.4 ± 8.8	94.2 ± 10.8	88.6 ± 9.5	<0.001
Insulin (μIU/dL)	6.3 ± 2.5	17.5 ± 7.0	8.1 ± 5.5	<0.001
hs-CRP (mg/L)	0.95 ± 1.9	1.10 ± 1.6	0.98 ± 1.9	n.s.
Adiponectin (μg/mL)	12.3 ± 5.5	9.2 ± 5.1	11.8 ± 5.5	<0.001
Lifestyles habits				
Unhealthy eating	139 (24.9)	20 (18.4)	159 (23.8)	n.s.
Physical inactivity	209 (37.4)	56 (51.4)	265 (39.7)	0.006[d]

[a]Mean ± S.D; [b]n (%); [c]Student's t-test, except as indicated. [d]Chi2 (Pearson).

Table 2: Association between IR and sex, obesity, sarcopenia, low adiponectin, life-style habits, and HFDM2 in adolescents ($n = 667$).

	Subjects with insulin resistance (HOMA-IR ≥ 2.6)		Crude OR[a]
	n	%	
Overall	109	16.32	
Obesity			
No	65	11.4	Ref. group
Yes	44	45.8	6.60 [4.09–10.6]***
Sarcopenia			
No	50	10.8	Ref. group
Yes	59	35.1	4.87 [3.17–7.50]***
Low adiponectin			
No	74	13.6	Ref. group
Yes	35	28.5	2.53 [1.59–4.01]***
Physical inactivity			
No	53	13.2	Ref. group
Yes	56	21.1	1.77 [1.17–2.67]***
Unhealthy food intake			
No	89	17.5	Ref. group
Yes	20	12.6	0.68 [0.40–1.14]***
Family history T2D[b]			
No	23	11.7	Ref. group
Yes	74	18.8	1.73 [1.10–2.88]***

[a]Statistical significance: * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$; obesity: BMI z-score ≥2 SD; sarcopenia: % FFMI ≤25th percentile; low adiponectin: adiponectin ≤7.9 μg/mL; physical inactivity: PA score ≤3; unhealthy food intake: eating test score ≤4.4; FHDM[b]:family history of type 2 diabetes in at least one first-degree relative ($n = 590$).

Table 3: Influence of FHDM, physical inactivity, sarcopenia, obesity, and low adiponectin over the risk of insulin resistance ($n = 590$).

	Model 1 OR [95% CI]	Model 2 OR [95% CI]	Model 3 OR [95% CI]	Model 4 OR [95% CI]
FHDM	1.74 [1.10–2.88]*	1.74 [1.10–2.94]*	1.72 [1.10–2.92]*	1.80 [1.10–3.08]*
Physical inactivity	1.77 [1.14–2.75]*	1.49 [0.94–2.37]	1.42 [0.89–2.26]	1.45 [0.90–2.34]
Sarcopenia	(···)	4.25 [2.67–6.75]***	2.27 [1.25–4.12]**	2.56 [1.21–4.07]**
Obesity	(···)	(···)	3.25 [1.71–6.19]***	2.92 [1.51–5.66]**
Low adiponectin	(···)	(···)	(···)	2.22 [1.30–3.77]**
Likelihood ratio (Chi2)	11.46**	48.5***	61.6***	69.8***
Hosmer-Lemeshow	<0.001 (0.99)	0.39 (0.99)	0.18 (0.99)	1.21 (0.98)
Correctly classified	83.6%	83.6%	84.2%	84.6%

OR [95% CI]: odd ratio [95% confidence interval]. Statistical significance: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. [···]: nonobserved. OR [95% CI]: odd ratio [95% confidence interval]. FHDM: family history of type 2 diabetes in at least one first-degree relative. Obesity: BMI z score ≥2 SD; sarcopenia: FFMI ≤25th percentile; physical inactivity: PA score ≤3; low adiponectin: adiponectin ≤7.9 μg/mL.

associated in the bivariate analyses with the outcome, IR. Those with physical inactivity had significantly higher odds of IR in Model 1. However, the association was lost when sarcopenia, obesity, and low adiponectin were entered in the regression equation. In a fully adjusted model, FHDM (OR: 1.79; 95% CI: 1.1–3.1), sarcopenia (OR: 1.9; 95% CI: 1.1–3.6), obesity (OR: 2.4; 95% CI: 1.2–4.9), and low adiponectin (OR: 2.3; 95% CI: 1.3–3.8) significantly increased the risk of IR.

3.2. Discussion. In this cohort of healthy Chilean adolescents of low to middle SES, the optimal cutoff point of HOMA-IR for IDF MetS diagnosis was 2.6 (sensitivity: 59% and specificity: 87%). Adolescents with HOMA-IR ≥ 2.6 showed a significantly higher prevalence of obesity (40.4%), abdominal obesity (61.5%), hypertriglyceridemia (20.4%), high blood pressure (18.4%), fasting hyperglycemia (19.3%), and MetS (33.0%). Thus, HOMA-IR of 2.6 or higher would find young individuals with greater biological risk.

In adolescents, HOMA-IR values ranging from 2.2 to 5.3 have been reported as cutoff for diagnosing MetS, but these studies vary greatly in their design, sample size, age and nutritional status of participants, and degree of pubertal development [14, 15, 19, 20, 27–29]. Our findings are similar to population-based studies in a number of other contexts [20, 28]. In Chinese children aged 6 to 18 years, 2.6 was the optimal cutoff point of HOMA-IR for MetS ATP III diagnosis [20]. In urban adolescents from India, aged 10 to 18 years, HOMA-IR value of 2.5 had the optimal sensitivity (>70%) and specificity (>60%) for MetS ATP III and IDF diagnosis [28]. This cutoff was most likely to detect MetS in adolescents from different BMI categories [28]. Other clinical studies also agree with our finding. In prepubertal overweight Brazilian children, HOMA-IR value of 2.5 showed optimal sensitivity (61%) and specificity (74%) for MetS diagnosis [30].

In this sample, FHDM, obesity, sarcopenia, and low adiponectin were all independently associated with a significant increased risk of IR. All these factors have been recognized as important determinants of IR and TD2 in pediatric populations [3, 4, 31–37]. In studies of twins, 50% of the variance in insulin sensitivity and secretion was attributed to genetic factors [31]. Furthermore, healthy children with FHDM were more likely than those without to be insulin resistant, with an impaired balance between insulin sensitivity and secretion [32]. Moreover, obesity is the most prevalent pathophysiological determinant of IR, whereas insulin sensitivity is inversely associated with BMI and percent body fat in child and adolescent populations [33]. Similarly, muscular strength has been identified as an independent and powerful predictor of better insulin sensitivity and, conversely, lower muscular strength and higher central adiposity are highly predictive of higher levels of IR in healthy children and adolescent [34]. Moreover, there is growing evidence that exercise exerts beneficial effects partly through alterations in the adipokine profile; that is, exercise increases secretion of anti-inflammatory adipokines, improves metabolic syndrome and insulin sensitivity, and reduces proinflammatory cytokines [35]. Finally, in children, adiponectin levels are lowered with MetS and obesity and inversely related to IR [36]. In both liver and skeletal muscle, adiponectin reduces triglyceride content and improves insulin signaling by increasing the gene expression involved in fatty acid oxidation and decreases the hepatic glucose production by inhibiting the expression of hepatic gluconeogenic enzymes [37]. Although IR has been shown to be linked with a chronic inflammatory state, we did not find significantly lower levels of hs_CRP in adolescents with HOMA-IR < 2.6. However, it is important to emphasize that the observed levels of hs_CRP in this sample overall were higher than those reported by others using adolescent participants as well as the same measurement methodology [38, 39]. Psychological factors related to stress, a diet high in saturated fat and refined sugars, and physical inactivity may be determinants of a systemic chronic inflammatory state [40–42]. These factors are usually more prevalent among low-SES groups [43, 44], and they all have been observed in this cohort. Whereas only 7.5% (95% CI: 5.5–9.5) of participants had healthy dietary habits, 19.3% (95% CI: 16.3–22.3) were considered to be physically active (data not shown), according to national references [16]. Similarly, results from a previous work suggest that adolescents, in our cohort, are exposed to a stressful environment [45].

This research has limitations that should be considered when interpreting its results. One limitation is that

we estimated sarcopenia from DEXA scans and calculated fat-free mass rather than a more direct method of assessing muscle mass [46]. Nonetheless, this methodology has better sensitivity for estimating the ratio between muscle and fat tissue compared to BMI [22, 47]. Another limitation is that our sample is not representative of the Chilean adolescent population. Our sample is composed of adolescents from low to middle SES living in urban Santiago. However, our findings may be equally relevant for a number of reasons. According to the Chilean National Health Survey, the prevalence of obesity, TD2, and CVD is significantly higher among low to middle SES individuals [48]. On the other hand, low to middle SES Chilean adolescents are highly exposed to insulin resistance risk factors including obesity, physical inactivity, low fitness, and processed food full of fat and sugar [10, 12, 25, 49–52]. Our study also makes an important contribution in confirming that obesity, sarcopenia, and FHDM might be considered relevant risk factors for IR in adolescents.

4. Conclusions

This research provides results that confirm that in adolescents a value of HOMA-IR $\geq$ 2.6 is associated with greater cardiovascular and metabolic risk [20, 28, 30]. We recommend this cutoff for diagnosis of IR in clinical practice. Adolescents with IR show a significantly higher prevalence of obesity, abdominal obesity, fasting hyperglycemia, and HFDM. In Chilean adult populations, fasting hyperglycemia, obesity, and self-reported FHDM were the best predictors of T2DM [53]. Obesity, sarcopenia, and FHDM might be considered relevant risk factors for insulin resistance. Our findings highlight the need for robust public policies and programs to reduce the risk for obesity and associated conditions in youth. For families with a history of diabetes mellitus, pediatricians should strongly counsel the youths and their parents to engage in healthy nutritional and physical activity practices in order to prevent the onset of early cardiometabolic risk.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

This research was carried out with financial support from the National Heart Lung and Blood Institute (NHLBI) and National Institutes of Health (USA), under Grant R01HL088530-2980925. Dr. Correa-Burrows was sponsored by the Advanced Human Capital Program (Grant code: 79140003) and from the National Council for Scientific Research and Technology (CONICYT) (Chile).

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Hindawi Publishing Corporation
Journal of Diabetes Research
Volume 2015, Article ID 129695, 8 pages
http://dx.doi.org/10.1155/2015/129695

Research Article

Association of the Genetic Polymorphisms in Transcription Factor 7-Like 2 and Peroxisome Proliferator-Activated Receptors-γ2 with Type 2 Diabetes Mellitus and Its Interaction with Obesity Status in Emirati Population

Habiba Al-Safar,[1] Ahmed Hassoun,[2] Shaikha Almazrouei,[1] Wala Kamal,[3] Bachar Afandi,[4] and Naushad Rais[3]

[1]*Department of Biomedical Engineering, Khalifa University of Science, Technology & Research, P.O. Box 127788, Abu Dhabi, UAE*
[2]*Dubai Diabetes Centre, Dubai Health Authority, Dubai, UAE*
[3]*School of Life Sciences, Manipal University, P.O. Box 345050, Dubai, UAE*
[4]*Diabetes Clinics, Tawam Hospital, Al-Ain, UAE*

Correspondence should be addressed to Naushad Rais; naushad@manipaldubai.com

Received 14 October 2014; Revised 27 April 2015; Accepted 27 April 2015

Academic Editor: Alfredo Vannacci

Background. Transcription factor 7-like 2 gene (*TCF7L2*) and peroxisome proliferator-activated receptors-γ2 (*PPAR-γ2*) have a profound effect on the incidence of type 2 diabetes mellitus (T2DM) and had previously been found to be associated with T2DM risk in various ppopulations. However, studies in the Arab population are inconsistent. We conducted a case control study to confirm the association of variants rs10885409 of *TCF7L2* and *Pro12Ala* (rs1801282) of *PPAR-γ2* with risk of T2DM and related complications in Emirati population of Arab origin. We also investigated the interaction of these associations with obesity status. *Methods*. DNA was extracted from the saliva samples of 272 T2DM patients and 216 nondiabetic Emiratis. Genotyping for rs10885409 (*TCF7L2*) and rs1801282 (*PPAR-γ2 P12A*) variants was accomplished with a TaqMan assay. The subgroups were constituted according to obesity status. *Results*. In the nonobese group, the rs10885409 C allele in the recessive model was significantly associated with the incidence of T2DM (OR 1.975 [95% CI 1.127–3.461], $P = 0.017$), but this association was not observed in the obese group or when BMI was not considered. *PPAR-γ2* risk allele Pro12 frequency (0.96) was similar in the groups tested and more than 90% population was homozygous for this allele. *Conclusions*. Our case-control study is the first of its kind in Emiratis which establishes *TCF7L2* rs10885409 C allele as a T2DM risk factor in Emiratis and this association is modulated by obesity status. We also confirmed that Pro12Ala mutation in *PPAR-γ2* is not associated with T2DM risk in this population.

1. Introduction

Insulin resistance in muscle and liver and β-cell failure represent the core pathophysiologic defects in type 2 diabetes mellitus (T2DM) [1]. Most patients with T2DM also develop serious complications because of chronic hyperglycemia, such as nephropathy, retinopathy, neuropathy, and cardiovascular diseases, like coronary artery disease, cerebrovascular, and peripheral vascular disease. T2DM involves a complex interaction between genetic variants and the environment, with obesity being a primary risk factor [2]. The correlations between body fat and insulin resistance have been very well established [3], and the associations between genetic polymorphisms and T2DM were demonstrated to be reliant on obesity status [4]. Evidence from various studies has shown that genetic susceptibility to T2DM is polygenic [5, 6], and Genome Wide Association Studies (GWAS) have reported more than 20 genetic loci associated with the risk of T2DM [7, 8]. The single nucleotide polymorphisms (SNPs) in some of these genes have only a small effect on the disease status, but few, like those at the transcription factor 7-like 2 gene (*TCF7L2*) and proliferator-activated receptors-γ2

(*PPAR-γ2*) have a profound effect on the T2DM prevalence, as has previously been reported in large scale studies or meta-analyses conducted in various populations and ethnic groups [9–11].

TCF7L2 variant rs7903146 a C-to-T (genomic position: 114748339) substitution in intron 3 and *PPAR-γ2 Pro12Ala* have been most extensively studied in all major ethnic groups and were found to be more consistently associated with the risk of developing T2DM in most of populations, such as Asians, Africans, and Caucasians [12, 13]. These associations were also observed in most of the Arab populations of MENA region like Tunisians, Lebanese, [14–18], Iranian [19, 20], and North Africans [21]. Although these polymorphisms were established as risk factor for T2DM in most of the populations studied so far, however, some of the studied polymorphisms seem not to be a major contributor to T2DM susceptibility in the Saudi and Emirati Arab populations [22–24]. There is considerable inter-population variation in the frequency of the risk allele (*Pro12*) which ranges from a high of 0.96–0.98 in populations including the Japanese, Chinese, and African Americans to 0.91 in Pima Indians and a low of 0.81 in the Finnish population [25]. *TCF7L2* is an important regulator of the proglucagon gene, which encodes *GLP-1* and is believed to play a role in glucose metabolism. Evidence also suggests that this transcription factor itself is critical for beta cell proliferation, antiapoptotic activity, and insulin secretion [26, 27]. GWAS also identified the *TCF7L2* genes to be associated with T2DM risk [28]. Moreover, in some studies the associations of SNPs in *PPAR-γ2* and *TCF7L2* have previously been shown to be modulated by obesity status [4]. *PPAR-γ2 Pro12Ala* polymorphism was found more associated with T2DM susceptibility in obese populations while *TCF7L2* rs7903146 T allele was shown to be more prevalent in T2DM nonobese population compared to T2DM obese individuals, suggesting a pivotal role of obesity status in genetic association with T2DM [4]. Therefore, inconsistence regarding the genetic association studies among the Arab populations might be partly explained by the obesity status.

Based on these findings, we decided to study the effect of obesity status on association of *PPAR-γ2 Pro12Ala* variant with T2DM risk in Emirati population. In addition, we have also chosen to evaluate genetic variant rs10885409 in *TCF7L2* which is C114798062T substitution and was reportedly associated with T2DM risk in North Indians [5] but has not been examined in earlier genetic epidemiologic studies in any other previously studied populations, including Arabs. We also studied the effect of these variants on diabetic complications, which had not been previously reported in any of the Arab populations studied to date.

2. Methodology

2.1. Subjects and Sample Collection. This study was conducted on four hundred and eighty-six ($n = 486$) unrelated Emiratis who were identified during their routine visit to a diabetes clinic in Dubai and Al Ain, United Arab Emirates, between the period of June 2012 and December 2013. The case group of 272 Type 2 Diabetes patients had mean age of 58 ± 12 years and consisted of 60% females. The control group consisted of 216 healthy individuals who had mean age 45 ± 16 years and was 68.37% female. The individuals selected for control group were nondiabetic, normotensive individuals with no indication of any complications. All participants gave their informed consent in writing. This study was approved by the Ethics Committees of the Dubai Health Authority and Al Ain Medical District Human Research Ethics Committee in the United Arab Emirates.

After obtaining an informed consent from the participant, one mL of saliva was collected by a registered nurse using Oragene kit OGR-500 (DNA Genotek, Ottwa, Canada). The genomic DNA was extracted from saliva using prepITTM•L2P (DNA Genotek, Ottwa, Canada) in accordance with the manufacturer's instructions. All the saliva samples were processed in the Molecular Cell Biology laboratory at Khalifa University, Abu Dhabi, for DNA extraction and genotyping. In addition, a clinical assessments and lifestyle questionnaire was completed at the clinic to study any correlation between lifestyle variables and genetic variation. An individual was classified as T2DM if the subject [1] was diagnosed with T2DM by a qualified physician, [2] was on a prescribed drug treatment regimen for T2DM, and [3] returned biochemical test results of a fasting plasma glucose level of at least 126 mg/dL based on the criteria outlined by the World Health Organization (WHO) consultation group report. The individuals with BMI more than 30 were considered obese and those with a BMI less than 30 were grouped into the nonobese population. According to JNC 8 classification, all the individuals with blood pressure more than 140/90 mmHg were considered hypertensive.

2.2. Genotyping for TCF7L2 rs10885409 and PPAR-γ2 Pro12Ala Variants. TaqMan assays were used for SNP genotyping (Applied Biosystems, Foster City, CA). These assays use fluorogenic probes in a $5'$ nuclease assay to identify differences in DNA sequences. The laboratory-designed probes were obtained from Applied Biosystems. All PCR reactions took place in optical 96-well reaction plates (Applied Biosystems) with a final reaction volume of 10 μL that contained 10 ng of genomic DNA, 5 μL of TaqMan Genotyping Master Mix (Applied Biosystems), and 0.5 μL of assay mix (20x). The PCR thermal cycling conditions were set as follows: 95°C for 10 min to activate DNA polymerase, followed by 40 cycles of 92°C for 15 s and 60°C for 1 min. All PCRs were performed using a 9700 fast thermal cycler (Applied Biosystems), and the end point fluorescence readings were obtained on a ViiA 7 Real-time PCR system (Applied Biosystems). Upon running 5% samples in duplicate, the genotyping success rates were found to be 99.9% for both SNPs. Negative control was used in each run for quality control purposes.

2.3. Statistical Methods. Statistical analyses were performed using STATA version 13 (STATA Corp., TX, USA). Student's t-test was used to compare the clinical parameters to different groups. The genotype frequencies were tested for the Hardy-Weinberg equilibrium using a χ^2 test. Fisher Exact test was

Table 1: Clinical and biochemical characteristics of T2DM patients and healthy subjects.

	T2DM patients $n = 272$	Nondiabetic subjects $n = 216$	P value
Age (years)	58 ± 12	45 ± 16	<0.0001
Male : female ratio	110 : 162	69 : 147	0.05
BMI	31.99 ± 6.3	29.97 ± 6.14	0.0009
Fasting glucose (mmol/L)	8.99 ± 7.23	5.63 ± 7.23	<0.0001
Systolic blood pressure	129.97 ± 17.24	122.50 ± 15.65	<0.0001
Diastolic blood pressure	70.0 ± 11.0	71.35 ± 11.45	0.6197
HB1AC	7.6 ± 1.6	5.59 ± 0.56	<0.0001
Cholesterol (mmol/L)	4.14 ± 1.05	4.49 ± 0.96	0.0001
Triglycerides (mmol/L)	1.46 ± 0.83	1.18 ± 0.76	0.0041
HDL (mmol/L)	1.22 ± 0.49	1.31 ± 0.51	0.1316
LDL (mmol/L)	2.30 ± 0.91	2.66 ± 0.93	0.0008

used to analyze the statistical significance of the difference in allelic distribution of various polymorphisms in patients and controls, with P values, odds ratios (ORs), and confidence intervals reported. The following gene transmission models were considered for *TCF7L2* to study the effect of T allele: (1) recessive (TTVs CT + CC) and (2) dominant (CC Vs CT + TT) effect of T allele for association analysis. A P value of < 0.05 was considered statistically significant.

3. Results

The demographic characteristics and clinical and biochemical parameters of T2DM patients and nondiabetic control patients are described in Table 1. The mean age and male/female ratios significantly differed in the affected individuals and controls (Table 1). The mean glucose level, mean arterial blood pressure, triglycerides, LDL, and total cholesterol by *TCF7L2* rs10885409 genotype did not significantly differ (CC Vs CT + TT) (Table 2). The allele and genotype frequencies of the rs10885409 SNP at *TCF7L2* and *PPAR-γ2 Pro12Ala* variant in control and T2DM groups without consideration of obesity status are summarized in Table 3. The distribution of the *TCF7L2* polymorphism (rs10885409) was consistent with the Hardy-Weinberg equilibrium in T2DM patients ($\chi^2 = 2.513$, $P = 0.2846$) as well as in nondiabetic controls ($\chi^2 = 1.296$, $P = 0.5230$). The frequencies of TT, CT, and CC genotypes for rs10885409 SNP at *TCF7L2* were 22.5, 44.6, and 32.9%, respectively, in T2DM cases and 23.3, 46.5, and 30.2%, respectively, in nondiabetic subjects. T allele was found with minor allele frequencies of 0.45 and 0.48 in T2DM cases and control groups, respectively. The genotype and allele frequencies were not statistically different between the groups. The genotyping results for *PPAR-γ2* demonstrated G allele (12Ala) as a minor allele with a frequency of 0.04 and which was found only in heterozygote form. The genotype and allele frequencies were almost similar in T2DM cases and controls. Deviations from the Hardy-Weinberg equilibrium were not observed in any of the groups ($\chi^2 = 0.669$, $P = 0.7156$ for control group).

Table 2: Clinical and biochemical characteristics in different genotypes.

	CC $n = 272$	TT + CT $n = 216$	P value
BMI	31.21 ± 5.6	31.15 ± 6.4	0.9399
Fasting glucose (mmol/L)	7.41 ± 3.15	7.42 ± 3.15	0.9829
Systolic blood pressure	128.68 ± 18.0	126.38 ± 16.71	0.2374
Diastolic blood pressure	71.76 ± 11.06	70.79 ± 11.29	0.4551
HB1AC	6.94 ± 1.64	7.36 ± 3.54	0.2427
Cholesterol (mmol/L)	4.18 ± 1.00	4.31 ± 1.06	0.2667
Triglycerides (mmol/L)	1.36 ± 0.87	1.37 ± 0.83	0.9144
HDL (mmol/L)	1.25 ± 0.37	1.25 ± 0.56	0.8946
LDL (mmol/L)	2.31 ± 0.87	2.49 ± 0.96	0.0888

3.1. Association of the TCF7L2 rs10885409 Variant with the Risk of T2DM and Related Complications in a Case-Control Study

3.1.1. All Control and T2DM Individuals. Association analyses demonstrated no significant association between the rs10885409 SNP of *TCF7L2* and T2DM susceptibility when the obesity status was not considered (Tables 3 and 4). Moreover, the genotype and allele frequency distributions among the T2DM case groups with or without hypertension or any of the complications related with T2DM did not significantly differ (Table 6).

3.1.2. Nonobese T2DM Association. When the data were analyzed considering the obesity status, the frequency of the CC genotype was found to be significantly higher (26.1 Vs 41.1, $P = 0.0176$) in nonobese (BMI < 30) T2DM patients, but not in the obese affected patients (BMI > 30) (Table 5). The nonobese subjects with CC genotypes were at an approximately two-fold higher risk of T2DM than those with CT or TT genotypes (OR 1.975 (95% CI 1.127–3.461), $P = 0.017$) (Table 5).

Table 3: Genotype and allele frequency distribution of rs10885409 of TCF7L2 and PPAR-γ P12A mutation without obesity consideration.

	Total N	T2DM patients N (%)	Nondiabetic subjects N (%)	P value
TCF7L2 genotypes				
TT	111	61 (22.5)	50 (23.3)	NS
CT	221	121 (44.6)	100 (46.5)	NS
CC	154	89 (32.9)	65 (30.2)	NS
Allele frequencies				
T	443	243 (0.45)	205 (0.48)	NS
C	529	299 (0.55)	225 (0.52)	NS
PPAR-γ genotypes				
CC	447	250 (91.9)	197 (91.2)	NS
CG	41	22 (8.1)	19 (8.8)	NS
GG	0	0	0	NS
Allele frequencies				
C	935	522 (0.96)	413 (0.96)	NS
G	41	22 (0.04)	19 (0.04)	NS

Table 4: Genotype and allele frequency distribution for *TCF7L2* rs10885409 variant and the risk of type 2 diabetes in the Emirati population in various groups based on obesity status.

	Without consideration of BMI			Obese (BMI >30)			Nonobese (BMI <30)		
	T2DM	Healthy	P	T2DM	Nondiabetics	P	T2DM	Nondiabetics	P
				Genotypes					
CC	89 (32.8%)	65 (30.2%)	0.557	42 (28.2%)	31 (35.2%)	0.3082	46 (41.1%)	30 (26.1%)	0.0176
CT + TT	182 (67.2%)	150 (69.8%)		107 (71.8%)	57 (64.8%)		66 (58.9%)	85 (73.9%)	
				Allele frequencies					
C	0.55	0.52	0.399	0.52	0.56	0.342	0.61	0.51	0.0376
T	0.45	0.48		0.48	0.44		0.39	0.49	

Table 5: Analysis of association of *TCF7L2* rs10885409 variant and T2DM in case-control study by obesity status.

Model	Without consideration of BMI			Obese (BMI >30)			Nonobese (BMI <30)		
	OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value
CC Vs CT + TT	1.128	0.767–1.660	0.539	0.722	0.410–1.269	0.258	1.975	1.127–3.461	0.017
TT Vs CT + CC	0.927	0.598–1.438	0.736	1.083	0.580–2.021	0.802	0.752	0.396–1.428	0.384
					Allele frequencies				
C Vs T	1.1232	0.8714–1.4477	0.37	0.843	0.580–1.226	0.372	1.495	1.029–2.170	0.035

Table 6: Analysis of association of *TCF7L2* rs10885409 variant and T2DM related complications in Emirati population in case-control study.

	Total N (n = 232)	With complications N = 105 (%)	Without complications N = 127 (%)	P value
		TCF7L2 genotypes		
TT	48	26 (4.8)	22 (17.3)	0.3135*
CT	115	44 (41.9)	71 (55.9)	
CC	69	35 (33.3)	34 (26.8)	
		Allele frequencies		
T	211	96 (0.46)	115 (0.45)	0.9257
C	253	114 (0.54)	139 (0.55)	

*CC Vs CT + TT.

Table 7: Biochemical parameters in different *TCF7L2* rs10885409 genotypes in nonobese Emirati T2DM cases and controls.

Genotypes	Cholesterol (mmol/L)		*P*	LDL (mmol/L)		*P*	HDL (mmol/L)		*P*	TGS (mmol/L)		*P*	HB1Ac (mmol/L)		*P*
	T2DM	Nondiabetics		T2DM	Nondiabetics		T2DM	Nondiabetics		T2DM	Nondiabetics		T2DM	Nondiabetics	
CC	4.12 ± 1.0	4.56 ± 0.68	0.1805	2.28 ± 0.94	2.57 ± 0.81	0.2700	1.3 ± 0.39	1.28 ± 0.39	0.8597	1.34 ± 0.84	1.37 ± 1.26	0.9157	7.62 ± 1.87	5.48 ± 0.44	**0.0003**
CT + TT	4.05 ± 0.83	4.55 ± 1.13	**0.0175**	3.86 ± 0.76	2.55 ± 0.98	**0.0001**	1.28 ± 0.84	1.42 ± 0.71	0.4050	1.4 ± 0.81	1.04 ± 0.44	**0.0221**	7.64 ± 1.96	5.76 ± 0.73	**0.0001**
P values	0.7565	0.9718		0.0001	0.9393		0.8946	0.4379		0.7375	0.1755		0.9611	0.2302	

4. Discussion

In this investigation, we carried out genetic association study of the SNPs in *PPAR-γ2* and *TCF7L2* with T2DM susceptibility and its interaction with the obesity status for the first time in the Emirati population. While many variants have been identified in *PPAR-γ2* gene, the most prevalent and best studied is the *Pro12Ala* polymorphism which has been shown to impair the function of the PPAR-γ2 isoform of the receptor and to be associated with obesity and/or diabetes and insulin sensitivity related phenotypes in different populations [9, 12, 13]. Grant et al. [29] found that common genetic variants of the *TCF7L2* gene were associated with T2DM risk, and these findings were consistently reproduced in several populations. Most of these studies focused on the genetic variant rs7903146, which was consistently found to be associated with a risk for T2DM in most populations studied to date. However, the scenario in the Arab population was a slightly different. Studies of two major Arab populations, Saudi Arabs and Emirati Arabs [22, 23], were not consistent with these findings. Saadi et al. [23] and Alsmadi et al. [22] already examined the variant s7903146 in Emirati and Saudi populations, respectively. We did not imitate their reports. Rather, we investigated another SNP rs10885409 that was found to be associated with T2DM in a previous study conducted in Indians Sikhs residing in the USA [9].

The genotyping results for *PPAR-γ2* showed *12Ala* allele as minor allele and we established the risk allele (*Pro12*) frequency in Emirati population among the highest observed so far and was comparable to Saudi [24], Japanese, Chinese, and African but failed to observe any association with T2DM risk. These findings are consistent with Saudi [24] and some Caucasian studies [30, 31]. Moreover, given the very high incidence of the *Pro12* allele in this population, the study size was extremely underpowered and the data could not be analysed for the association with incidence of T2DM related complications and interaction with obesity status.

The results of the genotyping for rs10885409 demonstrated that neither the T nor the C allele was associated with T2DM. Similarly, rs10885409 did not correlate with T2DM when the data for males and females were analyzed separately, ruling out the possibility of any gender-based association. We also observed that the genotype did not affect any of the clinical or biochemical parameters, such as the BMI, fasting glucose level, or lipid profile (Table 2), which are considered to be related to T2DM and were found significantly altered in the cases and controls (Table 1). The allele and genotype distribution were not in concordance with the only available study conducted in an Indian Sikh population on the same allele [9]. The C allele was established to be minor, with an allele frequency of 0.46 in nondiabetics and significantly higher frequency of 0.53 in T2DM cases, and the strongest association was suggested (1.64; 95% CI [1.20–2.24]; $P = 0.001$) in dominant models [9]. The data presented here and in the two previous studies in Arabs [16, 17] suggested that *TCF7L2* polymorphism is not related to T2DM risk in the Arabian Peninsula. A coding variant (*Pro477Thr*) in exon 14 of *TCF7L2* and the recently identified rs290487 variant of *TCF7L2* were also not associated with T2DM in Japanese [32] and Chinese [33] populations. However, rs7903146 was associated with T2DM in both of these populations [9, 10, 30], suggesting the possibility of allele-specific associations only. A literature exploration revealed inconsistencies in the reports in even the most thoroughly studied and widely accepted T2DM risk variant rs7903146, especially in Arab populations. In Arabian Peninsula, the life style is more westernized with reduced physical activity and excessive calorie intake. These divergent results of the association could be explained by the interaction between the BMI and environmental factors in modulating T2DM risk, as previous reports suggested that different genetic architectures could increase the T2DM susceptibility according to the obesity status [4]. In fact, the TCF7L2 rs7903146 T allele was associated with T2DM in nonobese Tunisian Arabs, whereas no effect was detected in overweight and obese individuals [31]. We analyzed our results while considering obesity. We divided our T2DM cases and controls into two groups each of BMI < 30 and BMI > 30. The obese group included 147 T2DM and 88 healthy subjects, and the nonobese group included 112 and 115 T2DM and control individuals, respectively. We observed the frequency of the CC genotype to be significantly higher (41.1 versus 26.1, $P = 0.0176$) in nonobese T2DM cases compared to matching nondiabetic subjects (0.61 versus 0.51, $P = 0.0376$), and the same was also true for the C allele (Table 4). The nonobese CC genotype carriers were found to be at high risk for T2DM (OR 1.975, 95% CI 1.127–3.461, $P = 0.017$) (Table 5); however, rs10885409 was not associated with T2DM risk in the obese group. These results were in complete concordance with those of the Tunisian study [20] and were sufficient to explain the inconsistencies that were observed regarding the effect of the *TCF7L2* variant on T2DM risk. Most of the studies were conducted without considering the obesity status, and our results strongly suggested that the genetic associations might be modulated by the presence and absence of obesity, especially in Arab populations.

The allelic and genotypic associations were also analyzed in diabetic patients with at least one complication and without any complications. We did not observe any significant differences in the allele and genotype frequencies between the two groups (Table 6), indicating that rs10885409 did not affect the presence of diabetes-related complications. Studies regarding the effect of variant rs10885409 on diabetes-related complications were not available, and the studies on rs7903146 were largely inconsistent [34, 35]. Unfortunately, we could not analyze this association for each complication separately or based on obesity status because of the very small sample size of the groups formed for this purpose. However, we observed significant differences in the biochemical parameters in different genotypes in nonobese subjects. The mean levels of cholesterol, LDL, and TGs were significantly higher in T2DM cases with either CT or TT genotypes compared to matching controls with the same genotype (Table 7). Interestingly, the LDL levels were significantly higher in T2DM subjects with the CT + TT genotype compared to T2DM cases with the CC genotypes ($P = 0.0001$) (Table 7).

In conclusion, our results show that the *Pro12Ala* mutation in the *PPAR-γ2* gene is unlikely to serve as clinically

useful predictor of T2DM and/or obesity in Emiratis. *TCF7L2* variation may be a risk factor for the occurrence of T2DM in Arab populations, but this association relies on the obesity status. Our case control study on rs10885409 established CC genotype as a risk factor in nonobese Emiratis. However, the association was absent when the obesity status was not considered and in the obese population with T2DM risk and related complications.

Conflict of Interests

The authors declare that there is no conflict of interests or financial interests associated with this paper.

Acknowledgments

The authors gratefully acknowledge the volunteers whose cooperation made this study possible. The authors also would like to thank Deeptha Murall from Manipal University, Dubai, for her specific technical support that has allowed for the statistical work to be carried out for this study. This work was funded by Khalifa University Internal Research Fund.

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Hindawi Publishing Corporation
Journal of Diabetes Research
Volume 2015, Article ID 760535, 11 pages
http://dx.doi.org/10.1155/2015/760535

Research Article

Edible Bird's Nest Prevents High Fat Diet-Induced Insulin Resistance in Rats

Zhang Yida,[1,2] Mustapha Umar Imam,[1] Maznah Ismail,[1,3] Der-Jiun Ooi,[1] Nadarajan Sarega,[1] Nur Hanisah Azmi,[1] Norsharina Ismail,[1] Kim Wei Chan,[1] Zhiping Hou,[1] and Norhayati Binti Yusuf[1]

[1]*Laboratory of Molecular Biomedicine, Institute of Bioscience, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia*
[2]*Cardiology Department, Affiliated Hospital of Chengde Medical University, Chengde, Hebei 067000, China*
[3]*Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia*

Correspondence should be addressed to Maznah Ismail; maznahis@upm.edu.my

Received 7 January 2015; Revised 10 March 2015; Accepted 17 March 2015

Academic Editor: Joseph Fomusi Ndisang

Edible bird's nest (EBN) is used traditionally in many parts of Asia to improve wellbeing, but there are limited studies on its efficacy. We explored the potential use of EBN for prevention of high fat diet- (HFD-) induced insulin resistance in rats. HFD was given to rats with or without simvastatin or EBN for 12 weeks. During the intervention period, weight measurements were recorded weekly. Blood samples were collected at the end of the intervention and oral glucose tolerance test conducted, after which the rats were sacrificed and their liver and adipose tissues collected for further studies. Serum adiponectin, leptin, F2-isoprostane, insulin, and lipid profile were estimated, and homeostatic model assessment of insulin resistance computed. Effects of the different interventions on transcriptional regulation of insulin signaling genes were also evaluated. The results showed that HFD worsened metabolic indices and induced insulin resistance partly through transcriptional regulation of the insulin signaling genes. Additionally, simvastatin was able to prevent hypercholesterolemia but promoted insulin resistance similar to HFD. EBN, on the other hand, prevented the worsening of metabolic indices and transcriptional changes in insulin signaling genes due to HFD. The results suggest that EBN may be used as functional food to prevent insulin resistance.

1. Introduction

The growing burden of cardiometabolic diseases, even in the face of increasing advances in medical sciences, is the driving factor behind the heightened interest in alternative therapies in the management of these diseases and associated problems [1, 2]. Additionally, rising obesity rates globally due to unhealthy lifestyle factors promote these rising disease trends; obesity promotes insulin resistance and eventually cardiometabolic diseases [3]. In fact, it is estimated that if persons at risk of insulin resistance and cardiometabolic diseases are accurately determined using sensitive diagnostic techniques, the numbers of those needing interventions to manage their conditions would be much higher than established figures [4]. There are different theories used to hypothesize the underlying mechanisms involved in the progression from obesity to insulin resistance and cardiometabolic diseases. Popularly, excess calories are thought to promote deposition of visceral fat around organs, with consequent changes in the adipose tissue metabolism in the body, and ultimately increase in insulin resistance especially in liver, as a result of glucolipotoxicity [5]. The ensuing insulin resistance causes disruption in the propagation of insulin signals on insulin-responsive cells. In fact, the perceived role of this phenomenon is the reason why therapeutic approaches to the management of insulin resistance and other associated cardiometabolic diseases involve the use of agents that promote insulin signaling.

Edible bird's nest (EBN) is traditionally consumed among Asians for its nutritional value. It is believed to enhance energy levels, prevent aging, and improve overall well-being. Furthermore, there are scientific reports of its antioxidative,

Table 1: Food composition and animal groups.

Animal group	Normal pellet	Cholesterol/cholic acid	Palm oil	Starch	Others
Normal	100%				
High fat diet	65%	5	20	10	
High fat diet + simvastatin	65%	5	20	10	Simvastatin (10 mg/kg)
High fat diet + 20% EBN	45%	5	20	10	20% EBN
High fat diet + 2.5% EBN	62.5%	5	20	10	2.5% EBN

EBN: edible bird's nest.

anti-inflammatory, and bone-strengthening effects [6–9]. However, its effects on insulin resistance and cardiometabolic indices have not been documented. In view of the large patronage of EBN by Asians, especially of Chinese origin [10], we decided to evaluate the effects of EBN consumption on cardiometabolic indices in high fat diet- (HFD-) fed rats. Based on the anti-inflammatory and antioxidant effects of EBN, we assumed it would have favorable effects on cardiometabolic indices, since both effects have been reported to favor insulin sensitivity. As the first study of its kind, we hypothesized that the results could provide the evidence for continued use of EBN as a supplement and may even pave way for evidence-based development of functional foods and nutraceuticals using EBN for managing cardiometabolic diseases.

2. Materials and Methods

2.1. Materials. Leptin, F2-isoprostane, and insulin ELISA kits were purchased from Elabscience Biotechnology Co., Ltd (Wuhan, China), while adiponectin ELISA kit was from Millipore (Billerica, MA, USA). Lipid profile kits were purchased from Randox Laboratories Ltd (Crumlin, County Antrim, UK). GenomeLab GeXP Start Kit was from Beckman Coulter Inc (Miami, FL, USA), and RNA extraction kit was from RBC Bioscience Corp. (Taipei, Taiwan). Simvastatin was from Pfizer (New York, NY, USA) and RCL2 Solution from Alphelys (Toulouse, France). Analytical grade ethanol was purchased from Merck (Darmstadt, Germany). Cholesterol and cholic acid were purchased from Amresco (Solon, OH, USA) and Santa Cruz Biotechnology (Santa Cruz, CA, USA), respectively. Standard rat pellet was from Specialty feeds (Glen Forrest, WA, USA), while palm oil was supplied by Yee Lee Edible oils Sdn. Bhd. (Perak, Malaysia). EBN, of *Aerodramus fuciphagus* (white nest swiftlet) origin, supplied by Blossom View Sdn. Bhd (Terrengganu, Malaysia) was cleaned under tap water for 5 mins, dried at room temperature, and ground into powder manually using mortar and pestle before incorporating it into rat pellet.

2.2. Bioactive and Proximate Analyses. The proximate analysis of EBN was done as reported in our previous publication [11], based on the official methods of Association of Official Analytical Chemists. Briefly, nitrogen content was determined using micro-Kjeldahl apparatus (Kjeltech 2200 Auto Distillation Unit, FOSS Tecator, Hoganas, Sweden), and then protein content was determined as N × 5.95. Furthermore, the ashing process was done by incinerating the sample in a furnace (Furnace 62700, Barnstead/Thermolyne, Dubuque, IA, USA) set at 550 C, while the fat content was determined as the dried ether extract of EBN. Then, carbohydrate content was determined using the following formula: (100% – protein content – moisture content – ash content – crude fat content). All results were expressed as percentage of dry weight. The amounts of major bioactives in EBN (sialic acid [SA], lactoferrin [LF], and ovotransferrin [OVF]) were analyzed using ELISA-based techniques (LF and OVF) and HPLC-DAD (SA). Briefly, EBN was ground to powder and dissolved in water at 37°C for 2 h on a shaking incubator (LSI-3016, Daihan Lab tech Co. Ltd, Korea) and finally filtered. The water extract was then used to detect LF and OVF concentrations using Chicken Lactoferrin and Ovotransferrin Elisa Kits, Biosource (San Diego, California, USA), according to manufacturer's instructions. Additionally, water extract of EBN was also analysed for SA content using HPLC-DAD as reported previously [12].

2.3. Animal Study. The Animal Care and Use Committee (ACUC) of the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, approved the use of animals in this study (Project approval number UPM/IACUC/AUP-R011/2014), and animals were handled as stipulated by the guidelines for the use of animals. Sprague Dawley rats (10-week old, 230–280 g, $n = 30$) were housed at the animal house (25 ± 2°C, 12/12 h light/dark cycle) and allowed to acclimatize for 2 weeks with free access to normal pellet and water. After acclimatization, rats were fed HFD containing 4.5% cholesterol and 0.5% cholic acid with or without treatment using simvastatin or EBN (Table 1), except the normal group ($n = 6$). Intervention lasted for another 12 weeks, after which rats were sacrificed and their organs harvested for further studies. Additionally, blood samples were collected at the end of the intervention for biochemical analyses.

2.4. Food Intake and Weight. Food intake was calculated by subtracting the leftover food from what was added the previous day. Weight was recorded after acclimatization and weekly thereafter until sacrifice.

2.5. Biochemical Analyses. Lipid profile analyses were performed using serum from blood collected at the beginning and end of the study by cardiac puncture after an overnight fast. Samples were analyzed using Randox analytical kits according to manufacturer's instructions using a Selectra XL

instrument (Vita Scientific, Dieren, The Netherlands). Blood glucose was measured using glucometer (Roche Diagnostics, Indianapolis, IN, USA), and homeostatic model assessment of insulin resistance (HOMA-IR), a measure of insulin sensitivity, was computed from the fasting plasma glucose and insulin levels using the formula, HOMA-IR = (fasting glucose level [mg/dL]/fasting plasma insulin [uU/mL])/2430 [13].

2.6. Serum Adiponectin, Leptin, F2-Isoprostane, and Insulin. Serum from blood collected in plain tubes was used for measurements of adiponectin, leptin, F2-isoprostane, and insulin using the respective ELISA kits according to the manufacturers' instructions. Absorbance was read on BioTeK Synergy H1 Hybrid Reader (BioTek Instruments Inc., Winooski, VT, USA) at the appropriate wavelengths (450 nm for insulin, leptin, and F2-isoproatane and 450 and 590 for adiponectin). The results were analyzed on http://www.myassays.com/ using four parametric test curve: adiponectin (R^2 = 0.9914), insulin (R^2 = 1), leptin (R^2 = 0.9996), and F2-isoprostane (R^2 = 1).

2.7. Gene Expression

2.7.1. Primer Design. *Rattus norvegicus* gene sequences from the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/nucleotide/) were used to design primers (Table 2) on GenomeLab eXpress Profiler software. In addition to the genes of interest, primers were also designed for housekeeping genes, while the internal control (Kanr) was supplied by Beckman Coulter Inc. Primers were tagged with an 18-nucleotide universal forward and 19-nucleotide universal reverse sequence, respectively. Primers were supplied by Integrated DNA Technologies (Singapore) and reconstituted in RNAse free water.

2.7.2. RNA Extraction, Reverse Transcription, and PCR. RNA was extracted from liver and adipose tissues using the total RNA isolation kit (RBC Biotech Corp., Taipei, Taiwan) according to the manufacturer's instructions. Reverse transcription (20 ng) and PCR were done according to the GenomeLab GeXP Start Kit protocol (Beckman Coulter, USA), using the conditions shown in Table 3.

2.7.3. GeXP Genetic Analysis System and Multiplex Data Analysis. PCR products (1 uL) were mixed with 38.5 μL sample loading solution and 0.5 μL DNA size standard 400 (GenomeLab GeXP Start Kit; Beckman Coulter, Inc, USA) on a 96-well sample plate and loaded on the GeXP genomelab genetic analysis system (Beckman Coulter, Inc, Miami, FL, USA), which separates PCR products based on size by capillary gel electrophoresis. Figure 1 shows a representative electropherogram. Results were analyzed with the Fragment Analysis module of the GeXP system software and normalized on the eXpress Profiler software.

2.8. Data Analysis. The means ± standard deviations (n = 6) of the groups were used for the analyses. One-way analysis of variance (ANOVA) was performed using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA) to assess the level of significance of differences between means with a cutoff of $P < 0.05$.

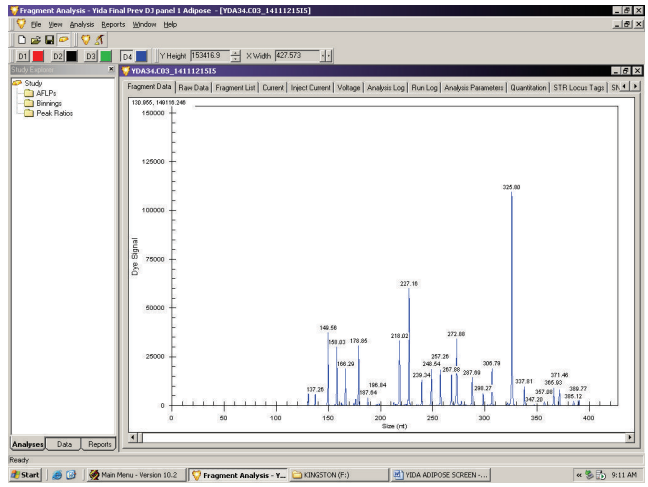

Figure 1: Representative electropherogram following gene expression analysis on GenomeLab GeXP genetic analysis system (Beckman Coulter Inc., USA). The genes and their expected sizes were Irs2-137; Slc2a2-149; Kcnj11-158; Insr-166; Glut4-178; Irs1-188; Gck-197; Mapk8-218; Pklr-227; Prkcd-239; B2m-248; Hprt1-257; Mapk1-268; Socs1-272; Rpl13a-287; Prkcz-298; Ikbkb-306; Kan(r)-325; Mtor-337; Pdx1-348; Pik3cd-357; Actb-365; Pik3r1-372; Pik3ca-385; Hk2-389.

3. Results and Discussions

3.1. Proximate and Bioactive Analyses. The proximate analysis of EBN showed that it contained mostly protein and carbohydrates (Table 3), in agreement with previous findings [10]. Additionally, it contained a significant amount of SA (11%) as bioactive, with lesser amounts of LF (1%) and OVF (0.4%). Previous reports have indicated that EBN is bioactive-rich [10], and it is likely that food synergy plays role in its overall effects [14]. The presence of any one bioactive compound may not explain the bioactivity of EBN, but the concentration of the leading bioactive compounds like SA may have an influence to a great extent, albeit with the contribution of other bioactives. Moreover, SA, LF, and OVF have all been reported to have varying functional effects [15, 16], and their synergism may even produce better. This is similar to the concept of bioactive-rich fraction we have advocated for recently, in which a lead bioactive compound in an extract produces better bioactivity in the presence of other bioactive compounds [17]. Therefore, in view of recent advocacy for the study of foods but not their individual constituents as the functional unit of nutrition [18], we decided to study the bioactivity of EBN as a whole.

3.2. Weight Changes. Figure 2 shows the changes in body weights of rats over 12 weeks of intervention. No statistically significant changes were observed but the changes in HFD-fed (untreated control) group (50% increase) were higher, in comparison with normal (47%), simvastatin (40%), 2.5% EBN (45%), and 20% EBN (43%) groups. Interestingly, as

Table 2: Names, accession number, and primer sequences used in the study.

	Accession number	Left sequence	Right sequence
Irs2	NM_001168633	AGGTGACACTATAGAATAAGGCACTGGAGCCTTAC	GTACGACTCACTATAGGGAGCAGCACTTTACTCTTTCAC
Kcnj11	NM_031358	AGGTGACACTATAGAATACTACTTCAGGCAAAACTCTG	GTACGACTCACTATAGGGAGAACTTTCCAATATTTCTTTT
Insr	NM_017071	AGGTGACACTATAGAATAAGCTGGAGGAGTCTTCAT	GTACGACTCACTATAGGGAAAGGGATCTTCGCTTT
Gck	NM_001270849	AGGTGACACTATAGAATAATCTTTTGCAACACTCAGC	GTACGACTCACTATAGGGATTGTTGGTGCCCAGA
Pklr	NM_012624	AGGTGACACTATAGAATATCGGAGGTGGAAATTG	GTACGACTCACTATAGGGACTCTGGGCCGATTTT
Prkcd	NM_133307	AGGTGACACTATAGAATATCAAGAACCACGAGTTCA	GTACGACTCACTATAGGGATCTTTCTGGAAGATGGTG
B2m*	NM_012512	AGGTGACACTATAGAATAATGCTTGCAGAGTTAAACA	GTACGACTCACTATAGGGATGCATAAAATATTTAAGGTAAGA
Hprt1*,#	NM_012583	AGGTGACACTATAGAATATCCTCATGGACTGATTATG	GTACGACTCACTATAGGGACTGGTCATTACAGTAGCTCTT
Mapk1	NM_053842	AGGTGACACTATAGAATACATTTTTGAAGAGACTGCTC	GTACGACTCACTATAGGGAAACTCTCTGGACTGAAGAAT
Prkcz	NM_022507	AGGTGACACTATAGAATACTTTAACAGGAGAGCGTACT	GTACGACTCACTATAGGGATATTGTCATGTTTCCGAGAT
Ikbkb	NM_053355	AGGTGACACTATAGAATACTTGAACTTAAAGCTGGTTC	GTACGACTCACTATAGGGAACATTTTACTGTTGTCAAAGAG
Kan(r)**			
Mtor	NM_019906	AGGTGACACTATAGAATATGGAACTTCGAGAGATGAG	GTACGACTCACTATAGGGATCACTTCAAACTCCACATAC
Actb*	NM_031144	AGGTGACACTATAGAATAAACTACATTCAATTCCATCA	GTACGACTCACTATAGGGATAAAACGCAGCTCAGTAAC
Pik3r1	NM_013005	AGGTGACACTATAGAATACATCAGTATTGGCTTACG	GTACGACTCACTATAGGGATCATTTACTTCTTCCCTTGA

*Housekeeping genes. #Normalization gene. Underlined sequences are left and right universal left and right sequences (tags). **Internal control supplied by Beckman Coulter Inc (Miami, FL, USA) as part of the GeXP kit. RT conditions were 48°C for 1 min; 37°C for 5 min; 42°C for 60 min; 95°C for 5 min and then hold at 4°C. PCR conditions were initial denaturation at 95°C for 10 min, followed by two-step cycles of 94°C for 30 sec and 55°C for 30 sec, ending in a single extension cycle of 68°C for 1 min.

Table 3: Proximate analyses and lactoferrin, ovotransferrin, and sialic acid concentrations of edible bird's nest (EBN).

Bioactive/nutrient	EBN
Lactoferrin	4.68 ± 0.4 μg/mg
Ovotransferrin	10.23 ± 0.8 μg/mg
Sialic acid	110.4 ± 0.8 μg/mg
Crude fat	0.54 ± 0.06%
Ash	4.0 ± 0.03%
Moisture	15.2 ± 0.02%
Carbohydrate	23.4 ± 0.29%
Crude protein	56.9 ± 0.27%

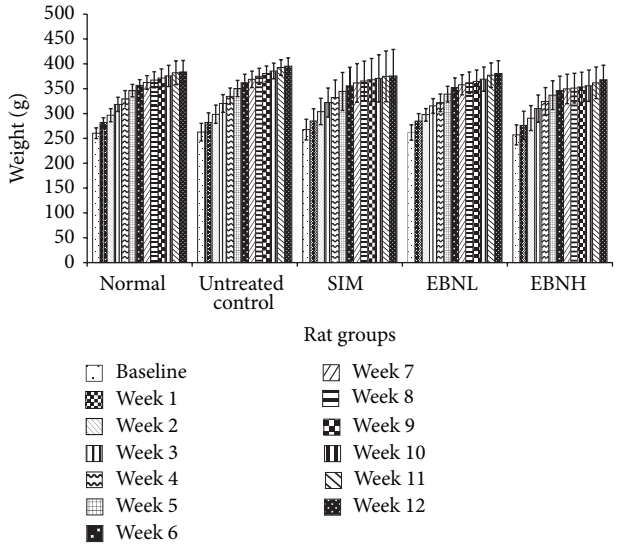

Figure 2: Effects of edible bird's nest (EBN) on body weight changes in high fat diet- (HFD-) fed rats over 12 weeks. The normal group received standard rat chow, while the other groups received HFD containing 4.5% cholesterol and 0.5% cholic acid (untreated control group), HFD containing 4.5% cholesterol and 0.5% cholic acid + 10 mg/kg/day simvastatin (SIM), HFD containing 4.5% cholesterol and 0.5% cholic acid + 2.5% EBN (EBNL, EBN low), or HFD containing 4.5% cholesterol and 0.5% cholic acid + 20% EBN (EBNH, EBN high).

shown in Table 3, calorie intake for the different groups was similar over the intervention period. The results indicated therefore that EBN had some weight-modulating properties, although the weight gain was lowest for simvastatin-treated group. Moreover, simvastatin is reported to have some weight reducing properties [19].

3.3. OGTT, Insulin, HOMA-IR, and Lipid Profile. Serum insulin levels at the end of intervention were not remarkably different between the groups except for the 2.5% EBN group, which was significantly lower ($P < 0.05$) than others (Table 4). However, absolute insulin levels may not reflect the state of the underlying insulin responsiveness since insulin resistance often starts with high insulin levels and ends up with lower levels. Therefore, we computed the HOMA-IR as a marker of insulin resistance that combines insulin levels

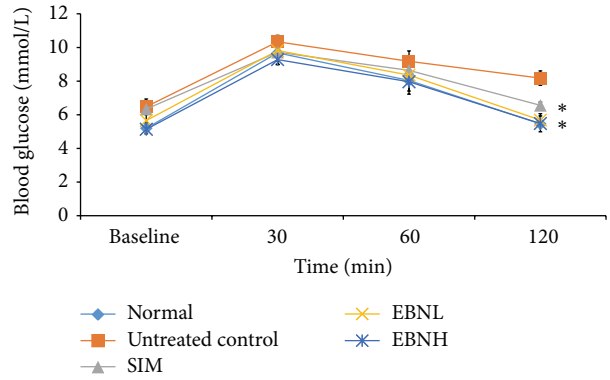

Figure 3: Effects of edible bird's nest (EBN) on oral glucose tolerance test in fed high fat diet- (HFD-) fed rats. Groupings are similar to Figure 2. * indicates significant difference ($P < 0.05$) in comparison with untreated control.

and fasting glucose levels. The data showed that untreated control and simvastatin groups had a tendency to cause insulin resistance. This mirrors earlier findings on the effects of HFD feeding [20] and simvastatin [21] on development of insulin resistance. EBN groups had lower HOMA-IR values in comparison with other groups, although not significantly different from normal (both EBN groups) and untreated control (20% EBN group) groups.

The cholesterol levels in the untreated control group were significantly increased in comparison with the normal group (Table 4). Moreover, worsening of lipid profile has been associated with insulin resistance [22]. The total cholesterol was significantly reduced by simvastatin and 20% EBN group ($P < 0.05$). As seen from other cholesterol indices in the table, simvastatin, which is used to manage hypercholesterolaemia was able to improve lipid profile but not as well as 20% EBN treatment. Furthermore, Figure 3 shows the OGTT results for the intervention groups. The glycemic response for the diabetic untreated group was higher than other groups ($P < 0.05$), while the normal and EBN groups were the lowest and significantly lower than simvastatin treated group ($P < 0.05$). Insulin regulates a number of metabolic changes in the body and derangements in its actions even before insulin resistance becomes overt can be detected using the OGTT. This is because the OGTT gives an indication of how a biological system will respond in the presence of glucose and indicates how well the postglucose insulin surge handles the glycemic load received in the blood stream [23]. In this study, the data showed that untreated control and simvastatin groups did not handle the glucose load in a manner befitting the levels of insulin observed in the serum. Therefore, in spite of the lack of difference in insulin levels between the groups, the OGTT data showed that the untreated control and simvastatin-treated groups will have abnormal glycemic responses compared with the normal and EBN groups because their bodies were tending towards insulin resistance.

3.4. Serum Adiponectin, Leptin, and F2-Isoprostane. Figure 4 shows the results for the serum levels of adiponectin, leptin, and F2-isoprostane. The results suggested worsened

Table 4: Food intake and biochemical parameters.

Rat groups	Food intake		Chol. (mmol/L)	Trig. (mmol/L)	LDL (mmol/L)	HDL (mmol/L)	LDL/HDL	TG/HDL	Insulin (pg/mL)	HOMA-IR
	g/kg/day	Kcal/kg/day								
Normal	64.34 ± 10.96	215.54 ± 33.5^{a}	1.55 ± 0.43^{a}	0.62 ± 0.15^{a}	0.28 ± 0.11^{a}	1.18 ± 0.35^{a}	0.24 ± 0.04^{a}	0.55 ± 0.15^{a}	495 ± 51.3^{a}	1.91 ± 0.23^{a,c,d}
Untreated control	48 ± 8.36	215.04 ± 37.45^{a}	7.47 ± 1.13^{b}	1.21 ± 0.38^{b}	4.98 ± 1.03^{b}	1.05 ± 0.13^{a}	4.77 ± 0.98^{b}	1.16 ± 0.33^{b}	513.3 ± 38.8^{a}	2.46 ± 0.22^{b}
SIM	48.14 ± 8.17	215.67 ± 36.60^{a}	4.99 ± 1.11^{c,d}	0.63 ± 0.18^{a}	3.6 ± 1.1^{b,c}	1.04 ± 0.17^{a}	3.46 ± 0.94^{b,c}	0.62 ± 0.22^{a,b}	602.1 ± 145.7^{a}	2.83 ± 0.79^{b,c}
2.5% EBN	48.23 ± 8.21	216.07 ± 36.78^{a}	6.04 ± 0.75^{b,c}	0.54 ± 0.1^{a}	4.52 ± 0.71^{b,c}	1.17 ± 0.18^{a}	3.94 ± 0.88^{b,c}	0.46 ± 0.08^{a}	414.5 ± 18.8^{b,c}	1.74 ± 0.09^{c,d}
20% EBN	48.33 ± 8.00	216.52 ± 35.84^{a}	4.17 ± 1.06^{d}	0.44 ± 0.1^{a}	2.98 ± 0.83^{c}	1.18 ± 0.29^{a}	2.63 ± 0.87^{c}	0.38 ± 0.08^{a}	426.7 ± 160.7^{a,c}	1.63 ± 0.71^{a,b,c}

Data represent mean ± SD ($n = 6$). Different alphabet in each column denotes significant difference ($P < 0.05$) in Tukey's multiple comparison test. Groupings are the same as Figure 2. HDL: high-density lipoprotein; HOMA-IR: homeostatic model assessment of insulin resistance; LDL: low-density lipoprotein; Chol.: cholesterol; SIM: Simvastatin; Trig.: triacylglyceride.

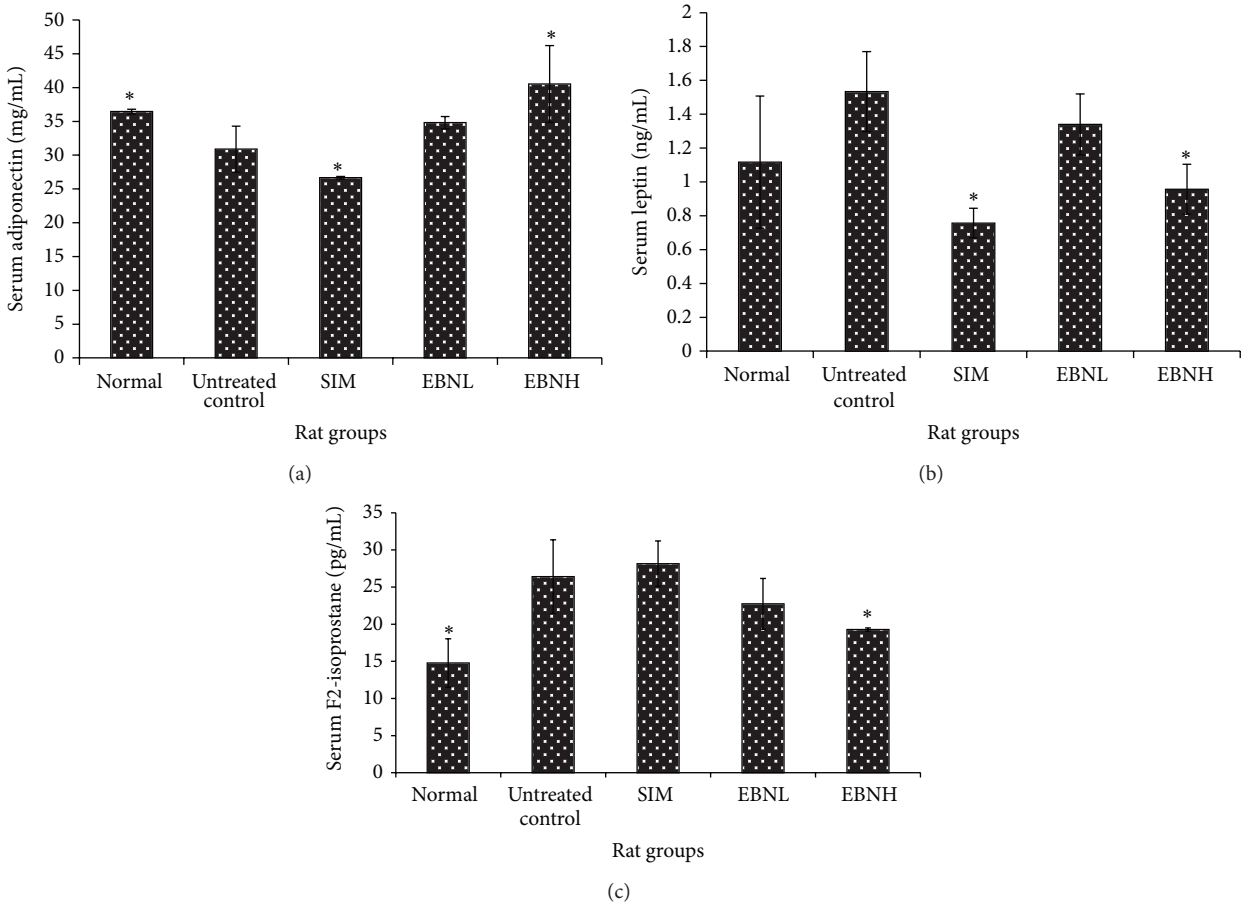

Figure 4: Effects of edible bird's nest (EBN) on (a) serum adiponectin, (b) serum leptin, and (c) serum F2-isoprostane in high fat diet- (HFD-) fed rats. Groupings are similar to Figure 2. $*$ indicates significant difference ($P < 0.05$) in comparison with untreated control.

metabolic indices (increased leptin and F2-isoprostane and decreased adiponectin) in the untreated control group in comparison with the normal group. The EBN groups showed dose-dependent improvements (decreased leptin and F2-isoprostane and increased adiponectin) in the metabolic indices although only 20% EBN group was significantly better than the untreated control group. Adiponectin and leptin are adipokines that have an inverse relationship and have both been implicated in the development of insulin resistance. Low levels of adiponectin and high levels of leptin are indicative of a tendency for insulin resistance, while interventions that reverse these trends are reported to improve insulin sensitivity [24]. Furthermore, F2-isoprostane is a marker of oxidative stress, which is also linked with insulin resistance [25]. In fact, oxidative stress is hypothesized to precede insulin resistance [26], while antioxidants and interventions that lower oxidative stress levels are thought to improve insulin sensitivity [27]. Based on the trends observed in the present study, therefore, it can be argued that EBN prevented HFD-induced insulin resistance in rats, partly through its ability to reduce oxidative stress.

3.5. Hepatic and Adipose Tissue mRNA Levels of Insulin Signaling Genes. The data thus far indicated that EBN is able to prevent insulin resistance in rats fed HFD over 12 weeks. Additionally, the data showed that although simvastatin is able to produce lower levels of cholesterol, it, in fact, increases insulin resistance, in agreement with previous reports [21]. Based on the fact that insulin levels were similar between the groups in this study, but there were significant differences in insulin sensitivity, we hypothesized that changes in insulin sensitivity may have been mediated at insulin signaling level. We, therefore, determined the effects of our interventions on transcriptional regulation of insulin signaling genes (Table 2) in hepatic and adipose tissues.

The expressions of the insulin signaling genes in hepatic and adipose tissues were characteristic of insulin resistance in the untreated control group; downregulation of the insulin receptor (Insr), insulin receptor substrate (IRS) 2, and phosphoinositide-3-kinase (PI3K) observed in the liver and adipose tissues in this group are suggestive of insulin resistance (Figure 5) [28–30]. Activation of Insr by insulin will normally initiate a cascade that involves activation of IRS and eventually PI3K, which mediate the intracellular actions of insulin. Transcriptional disruption of this insulin-initiated cascade forms part of the basis for obesity-induced insulin resistance [31].

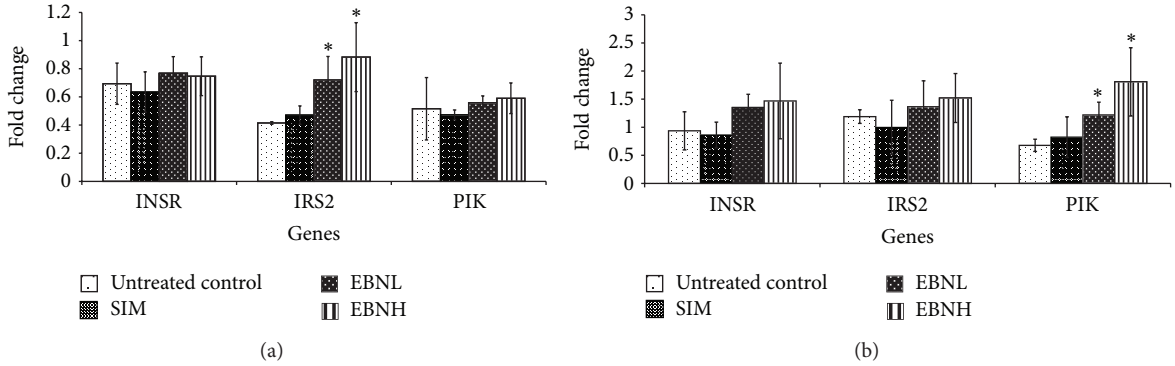

Figure 5: Effects of edible bird's nest (EBN) on (a) hepatic and (b) adipose tissue mRNA levels of insulin receptor (Insr), insulin receptor substrate (Irs) 2 and Phosphoinositide-3-kinase (PI3K) in high fat diet- (HFD-) fed rats. Groupings are similar to Figure 2. ∗ indicates significant difference ($P < 0.05$) in comparison with untreated control.

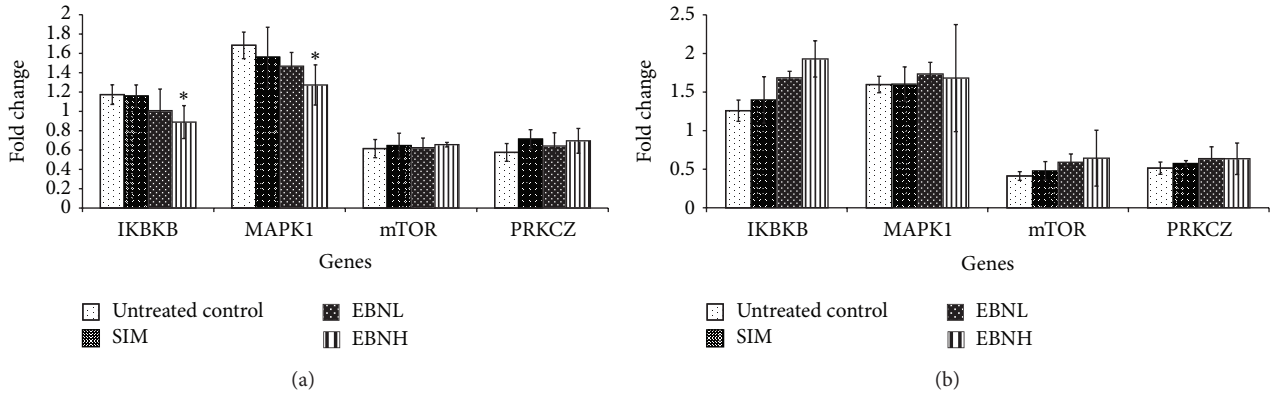

Figure 6: Effects of edible bird's nest (EBN) on (a) hepatic and (b) adipose tissue mRNA levels of mammalian target of rapamycin (mTOR), protein kinase C zeta (Prkcz), inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta (IKBKB), and mitogen-activated protein kinase (MAPK) 1 in high fat diet- (HFD-) fed rats. Groupings are similar to Figure 2. ∗ indicates significant difference ($P < 0.05$) in comparison with untreated control.

Additionally, upregulation of mitogen-activated protein kinase (MAPK) [32] and inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta (Ikbkb) [33] and downregulation of mammalian target of rapamycin (mTOR) [34] and protein kinase C, zeta (Prkcz) [35], as seen with the untreated control group (Figure 6) are thought to promote phosphorylation of IRS with consequent increase in insulin resistance due to disruption of IRS-mediated insulin action via activation of PI3K [28, 30]. Intervention with EBN upregulated the expression of Insr, IRS2 and PI3K in both liver and adipose tissues, but the difference was only significant for IRS2 in the liver and PI3K in the adipose tissue (Figure 5). These, however, suggest that EBN prevented HFD-induced insulin resistance through transcriptional regulation of insulin signaling genes. Moreover, EBN upregulated mTOR and Prkcz in the liver and adipose tissue but only caused downregulation of MAPK and Ikbkb in the liver indicating that the transcriptional changes induced by EBN had differential effects on insulin signaling genes in liver and adipose. Therefore, slightly different mechanisms may be involved in its enhanced insulin signaling in different tissues.

The activities of glucokinase (Gck) and pyruvate kinase (Pk) are affected in insulin resistance, decreasing the chances of intracellular glucose phosphorylation and its commitment to glycolysis [36]. In the adipose and liver tissues of untreated control group, we observed downregulation of the Gck and Pk genes, in line with increased insulin resistance (Figure 7). The levels of these genes are believed to directly influence the levels of cellular adenosine triphosphate (ATP) and consequently the activity of the potassium inwardly rectifying channel, subfamily J, member 11 (KCNJ11) gene, which regulates the ion channels involved in glucose sensing [37]. In this study, we observed downregulation of the KCNJ11 gene in both liver and adipose tissues, suggesting that the changes in Gck and Pk expression may have affected its expression through their effects on cellular ATP levels. EBN intervention was able to upregulate expressions of Gck, Pk, and KCNJ11 in both liver and adipose tissues.

Based on the patterns of expression in the liver and adipose tissues, we propose that EBN may be exerting its effect on insulin sensitivity through increased expression and likely activity of several genes involved in the insulin signaling

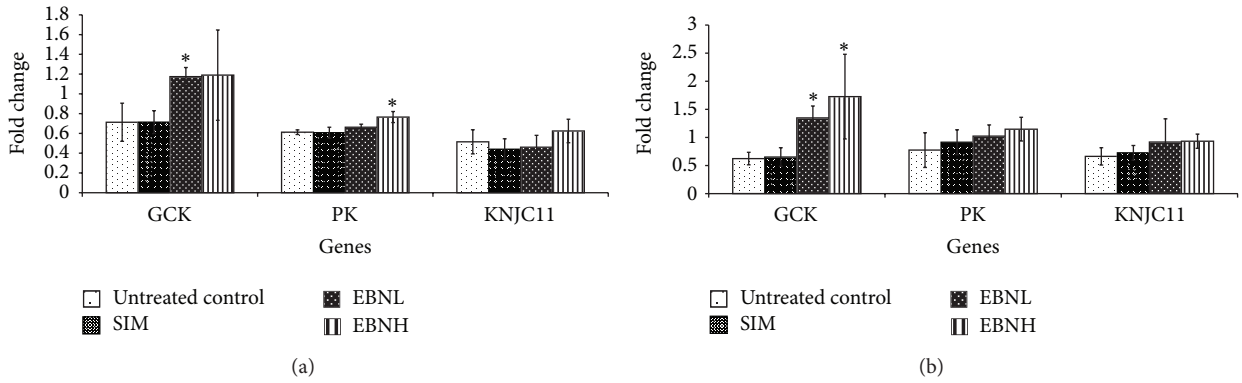

Figure 7: Effects of edible bird's nest (EBN) on (a) hepatic and (b) adipose tissue mRNA levels of Glucokinase (Gck), potassium inwardly rectifying channel, subfamily J, member 11 (KCNJ11), and pyruvate kinase-liver isoform (L-Pk) in high fat diet- (HFD-) fed rats. Groupings are similar to Figure 2. $*$ indicates significant difference ($P < 0.05$) in comparison with untreated control.

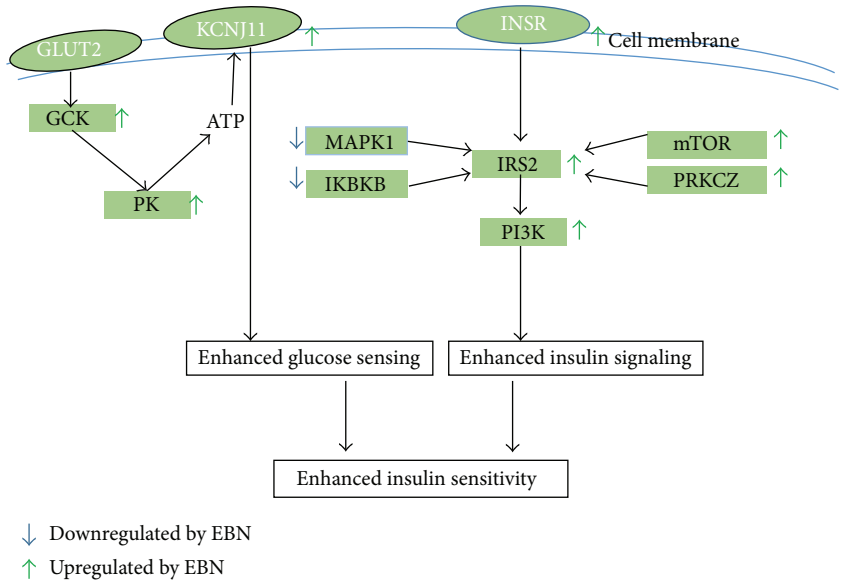

Figure 8: Proposed schematic showing targets of edible bird's nest (EBN) action in the insulin signaling pathway. EBN prevents insulin resistance in high fat diet rats by influencing the transcriptional regulation of multiple genes.

pathway in the liver and adipose tissues (Figure 8). Although simvastatin is able to lower cholesterol levels (Table 4), its effects on insulin signaling genes (Figures 5, 6, and 7) tended towards insulin resistance, in agreement with previous reports. Liver and adipose tissues are involved in development of insulin resistance, and in fact they have been proposed to be the organs from where the problem is initiated. Therefore, the enhanced sensitivity of insulin in these tissues suggests that EBN is effective at preventing insulin resistance. Furthermore, we hypothesize that synergism of multiple bioactives in EBN is contributing to the overall bioactivity observed.

4. Conclusions

In this study, we demonstrated that HFD will induce insulin resistance (higher OGTT, leptin and F2-isoprostane, and lower adiponectin levels), partly through transcriptional modulation of insulin signaling genes. Additionally, simvastatin was shown to further promote insulin resistance. EBN however is able to prevent insulin resistance by preventing some of the transcriptional changes on insulin signaling genes induced by HFD. There is need to further evaluate the potential use of EBN in the management of insulin resistance in already established insulin-resistant conditions.

Abbreviations

EBN: Edible bird's nest
Gck: Glucokinase
HFD: High fat diet
HOMA-IR: Homeostatic model assessment of insulin resistance
Ikbkb: Inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta

Insr: Insulin receptor
IRS: Insulin receptor substrate
KCNJ11: Potassium inwardly rectifying channel, subfamily J, member 11
Mtor: Mammalian target of rapamycin
MAPK: Mitogen-activated protein kinase
OGTT: Oral glucose tolerance test
PI3K: Phosphoinositide-3-kinase
Pk: Pyruvate kinase
Prkcz: Protein kinase C, zeta.

Conflict of Interests

The authors declare no conflict of interests.

Acknowledgments

The authors thank Ministry of Science, Technology and Innovation (MOSTI), Malaysia for sponsoring this research (e-Sciencefund 02-01-04-SF1453) and the staff of the Laboratory of Molecular Biomedicine for their assistance during the study.

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Hindawi Publishing Corporation
Journal of Diabetes Research
Volume 2015, Article ID 807891, 9 pages
http://dx.doi.org/10.1155/2015/807891

Clinical Study

Effect of Rosiglitazone and Insulin Combination Therapy on Inflammation Parameters and Adipocytokine Levels in Patients with Type 1 DM

Metin Guclu,[1,2] Ozen Oz Gul,[2] Soner Cander,[1,2] Oguzkaan Unal,[2,3] Guven Ozkaya,[4] Emre Sarandol,[5] and Canan Ersoy[2]

[1]*Division of Endocrinology, Sevket Yılmaz Research and Education Hospital, 16310 Bursa, Turkey*
[2]*Department of Endocrinology and Metabolism, School of Medicine, Uludağ University, 16210 Bursa, Turkey*
[3]*Acibadem Private Hospital, 16210 Bursa, Turkey*
[4]*Department of Biostatistics, School of Medicine, Uludağ University, 16210 Bursa, Turkey*
[5]*Department of Biochemistry, School of Medicine, Uludağ University, 16210 Bursa, Turkey*

Correspondence should be addressed to Metin Guclu; dr.metinguclu@gmail.com

Received 18 January 2015; Revised 10 March 2015; Accepted 17 March 2015

Academic Editor: Joseph Fomusi Ndisang

Aim. To investigate the efficacy of combined therapy of insulin and rosiglitazone on metabolic and inflammatory parameters, insulin sensitivity, and adipocytokine levels in patients with type 1 diabetes mellitus (type 1 DM). *Material and Methods.* A total of 61 adults with type 1 DM were randomly and prospectively assigned in open-label fashion to take insulin and rosiglitazone 4 mg/day ($n = 30$) or insulin alone ($n = 31$) for a period of 18 weeks while undergoing insulin therapy without acute metabolic complications. *Results.* Combination therapy did not significantly improve metabolic and inflammatory parameters, insulin sensitivity, and adiponectin levels. While leptin and resistin levels decreased in both groups (group 1: resistin 6.96 ± 3.06 to 4.99 ± 2.64, $P = 0.006$; leptin 25.8 ± 17.6 to 20.1 ± 12.55, $P = 0.006$; group 2: resistin 7.16 ± 2.30 to 5.57 ± 2.48, $P = 0.031$; leptin 16.72 ± 16.1 to 14.0 ± 13.4, $P = 0.007$) Hgb and fibrinogen levels decreased only in group 1 (Hgb 13.72 ± 1.98 to 13.16 ± 1.98, $P = 0.015$, and fibrinogen 4.00 ± 1.08 to 3.46 ± 0.90, $P = 0.002$). Patients in both groups showed weight gain and the incidence of hypoglycemia was not lower. *Discussion.* The diverse favorable effects of TZDs were not fully experienced in patients with type 1 DM. These results are suggesting that insulin sensitizing and anti-inflammatory characteristics of TZDs were likely to be more pronounced in patients who were not totally devoid of endogenous insulin secretion.

1. Introduction

There has been a progressive increase in the incidence of type 1 DM, and several advances in its treatment have been achieved. As a result, an increasing number of patients, who are older and have longer disease durations, are more severely affected by chronic complications [1–3]. Chronic subclinical inflammation, impaired fibrinolytic system activity, and elevated procoagulant factor levels form the basis of atherosclerotic diseases. Consequently, DM has been regarded as a major risk factor for cardiovascular diseases [4, 5]. Insulin resistance is characterized by limited stimulation of glucose metabolism in muscle and the liver and has been described in patients with poorly controlled type 1 DM [6–8].

Fatty tissue releases a number of adipocytokines associated with neuroendocrine and immune functions. Cytokines such as leptin, resistin, and adiponectin released from these tissues critically impact nutritional status, body fat distribution, metabolic parameters, inflammatory status, atherosclerotic alterations, and insulin resistance [9]. The levels of adiponectin, which is regarded as an antidiabetic, anti-inflammatory, and antiatherogenic cytokine, are reported to be depressed in patients with type 2 DM [10, 11]. However, in some studies, increased adiponectin levels have been

reported in patients with type 1 DM [12]. Resistin, on the other hand, impairs cellular glucose intake, because it is stimulated by insulin; as a result, hepatic glucose production is increased, leading to impaired glucose tolerance and eventual development of insulin resistance. Owing to its augmenting effect on the production of adhesion molecules, resistin is considered to be having proinflammatory effects in the vascular endothelium [13, 14]. Leptin has been shown to have important effects on both body energy balance and fat distribution [15, 16].

Glucose uptake rates by peripheral tissues, which can be stimulated by insulin in skeletal muscles, decrease over time in patients with type 1 DM and poor glycemic control. These patients have significant hepatic insulin resistance, and the effects of insulin are impaired because of plasma free fatty acids (FFAs) [17–19]. Despite recent advances in the management of DM, it has been suggested that cardiovascular disease-related mortality rates increase as more intensive therapies are required to ensure tight blood glucose control. Therefore, combination therapies are required to ensure tight glycemic control, minimize the risk of macrovascular disease, and reduce other cardiovascular risk factors [20].

Thiazolidinediones (TZDs) act by binding to "nuclear peroxisome proliferator activated receptor-gamma" (PPAR-γ), which is chiefly expressed in fatty tissue, and mediate their effects by activating the transcription of the genes that influence adipocyte differentiation as well as glucose and lipid metabolism [21–23]. TZDs decrease the triglyceride concentration in β-cells, leading to improved β-cell function. TZDs, apart from their direct effect on fatty tissue, might influence the release of adipocyte-derived signal factors that determine the insulin sensitivity of muscles, such as FFAs, adiponectin, leptin, and tumor necrosis factor-alpha (TNF-α). In addition to their favorable effects on glycemic control, TZDs directly influence vessel walls, decrease vasoconstriction, and inhibit inflammation. Therefore, they inhibit insulin resistance and slow down the atherosclerotic process [24–27]. In addition, it has been shown that TZDs could inhibit hyperglycemia-induced reactive oxygen species production from mitochondria (mtROS) by activating the PPAR-γ coactivator-1 alpha (PGC-1α) pathway which could contribute to the prevention of diabetic vascular complications [28, 29]. Agonists of PPAR-gamma and PPAR-alpha have been shown to upregulate the heme-oxygenase- (HO-) system which has been shown to increase insulin sensitivity, improve glucose/lipid metabolism, suppress inflammation/oxidative stress, decrease immune response, and modulate cell-growth/differentiation [30]. It has been also shown that there were beneficial effects of the HO-system in the pathogenesis of type 1 diabetes and related cardiometabolic complications [31].

Although, euglycemic-hyperinsulinemic clamp study is accepted standard for measurement of insulin sensitivity in patients with type 1 DM, it is not practical for use and is labor-intensive. Estimated glucose disposal rate (eGDR) is a derived measure of insulin resistance and can be calculated using routine clinical measures such as waist circumferences or waist-to-hip ratio, presence of hypertension, and HbA1c levels. As an insulin sensitivity index, it is well correlated with results obtained from clamp studies and it should be emphasized that lower eGDR levels indicate greater insulin resistance [32, 33].

The purpose of the present study was to investigate the efficacy of combined therapy of insulin and the oral antihyperglycemic agent rosiglitazone, a PPAR-γ agonist, on blood glucose regulation, total administered daily insulin dose, metabolic parameters, eGDR, FFAs, inflammatory indicators, and adipocytokine levels in patients with type 1 DM and poor glycemic control despite intensive insulin therapy.

2. Materials and Methods

The study was conducted between March 2007 and January 2008 at the Clinic of Endocrinology and Metabolism Diseases, following the approval of the Uludağ University Medical School Ethics Committee. After providing written consent, the patients were considered eligible based on the following criteria: age 18–65 years, diagnosis of type 1 DM, and a glycosylated hemoglobin (HbA1c) level >6.5% despite 40-unit (U) intensive insulin therapy, on average, for ≥6 months. Exclusion criteria were renal failure (glomerular filtration rate <75 mg/min or creatinine >1.5 mg/dL), chronic hepatic disease or aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels 2.5 times the normal values, current antidiabetic therapy other than insulin therapy, known history of rosiglitazone allergy, stage II–IV heart failure according to the New York Heart Association (NYHA) classification [34], inability of the patient to enforce strict lifestyle changes, medical nutrition therapy or self-monitoring of blood glucose levels, ongoing or planned pregnancy, and current lactation.

2.1. Study Protocol. This prospective, open-label, randomized trial investigated the effectiveness of 4 mg/day rosiglitazone for 18 weeks in patients undergoing insulin therapy and without acute metabolic complications in combination with strict lifestyle changes and effective medical nutrition therapy. The patients attended a screening visit (Visit 1) two weeks before randomization, and they were classified into two open-label groups, such that clinical and demographical characteristics were similar in the two groups. The patients were evaluated for lifestyle changes, diet and exercise compliance, and insulin requirements. Their therapies were modified, and the randomization visit (Visit 2) occurred two weeks later. The patients in both groups underwent insulin titration at Visit 2, and the patients in one group had 4 mg/day rosiglitazone added to their ongoing therapy (group 1), while the patients in the other group were monitored from that point with their most recent insulin titration (group 2). To reduce the risk of side effects, 4 mg rosiglitazone was initially administered in group 1. Then, the patients attended a control visit (Visit 3) four weeks after randomization and a final visit (Visit 4) after 16 weeks. During each of the four visits, variables within six different categories were monitored, including a detailed physical examination; self-monitoring of BG levels; glycemic control; therapy alterations and insulin requirements; adverse events; and biochemical, hematological, and inflammatory parameters, including adipocytokine levels.

2.2. Glycemic Control and Insulin Titration. In order to measure the direct effect of rosiglitazone, administered insulin doses were not changed unless patients developed acute metabolic complications. Recurrent hypoglycemia, presence of symptomatic hyperglycemia, diabetic ketosis (DK), diabetic ketoacidosis (DKA), and nonketotic hyperosmolar syndrome (NKHS) were considered metabolic complications. While patients with DK, DKA, NKHS, or major hypoglycemia were planned to be excluded from the study and hospitalized if necessary, patients with symptomatic hyperglycemia and recurrent minor hypoglycemia underwent insulin titration. Patients with symptoms of polydipsia, polyuria, weight loss, and nocturia were considered to be having symptomatic hyperglycemia if they also had mean BG levels >276 mg/dL; minor hypoglycemia if they were aware enough of their condition to administer self-therapy, were symptomatic, and had BG levels <56 mg/dL; or major hypoglycemia if they were unconscious, were unable to treat themselves, and recovered after therapy administered by others at home or at hospital.

2.3. Laboratory Methods. Blood samples were analyzed for fasting plasma glucose (FPG), urea, creatinine (Cr), AST, ALT, total cholesterol (total-C), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglycerides (TG) using an autoanalyzer (Aeroset System Operations Manual; Abbot Laboratories, Abbott Park, IL, USA). LDL-C levels in patients with TG values <400 mg/dL were calculated using the Friedewald formula as follows: LDL-C = total-C − [(TG/5) + HDL-C].

HbA1c levels were measured using high performance liquid chromatography (HPLC; BIO RAD Diagnostic Group, Hercules, CA, USA). Spot urine albumin excretion was measured by chemiluminescence immunoassay (Immulite 2500 Analyzer; Siemens, CA, USA), and creatinine levels were measured spectrophotometrically (Aeroset System Operations Manual; Abbot Laboratories, Abbott Park, IL, USA); in addition, the albumin/creatinine ratio (ACR) was calculated.

The eGDR was calculated as follows: $21.158 + (-0.009 \times \text{WC}) + (-3.407 \times \text{HTN}) + (-0.51 \times \text{HbA1c})$, where the WC indicates the waist circumferences and HTN indicates blood pressure and is expressed as 0: no, 1: yes. The range of clamp-measured glucose disposal rates in the eGDR validation study was 3.8 to 13.4, with a range of ~9 to 11 in those with normal insulin resistance [32].

FFA measurements were conducted using Wako NEFA-HR [2] (Wako Chemicals, GmbH, Neuss, Germany) and an *in vitro* enzymatic calorimetric assay method. The measureable range was 0.01–4.00 mEq/L.

White blood cell (WBC), hemoglobin (Hgb), hematocrit (Hct), and platelet (PLT) measurements were conducted using Cell-Dyn 3700 (MAPSS Laser Differential; Abbott Laboratories, Abbott Park, IL, USA).

Blood samples were collected as indicated and placed in Westergren tubes to establish erythrocyte sedimentation rates (ESRs) in a 1-hour period. The results were recorded as ESR in mm/h.

Fibrinogen was measured using the Coagulation System, Dade Behring BNII (Dade Behring Inc., Marburg, Germany). The measurable range was <1.8–3.5 mg/L.

The solid phase enzyme linked immunosorbent assay (ELISA) method was used with a High Sensitivity CRP Enzyme Immunoassay (DRG International Inc., Mountainside, NJ, USA) for the measurement of high-sensitivity C-reactive protein (hs-CRP). The measurable range was <0.1–10 mg/L, and intra- and interassay coefficients were 2.5% and 2.3%, respectively.

The solid phase ELISA method and a DRG Leptin Enzyme Immunoassay Kit (DRG GmbH, Marburg, Germany) were used to measure leptin. The expected results were 3.84 ± 1.79 ng/mL for men and 7.36 ± 3.73 ng/mL for women. The intra- and interassay coefficients were 5.95% and 8.66%, respectively.

Resistin was measured using the ELISA method with Human Resistin ELISA (BioVendor, Brno, Czech Republic). The normal range was considered to be 8.1 ± 4.0 ng/mL. The intra- and interassay coefficients were 2.8% and 5.1%, respectively.

Adiponectin was measured using the ELISA method with a High Sensitivity Human Adiponectin ELISA kit (BioVendor, Brno, Czech Republic). The normal range was dependent upon BMI and was 9.5 ± 3.9 μg/mL for men and 13.2 ± 6.1 μg/mL for women. The intra- and interassay coefficients were 4.1% and 4.0%, respectively.

2.4. Statistical Analysis. According to pilot study, a sample size of 30 patients per group was calculated to give 80% power to detect a difference of 6 in change from baseline in mean adiponectin between groups at the 5% 2-sided significance level, assuming 8 as the common SD. Statistical analyses were conducted using SPSS for Windows (Version 22.0; SPSS Inc., Chicago, IL, USA). Continuous variables are expressed as the mean ± standard deviation or median (minimum-maximum), as appropriate; categorical variables are expressed as frequencies (n, %). A one-way ANOVA was used to compare mean values for normally distributed variables when there were more than two independent groups, and the Kruskal-Wallis test was used when the assumptions for parametric tests were not met. The Mann-Whitney U test was used for nonparametric comparison of two groups. Paired data were analyzed using paired t-test and the Wilcoxon signed rank test when data were not normally distributed. For measurement at last visit, percent changes were calculated according to baseline measurement. These percent changes were compared. Pearson's chi-squared was used for comparison of the frequencies. $P < 0.05$ was considered statistically significant in all tests.

3. Results

The demographic characteristics and baseline glycemic levels are shown in Table 1.

There were no significant differences between the groups in sex, mean age, disease duration, BW, BMI, waist circumferences, waist-to-hip ratios, FPG, HbA1c values, and eGDR in the baseline and mean P value was higher than 0.05 for all parameters mentioned above. eGDR was 8.53 ± 2.70 mg/kg/min in group 1 and 9.25 ± 3.18 mg/kg/min in group 2. Patients in group 1 had higher FPG and HbA1c and

Table 1: Comparison of the demographic characteristics and baseline glycemic parameters between the groups.

Characteristics	Group 1 Insulin + Ros ($n = 30$)	Group 2 Insulin alone ($n = 31$)	P
Sex (women/men)	18/12	17/14	0.570
Age (years)	27.55 ± 8.48	27.09 ± 5.38	0.734
Diabetes duration (years)	10 ± 4.95	9.6 ± 4.92	0.814
BW (kg)	66.59 ± 8.6	63.13 ± 8.2	0.768
BMI (kg/m^2)	24.17 ± 2.62	22.97 ± 2.74	0.606
WC (cm)	83.80 ± 8.26	78.71 ± 9.11	0.135
WHR	0.85 ± 0.06	0.80 ± 0.06	0.773
FPG (mg/dL)	249.1 ± 69.5	223.2 ± 78.5	0.167
HbA1c (%)	9.22 ± 1.77	8.75 ± 1.14	0.886
eGDR (mg/kg/min)	8.53 ± 2.70	9.25 ± 3.18	0.120

BW: body weight; BMI: body mass index; WC: waist circumference; WHR: waist-to-hip ratio; SBP: systolic blood pressure; DBP: diastolic blood pressure; FPG: fasting plasma glucose; HbA1c: glycosylated hemoglobin; eGDR: estimated glucose disposal rate.

slightly low eGDR, in other words more insulin resistant than group 2.

The comparisons between the final and baseline values for each group are shown in Table 2.

The total number of patients in each group that completed the study was 28, as two patients in each group were excluded owing to acute metabolic complications and one patient in group 2 was excluded owing to noncompliance with the visit schedule. During the follow-up period, the changes in BW and BMI were statistically significant in both groups ($P < 0.05$ for group 1 and $P < 0.01$ for group 2), whereas the changes in WC and waist-to-hip ratio (WHR) were not significant.

Systolic blood pressure (SBP) significantly decreased in group 2; the changes in diastolic blood pressure (DBP) were similar in both groups. Although the final FPG and HbA1c values were lower than the baseline values in both groups, the changes were statistically significant only in group 2. No statistically significant differences were found in eGDR during the study period in both groups. eGDR was 8.53 ± 2.70 mg/kg/min at baseline and 8.36 ± 2.45 mg/kg/min at final visits in group 1. It was 9.25 ± 3.18 mg/kg/min at baseline and 9.22 ± 3.20 at final visits in group 2. P value was 0.185 for group 1 and 0.235 for group 2 in terms of eGDR changes between two visits. Urea, Cr, spot urine ACR, AST, and ALT levels did not significantly change in either group. Of the total-C, HDL-C, LDL-C, TG, and FFA levels, only the HDL-C levels in group 2 significantly changed ($P = 0.038$).

While Hgb and HCT values did not change significantly in group 2, a significant decrease in Hgb levels was observed in group 1. Of the ESR, fibrinogen, and hs-CRP levels, only fibrinogen levels significantly decreased in group 1, and the changes in the other parameters were not significant for either group.

Although the resistin and leptin levels decreased significantly and changes were statistically significant in both groups, adiponectin levels increased but the change was not significant. The total daily insulin doses administered during the follow-up period are also shown in Table 2. The changes observed both between and within the groups in total daily insulin doses per kilogram of BW were not significant.

Comparisons between the groups for the final and baseline values of inflammatory markers, adipocytokine, and FFA levels are shown in Table 3.

The differences between the groups in ESR and hs-CRP levels were not significant at both baseline and final visits. However, while the difference in fibrinogen levels was significant at the baseline visit, the values at the final visit were not different owing to the decrease in fibrinogen levels in group 1. Also, the differences between the groups in resistin, leptin, and adiponectin levels were not significant at both baseline and final visits.

Diabetes-related complications experienced by the patients over the 18-week follow-up period are shown in Table 4.

The mean hypoglycemia frequency was calculated by dividing the number of total hypoglycemia episodes for all of the patients over the 18-week period by the number of patients, and the mean change in BW for the same period was calculated similarly. The number of patients with acute complications or major hypoglycemic events was expressed as the total frequency for the group. Two patients in group 1 experienced major hypoglycemic events, while two patients in group 2 had to be hospitalized due to DKA (Table 4). While 5.35 minor hypoglycemia episodes were experienced per patient over the 18 weeks in group 1, the mean weight gain was 2.58 ± 3.10 kg during the same period. The patients in group 2, on the other hand, experienced 4.61 minor hypoglycemic episodes per patient, and the mean weight gain was 1.47 ± 1.53 kg during the same period. The P value for minor hypoglycemia was 0.437, and it was 0.142 for weight gain. Therefore, the difference between the groups with respect to either parameter was not statistically significant. Because of the low number of major hypoglycemia events and acute metabolic complications, statistical comparisons were not possible.

4. Discussion

The combined therapy approaches used in type 2 diabetes management have also become increasingly common in type 1 diabetes management. Furthermore, studies have demonstrated that insulin resistance is not exclusively observed in patients with type 2 diabetes and that it is also a critical factor for patients with type 1 diabetes because it can be overlooked in patients without adequate BG regulation [32, 35–38]. The primary goals when introducing insulin-sensitizing agents in combined therapy are ensuring good glycemic control, decreasing insulin demand, achieving favorable effects on cardiovascular risk factors, and minimizing alterations in BW. Strowig and Raskin reported improved glycemic control without an increase in insulin demand in 25 overweight adult patients with type 1 diabetes following therapy with

Table 2: Comparison between the baseline and final (week 16) values for each group.

Parameters	Group 1 Insulin + rosiglitazone			Group 2 Insulin alone		
	Baseline ($n = 30$)	Final ($n = 28$)	P	Baseline ($n = 31$)	Final ($n = 28$)	P
BW (kg)	66.59 ± 8.6	69.96 ± 9.29	**0.003**	63.13 ± 8.2	65.18 ± 8.20	**0.001**
BMI (kg/m^2)	24.17 ± 2.62	25.69 ± 2.57	**0.002**	22.97 ± 2.74	23.76 ± 2.73	<**0.001**
WC (cm)	83.80 ± 8.26	86.61 ± 8.53	0.107	78.71 ± 9.11	80.78 ± 8.09	0.109
WHR	0.85 ± 0.06	0.85 ± 0.05	0.792	0.80 ± 0.06	0.82 ± 0.06	**0.048**
SBP (mmHg)	114 ± 13.63	111.6 ± 13.7	0.604	116 ± 9.37	110.5 ± 9.62	**0.030**
DBP (mmHg)	75.25 ± 9.24	70.83 ± 9.11	0.061	75.95 ± 7.51	72.63 ± 7.70	0.174
FPG (mg/dL)	249.1 ± 69.5	219.7 ± 97	0.460	223.2 ± 78.5	178 ± 91	**0.006**
HbA1c (%)	9.22 ± 1.77	9.09 ± 1.40	0.239	8.75 ± 1.14	8.46 ± 1.19	0.050
eGDR (mg/kg/min)	8.53 ± 2.70	8.36 ± 2.45	0.185	9.25 ± 3.18	9.22 ± 3.20	0.235
Urea (mg/dL)	25.50 ± 7.17	26.66 ± 6.2	**0.032**	25.52 ± 6.03	27.84 ± 4.96	**0.042**
Cr (mg/dL)	0.83 ± 0.16	0.82 ± 0.26	0.935	0.86 ± 0.12	0.90 ± 0.31	0.602
ALT (IU/L)	18.25 ± 11.4	13.55 ± 4.3	**0.033**	15.85 ± 9.85	17.52 ± 13.3	0.912
Total-C (mg/dL)	172.8 ± 36.2	169.0 ± 31.8	0.935	177.3 ± 33.8	170.1 ± 34.5	0.678
HDL-C (mg/dL)	45 ± 5.90	47.80 ± 9.37	0.285	47.00 ± 9.35	51.47 ± 10.60	**0.038**
LDL-C (mg/dL)	105 ± 27.12	98.86 ± 27.89	0.688	111.15 ± 24.17	107.45 ± 30.1	0.668
TG (mg/dL)	113 ± 77.91	111.20 ± 52.41	0.971	79.47 ± 42.95	81.57 ± 27.14	0.821
FFA (mEq/L)	0.57 ± 0.43	0.53 ± 0.28	0.913	0.49 ± 0.25	0.47 ± 0.26	0.601
ACR (mg/min)	35.50 ± 42.91	23.57 ± 17.65	0.136	19.36 ± 19.00	12.54 ± 10.72	0.667
Hgb (g/dL)	13.72 ± 1.98	13.16 ± 1.98	**0.015**	13.74 ± 1.64	13.61 ± 1.04	0.838
HCT (%)	40.55 ± 5.48	39.06 ± 5.42	0.067	40.53 ± 4.44	40.65 ± 2.69	0.428
Resistin	6.96 ± 3.06	4.99 ± 2.64	**0.006**	7.16 ± 2.30	5.57 ± 2.48	**0.031**
Leptin	25.8 ± 17.6	20.1 ± 12.55	**0.006**	16.72 ± 16.1	14.0 ± 13.4	**0.007**
Adiponectin	17.48 ± 10.71	19.81 ± 11.21	0.145	11.90 ± 5.23	15.98 ± 9.47	0.948
Insulin dose (units/day)	64.45 ± 16.31	65.88 ± 15.29	NS	53.33 ± 13.45	52.78 ± 11.07	NS

BW: body weight; BMI: body mass index; WC: waist circumference; WHR: waist-to-hip ratio; SBP: systolic blood pressure; DBP: diastolic blood pressure; FPG: fasting plasma glucose; HbA1c: glycosylated hemoglobin; eGDR: estimated glucose disposal rate; CR: creatinine; AST: aspartate aminotransferase; ALT: alanine aminotransferase; Total-C: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; TG: triglycerides; FFA: free fatty acids; ACR: albumin/creatinine ratio; Hgb: hemoglobin; Hct: hematocrit.

Table 3: Comparisons between the groups for inflammatory marker, FFA, and serum adipocytokine levels at baseline and at the final visit (week 16).

Parameters	Randomization visit			Final visit		
	Group 1 Insulin + Ros	Group 2 Insulin only	P	Group 1 Insulin + Ros	Group 2 Insulin only	P
ESR (mm/h)	11.85 ± 11.36	10.89 ± 10.39	0.969	14.44 ± 13.14	11.00 ± 8.76	0.599
Fibrinogen (g/L)	4.00 ± 1.08	3.46 ± 0.90	0.047	3.46 ± 0.90	3.11 ± 0.92	0.271
hs-CRP (mg/L)	2.32 ± 2.64	1.41 ± 1.69	0.361	1.76 ± 1.14	1.76 ± 2.31	0.443
FFA (mEq/L)	0.57 ± 0.43	0.49 ± 0.25	0.917	0.53 ± 0.28	0.47 ± 0.26	0.518
Resistin (ng/mL)	6.96 ± 3.06	7.16 ± 2.30	0.489	4.99 ± 2.64	5.57 ± 2.48	0.650
Leptin (ng/mL)	25.8 ± 17.6	16.72 ± 16.1	0.130	20.1 ± 12.55	14.0 ± 13.4	0.724
Adiponectin (μg/mL)	17.48 ± 10.71	11.90 ± 5.23	0.760	19.81 ± 11.21	15.98 ± 9.47	0.091

Ros: rosiglitazone; ESR: erythrocyte sedimentation rate; hs-CRP: high-sensitivity C-reactive protein; FFA: free fatty acids.

Table 4: Complications observed in the groups.

Complication	Group 1 Insulin + Ros	Group 2 Insulin	P
Minor hypoglycemia events experienced per patient	5.35	4.61	0.437
Major hypoglycemia events	2	—	NA
Acute metabolic complication DK/DKA/NKNAHS	—	2	NA
Mean weight gain (kg)	2.58 ± 3.10	1.47 ± 1.53	0.142

Ros: rosiglitazone; DK: diabetic ketosis; DKA: diabetic ketoacidosis; NKNAHS: nonketotic nonacidotic hyperosmolar syndrome; NA: not available.

4 mg rosiglitazone twice a day for 8 months [39]. Although patients in both groups in the present study had decreased FPG and HbA1c levels, the changes were not significant. Baseline glucose and HbA1c values were higher in the patients receiving rosiglitazone; however, the differences in the changes between the groups were not significant. In a study conducted by Stone et al. [40], 36 patients with type 1 diabetes whose daily insulin requirement was >1.1 U/kg were administered 8 mg/day rosiglitazone. The investigators reported that the change in HbA1c levels was not significant when compared with the placebo group.

For decades, type 1 diabetes has been traditionally known as insulin-dependent, while type 2 has been known as noninsulin-dependent diabetes. However, it is becoming increasingly clear that insulin deficiency and insulin resistance are manifested in both forms of diabetes at different stages. Unlike other studies [39, 40] which investigate the effects of TZDs on insulin resistance and consist of obese or overweight patients with type 1 DM, almost all of the patients were lean in present study. eGDR is a validated clinical tool for estimating insulin sensitivity in patients with type 1 DM. It was near normal at baseline and did not change significantly during our study period in both groups. This result was important to show effects of rosiglitazone, except insulin-sensitizing characteristics. Thus, it can be thought that the effects of rosiglitazone observed in present study were not associated with insulin sensitivity. One of the known major effects of these drugs is on oxidative stress and mitochondrial ROS production. These effects may be key factors during the development of diabetic vascular complications. It has been shown that TZDs could inhibit hyperglycemia-induced mtROS and contribute to the prevention of diabetic vascular complications [41]. Although there were some studies investigating these effects in patients with type 2 DM, it is unclear in patients with type 1 DM. It is obvious that there is a need to investigate this effect in prospective cohort studies composed of patients with type 1 DM. In addition, agonists of PPAR-gamma might increase insulin sensitivity via upregulating heme-oxygenase-system. The HO-system and related products have been shown to decrease inflammation and enhance insulin sensitivity. More importantly, in experimental models of type 1 DM, upregulating HO-system caused increase in pancreatic beta cell insulin production. Beneficial effects of TZDs on insulin resistance and inflammation resulting from HO-system have been studied in patients with both type 2 and type 1 DM [30, 31, 42]. These developments may offer new options, either prevention of disease or development of the complications.

We observed significant BW and BMI changes in all patients at the final visit compared with baseline values. However, weight gain did not significantly differ between the baseline and final visits. The most significant side effect of TZDs, particularly when combined with sulfonylurea and insulin, is weight gain. TZDs increase overall fat tissue, and the most affected area is subcutaneous fat tissue. Another major cause of TZD-related weight gain is increased water and salt retention, which leads to increased plasma volume. Consequently, these drugs might lead to complications in patients with heart failure. Edema is caused by depressed renal sodium excretion and free water retention [43–45]. Sotton et al. reported a 10% increase in left ventricular mass without significant alterations in cardiac structure and function in patients undergoing rosiglitazone therapy. They attributed it mainly to the increase in plasma volume [46]. Although we observed weight gain in our patient groups in this present study, no patient had heart failure. Moreover, peripheral edema or dyspnea was not among the adverse events reported by our patients. This could be attributed to the relatively young mean age in our sample or to the fact that heart failure at baseline was among the exclusion criteria. Furthermore, no patient in this cohort had diabetic nephropathy that could lead to edema, which might have decreased the risk for edema.

The hypoglycemic effects of TZDs include increased insulin sensitivity that is mediated through TG and FFA metabolism and is associated with the agonistic effects of PPAR-γ. Increased FFA levels lead to insulin resistance and fasting plasma FFA levels in patients with type 2 diabetes administered TZD might decrease by 20–30%. PPAR-γ activation impairs TG and fatty acid synthesis, leading to decreased very-LDL-C and HDL-C synthesis as well as increased LDL-C and total-C levels [47, 48]. The patients in the present study with BMI and WC levels in the normal range had baseline TG and HDL-C levels within the desired range, and the LDL-C levels in all patients were close to 100 mg/dL. While slight decreases in total-C, LDL-C, TG, and FFA levels were noted in the patients receiving rosiglitazone at the end of the present study, HDL-C levels had increased slightly. Patients receiving only insulin had similar values to those in the combined therapy group except for slightly higher TG levels, although this was not statistically significant. Comparisons within and between the groups did not show significant differences. We suggest that the strict enforcement of lifestyle changes during the follow-up period might have contributed to these results.

TZDs have been demonstrated to have significant anti-inflammatory characteristics in studies conducted on patients with type 2 diabetes. Calkin et al. reported that rosiglitazone reduces diabetes-related atherosclerosis and that this effect is possibly associated with oxidative stress and inflammation, independent of metabolic effects, unrelated to the insulin dose [49]. In particular, the Diabetes Control and Complications Trial, as well as a number of other studies, revealed

that hs-CRP levels increased in patients undergoing intensive therapy. Furthermore, hs-CRP and increased fibrinogen levels are independent risk factors for coronary heart disease, and a number of studies have reported elevated fibrinogen levels in patients with diabetes [50, 51]. The ESR levels in the present study were within the normal range at baseline, and they continued to be so until the end of study. While hs-CRP levels decreased with respect to baseline values in the patients receiving rosiglitazone in the present study, they increased slightly in the patients receiving insulin alone. To the best of our knowledge, this is the first study to measure fibrinogen levels in patients with type 1 diabetes after rosiglitazone administration. The levels decreased significantly in these patients, while the patients undergoing therapy with insulin alone showed minimal decreases in fibrinogen levels. Independent of the improvement in BG regulation, levels of hs-CRP and fibrinogen, which are conventional inflammatory markers, decreased significantly following rosiglitazone therapy in the present study.

Plasma leptin levels are positively correlated with female sex, BMI, and age but not with diabetes duration, HbA1c, or total insulin dose per kilogram [52]. Most patients with type 1 diabetes are either underweight or in the normal BW range. Data concerning leptin levels in this group of patients vary, and exogenous insulin therapy leads to elevated leptin levels in patients with type 1 diabetes [53, 54]. Resistin, which is released from adipose tissue, is directly associated with insulin resistance factors such as WC and WHR. Shalev et al. reported increased serum resistin levels in patients with type 1 diabetes and reported that levels returned to the normal range after pancreas transplant [55, 56]. The patients in the present study had normal BW, BMI, and WC. The leptin levels were within the normal range at both baseline and final visits. While no significant differences were observed between the groups at the baseline or final visit or in the change between the visits, the within-group changes during the follow-up period were significant, despite the weight gain. Resistin levels were low in both groups, and the change in the patients receiving rosiglitazone was significant, despite weight gain, and was associated with the favorable effects of rosiglitazone.

Adiponectin has been shown to have positive effects on cardiometabolic risk, and adiponectin levels are negatively correlated with insulin resistance and weight gain. Moreover, good glycemic control increases adiponectin levels, whereas poor glycemic control decreases adiponectin levels [57, 58]. In the CACTI trial, Maahs et al. reported negative correlations between adiponectin levels and male sex, central adiposity, SBP, DBP, daily insulin dose, HbA1c, fibrinogen, albumin excretion rate, and TG levels; positive correlations were noted with type 1 diabetes, HDL-C, and homocysteine [59]. In the present study, adiponectin levels increased parallel to glycemic improvement both in patients undergoing combined therapy and in those receiving insulin therapies alone. Thus, the increase was independent of the therapy.

In conclusion, the diverse favorable effects of TZDs previously reported in patients with type 2 diabetes were not fully experienced in patients with type 1 diabetes in the present study. The addition of 4 mg/day rosiglitazone to intensive insulin therapy did not significantly improve glycemic parameter, lipid parameter, FFA, ESR, hs-CRP, leptin, or resistin levels. Only fibrinogen levels were significantly different between the groups. Patients receiving rosiglitazone showed weight gain, a major side effect of TZDs, whereas insulin sensitivity was not significantly different and the incidence of hypoglycemia was not lower. HbA1c, FPG, and total daily insulin doses decreased significantly after combination therapy with TZD and insulin in patients with type 2 diabetes mellitus, suggesting that the insulin-sensitizing characteristics of TZDs are likely more pronounced in patients who are not totally devoid of endogenous insulin secretion.

We acknowledge the limitations of the current study including single centre experience and small sample size. Patients treated in our series did not appear to have driven any meaningful benefit from combination therapy. Due to small number of patients included in study, prospective clinical trials are however required to define optimal treatment regimens. Clinical prognostic factors evaluated in our series may be useful for stratification and eligibility considerations in future clinical trials.

Conflict of Interests

The authors declare that they have no conflict of interests.

Authors' Contribution

This study was designed by Metin Guclu and Canan Ersoy. Patient selection and follow-up visits were organized by Metin Guclu, Ozen Oz Gul, Soner Cander, and Oguzkaan Unal. Statistical analyses were done by Guven Ozkaya. Biochemical analyses except routine metabolic investigation such as measurements of adipocytokines were performed by Emre Sarandol. The paper was written by Metin Guclu.

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Hindawi Publishing Corporation
Journal of Diabetes Research
Volume 2015, Article ID 490842, 14 pages
http://dx.doi.org/10.1155/2015/490842

Review Article

GDF-15 as a Target and Biomarker for Diabetes and Cardiovascular Diseases: A Translational Prospective

Ramu Adela and Sanjay K. Banerjee

Drug Discovery Research Center, Translational Health Science and Technology Institute (THSTI), Faridabad, Haryana 122014, India

Correspondence should be addressed to Sanjay K. Banerjee; skbanerjee@thsti.res.in

Received 11 December 2014; Revised 19 January 2015; Accepted 20 January 2015

Academic Editor: Joseph Fomusi Ndisang

Growth differentiation factor-15 (GDF-15) is a stress responsive cytokine. It is highly expressed in cardiomyocytes, adipocytes, macrophages, endothelial cells, and vascular smooth muscle cells in normal and pathological condition. GDF-15 increases during tissue injury and inflammatory states and is associated with cardiometabolic risk. Increased GDF-15 levels are associated with cardiovascular diseases such as hypertrophy, heart failure, atherosclerosis, endothelial dysfunction, obesity, insulin resistance, diabetes, and chronic kidney diseases in diabetes. Increased GDF-15 level is linked with the progression and prognosis of the disease condition. Age, smoking, and environmental factors are other risk factors that may increase GDF-15 level. Most of the scientific studies reported that GDF-15 plays a protective role in different tissues. However, few reports show that the deficiency of GDF-15 is beneficial against vascular injury and inflammation. GDF-15 protects heart, adipose tissue, and endothelial cells by inhibiting JNK (c-Jun N-terminal kinase), Bad (Bcl-2-associated death promoter), and EGFR (epidermal growth factor receptor) and activating Smad, eNOS, PI3K, and AKT signaling pathways. The present review describes the different animal and clinical studies and patent updates of GDF-15 in diabetes and cardiovascular diseases. It is a challenge for the scientific community to use GDF-15 information for patient monitoring, clinical decision-making, and replacement of current treatment strategies for diabetic and cardiovascular diseases.

1. Introduction

Prevalence of diabetes is reaching epidemic proportions in young people due to increase in life expectancy, sedentary life style, and obesity. Adults with diabetes and obesity are more prone to cardiovascular complications (World health statistics 2014). As per the International Diabetic Federation (IDF) diabetes atlas (Sixth edition 2013), the number of people with diabetes is 382 million and it is going to rise to 592 million by 2035. Global burden of diabetes is huge and 548 billion dollars was spent in 2013. In India, approximately 65.1 million people are with diabetes [1]. The prevalence, incidence, and mortality of cardio vascular diseases are 2–8-fold higher in persons having diabetes than those without diabetes [2].

Diabetes is characterized by high glucose level in blood due to either less insulin secretion from pancreas or developing insulin resistance in skeletal muscle. Diabetes is categorized into many types; however, two major types of diabetes are type 1 diabetes (T1DM) and type 2 diabetes (T2DM). T1DM is an autoimmune disease and result of autoimmune destruction of β cells. Thus patients with T1DM are not able to secrete sufficient insulin in blood or totally lose insulin secretary capacity. T2DM is the commonest form and it is characterized by insulin resistance mostly in skeletal muscle and deficiency of insulin release at end stage. In general, T2DM causes elevation of blood glucose level and other components of metabolic syndrome. Parameters of metabolic syndrome are elevated blood pressure, elevated triglycerides, reduced high density lipoprotein levels, and

abdominal obesity. An increase in adipose tissue (abdomen obesity) results in elevation of adipokines, that is, free fatty acids (FFA), tumor necrosis factor (TNF-α), C-reactive protein (CRP), interleukin-6 (IL-6), plasminogen activator inhibitor-1 (PAI-1), adiponectin, and leptin. Adipocytokines integrate the endocrine, autocrine, paracrine signals to mediate the insulin sensitivity, oxidative stress, energy metabolism, blood coagulation, and inflammatory responses. Elevated levels of FFA induce insulin resistance and increase fibrinogen and PAI-1. In the long run, high FFA and glucose together impair beta cell function through lipotoxicity and glucotoxicity and develop macro- and microvascular complications [3, 4]. Recently GDF-15 was identified as one of the important plasma markers, which correlates with cardiometabolic syndrome.

Growth differentiation factor-15 (GDF-15) is a member of the transforming growth factor-β (TGF-β)/bone morphogenetic protein (BMP) super family. GDF-15 is also known as macrophage inhibiting cytokine 1 (MIC-1), placental transformation growth factor (PTGF-β), prostate derived factor (PDF), placental bone morphogenetic protein (PLAB), NSAID activated gene-1 (NAG-1), and PL74 [5, 6]. Initially GDF-15 was reported to inhibit TNF-α production in lipopolysaccharide-stimulated macrophages and thus named as macrophage inhibitory cytokine-1(MIC-1) [7]. However, Subsequent studies did not confirm the same concept of macrophage suppression [8].

GDF-15 is produced as $a \approx 40$ kDa propeptide form. The N terminus is cleaved and released as $a \approx 30$ kDa disulphide linked dimeric active protein form [9]. GDF-15 is a growth factor whose expression increases with age. Biologic age is related to the several markers such as oxidative stress, protein glycation, inflammation, and hormonal changes. Many of these stresses induce GDF-15 expression by either p53 or early growth response protein -1 (EGR-1) transcription factors [10–12]. GDF-15 levels are also affected by environmental factors independently of genetic background. One study found that GDF-15 level is a novel and powerful predictor of all-cause mortality in general population and independent of several markers associated with mortality risk including age, body mass index (BMI), smoking history, IL-6, CRP, and telomere length [13].

Higher level of GDF-15 is associated with increased cardiovascular and noncardiovascular mortality; it plays pivotal role in development and progression of cardiovascular diseases such as heart failure, coronary artery diseases, atrial fibrillation, diabetes, cancer, and cognitive impairment (Figure 1) [14, 15]. Increased GDF-15 expression is a feature of many cancers including breast, colon, pancreas, and prostate. Many epithelial tumor cell lines secrete high levels of GDF-15. Several studies showed that higher expression of GDF-15 mRNA and protein was found in cancer biopsies [16–18]. High expression of GDF-15 in tumor is also associated with an increase in serum GDF-15 levels, suggesting the use of serum GDF-15 measurement for the diagnosis and management of cancer [9, 18–20]. In this present review, we described all studies on GDF-15 that reports its role in diabetes and cardiovascular diseases. We explained how GDF-15 could be used as a prognostic and diagnostic biomarker for cardiometabolic diseases. We have also looked into the potential of GDF-15 as a novel target for diabetes and cardiovascular diseases.

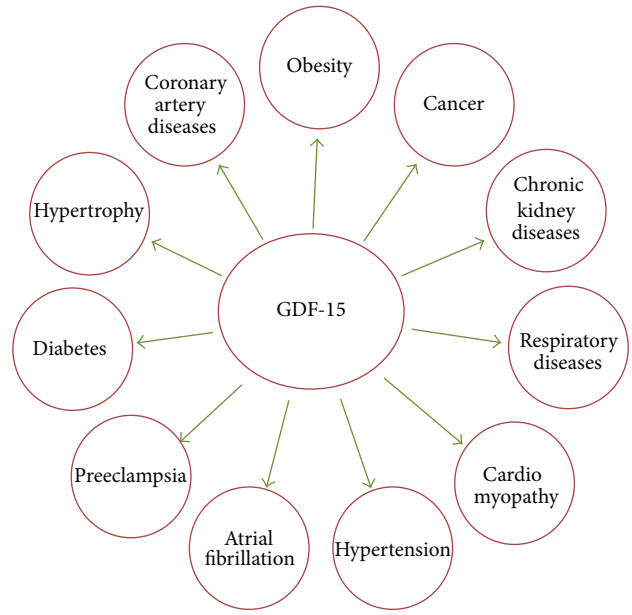

Figure 1: Role of GDF-15 in different diseases conditions. GDF-15 plays an important role to modulate metabolic, cardiovascular, obesity, cancer, and chronic disease.

2. GDF-15 Expression and Release

GDF-15 is highly expressed in the placenta and prostate but also expressed in heart, pancreas, liver, kidney, and colon [6, 46, 47]. It is a stress-induced cytokine and also releases from macrophages [48], vascular smooth muscle cells [49], cardiomyocytes, adipocytes [50], and endothelial cells [51] after tissue injury, anoxia, and proinflammatory cytokines responses. GDF-15 plays a role as an endocrine factor if present in circulation [52]. GDF-15 highly expressed in response to different kinds of cytokines and growth factors like interleukin-1β (IL-1β), TNF-α, angiotensin II, macrophage colony stimulating factor (M-CSF), and TGF-β. Tumor suppressor protein p53 also induces GDF-15 and thus acts as a growth inhibitory molecule in tissue [7, 46, 47, 53]. GDF-15 expression is highly induced in cardiomyocytes after ischemia/reperfusion [53]. Increased expression of GDF-15 was observed in the mouse and human heart within hours after myocardial infarction and remains elevated in the infarcted myocardium for several days. Cardiomyocytes in the infarct border zone have been identified as the main source of GDF-15 [54]. There is a controversy regarding the production sites of GDF-15 during heart failure conditions. Although GDF-15 is strongly released from the infracted human heart [54], it may also be released from macrophages [48]. Lok et al. reported that there is no evidence for the myocardial expression of GDF-15 in patients having advanced nonischemic heart failure. However, the circulating GDF-15 levels were increased the same as cardiac troponin and natriuretic peptides levels in serum. This study indicates that

Table 1: List of human studies dealing with GDF-15 levels in obesity and diabetes.

Disease/population/follow-up period	Sample size	Major findings	Reference
T1DM patients with diabetic nephropathy (8.1 years)	451	GDF-15 can be used to detect faster deterioration of kidney function	[21]
Obese nondiabetic (XENDOS) trial (4 years follow Up period)	496	GDF-15 is altered among patients having abdominal obesity and insulin resistance and independently associated with future insulin resistance and abnormal glucose control	[22]
Morbidly obese patients	118	GDF-15 changes following bariatric surgery suggest an indirect relationship between GDF-15 and insulin resistance	[23]
Type 2 diabetes (whitehall II study) (11.5 ± 3.0 years follow up period)	552	Baseline GDF-15 concentrations were increased in individuals before type 2 diabetes manifestation	[24]
Patients with obesity and/or obesity and type 2 diabetes mellitus	54	Elevated GDF-15 levels in patients with obesity are further increased by the presence of T2DM	[25]
Preeclampsia and diabetic pregnancies	267	GDF-15 is dysregulated, both in preeclampsia and in diabetic pregnancies	[26]
Patients with T2DM included in screened for the presence of diabetic cardiomyopathy (DC)	T2DM ($n = 213$)	GDF-15 represents a useful and novel tool to screen diabetic cardiomyopathy (DC) in patients with type 2 DM	[27]
Diabetic nephropathy	T2DM ($n = 30$), microalbuminuria ($n = 20$), macroalbuminuria ($n = 30$) patients	Suggesting its value in early diagnosis, evaluation, and prediction of the outcomes of type 2 diabetic nephropathy	[28]

GDF-15 may also be released outside of the heart [55, 56]. Strelau et al. reported that GDF-15 is highly expressed in the central nervous system (CNS) and peripheral nervous system (PNS), mainly in the choroid plexus and is secreted into the cerebral spinal fluid (CSF) [57]. Wiklund et al. have stratified the blood GDF-15 levels into three categories, that is, normal (<1200 pg/mL), moderately elevated (1200–1800 pg/mL), and highly elevated (>1800 pg/mL). They reported that 61% people survived when GDF-15 levels were more than 1800 pg/mL^{-1} [13].

3. GDF-15, Obesity, and Diabetes

Obesity is a risk factor for diabetes and cardiovascular diseases. Excess body weight is associated with increased health problems and cause increased cardiovascular morbidity and mortality [58]. GDF-15 released from macrophages, liver and white adipose tissue may act as a metabolic regulator. GDF-15 acts as adipokine like adiponectin and leptin [50] and thus has also been termed as cardiokine [59]. Adipokines, in general, regulate the lipid and glucose metabolism, increase insulin sensitivity, regulate food intake and body weight, and protect from chronic inflammation in adipose tissue [3]. Recently Macia et al. found that GDF-15 decreases food intake, body weight, and adiposity and improves glucose tolerance in normal and obesogenic diets [60]. Several human studies dealing with GDF-15 levels in obesity and diabetes are shown in Table 1. Serum GDF-15 levels were increased in obese and type 2 diabetic women and correlated with body mass index (BMI), body fat, glucose, and C-reactive proteins [50]. Vila et al. reported that median interquartile range (IQR) plasma GDF-15 was 427 (344–626) ng/mL in obese patients as compared to the controls 309 (275–411) ng/mL. Increased GDF-15 levels are strongly associated with waist to height ratio, age, arterial blood pressure, triglycerides, creatinine, glucose, insulin, glycated hemoglobin (HbA1c), and C-peptide. Age, insulin resistance, and creatinine were independent predictors of GDF-15 in obese patients [23]. Recently Chrysovergis et al. reported that GDF-15 is a novel therapeutic target in preventing and treating obesity and insulin resistance by modulating metabolic activity through increased expression of key thermogenic and lipolytic genes in brown adipose tissue (BAT) and white adipose tissue (WAT) [61].

Hyperglycemia is one of the main chronic symptoms of diabetes. In hyperglycemic conditions, increased reactive oxygen species (ROS) formation leads to cellular injury and cell death [61, 62]. Increased ROS generation in HUVEC cells can cause apoptosis by inhibiting the PI3 K/AKT/eNOS/NO pathway and activation of NF-κB/JNK/caspase-3 pathway [63]. Li et al. proved that increased GDF-15 protects endothelial cells against high glucose induced cellular injury by activating PI3 K/AKT/eNOS signaling pathway and attenuating NF-κB/JNK activation. Nitric oxide production was significantly lower in GDF-15 siRNA transfected HUVEC cells. This study concluded that GDF-15 plays protective role against cell apoptosis through PI3 K/Akt/eNOS pathway but not ERK1/2 and SMAD2/3 (Figure 2) [5]. They found that high glucose increases GDF-15 expression and its secretion, which modulates cell apoptosis in negative feedback manner [5]. As discussed before, GDF-15 is expressed by the adipose tissue through p53, a transcriptional factor that links GDF-15

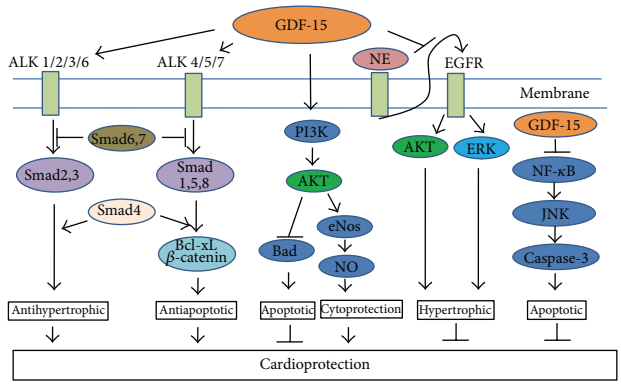

FIGURE 2: GDF-15 regulates signaling pathways essential for cardioprotection. GDF-15 shows cardioprotective effect through activation of ALK type 1 receptors (ALK 1–7) and phosphorylation of Smad2/3 and Smad1/5/8. After phosphorylation, Smad translocates to the nucleus in the form of heteromeric complex with Smad 4 and activates antihypertrophic pathway. GDF-15 also activates PI3 K/AKT/eNOS/NO pathway and shows cardioprotection. GDF-15 inhibits epidermal growth factor receptor (EGFR) transactivation and NF-κB/JNK/caspase-3 pathway to show its cardioprotective effect.

with obesity and insulin resistance. Expression of GDF-15 is controlled by both p53 dependent and independent mechanisms [46, 64]. Li et al. explained that increase in GDF-15 expression by high glucose in HUVEC cells was p53 dependent pathway. Inhibition of high glucose-induced p53 accumulation by p53 siRNA abolished GDF-15 induction [5]. Obesity promotes p53 activation in adipose tissue and leads to increased production of proinflammatory cytokines, insulin resistance, and diabetes. GDF-15 expression is rapidly induced by proinflammatory cytokines and thus serves as a marker for inflammation in adipose tissue [22, 25, 50, 65]. Insulin resistance and increased GDF-15 both are associated with endothelial dysfunction. The endothelial dysfunction may lead to metabolic derangement, inflammation. and vascular injuries and is associated with increased cardiovascular complications [22]. Increased glucose level in urine and decreased expression of glucose transporters (Glut 1, Glut 2, SGLT 1, and SGLT 2) was observed in type 2 diabetic GDF-15 knockout mice. Similar increased urinary volume was observed in streptozotocin (STZ) induced mice without any alteration of glucose transporters. GDF-15 expression was upregulated within the first 7 days of STZ induced diabetic rats and mice [66]. Khan et al. explained in their study that GDF-15 but not NT-proBNP is raised in patients who have diabetes. Diabetes independently influences the levels of GDF-15 [29]. XENDOS study reported that plasma GDF-15 levels are positively associated with HOMA-IR, an index of insulin resistance [22].

Activated macrophages will secrete proinflammatory, chemotactic cytokines, and chemokines that impair β cells function, insulin sensitivity, and infiltration of monocytes into the tissues [24, 67]. Several studies reported that numbers of macrophages were increased in pancreatic islets in type 2 diabetic patients [68]. GDF-15 concentration in plasma was increased in individuals with early stages of T2DM manifestation. Dostalova et al. reported that serum concentration of GDF-15 was increased approximately 1.2- and 2-fold in obese and T2DM women patients, respectively, compared to control subjects. Serum GDF-15 levels are positively correlated with body weight, body fat, triglyceride, glucose, HbA1c, and C-reactive protein [25]. Sugulle et al. observed that plasma GDF-15 levels was elevated in preeclampsia (5978 median (3822–15652 IQR) ng/L) and superimposed preeclampsia in diabetes mellitus (6002 (4230–11830) ng/L) compared to the control subjects (3710 (1860–6266) ng/L) [26]. However some group of scientists believed that it is not associated with the incident type 2 diabetes rather its increase in plasma might be part of anti-inflammatory response for the onset of diabetes [24]. Scientific data showed that serum GDF-15 could be a potential marker to identify individuals who are at risk for diabetes and obesity. However, a longitudinal study should be done where we can identify the early stage of a disease when GDF-15 level starts to increase in serum. More research needs to be carried out to find if administration of GDF-15 has any role to reduce inflammation or early pathological changes in diabetes and obesity.

4. GDF-15 and Cardiovascular Diseases

Cardiovascular (CV) diseases, that is, atherosclerosis, hypertension, hypertrophy or heart failure, myocardial infarction (MI), coronary artery disease (CAD), or stroke, are the most prevalent diseases and major cause of the death worldwide [69]. Aging, diabetes, and other risk factors will increase the disease progression by inducing left ventricular hypertrophy, endothelial dysfunction, hypertension, and vascular diseases [65]. GDF-15, the first TGF-β protein family, plays a cardioprotective role in the adult heart through activation of Smad2, Smad3, and ALK4/5/7 receptors [70]. GDF-15 is not expressed in heart under normal physiological conditions but increases rapidly in response to cardiovascular injury, such as pressure overload, heart failure, ischemia/reperfusion, and atherosclerosis [54, 71]. Several human studies dealing with GDF-15 levels in cardiovascular diseases are shown in Table 2. GDF-15 showed antiapoptotic effect against ischemia reperfusion (I/R) and reduced the size of myocardial infarction (MI). GDF-15 activates Smad1 and reduces apoptotic cell death through upregulation of Bcl-xL and β-catenin. Similarly, BMP-2 also exerts antiapoptotic effect through activation of Smad1. GDF-15 and BMP-2 show similarities in their primary structure and Smad activation. GDF-15 is more close to the BMP-2 family than the TGF-β subfamily. BMP-2 activates ALK-2/3/6 and phosphorylates Smad1/5. Similarly, GDF-15 activates type I receptors and Smad1/5 [70]. All these signaling pathways regulated by GDF-15 are responsible for cardioprotection (Figure 2).

GDF-15 predicts adverse outcomes in patients with acute chest pain, MI, or chronic angina [29, 30, 45, 72]. GDF-15 is an emerging biomarker, as it is elevated in early subclinical disease and has prognostic utility for cardiovascular events and mortality [37]. Recent findings showed that GDF-15 levels were associated with lower left ventricular ejection fraction

Table 2: List of human studies dealing with GDF-15 levels in cardiovascular diseases.

Disease/population/follow-up period	Sample size	Major findings	Reference
Acute myocardial infarction [AMI]	1142	GDF-15 is a prognostic marker of death and HF in patients with AMI Multimarker approach with GDF-15 and NT-pro-BNP is more informative than either marker alone and may be useful for risk stratification in AMI patients	[29]
Acute coronary syndrome [ACS] (PROVE IT-TIMI 22)	3501	GDF-15 is altered with recurrent events after ACS. GDF-15 may be used as a prognostic marker in ACS	[30]
Human model of acute muscle wasting following cardiac surgery	42	GDF-15 is a potential novel factor associated with muscle atrophy, which may become a therapeutic target in patients with ICU acquired paresis and other forms of acute muscle wasting	[31]
Non–ST-elevation ACS (FRISC-II) trial (2 years)	2079	GDF-15 is a potential tool for risk stratification and therapeutic decision making in patients with non-ST-elevation acute coronary syndrome	[32]
General adult population (Dallas Heart Study) (7.3 years follow up period)	3219	GDF-15 is independently marker for subclinical coronary atherosclerosis and mortality	[33]
Framingham Offspring cohort participants (9.5 years follow up period)	2614	Higher circulating GDF-15 was observed with incident renal outcomes and improves risk prediction of incident chronic kidney diseases (CKD)	[34]
Hypertensive left ventricular hypertrophy (H-LVH), hypertensive cardiomyopathy (HCM)	149	GDF-15 might be a useful biomarker for discriminating HCM from H-LVH	[35]
Patients with preclinical diastolic dysfunction or heart failure with normal ejection fraction (HFnEF)	119	GDF-15 levels are elevated in subjects with HFnEF and can differentiate normal diastolic function from asymptomatic LVDD	[36]
Patients with stable ischemic heart disease (Heart and Soul study) (8.9 yrs follow-up period)	984	Higher GDF-15 level was observed with major cardiovascular (CV) events in patients with stable ischemic heart disease	[37]
Untreated hypertensive patients	299	Plasma GDF-15 level was increased with LVH in hypertensive patients	[38]
71-year-old men (ULSAM study)	940	In elderly men, GDF-15 improves progression of both cardiovascular, cancer mortality, and morbidity beyond established risk factors and biomarkers of cardiac, renal dysfunction, and inflammation	[39]
Heart failure (Val-HeFT study)	1734	Providing independent prognostic information in heart failure	[40]
Coronary artery diseases (CAD)	CAD ($n = 348$) and ($n = 205$) controls	Significant differences of GDF-15, IMA, and PAPP-A in patients with CAD. GDF-15 might be associated with severity of CAD	[41]
Coronary Artery Bypass Grafting with Cardiopulmonary Bypass	34 patients	GDF-15 levels were increased substantially and it is associated with the renal and cardiac biomarkers	[42]
Patients on maintenance hemodialysis	Hemodialysis ($n = 87$), and controls ($n = 45$)	Relation between GDF-15, mortality, and carotid artery thickening suggests that GDF-15 may be a novel marker of atherosclerosis, inflammation, and malnutrition in hemodialysis patients	[43]
ST segment elevation myocardial infarction (STEMI) (3 years)	Patients with STEMI ($n = 216$)	High GDF-15 level is a strong predictor of death and heart failure in patients with STEMI. Although patients with higher GDF-15 levels tend to have lower LV ejection fraction	[44]
Acute chest pain (APACE study)	646	GDF-15 is a better predictor of mortality than of nonfatal CV events	[45]

(LVEF), worse diastolic function, greater inducible ischemia, and lower exercise capacity. GDF-15 is also correlated with NT-proBNP, reduced plaque burden, left ventricular mass, concentric left ventricular hypertrophy, coronary artery disease, and heart failure [37, 73]. Role of GDF-15 in different cardiovascular diseases condition is described below.

4.1. Hypertrophy. Cardiac hypertrophy is typically characterized by enlargement of the heart associated with an increase in cardiomyocyte cell size in response to physiological stimuli such as exercise and pathophysiological stimuli such as hypertension, ischemic heart diseases, valvular insufficiency, infectious agents, or mutations in sarcomeric genes [71]. Hypertensive patients are more prone to left ventricular hypertrophy (LVH). LVH is an early change for the cardiac damage in hypertension [74]. The prevalence of LVH in hypertensive patients was about 25% to 35% in China [75, 76]. It has been shown that LVH increases the risk of stroke, coronary heart disease, congestive heart failure, arrhythmias, and sudden cardiac death. All these are associated with cardiovascular morbidity and mortality, as all-cause mortality [77, 78]. Serum GDF-15 levels in hypertensive patients were significantly higher than in healthy volunteers and positively correlated with the thickness of the posterior wall of the left ventricle, interventricular septum, and left ventricular mass, as well as the serum level of norepinephrine [79]. Plasma GDF-15 levels in hypertensive patients with LVH was higher than those hypertensive patients without LVH. They observed positive correlation between plasma GDF-15 levels and LVH in hypertensive patients which indicates that GDF-15 may be involved in the development of LVH in hypertension [79]. Hantani et al. found that GDF-15 might be a useful biomarker for discriminating hypertrophic cardiomyopathy (HCM) from hypertensive left ventricular hypertrophy (H-LVH). It was also observed that serum GDF-15 levels were significantly higher in patients with H-LVH than with HCM, and thus GDF-15 is an independent predictor of H-LVH. The data suggest that GDF-15 levels may help to introduce different treatment strategies for treating HCM and H-LVH [80]. One of the recent studies shows that GDF-15 is an autocrine/paracrine factor that attenuates the cardiac hypertrophy in experimental models via SMAD and kinases (PI3 K and ERK) signaling pathways. This study indicates that GDF-15 works through activation of SMAD protein and kinases, that is, PI3 K and ERK mechanism [71].

Intracellular mechanism of TGF-β family is divided into Smad dependent and Smad independent pathway. This intracellular mechanism is determined by the type 1 receptors (ALK1 to 7). GDF-15 activates type 1 receptor and phosphorylates Smad2/3 and Smad1/5/8, which translocate to the nucleus in the form of heteromeric complex with Smad4 [70]. Smad4 is a common transcriptional mediator of the Smad dependent pathway. Wang et al. reported that heart specific deletion of *smad4*$^{-/-}$(*smad4*$^{-/-}$ mice) showed greater cardiac hypertrophy and heart failure [55]. Xu et al. suggested that Smad dependent pathway can inhibit apoptosis and shows protection against hypertrophy and fibrosis [79]. Xu et al. proposed a protective mechanism of GDF-15 against cardiac hypertrophy and cell death through Smad protein activation. It was explained in the study that GDF-15 shows TGF-β/activin-like response through Smad2/3. While Smad2 overexpression showed the similar beneficial effects of GDF-15, overexpression of Smad6 or Smad7 reversed its antihypertrophic effects [78]. GDF-15 treatment transiently activates the Akt and ERK1/2 signaling [71]. Akt activation regulates cardiomyocyte viability [53] whereas ERK1/2 signaling regulates the cell survival [81]. Both pathways are cardioprotective in nature, thus, most of the data confirm that GDF-15 is a novel antihypertrophic as well as cardioprotective regulatory factor [29, 71].

GDF-15 was found to inhibit myocardial hypertrophy through a Smad2/3 pathway in a pressure-induced hypertrophy model [71]. Similarly Xu et al. reported that GDF-15 protects the heart from norepinephrine (NE) induced hypertrophy through Smad independent pathway [71]. Different Smad independent pathways through which GDF-15 works are MAPKs, TAK-1, and PI3 K/AKT pathways [82]. GDF-15 also inhibits norepinephrine-induced myocardial hypertrophy by inhibition of epidermal growth factor receptor (EGFR) transactivation and phosphorylation of downstream kinases, that is, Akt and extracellular signal-regulated kinases (ERK) (Figure 2) [79]. In contrast, GDF-15 results in prohypertrophic effect in cardiomyocytes through a Smad1 pathway [83].

Recently, one study observed that GDF-15 is a novel promising biomarker in heart failure with normal ejection fraction (HFnEF). It is elevated in subjects with either mild or moderate to severe left ventricular diastolic dysfunction (LVDD) regardless of the presence of CAD or other established risk factors frequently associated with HFnNF [84]. Lok et al. reported for the first time that highly elevated GDF-15 levels can be reversible in some extent, after measuring GDF-15 before and after intervention with left ventricular assist device (LVAD) in New York Heart Association (NYHA) class IV, nonischaemic, and nonvalvular HF patients. They found that the GDF-15 levels were gradually reduced after implantation. This finding suggests that GDF-15 could be used as prognostic marker to measure the response to a potentially life-saving therapeutic intervention such as LVAD implantation [56]. Other studies like valsartan heart failure trial (Val-HeFT trial) indicated that higher GDF-15 levels are associated with many pathological processes and then linked to the severity and progression of heart failure (HF), including neurohormonal activation, inflammation, myocyte death, and renal dysfunctions. Higher GDF-15 levels are associated with adverse outcomes independent of established clinical and biochemical risk factors [40, 65]. Recently, Chen et al. demonstrated that olmesartan prevents cardiac rupture in mice with myocardial infarction (MI) through inhibition of apoptosis and inflammation and is associated with downregulation of p53 activity and upregulation of myocardial GDF-15 [85]. In contrast, irbesartan (AT1 receptor blocker) significantly reduced angiotensin II induced GDF-15 expression in cardiomyocytes [86]. The above studies indicate that angiotensin receptor blockers regulate GDF-15 expression. However, more therapeutic intervention studies are needed to understand whether GDF-15 can be used as

a prognostic marker for therapeutic intervention for different cardiovascular disorders.

4.2. Atherosclerosis. Development and progression of atherosclerotic plaques are driven by endothelial dysfunction, oxidized low-density lipoprotein (oxLDL) deposition in the subendothelial space, recruitment of inflammatory monocytes to the arterial vessel wall, their differentiation into activated macrophages, and subsequent transformation into cholesterol-laden foam cells in the subendothelial space [87]. It has been shown that GDF-15 inhibits proliferation of endothelial cells (ECs) *in vitro* and *in vivo*. A recent study demonstrated that GDF-15 at high concentration (50 ng/mL) inhibits EC proliferation, whereas, at lower concentrations (5 ng/mL), GDF-15 caused endothelial cell proliferation and was found proangiogenic [50, 88–90].

Transforming growth factor betas (TGFβs) have been involved in many of the pathophysiological process mainly in the vascular diseases. It will act as inflammatory markers in advanced stage of atherosclerosis and play a role in pathogenesis of ischemic heart diseases. TGFβs involved in the pathogenesis of atherosclerosis by activating proteolytic mechanism of activated macrophages [91–94]. These activated macrophages will undergo apoptosis in lipid rich plaque condition. This phenomenon pointing out that lipid content and inflammatory cell viability may be responsible for the thrombogenicity [95]. Activation of caspase 3, induction of manganese superoxide dismutase (MnSOD), and increase in expression of p53 were seen in human atherosclerotic plaques [96–100]. Signal transduction of oxLDL and its mediator's ceramide and TNF-α induce apoptosis in human activated macrophages [97, 98, 101–103]. Poly(ADP-ribose) polymerase (PARP), c-Jun-AP-1, and apoptosis inducing factor (AIF) were detected in apoptotic cells [49, 97–101, 103]. Recently, Schlittenhardt et al. found that GDF-15 is expressed in macrophages after stimulation by several biological mediators, including tumor necrosis factor α (TNF-α), C6-ceramide, interleukin-1 (IL-1), macrophage-colony stimulating factor (M-CSF), oxLDL, and hydrogen peroxide. In human atherosclerotic carotid arteries, GDF-15 (immunoreactivity) was exclusively localized in activated macrophages and colocalized with oxLDL, MnSOD, AIF, caspase-3 (CPP32), PARP, c-Jun/AP-1, and p53. GDF-15 is supposed to contribute to modulation of apoptosis and inflammatory processes of activated macrophages. All data suggested that increased expression of GDF-15 is associated with the development and progression of atherosclerotic plaques, possibly through the regulation of apoptotic processes [49]. In support of previous statement another study demonstrated that GDF-15 deficiency attenuates early atherogenesis and improves plaque stability by attenuating CCR2 (C-C chemokine receptor type 2) mediated macrophage chemotaxis. Similarly, deficiency of GDF-15 in leukocytes improves atherosclerotic plaque stability by impairing macrophage migration and inducing collagen deposition. A novel function of GDF-15 is to regulate the CCR2-dependent macrophage chemotaxis and proceeds via TGF-β receptor II and its downstream effector GRK-2 [104].

Another function of GDF-15 is to control inflammatory process in cells. GDF-15 deficiency attenuates atherosclerosis by regulating interleukin-6 dependent inflammatory response to vascular injury. GDF-15 deficiency results in inhibition of atherosclerosis in mice despite an inhibition of apoptotic processes and an increase in cell density in atherosclerotic lesions. This implicates that inhibition of apoptosis acts as antiatherogenic. Regulation of apoptosis through GDF-15 may be a therapeutic strategy to control atherosclerosis and plaque progression [87]. Triglyceride-rich lipoproteins upregulate GDF-15 by >5-fold in human smooth muscle cells of coronary arteries [48]. This increased GDF-15 may trigger the prognosis of the diseases. Overall GDF-15 levels are increased in cardiovascular disease patients [105] and enhance the risk of atherosclerosis. However, GDF-15 deficiency in leukocytes protects against atherosclerosis [104].

4.3. Coronary Artery Diseases and Myocardial Infarction. Coronary artery disease (CAD) is a chronic degenerative condition. CAD is a combination of different clinical syndromes including stable angina, acute coronary syndrome (ACS), heart failure, arrhythmia, and death. Myocardial infarction, a condition associated with coronary artery disease, contributes to deaths [106]. Circulated levels of GDF-15 levels are increased in patients who are admitted to the hospital with an acute coronary syndrome. This was proved in "non-ST segment elevation ACS" patients in GUSTO 4 trial [106]. People with elevated levels of GDF-15 (>1800 ng/L) had a high risk for mortality within one year [105]. However, increased GDF-15 level has beneficial role during invasive strategy. In the "Fast Revascularization during in Stability in Coronary artery disease II" (FRISC-II) trial with GDF-15 level <1200 ng/L did not show any benefit from the invasive strategy even though they had ST segment depression or a troponin T level >0.01 μg/L. Patients with GDF-15 levels >1200 ng/L, especially those with 1800 ng/L experienced significant reduction in the combined end point of death or myocardial infarction by the routine invasive strategy [32].

A recent Dallas Heart Study suggests that higher GDF-15 is associated with prevalent coronary artery calcium (CAC) and cardiovascular mortality. People having GDF-15 concentrations ≥1800 ng/L were at increased risk for all-cause and cardiovascular death compared to those with <1200 ng/L. Increasing GDF-15 levels were associated with increasing age, diabetes, renal dysfunction, and inflammatory marker (CRP). Increasing GDF-15 was significantly correlated with black race, smoking, and hypertension. Increasing NT-proBNP concentrations are less associated with increasing GDF-15 levels, but there were no associations with BMI and sex differences [33].

Recently, elevated circulating GDF-15 levels that measured in individuals with acute myocardial infarction have been correlated with inflammatory biomarkers, suggesting a link between GDF-15 and inflammation [30, 32]. After cardiac surgery acute muscle wasting will occur because of imbalance between muscle atrophy and hypertrophy. Bloch et al. found that GDF-15 may be responsible for the skeletal muscle wasting in humans [31].

Recent study demonstrated that GDF-15 protects against fatal cardiac rupture in a mouse model of myocardial infarction. Induction of GDF-15 locally in the infarcted heart reduces the cardiac rupture by acting as an anti-inflammatory cytokine and represses myeloid cell recruitment into the infarcted area [107]. GDF-15 also inhibits chemokine-triggered $\beta 2$ integrin activation on myeloid cells by activating Cdc42 and inhibition of small GTPase Rap1. Thus, GDF-15 is an inhibitor of leukocyte integrin that is one of the essential components to induce cellular injury after myocardial infarction [107].

In summary, GDF-15 is a very promising diagnostic marker for mild to moderate heart failure with normal ejection fraction or the absence of coronary artery diseases. However, more studies need to be done to distinguish different kinds of heart failure using GDF-15. Higher level of GDF-15 can predict the mortality for CAD patients. Some studies also showed its potential to use GDF-15 as a prognostic marker for therapeutic intervention for different cardiovascular disorders. Although the use of GDF-15 as a biomarker for cardiovascular disease is well established, its therapeutic application is debatable. While GDF-15 can show protection against cardiac hypertrophy, its increased expression is associated with the development and progression of atherosclerotic plaques. Further research is essential before considering GDF-15 as therapeutic intervention against cardiovascular diseases.

5. GDF-15 and Kidney Disease: Linkage between Diabetes and Cardiovascular Diseases

Diabetic nephropathy is a progressive kidney disease and a well-known complication of long standing diabetes [108]. Myocardial damage is directly associated with the development of proteinuria and focal glomerulosclerosis [109]. Increase in circulating troponins was observed commonly in patients with chronic kidney diseases (CKD) and are associated with the kidney disease progression and death. The association of circulating troponin and kidney damage is related to cardiac injury, rather than diminished clearance [110]. Higher plasma GDF-15 was associated with incident of CKD and indicates rapid decline in renal function [111]. Higher levels of GDF-15 were predictive of deterioration of kidney function [21]. Studies on renal injury in animal models suggest two possible reasons for increase in GDF-15 levels during renal diseases; either GDF-15 is less cleared from the circulation by the kidneys or synthesis of GDF-15 is increased in renal diseases, or both [112, 113]. In diabetic renal injury, increases in urinary GDF-15 were associated with proximal tubule injury [114]. Thus the hypothesis of less clearance of GDF-15 from kidney is not true. Renal GDF-15 expression also appears to be upregulated in response to metabolic acidosis [113] and kidney injury [113]. van Huyen et al. found that higher levels of GDF-15 are a predictive marker of cardiovascular mortality in patients with diabetic nephropathy besides other well-known cardiovascular risk factors like NT-proBNP and glomerular filtration rate (GFR) [113]. Plasma GDF-15 levels were also increased with the mogensen stage in type 2 diabetic nephropathy, and, thus, it is an independent risk factor for increased microalbuminuria (mAlb). It is significantly correlated with mAlb and eGFR, and thus GDF-15 would be useful in early diagnosis, evaluation, and prediction of the outcomes of type 2 diabetic nephropathy [28]. Although some studies have been performed to find the correlation of plasma and urine GDF-15 levels with kidney disease, there is no study to look into its potential as prognostic marker for kidney disease after intervention.

6. GDF-15 and Nitric Oxide: Cross Talk in Diabetes and Cardiovascular Diseases

Increased levels of GDF-15 were associated with reduced endothelium-dependent vasodilation in resistance vessels [73]. GDF-15 shows protective effect against high glucose induced endothelial cell injury by activation of PI3 K/AKT/eNOS signaling pathway. GDF-15 is important to release NO level in endothelial cells. In a recent study, nitric oxide production was significantly lower in GDF-15 siRNA transfected HUVEC cells. On the other hand NO is also responsible to increase GDF-15 gene expression [5]. Nitric oxide is responsible to alter the gene expression through cGMP dependent and cGMP independent signaling pathway. In cGMP independent pathway, nitric oxide reacts with superoxide to form peroxynitrate. Kempf et al. observed that nitric oxide increased GDF-15 expression in cardiomyocytes through superoxide/peroxynitrate dependent pathway, a c-GMP independent pathway [54]. Endothelial dysfunction or injury due to diabetes and smoking may induce inflammation and generate oxidative stress within the vessel wall [115]. Several authors mentioned that oxidative stress and proinflammatory cytokines can induce GDF-15 expression in macrophages and different other cells. Thus increased GDF-15 is linked to oxidative stress, inflammation, and endothelial dysfunction [34, 49, 73, 116–118]. Further research is still needed to understand whether GDF-15 can modulate NO levels or vice-versa in other nonendothelial cells.

7. GDF-15 a Potential Biomarker

In the last two decades, we have enormous improvement in the biomarker discovery but only few biomarkers gained wide spread use in clinical practice such as troponin T, troponin I, Nt-proBNP, and B-type natriuretic peptide (BNP) [14]. Recently, Kahli reported that GDF-15 levels increased gradually during and after coronary bypass grafting. This study concluded that GDF-15 levels might be used as a marker of cardiac injury and renal dysfunction [42]. Zhang et al. performed a study to find out multimarkers as predictors of cardiovascular events in patients with mild to moderate coronary artery lesions. This study examined nine plasma inflammatory cytokines, that is, cathepsin S, chemokine (C-X-C motif) ligand 16 (CXCL16), sopluble CD40 ligand, IL-10, placental growth factor, GDF-15, MMP-9, monocyte chemo attractant protein-1, and hs-CRP in 964 patients having

Table 3: GDF-15 patents related to diabetes, cardiovascular diseases, and chronic kidney diseases.

Patent	Applicant	Title
WO2011144571A2	F. Hoffmann-La Roche Ag	GDF-15 based means and methods for survival and recovery prediction in acute inflammation
WO2012138919A2	Amgen Inc.	Method of treating or ameliorating metabolic disorders using GDF-15
WO2012146645A1	F. Hoffmann-La Roche Ag	Diagnosis of kidney injury after surgery
EP2336784A1	Roche Diagnostics GmbH	GDF-15 and/or Troponin T for predicting kidney failure in heart surgery patients
EP2388594A1	Roche Diagnostics GmbH	GDF-15 based means and methods for survival and recovery prediction in acute inflammation
WO2009141357A1	Roche Diagnostics Gmbh, F. Hoffmann-La Roche Ag	GDF-15 as biomarker in type 1 diabetes
US 8,771,961 B2	Roche Diagnostics Operations, Inc.	Monitoring myocardial infarction and its treatment
EP1884777A1	Medizinische Hochschule Hannover	Means and methods for assessing the risk of cardiac interventions based on GDF-15
EP2439535A1	F. Hoffmann-La Roche AG	Diagnosis of diabetes related heart disease, GDF-15 and Troponin as predictors for the development of type 2 diabetes mellitus
WO2013113008A1	Amgen Inc.	GDF-15 polypeptides-ameliorating metabolic disorders
WO2010048670A1	St. Vincent's Hospital Sydney Limited	Method of prognosis in chronic kidney disease
WO2011073382A1	Roche Diagnostics Gmbh	GDF-15 and/or troponin T for predicting kidney failure in heart surgery patients

mild to moderate lesions, and assessed their association with risk of cardiovascular events during 3 years of their follow-up study. It was concluded that cathepsin S, sopluble CD40 ligand, placental growth factor, and GDF-15 were instructive biomarkers for predicting cardiovascular diseases. This study showed that multimarkers approach is useful to significantly predict cardiovascular diseases progression than the individual marker approach [119].

Similarly, Schnabel et al. investigated 12 biomarkers including GDF-15, related to inflammation, lipid metabolism, renal function, and cardiovascular function and remodeling. These markers are C-reactive protein, GDF-15, neopterin, apolipoproteins AI, B100, cystatin C, serum creatinine, copeptin, C-terminal-proendothelin-1, midregional-proadrenomedullin (MR-proADM), midregional-proatrial natriuretic peptide (MR-proANP), and N-terminal-pro-B-type natriuretic peptide (Nt-proBNP). Blood was collected from 1781 stable angina patients in relation to nonfatal myocardial infarction and cardiovascular death ($n = 137$). The study concluded that Nt-proBNP, GDF-15, MR-proANP, cystatin C, and MR-proADM are the strongest predictors of cardiovascular outcome among patients with stable angina [120].

Similar to other biomarkers, GDF-15 can also be used for diagnosis of diseases and help to select the therapy. GDF-15 diagnosis method is patented for diagnosing any subject suffering from an acute inflammation. GDF-15 is also patented for the diagnosis of kidney injury after surgery, prediction of kidney failure after heart surgery, and detection the prognosis of chronic kidney diseases. GDF-15 is patented as a biomarker for the type 1 diabetes and diabetes related heart diseases. Besides using GDF-15 as a biomarker, GDF-15 polypeptide itself is patented to treat or ameliorate metabolic disorders. Table 3 described briefly the list of patents with GDF-15 that used as biomarker for diabetes, cardiovascular disease, and kidney disease.

8. Conclusion

Previous studies revealed that GDF-15 could be a prognostic and diagnostic marker for the cardiovascular and diabetic diseases. Proper reference ranges of GDF-15 need to be established to identify the disease severity and risk stratification of the diseases. However, before accepting as a clinically useful biomarker, the following questions need to be answered. (1) Whether GDF-15 measurement can support therapeutic management? (2) Can it be used for the routine clinical practice or clinical measurement? (3) Whether GDF-15 level can give any diagnostic and prognostic information? (4) Whether it can be used to take clinical decision for any particular diseases like B-type natriuretic peptide (BNP) for the heart failure and troponin for the acute coronary syndrome (ACS). (5) GDF-15 can be used as a single marker or multi marker approach along with other individual marker. There is very little information regarding pathophysiological role of GDF-15 in diabetes, CAD, hypertension, and diabetes associated with cardiovascular diseases. More intervention studies like AT1 receptor antagonist need to be carried out to bring GDF-15 as a prognostic marker for diabetic and cardiovascular diseases. Further understanding regarding the signaling pathways of GDF-15 may help to discover novel therapies against diabetes and cardiovascular complications.

Abbreviations

ACS:	Acute coronary syndrome
AKT:	Serine/threonine kinase (protein kinase B)
AIF:	Apoptosis inducing factor
AP-1:	Activator protein-1
APACE:	Acute coronary syndrome evaluation
Bcl-xL:	B-cell lymphoma-extra large
BMI:	Body mass index
BMP-2:	Bone morphogenetic protein-2
BNP:	B-type natriuretic peptide
CAC:	Coronary artery calcium
CAD:	Coronary artery diseases
Cdc42:	Cell division control protein 42
CKD:	Chronic kidney diseases
CCR2:	C-C chemokine receptor type 2
cGMP:	Cyclic guanosine monophosphate
CXCL16:	Chemokine (C-X-C motif) ligand 16
EGR-1:	Early growth response protein-1
ERK:	Extracellular signal-regulated kinases
eNOS:	Endothelial nitric oxide synthase
GRK-2:	G protein-coupled receptor kinase 2
HbA1c:	Glycated hemoglobin
H-LVH:	Hypertensive left ventricular hypertrophy
hs-CRP:	High-sensitivity C-reactive protein
HUVEC:	Human vascular endothelial cells
ICU:	Intensive care unit
IDF:	International diabetic federation
IL-10:	Interleukin-10
IMA:	Ischemia modified albumin
JNK:	Jun-N-terminal kinase
LVAD:	Left ventricular assist device
LVH:	Left ventricular hypertrophy
mAlb:	Microalbuminuria
MMP-9:	Matrix metalloproteinase 9
MR-proADM:	Midregional-proadrenomedullin
MR-proANP:	Midregional-proatrial natriuretic peptide
NF-κB:	Nuclear factor kappa B
Nt-proBNP:	N-terminal-pro-B-type natriuretic peptide
oxLDL:	Oxidized low-density lipoprotein
PI3 K:	Phosphoinositide 3-kinase
PARP:	Poly (ADP-ribose) polymerase
PAI-1:	Plasminogen activator inhibitor-1
PAPP-A:	Pregnancy associated plasma protein-A
T1DM:	Type 1 diabetes mellitus
T2DM:	Type 2 diabetes mellitus.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Hindawi Publishing Corporation
Journal of Diabetes Research
Volume 2015, Article ID 616747, 6 pages
http://dx.doi.org/10.1155/2015/616747

Research Article

NLRP3 Inflammasome Polymorphism and Macrovascular Complications in Type 2 Diabetes Patients

Jasna Klen,[1] Katja Goričar,[2] Andrej Janež,[3] and Vita Dolžan[2]

[1]*General Hospital Trbovlje, Rudarska cesta 9, SI-1420 Trbovlje, Slovenia*
[2]*Pharmacogenetics Laboratory, Institute of Biochemistry, Faculty of Medicine, University of Ljubljana, Vrazov trg 2, SI-1000 Ljubljana, Slovenia*
[3]*Department of Endocrinology, Diabetes and Metabolic Diseases, University Medical Center Ljubljana, Zaloška Cesta 7, SI-1000 Ljubljana, Slovenia*

Correspondence should be addressed to Vita Dolžan; vita.dolzan@mf.uni-lj.si

Received 19 January 2015; Accepted 17 March 2015

Academic Editor: Joseph Fomusi Ndisang

Background. It is generally accepted that poor glycemic control, arterial hypertension and/or hyperlipidemia, and the associated oxidative stress may contribute to the development of macro- and microvascular complications in type 2 diabetes (T2D). Such metabolic damage signals may activate inflammasome and trigger chronic inflammation. We investigated common polymorphisms in inflammasome coding genes and the risk for macro- and microvascular complications in T2D. *Methods*. In total 181 clinically well-characterised T2D patients were genotyped for *NLRP3* rs35829419 and *CARD8* rs2043211. Risk for diabetic complications was assessed using logistic regression. *Results*. Patients with median duration of T2D 11 (6–17) years had relatively well controlled blood glucose and lipid levels and blood pressure on the prescribed treatment regimen. Duration of T2D and plasma cholesterol levels were the most important clinical risk factors for macrovascular complications ($P = 0.007$ and $P = 0.031$). *NLRP3* rs35829419 was associated with increased risk for macrovascular complications ($P = 0.004$), with myocardial infarction in particular ($P = 0.052$). No association was observed between *CARD8* polymorphism and any of T2D complications. *Conclusions*. Our preliminary data suggest the role of *NLRP3* polymorphism in diabetic macrovascular complications, especially in myocardial infarction.

1. Introduction

Macro- and microvascular complications are common in long-standing type 2 diabetes (T2D) and diminish the quality of life and life-time expectancy of patients [1, 2]. In addition to genetic and lifestyle factors, poor glycemic control, arterial hypertension, and/or hyperlipidemia are predisposing factors for the development of these complications [3, 4]. High blood glucose levels and dyslipidemia may stimulate the production of reactive oxygen species (ROS), leading to increased oxidative stress and chronic inflammation that appears to be the major driver of molecular processes leading to late diabetic complications [5–8]. Recent studies suggested the involvement of inflammatory pathways that may be triggered by inflammasome, a crucial complex that senses and is activated by metabolic damage signals [9, 10].

Inflammasomes are a large family of multiprotein complexes that can be activated by pathogen-associated or damage-associated molecular patterns (PAMPs or DAMPs, resp.), among others also such as ROS, cholesterol crystals, and possibly high glucose levels. NLRP3 inflammasome is composed of the NLRP3 scaffold protein, CARD containing adaptor protein, and caspase-1 [11]. Activation of inflammasome leads to assembly of the multiprotein complex that cleaves and activates caspase-1, resulting in cleavage of pro-interleukin-1β (pro-IL-1β) and release of IL-1β that triggers downstream inflammatory response [9, 10].

NLRP3 inflammasome has been suggested as a link between insulin resistance, obesity, circulating immune markers, immunogenetic susceptibility, macrophage function, and chronic inflammation [12]. Genetic variations leading to the altered production or function of inflammasome

and inflammatory cytokines were linked to various inflammatory diseases, including obesity, insulin resistance, and T2D [13, 14]. Two inflammasome related single nucleotide polymorphisms (SNPs) have been most frequently studied in Caucasians. *NLRP3* rs35829419 (p.Gln705Lys) is a gain-of-function polymorphism associated with increased production of IL-1β [15]. On the other hand, *CARD8* rs2043211 (p.Cys10Ter) results in nonfunctional protein and leads to loss of CARD-8 inhibition of caspase-1. Both SNPs were associated with proinflammatory phenotype [16–19]. Another common SNP associated with mRNA stability, *NLRP3* rs10754558, was linked to insulin resistance and increased risk for T2D in Chinese Han population [13].

The aim of our study was to investigate if *NLRP3* and *CARD8* polymorphisms play a role in the risk for macro- and microvascular complications in T2D.

2. Patients and Methods

We performed a retrospective study that included T2D patients aged between 18 and 75 years. All the patients were treated and followed up at the outpatient clinic at the General Hospital Trbovlje, Slovenia. Patients with diabetes type 1, gestational diabetes, other types of diabetes, active cancer, addiction to alcohol or illegal drugs, and dementia or severe psychiatric disorders were not eligible for the study. Patients were included as they were coming for their regular follow-up visits and were receiving treatment according to the established clinical guidelines. At inclusion they all underwent a complete physical examination and laboratory evaluation. Blood pressure, body height, and body weight were measured at every follow-up visit and the body mass index (BMI) was calculated. Information on the history of diabetes, presence of arterial hypertension, hyperlipidemia and chronic macrovascular and microvascular diabetic complications, smoking status, and other medications was obtained from the medical records and from the interview at the inclusion in the study. Plasma lipid levels, urea and creatinine, and urine albumin and albumin/creatinine ratio were determined at least once per year. All patients were referred to consulting ophthalmologist for screening for diabetic retinopathy at least once a year. Echosonography and exercise stress test (cycloergometry) were performed at the first visit and also at any complaints suggestive for ischemic heart disease.

The study was approved by the National Ethics Committee and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all subjects.

All the laboratory parameters were measured using standard laboratory procedures at the biochemistry laboratory of the General Hospital Trbovlje, Slovenia [20]. Genomic DNA was isolated from peripheral blood leukocytes using Qiagen FlexiGene kit (Qiagen, Hilden, Germany). DNA extraction and genotyping analyses were performed at the Pharmacogenetics Laboratory, Institute of Biochemistry, Faculty of Medicine, University of Ljubljana. Genotyping of *NLRP3* rs35829419 (c.2113C>A, p.Gln705Lys) and *CARD8* rs2043211 (c.304A>T, p.Phe102Ile, p.Cys10Ter) was carried out using a fluorescence-based competitive allele-specific (KASPar) assay according to the manufacturer's instructions (KBiosciences, Herts, UK). Genotyping was performed blindly to any clinical data and was randomly repeated in 20% of samples to check for genotyping reliability.

Median and interquartile range were used to describe central tendency and variability of continuous variables, while frequencies were used to describe the distribution of categorical variables. Standard chi square test or Mann-Whitney test was used to compare clinical characteristics between different patient groups. Risk for macro- and microvascular complications was assessed using logistic regression. All statistical analyses were performed using IBM SPSS Statistics 19.0 (IBM Corporation, Armonk, NY, USA). Dominant genetic model was used in all analyses and the level of statistical significance was set at 0.05.

3. Results

In total 181 patients (105 males and 76 females) with T2D were included in the study. The median (25–75% range) age of the patients was 64 (58.5–70.5) years and duration of T2D was 11 (6–17) years. On the prescribed treatment regimen most of the patients achieved relatively good blood glucose control with the average HbA1c 6.9% (6.3–7.6), well controlled blood pressure, and plasma lipid levels (Table 1).

In total 46 (25.4%) patients suffered from macrovascular complications: 10 (5.5%) had peripheral arterial occlusive disease (PAOD), 16 (8.85%) ischemic cerebrovascular disease (ICD), and 32 (17.7%) myocardial infarction (MI). Only 34 (18.8%) patients had microvascular complications: 25 (13.8%) suffered from end-stage kidney failure due to diabetic nephropathy, 13 (7.2%) from neuropathy, and 15 (8.3%) from retinopathy. Duration of T2D and plasma cholesterol levels were the most important clinical risk factors for macrovascular complications in logistic regression analysis (OR = 1.06; 95% CI = 1.02–1.11; $P = 0.007$ and OR = 0.67; 95% CI = 0.46–0.96; $P = 0.031$, resp.). The most important clinical risk factor for microvascular complications was duration of T2D (OR = 1.12; 95% CI = 1.07–1.17; $P < 0.001$).

All the patients were genotyped for *CARD8* rs2043211 and *NLRP3* rs35829419 polymorphisms; however, CARD8 genotype data could not be obtained for one patient. In total 89 (49.4%) patients were homozygous for the wild type *CARD8* AA allele, 70 (38.9%) carried one, and 21 (11.7%) carried two polymorphic T alleles. *NLRP3* polymorphism was less common: 160 (88.4%) were homozygous for the wild type CC genotype, while 21 (11.6%) patients carried one of the polymorphic A alleles: 20 (11.0%) carried one and 1 (0.6%) carried two A alleles. All genotype frequencies were in agreement with HWE ($P = 0.213$ and 0.666 for *CARD8* and *NLRP3,* resp.).

We observed an association between *NLRP3* rs35829419 polymorphism and the risk for macrovascular complications (OR = 3.93; 95% CI = 1.54–10.0; $P = 0.004$) (Table 2). This association remained significant after adjustment for duration of T2D and plasma cholesterol levels (OR = 4.36; 95% CI = 1.58–12.01; $P = 0.004$). Although carriers of one or

Table 1: Patients characteristics.

	All patients ($N = 181$)	Macrovascular complications ($N = 46$)	P^a	Microvascular complications ($N = 34$)	P^b
Male gender[c]	105 (58.0)	29 (63.0)	0.423[d]	23 (67.6)	0.206[d]
Age (years)	64.0 (58.5–70.5)	64.0 (58.8–72.3)	0.495	64.0 (58.0–75.3)	0.251
Duration of T2D (years)	11.0 (6.0–17.0)	16.0 (7.0–23.5)	0.003	20.5 (11.0–27.0)	0.000
HbA1c (%) [mmol/mol]	6.9 (6.3–7.6) [52 (45–60)]	6.7 (5.9–7.4) [50 (41–57)]	0.143	6.5 (5.8–7.7) [48 (40–61)]	0.020
BMI (kg/m^2)	30.0 (28.0–33.3)	30.0 (27.0–33.1)	0.741	29 (25.8–32.4)	0.191
Blood pressure systolic (mmHg)	135.0 (130.0–145.0)	140.0 (128.8–150.5)	0.257	139.0 (119.3–149.0)	0.759
Blood pressure diastolic (mmHg)	80.0 (70.0–80.0)	75.0 (65.8–80.0)	0.014	72.0 (63.0–80.0)	0.000
Total cholesterol (mmol/L)	4.2 (3.5–5.0)	3.9 (3.1–4.5)	0.004	3.7 (2.9–4.7)	0.002
LDL cholesterol (mmol/L)	2.4 (1.9–3.1)	2.3 (1.5–2.7)	0.047	2.0 (1.5–2.4)	0.004
HDL cholesterol (mmol/L)	1.1 (1.0–1.4)	1.1 (0.9–1.3)	0.009	1.1 (0.9–1.4)	0.323
TAG (mmol/L)	1.6 (1.2–2.4)	1.7 (1.1–2.2)	0.587	1.3 (0.9–2.1)	0.046

[a]Comparison between patients with and without macrovascular complications. [b]Comparison between patients with and without microvascular complications. Data are shown as median (25%–75%), except for [c]N (%). P values were calculated using Mann-Whitney test, except for [d]chi square test.

Table 2: The association of *NLRP3* rs35829419 with the risk for macro- and microvascular complications in type 2 diabetes patients.

T2D complications	All patients				Male patients			
	CC N (%)	CA + AA N (%)	OR (95% CI)	P	CC N (%)	CA + AA N (%)	OR (95% CI)	P
Macrovascular	35 (21.9)	11 (52.4)	3.93 (1.54–10.00)	0.004	21 (23.1)	8 (57.1)	4.44 (1.39–14.25)	0.012
PAOD	7 (4.4)	3 (14.3)	3.64 (0.86–15.34)	0.078	4 (4.4)	3 (21.4)	5.93 (1.17–30.06)	0.032
ICD	12 (7.5)	4 (19.1)	2.90 (0.84–10.01)	0.092	9 (9.9)	3 (21.4)	2.48 (0.58–10.60)	0.219
MI	25 (15.6)	7 (33.3)	2.70 (0.99–7.36)	0.052	14 (15.4)	5 (35.7)	3.06 (0.89–10.48)	0.076
Microvascular	28 (17.5)	6 (28.6)	1.89 (0.67–5.29)	0.228	17 (18.7)	6 (42.9)	3.26 (1.00–10.65)	0.050
End-stage kidney failure	20 (12.5)	5 (23.8)	2.19 (0.72–6.63)	0.166	11 (12.1)	5 (35.7)	4.04 (1.14–14.27)	0.030
Retinopathy	12 (7.5)	3 (14.3)	2.06 (0.53–7.98)	0.298	9 (9.9)	1 (7.1)	0.70 (0.08–6.00)	0.746
Neuropathy	12 (7.5)	1 (4.8)	0.61 (0.08–4.97)	0.646	8 (8.8)	3 (21.4)	2.83 (0.65–12.29)	0.165

PAOD: peripheral arterial occlusive disease; 16 (8.85%) ICD: ischemic cerebrovascular disease; MI: myocardial infarction.

two polymorphic *NLRP3* rs35829419 alleles had higher risk for MI, PAOD, and ICD, these associations were statistically nonsignificant ($P = 0.052$, $P = 0.078$, and $P = 0.092$, resp., Table 2). When stratified by sex, a significant association of *NLRP3* rs35829419 with macrovascular complications was observed only in males (OR = 4.44; 95% CI = 1.39–14.25; $P = 0.012$) (Table 2). Additionally, significant association with PAOD was observed among males (OR = 5.93; 95% CI = 1.17–30.06; $P = 0.032$). This association could not be tested in females as only 3 female patients had PAOD and all were *NLRP3* CC homozygous.

No association was observed between the investigated *CARD8* rs2043211 and the risk for macrovascular complications in univariable (OR = 0.97; 95% CI = 0.50–1.90; $P = 0.930$) or multivariable analysis (OR = 1.02; 95% CI = 0.50–2.08; $P = 0.951$). We also observed no associations of *CARD8* rs2043211 with individual macrovascular complications (data not shown).

The presence of individual microvascular complications was also investigated, but no association was observed between *CARD8* or *NLRP3* polymorphisms and these complications (Table 2). However, male carriers of at least one polymorphic *NLRP3* rs35829419 allele tended to have more microvascular complications ($P = 0.050$) and had increased risk for end-stage kidney failure ($P = 0.030$, Table 2).

4. Discussion

To our knowledge, this is the first study that investigated the influence of genetic variability in inflammasome on T2D long-term complications.

We observed that polymorphic *NLRP3* rs35829419 A allele is associated with increased risk for development of any macrovascular complications in long-term T2D patients. Despite the small numbers of individuals with a particular complication, *NLRP3* polymorphism showed statistically nonsignificant association with MI, PAOD, and ICD. These preliminary data are concordant with previous reports on association of *NLRP3* and *CARD8* polymorphism with the proinflammatory phenotype. In a large cohort of healthy blood donors higher IL-1β levels were observed in carriers of polymorphic alleles of these genes than in noncarriers [16]. Our results are also in agreement with reports on potential role of NLRP3 inflammasome in MI [21]. Recent

genome-wide association study [18] as well as a large cardiovascular candidate gene study [22] observed association of *NLRP3* locus with higher fibrinogen levels that may lead to higher atherothrombotic risk. Inflammasome proteins were indeed shown to be expressed not only in neutrophils and macrophages, but also in vascular endothelia and cardiac fibroblasts [23]. Knockout mice showed declined inflammatory responses and were more protected against myocardial damage after MI [23].

Contrary to our findings, a large First-ever myocardial Infarction study in Ac-county (FIA) showed no association between *NLRP3* rs35829419 and MI. However, they observed a gender-specific effect of *NLRP3* rs35829419: polymorphic A allele conferred protection from development of MI in females but was associated with increased CRP levels as a marker of inflammation in males [17]. In our study group, polymorphic *NLRP3* A allele conferred to increased risk of macrovascular complications among males. The risk was highest for PAOD, while a tendency of increased risk of MI was also observed. It was shown that *NLRP3* may play a role in vascular lipid deposition and atherosclerosis [24].

We observed no associations between any complications and *NLRP3* polymorphisms among females; however, in our study group fewer females than males presented with complications. The discrepancy between our results and the FIA study may be due to the differences in the study design between the two studies: while FIA was a large population based study with cases and controls carefully matched for age and gender [17], our study included patients with T2D as they were coming for their regular follow-up visits and patients with and without complications were not selected for age or gender. As the number of patients with complications was limited, any protective effect of *NLRP3* rs35829419 polymorphism in females may have been missed in our study due to the small numbers. We also have to note that both studies recruited patients from a small geographical area and other factors contributing to MI, such as lifestyle and physical activity, may differ between the two countries. The authors of the FIA study acknowledge that differences in lifestyle factors, including type of food intake and physical activity, between males and females of northern Sweden may contribute to the gender-specific genetic association and MI observed in their study [17].

In our study males also showed some tendency for increased risk of microvascular complications among polymorphic *NLRP3* rs35829419 A allele carriers, in particular in end-stage kidney failure. Inflammasome was also implicated in inflammatory, autoimmune, and obstructive kidney disease and in ischemia-reperfusion type kidney injury, mostly in rat or mouse models, but the role of NLRP3 inflammasome in diabetic nephropathy remains to be elucidated [25]. Serum IL-18 levels were elevated in patients with diabetic nephropathy, but not in other diabetic complications [26].

We observed no association between *CARD8* rs2043211 and any long-term T2D complications. To our knowledge this is the first study that investigated CARD8 polymorphism in T2D. So far, CARD8 polymorphism was associated with psoriasis [27] and rheumatoid arthritis susceptibility in some [28], but not all, studies [29], but was not associated with juvenile idiopathic arthritis [30]. *CARD8* polymorphism did not influence cytokine profiles in a cohort of healthy blood donors [16]. Recently, *CARD8* rs2043211 T allele was found to be significantly associated with a protective effect of ischemic stroke, but not with coronary artery disease [31].

Due to its exploratory nature the present study has several limitations. The study was retrospective in nature and limited to a single regional diabetic outpatient clinic; therefore the number of patients with a particular long-term T2D complication was relatively small. Nevertheless all the patients were clinically well characterized and were followed up by the same diabetologist; therefore, the discrepancies in clinical data collection procedures were minimalized. Furthermore all the patients were recruited in a small geographic area with ethnically homogeneous population; therefore, there was no bias due to genetic heterogeneity [32].

As diabetic complications are multifactorial, we carefully checked for known clinical parameters as possible covariates that could influence our findings. Glycemia was well controlled in all patients, while blood pressure and dyslipidemia were even more tightly controlled in patients with complications to reduce the risk of diabetes-related mortality [33, 34].

NLRP3 polymorphism could be used as a molecular marker to identify patients in which pharmacological approach could be used to counteract the proinflammatory phenotype [35]. Furthermore components of NLRP3 inflammasome and downstream signalling pathways are potential novel targets for pharmacological prevention of late complications of T2D [36], as clinical trials targeting other inflammatory cytokines such as IL-6 and TNFa have not been successful [37, 38].

In conclusion, our preliminary data suggest the role of NLRP3 polymorphism in diabetic macrovascular complications, especially in MI. Validation in larger patient cohort is required.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

The authors wish to acknowledge the clinical nurse Mrs. Nevenka Kirič for her support with clinical part of the study and Mrs. Savica Soldat, B.S., for her expert technical assistance. This work was supported by the Ministry of Education, Science, and Sport of the Republic of Slovenia (Grant nos. P1-0170 and P3-0298).

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Hindawi Publishing Corporation
Journal of Diabetes Research
Volume 2015, Article ID 150176, 10 pages
http://dx.doi.org/10.1155/2015/150176

Review Article

Study of Protein Biomarkers of Diabetes Mellitus Type 2 and Therapy with Vitamin B1

Samreen Riaz

Department of Microbiology and Molecular Genetics, University of the Punjab, Lahore 54590, Pakistan

Correspondence should be addressed to Samreen Riaz; samreen.mmg@pu.edu.pk

Received 15 September 2014; Revised 27 January 2015; Accepted 3 February 2015

Academic Editor: Joseph Fomusi Ndisang

In the present research work, the levels of protein biomarkers specific to diabetes mellitus type 2 in the Pakistani population using proteomic technology have been identified and characterized and effect of high dose thiamine has been seen on the levels of these marker proteins. Diabetic patients and normal healthy controls were recruited from the Sheikh Zayed Hospital, Lahore, Pakistan. Total biochemical assays and proteins were estimated by modern proteomic techniques. Some proteins were up- and downregulated in diabetic samples as compared to control and decreased after thiamine therapy, while other protein markers did not show a significant change after the thiamine therapy. The effect of high dose thiamine on the levels of these identified protein biomarkers in the human urine has also been observed. Assessment of the levels of these biomarkers will be helpful in not only early diagnosis but also prognosis of diabetes mellitus type 2.

1. Introduction

Diabetes mellitus (DM) is a metabolic and multifactorial syndrome with disordered metabolism and hyperglycemia. On the etiological basis, factors which contributed to the DM and hyperglycemia are reduced secretion of insulin, inherited or acquired insulin deficiency, ineffectiveness of insulin, and low glucose utilization with high production of glucose. The root causes of diabetes are very much complex. Most of the cases start with two processes, metabolic and autoimmune. Some risk factors which contributed to long-term complications for DM are diet, overweight, obesity, smoking, alcohol, level of physical activity, hormones, some medical treatments, viruses, vascular or cardiovascular disease, atherosclerosis, heart conditions, stroke, kidney disease, eye diseases, nerve damage, impaired thinking, infections and wounds, cancer, musculoskeletal disorders, pregnancy complications, emotional difficulties, insulin shock, diabetic ketoacidosis, and hyperosmolar hyperglycemic nonketotic state. The uncontrollable risk factors like genetics and age cannot be altered by people. People can lower the controllable risk factors like exercise and diet through improved health habits and can reduce their risk of developing diabetes [1, 2].

There are several types of DM which exist and are caused by genetic or environmental factors and lifestyle choices. DM is classified into different types on the basis of pathogenic process. The two important types of DM are known as type 1 and type 2. Type 1 DM is juvenile diabetes and is called as insulin dependent diabetes mellitus (IDDM) in which the pancreas fails to produce insulin due to autoimmune beta-cell destruction. It is usually diagnosed in young age like in children, adolescents, and young adults. Type 2 DM is adult-onset diabetes and is called as noninsulin dependent diabetes mellitus (NIDDM) which results from the body tissues and cells inability to respond properly to the action of insulin. There are some other abnormalities like genetic and metabolic abnormalities which are produced in response to insulin action and secretion. Type 2 DM usually occurs in adulthood and develops more with the age and sometimes it is also observed in children and some adolescents having obesity. There are some other types of DM in which specific genetic defects, metabolic and mitochondrial abnormalities, and some conditions that impair glucose tolerance are included [1, 2].

Diabetes is one of the most widely occurring human ailments and the world wide prevalence has risen over

the past two decades. According to new publications of some health agencies like World Health Organization (WHO) and International Diabetes Federation (IDF), diabetes becomes an epidemic which is not controlled like other major diseases, for example, cancer and cardiovascular diseases, and becomes sixth leading cause of death worldwide. Humans are not the only species that can develop DM. This disease occurs also in some animals like dogs, cats, and others. Type 2 DM is much more common than type 1 DM and makes up about 90% of all cases of diabetes. It is more common in the developing countries like Pakistan than developed countries. The incidence of this disease in any developed or developing country is difficult to judge. It is quite obvious that the disease is multiplying geometrically more due to genetic and environmental factors [3].

Vitamin is an organic nutrient which is essential and is required in tiny amounts. A vitamin cannot be synthesized by the human body. There are two main types of vitamins: fat soluble and water soluble vitamins. The water soluble vitamins must be eaten more regularly than fat soluble vitamins. Thiamine (vitamin B_1) is a water soluble vitamin. It has been used singly and in the compound form as a member of B complex family. It has important role in carbohydrates and fat metabolism and is essential for normal growth and development of the human body. It also maintains proper function of the heart, nerves, and digestive system. It occurs as a part of our diet and is present in some diets like cereals, fortified bread, rice, nuts, meats, and legumes. Recommended intake of thiamine for men is 1.4 mg/day, for woman is 1.1 mg/day, for pregnant women is 1.5 mg/day, and for breast feeding is 1.6 mg/day. 100 g corn flakes or 3-4 dL soya milk or 300 g ham covers the daily need. It occurs as a part of our diet. Its deficiency results in a disease called beri beri in which cardiovascular, neurological, and dermatological complications arise. Thiamine deficiency was treated with 50–100 mg of thiamine per day for several days followed by 5–10 mg of oral thiamin per day which was given in parenteral. Treatment is successful in about 50% of patients and replacement of other vitamins as needed. Toxicity of thiamine is not known or reported; it is generally safe. However aside of this restricted use and as a general tonic, it has never been administered as therapy for the diseases for many years [4].

Biomarker is a substance which is used as an indicator for pathological state of disease and a characteristic that is estimated and evaluated for normal, pathological, and pharmacologic responses to a therapeutic intervention. Biomarkers can be used in laboratory for drug discovery, diagnosing, classification, and grading the severity of disease in both laboratory and clinical settings. Biomarkers have a potential for understanding the relationship between disease and health. There are different types of biological markers like protein and genetic and metabolite markers. Biomarkers can also be used for the identification, characterization, and expression of the proteins for biological systems. There are some variations in the patterns of protein expression in normal healthy controls as compared with diseased person. Certain levels of proteins can be up- and downregulated during the progression of disease. Detection of these differences in protein levels is an important area of research in the field of proteomic study. Some of the disease areas of protein biomarkers are, for example, cancer, diabetes, and cardiovascular and neurological diseases. The protein biomarkers are very useful for diagnosis and prognosis of acute and chronic type of mortality in patients having diabetes, various forms of cancer, and other syndromes (Graves and Haystead, 2002).

To identify the protein biomarkers, the proteomic approaches were used that can be exploited for potential diagnostic and prognostic biomarkers in the short and long term of different diseases. Proteomics is the large-scale study of *protein* with their structures, functions, and information coded in a cell which is expressed and regulated at the protein level to achieve the function of an organism. Clinical proteomics aims to discover protein biomarkers which may be a potential target for pharmaceutical field, disease diagnosis, and risk assessment. The goals of proteomics are to apply the proteomic technologies in the clinical research, public health, and environmental, agricultural, and veterinary research [5].

There has been a great interest in the proteomic analysis of plasma and serum for the identification and characterization of protein biomarkers of different diseases. In the modern era for the protein identification, one of the most important developments and technologies is the proteomics. Identification and characterization of low molecular weight proteins in the human plasma/serum and protein profile of human urine have been done by the proteomics. There are many conventional and advanced proteomic technologies that separate the proteins or peptides prior to mass spectrometry (MS) analysis. Ever since O' Ferral introduced the high resolution two-dimensional gel electrophoresis system in 1975, which became the most commonly used technique to analyze protein components of complex mixtures like bacterial extracts and blood plasma. In many ways, the two-dimensional gel electrophoresis was commonly used as a first step for protein identification by mass spectrometry. This approach has made it possible to carry out global protein analysis of living organisms that helps in examining the proteome [6].

Despite considerable reproducibility, this technique could not gain the attention of researchers mainly due to the limitations of this being a labor intensive and qualitative method. Advanced technology in the proteomic filed for the protein separation is the commercial instrument ProteomeLab PF2D from Beckman Coulter. This is the 2D liquid chromatography system (2D-LC) which separates proteins in the first dimension (chromatographic focusing) on the basis of isoelectric points (pI) of protein followed by the second dimension that is reversed phase high performance liquid chromatography (RP-HPLC), where proteins are further fractionated on the basis of hydrophobicity. The assessment, optimization, and separation from the biofluids like plasma, serum, urine, cerebrospinal fluid, and saliva have been observed by the PF2D [7].

The identification and characterization of protein biomarkers have been achieved by the proteomics coupled with mass spectrometry (MS) analysis. MS technology is highly sensitive and is the most important developments in identification of the proteins. For the detection and

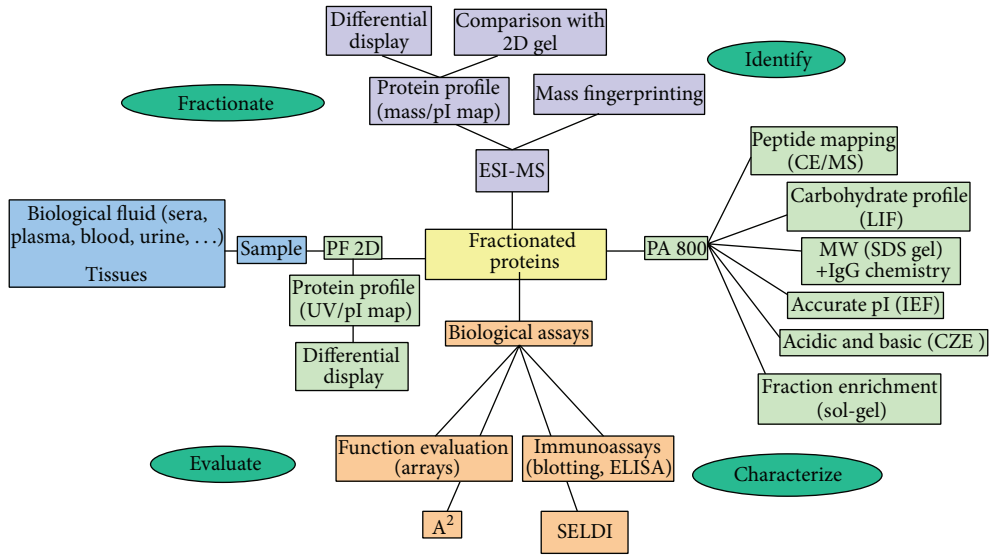

Figure 1: Schematic diagram of protein biomarkers from identification to characterization.

characterization of various biomolecules like proteins, peptides, oligosaccharides, and oligonucleotides, the MS technology was used with the molecular mass range between 400 and 350,000 dalton. It detects very low quantities of sample up to 10^{-15} to 10^{-18} mole with an accuracy of 0.1–0.01%. Matrix assistant laser desorption ionization/time of flight (MALDI-TOF MS) is relatively simple to operate. It has good mass accuracy with increased resolution and high sensitivity and is used in the field of proteomics very widely to identify and characterize the protein biomarkers through peptide mass fingerprinting that can be helpful to identify proteins involved in global diseases like diabetes and others. In the method of MS, selected proteins of choice were digested with a specific proteolytic enzyme and the resulting peptides were analyzed for further studies. The information obtained from this can be used for identification and confirmation of the proteins by searching different protein databases. The process of identification and characterization of disease protein biomarkers was summarized and shown in Figure 1 [8].

2. Review of the Literature

A large number of people with diabetes especially type 2 grow worldwide. So, this disease takes an ever-increasing proportion of national health care budgets. Without primary prevention, the diabetes epidemic will continue to grow. Immediate action is needed to stem the tide of diabetes. There is also a need of introducing cost-effective treatment strategies to control this epidemic. In the following sections, identification, purification, characterization of protein biomarkers for early diagnosis of pathological states like diabetes mellitus, and role of thiamine on the levels of these marker proteins in diabetes type 2 were described. Research work has been done for the search of protein biomarkers for monitoring and predicting the diabetes mellitus. Several modern techniques mainly involving protein characterization by mass spectrometry and proteomic profiling of plasma/serum samples by proteomics have also been described. Animal studies with high dose thiamine therapy have been shown to reduce diabetic nephropathy (microalbuminuria) and lipid disturbances. Therefore a clinical trial of high dose thiamine therapy has been planned in our type 2 diabetic Pakistani population with a hope to find protein biomarker and role of high dose thiamine on the levels of these biomarkers in the patients having diabetes type 2.

2.1. Diabetes Mellitus (DM). Diabetes results in a condition with abnormal or elevated levels of glucose in the bloodstream. This can cause severe acute and chronic complications like forming brain damage to amputations and heart disease. Dysregulation of multiple glucoregulatory hormones (insulin and glucagons) that maintain glucose homeostasis result in the form of diabetes. The imbalances in these hormones lead to long term elevated levels of glucose and some of microvascular and macrovascular complications like retinopathy, nephropathy, and neuropathy [3].

2.1.1. Regulation of Blood Glucose Level in the Body. The blood sugar at normal levels in the human body is maintained and brought by a regulatory mechanism which is very effective and efficient. In this mechanism, the main organs are liver, autonomic nervous system, and certain glands of internal secretion called endocrine glands. To maintain the homeostasis, the blood glucose is vital for the life of the human being. The important and dominant tissues are liver which respond to the signals that indicate low or high levels of blood glucose. The vital function of liver is to produce glucose for the circulation in the blood. Both low and high levels of blood glucose triggered the hormonal responses which initiate the pathways to restore the glucose homeostasis. Reduced levels of blood glucose trigger release of glucagons

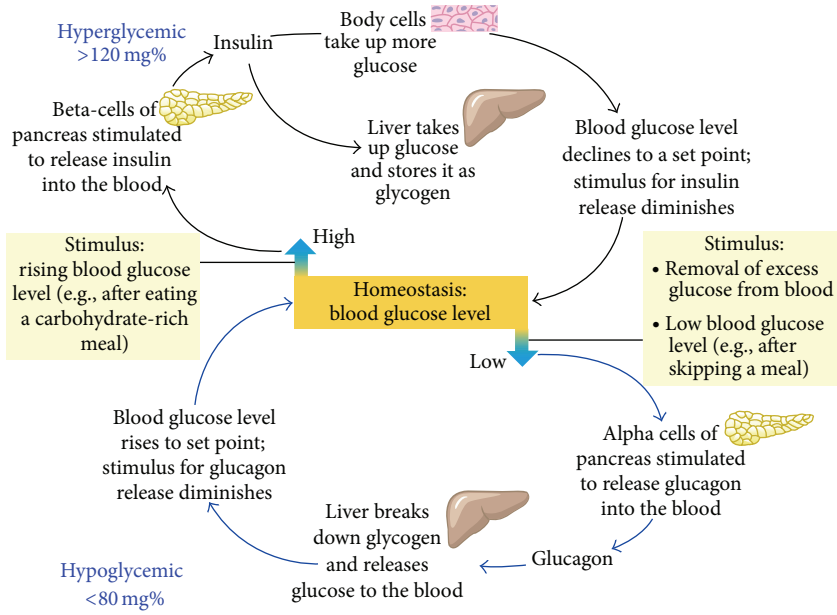

Figure 2: Glucose homeostasis in the human body (adapted from [9]).

from the beta-cells of pancreas, while the increased levels of blood glucose trigger release of insulin from the pancreatic beta-cells. DM results from the dysregulation of multiple glucoregulatory hormones (insulin and glucagons). Any defect in glucoregulatory hormones production leads to improper regulation of glucose in the blood and results in diabetes [9]. This is the glucose homeostasis in which blood glucose levels maintained in the body and dysregulation cause hyper- and hypoglycemia as shown in Figure 2.

2.1.2. Insulin and Its Mechanism of Action in DM. Insulin is a peptide hormone synthesized as precursor of polypeptide preproinsulin with single chain 86 amino acids and is secreted by the beta-cells of the islets of langerhans of pancreatic cells. It maintains the normal levels of glucose in the blood and has mitogenic effects. It facilitates the glucose uptake in the cells, regulates the carbohydrate, lipid, and protein metabolisms, and promotes the cell division and growth. Glucose is the principal stimulus for the secretion of the insulin hormone. Glucose levels >3.9 mmol/L stimulate insulin synthesis. Insulin with its counter regulatory hormone glucagon regulates the concentrations of blood glucose. Beta-cells of pancreases secrete 0.25–1.5 units of insulin per hour during the state of fasting. It is also sufficient to enable the entry of glucose insulin dependent into cells. Any defect in insulin production leads to improper regulation of glucose in the blood and results in diabetes. Similarly, postprandial glucagon secretion is abnormally increased in the patients with type 1 and type 2 diabetes. This abnormal secretion of glucagon leads to excess production of hepatic glucose. It has also played important role in postprandial hyperglycemia in the patients with diabetes mellitus [10].

Glucose regulates the insulin secretion by the pancreatic beta-cell through glucose transporters. There are different types of glucose transporters called GLUT1, GLUT2, GLUT3, and GLUT4. Glucose is transported by the glucose transporter GLUT2 in the body. The glucose metabolism in the beta-cells changes the ion channel activity which leads to secretion of insulin. The sulfonylurea receptor (SUR) is the binding site for the drugs that act as secretagogues for the hormone insulin. Glucose stimulation of insulin and its transport also initiate by the GLUT2 into the beta-cell. Phosphorylation of glucose by the enzyme glucokinase controls the insulin secretion regulated by glucose. The metabolism of glucose-6-phosphate via glycolysis produces the ATP which inhibits the activity of an ATP-sensitive potassium channel. Inhibition of this channel initiates the beta-cell membrane depolarization and opens calcium channels that stimulate insulin secretion as shown in Figure 3. Dearrangement in the normal secretary patterns is one of the earliest signs of beta-cell dysfunction in diabetes [9].

Low insulin levels increase the production of glucose by promoting the hepatic gluconeogenesis and glycogenolysis in the fasting state. Glucagons stimulate the glycogenolysis and gluconeogenesis by the liver and the renal medulla. Reduced insulin levels declare the glycogen synthesis and low glucose uptake in insulin sensitive tissues. This also promotes the mobilization of precursors which were stored. Postprandially, glucose load elicits an increase in insulin and decrease in glucagons leading to a reversal of these processes. The main potion of postprandial glucose is utilized by skeletal muscle and insulin stimulated glucose uptake. Other tissues like brain also utilize glucose in an insulin independent manner [11].

2.1.3. Role of Insulin in Type 2 DM. Type 2 DM is a heterogeneous group of disorders and is characterized by the resistance and impaired secretion of insulin and high

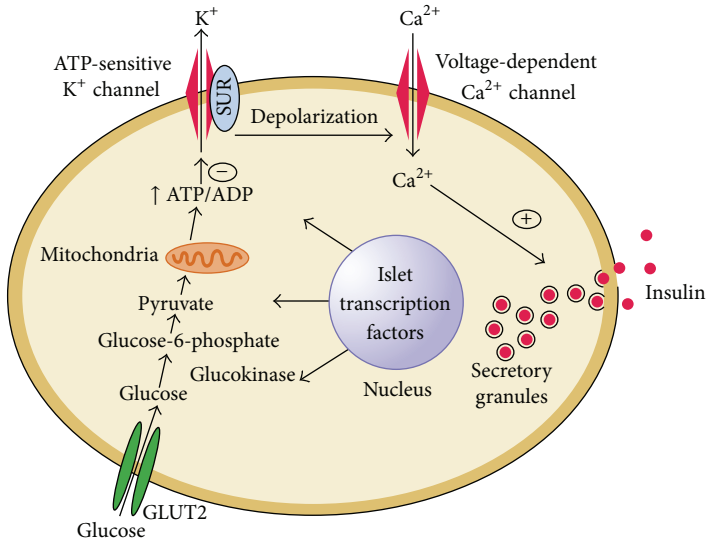

Figure 3: Diabetes and abnormalities in glucose-stimulated insulin secretion (adapted from [9]).

levels of glucose production. There are some metabolic and genetic defects in the action or secretion of insulin which give rise to hyperglycemia in type 2 DM. Obesity is very common in type 2 diabetes where the adipocytes cells secret a number of biological proteins (leptin, TNF-alpha, resistin, and adiponectin) that modulate insulin secretion or action and may contribute to insulin resistance. The pancreatic islets are unable to sustain the hyperinsulinemia state in the progression of insulin resistance and hyperinsulinemia in certain individuals. Impaired glucose tolerance characterized by elevations in postprandial glucose was developed. Reduced insulin secretion and increased hepatic glucose production lead to hyperglycemia in fasting diabetes. Ultimately the beta-cell failure may increase the release of inflammatory protein markers such as Interleukin-6 (IL-6) and C-reactive protein (CRP) in type 2 DM [11].

The molecular mechanism of insulin resistance can be explained briefly as levels of insulin receptors and tyrosine kinase activity in skeletal muscles are reduced. These alterations are most likely to cause hyperinsulinemia. Therefore, postreceptor defects play the important and dominant role in insulin resistance. Polymorphism in insulin receptor substrates (IRS) may also be linked with intolerance of glucose, which raised the possibility that polymorphism in different postreceptors molecules combined and created a state of insulin resistance. The pathogenesis of insulin resistance is focused on signaling defect of a phosphatidylinositol-3-kinase (PI-3 kinase). Among other abnormalities, it also lowers the translocation of GLUT4 to the membrane of plasma. The insulin receptor has tyrosine kinase activity which has intrinsic property. These may interact with the proteins of IRS. Many docking proteins bind to these proteins and stimulated the metabolic action of insulin and PI-3-kinase pathway. As a result, insulin gives rise to elevation in glucose transport via PI-3-kinase pathway and stimulates the translocation of intracellular vesicles which has glucose transporters as GLUT4 to the plasma membrane as shown in Figure 4. There are some pathways of insulin signal transduction which are not resistant to effect of insulin like the cell growth/differentiation and mitogenic activated protein (MAP) kinase pathways. As a result, hyperinsulinemia elevated the insulin action through these pathways and accelerated the diabetes and other conditions like atherosclerosis. Free fatty acids can impair the utilization of glucose in the muscles. It also promotes the production of glucose by the liver and impairs the function of beta-cells in the case of obese type 2 DM [9].

2.2. Protein Biomarkers for DM. There are varying reports on the role of protein biomarker for the identification of different diseases like cancer, diabetes, and others. There are some proteins which may be up- and downregulated in the serum/plasma and urine of diabetes mellitus especially in type 2.

2.2.1. Identification and Characterization of Protein Biomarkers. The protein biomarkers are very helpful for predicting long-term mortality in patients with diabetes, cancer, and coronary syndromes. Searching for novel biomarkers can be done using tissues and/or biofluids (blood, serum, plasma, and urine). The urine is an ideal biofluid for biomarker discovery in kidney diseases and diabetes mellitus. Urine samples obtained from patients with other diseases or disorders that have clinical, biochemical, and metabolic profiles similar to those of the disease of interest must be included as the other controls. Finally, a single ideal biomarker may not exist for each disease. Therefore, evaluating a panel of multiple biomarkers may be required.

Recently, Thongboonkerd [12] has extensively applied proteomics to biomarker discovery in several diseases with the hope of finding novel biomarkers for earliest diagnosis of the diseases at their very beginning phase and for prediction of therapeutic response, survival, and recurrence.

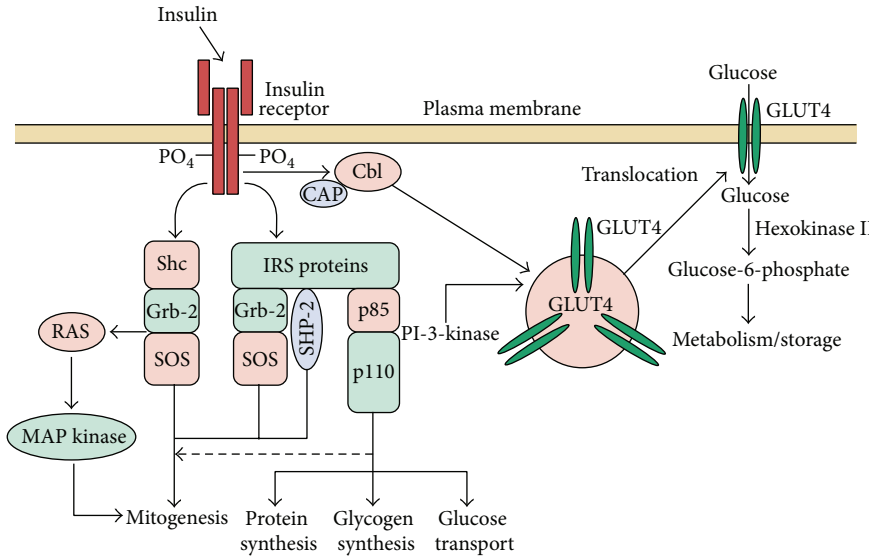

Figure 4: Insulin signal transduction pathway in skeletal muscle (adapted from [9]).

A two-dimensional liquid phase chromatographic separation followed by the mass spectrometry method is involved for proteomics studies and biomarker identification of different diseases. Proteomic analyses using prefractionation strategies were used to identify the biomarker for early detection, diagnosis, prognosis, tumor responses, and disease recurrence for predicting and monitoring biological markers. Proteome analysis of human serum proteins and efficient prefractionation of low abundance proteins in human plasma were done. The construction of two-dimensional map was demonstrated which displayed nearly 3700 chromatographically separated proteins which included 235 more distinct proteins [13]. Complex protein mixture analysis by an all-liquid-phase 2D mapping technique has been done by ProteomeLab PF 2D that is a novel protein profiling and mapping technology. It is also a new powerful way to analyze complex protein mixture in tissues and cells [14]. Detection of electrophoretically derived protein mass based on MS/MS was used as additional constraints in proteomic analysis of human serum [15].

2.2.2. Serum Protein Biomarkers Reported in DM. The proteomics is a valid approach to screen for novel protein biomarkers in animal and human models of obesity and type 2 diabetes. Proteins are reported as biomarkers in the biofluids, tissues, and cells especially in type 2 DM. There are various forms of apolipoproteins which have been identified as protein biomarkers. One of them is apolipoprotein A1 (apoA-I), which is the main component of *high density lipoproteins* (HDL) present in *plasma*. In case of excretion, HDL promotes *cholesterol* efflux from tissues to the liver for excretion (Yui et al., 1998). The level of apoA-1 is positively correlated with HDL cholesterol in the serum and negatively correlated with low density lipoproteins (LDL) cholesterol. Clay et al. [16] have studied the apolipoprotein A-II (ApoA-II) that is in forms of 20% of HDL cholesterol and in human it is present about two-thirds of HDL in humans.

Apolipoprotein H (Apo-H) is also called β2-glycoprotein I. It is a plasma glycoprotein present in circulating and free protein or associated with lipoproteins. It has an important role in blood coagulation and clearance of apoptotic bodies from the circulation [17]. The ApoA-I-CIII-AIV levels in type 2 DM and coronary heart disease were studied. The determination of a novel susceptible haplotype was investigated after the study. Apolipoprotein B (Apo-B) is the primary *apolipoprotein* of *LDL*. It is also called bad cholesterol and is responsible for carrying cholesterol to *tissues* [18]. In 2002, Bach-Ngohou and coworkers [19] studied the apolipoprotein E (Apo-E) which is important for the normal *catabolism* of *triglyceride*-rich *lipoproteins*. It is important in lipoprotein metabolism and has a role in *cardiovascular disease*. The serum levels of some protein biomarkers like clusterin and apolipoprotein J were elevated significantly in type 2 DM and during development of coronary heart disease or at myocardial infarction [20]. Nakanishi et al. [21] observed the diabetic macular edema after proteomic analysis of vitreous and recorded six proteins including pigment epithelium derived factor (PEDF), ApoA-4, ApoA-1, *thyroid hormone receptor interactor 11* (Trip-11), retinol binding protein 4 (RBP4), and vitamin D binding protein (VDBP) and only Apo H is present in nondiabetic controls.

Borth in 1992 [22] studied the alpha 2-macroglobulin (α2-M) which is an inhibitor for the proteinase enzyme in the blood and tissue. It acts as a binding protein for numerous cytokines and growth factors and also acts as a leptin-binding protein in human plasma. Measurement of levels of protein human α_1-microglobulin (α_1m) was studied in the serum and urine of patients with various liver diseases [23]. Transthyretin and its miracle function and pathogenesis were also observed in one recent research work [24]. In addition, protein biomarker for diabetic nephropathy such as microalbuminuria is also a known predictor for the coronary heart and peripheral vascular diseases and high risk of

Table 1: Serum proteins identified in diabetes mellitus.

Serial number	Category of protein	Name of protein	Expression in diabetics
1	Cytokines and cytokine-related proteins	Leptin	+
		TNF-alpha	+
		IL-6	+
2	Other immune-related proteins	MCP-1	+
3	Proteins involved in fibrinolytic system	PAI-1	+
		Tissue factor	+
4	Complement and complement-related proteins	Adipsin (complement factor D)	+
		ASP	+
		Adiponectin	−
5	Lipids and proteins for lipid metabolism or transport	Lipoprotein lipase (LPL)	+
		Apolipoprotein E	+
		Apolipoprotein A1,	−
		Apolipoprotein A2	−
		Apolipoprotein B	+
		Apolipoprotein H	−
		Apolipoprotein C1, C2	−
		NEFAs	+
		Cholesterol ester transferase protein (CETP)	+
6	Inflammatory proteins	C-reactive protein (CRP)	+
		α-tumor necrosis factor (αTNF)	+

mortality in patients of type 2 DM [25]. In 1989, Semenkovich and his coworkers [26] observed that the lipoprotein lipase is an important enzyme for lipid homeostasis in humans. It provides intravascular release of fatty acid from circulating triacylglycerol. Lipoprotein lipase is the primary enzyme that converts lipoprotein triglyceride (TG) to free fatty acids. Tumor necrosis factor-α (TNF-α) is a pleiotropic cytokine and has role in immunity and inflammation and during chronic illness. Its elevated secretion contributed to hemorrhage, necrosis, and death [27]. Leptin was adipokines and has a role in modulating adiposity. This obesity-related hormone is a molecule which regulates the energy balance and body weight [28].

C-reactive protein (CRP) is a protein of acute phase condition and a strong biomarker of inflammation in the progression of various diseases like coronary heart disease, cancer, diabetes, and others. Genetic variation and levels of CRP and incidence of diabetes especially risk of developing type 2 diabetes mellitus have been studies. The concentration of CRP in the blood of normal healthy control human beings ranges from 0 to 1.0 mg/dL. In the acute phase condition of inflammation the CRP levels may rise up to the 1000-fold [29]. In 2002, Lindsay and coworkers [30] demonstrated that some of the proteins are downregulated in DM likeone of the adipokines adiponectin in Indian population. It is a collagen-like plasma protein produced and secreted by adipose tissue. It has compelling antiatherogenic and insulin-sensitizing properties.

Few years later, Martín-Gallán et al. [31] studied that some protein biomarkers of diabetes are associated in young diabetic patients with oxidative stress and antioxidant status. Proteomic analysis of proteins was done and involved in insulin resistance and type 2 DM with the b3-Adrenergic receptor [32]. Proteomics detects oxidatively induced protein carbonyls in muscles of a diabetic rat. A number of proteins, including mitochondrial ATP synthase, desmin, actin, and myosin, are found carbonylated [33]. Festa et al. 2002 [34] have described that the elevated levels of acute-phase proteins and plasminogen activator inhibitor-1 (PAI-1) in type 2 diabetes with insulin resistance were observed. In type 2 DM, the intramuscular heat shock protein 72 and heme oxygenase-1 mRNA were decreased [35]. In literature, diabetes is associated with pancreatic cancer in 80% cases. Tumor-derived peptide with an S-calcium binding protein is involved in the pancreatic cancer associated with diabetes which are the studies of Bassoa and coworkers [36]. Lipids and lipoproteins have been studied in patients with type 2 DM. Recently, obesity and its related proteins like leptin were studied in the diabetic population of Pakistan [37]. List of identified proteins in human serum of patients having diabetes mellitus is given in Table 1.

2.2.3. Urinary Protein Biomarkers Reported in DM. Urinary protein profiling can reveal changes in excretion rates of specific proteins that can have predictive value in the clinical arena, for example, in the early diagnosis of disease, classification of disease with regard to likely therapeutic responses, assessment of prognosis, and monitoring response to therapy.

Yang et al. in 2005 [38] have described that RBP4 protein is an important biomarker for insulin resistance in diabetes. In one such study which was carried out by Metz and coworkers [39], the five protein biomarkers were identified as 2-fold upregulated in diabetes which were alpha-2-glycoprotein (zinc), corticosteroid-binding globulin,

Table 2: Urinary protein identified in diabetes mellitus.

Serial number	Category of protein	Name of protein	Expression in diabetics
1	Defense response	α1-antitrypsin	+
		Complement factor H, C3, B, I, C7, 9	+
		Alpha-1-antichymotrypsin precursor	+
		Antithrombin-III	+
		Alpha-2-glycoprotein 1, zinc	+
		Ig gama 1 chain C region	+
		Alpha and beta-2-microglobulin	+
		Alpha-2-antiplasmin precursor	+
		Vitronectin precursor	+
2	Transport	Serotransferrin precursor	−
		Ceruloplasmin precursor	+
		Hemopexin	+
		AMBP protein	+
		Albumin	−
		Haptoglobin precursor	+
		Transthyretin precursor	−
		VDBP	−
3	Metabolism	ApoA-1, ApoA-II precursor, Apo-D	−
		Alpha-1B-glycoprotein	+
		Beta-2-glycoprotein 1 precursor	+
		Prostaglandin H2 D-isomerase precursor	+
		Alpha-2-HS-glycoprotein precursor	+
		E-cadherin	+
		Dystroglycan precursor	+
		Fibrinogen beta chain precursor	+
4	Signal transduction	Kininogen precursor	+
		B-factor, properdin	+
		Clusterin	−
		Angiotensinogen	+
		Sulfated glycoprotein 2	+
		retinol-binding protein 4	+
		Epidermal growth factor	+
5	Cell development	Lumican precursor	+

and lumican. The clusterin and serotransferrin were 2-fold downregulated in diabetic samples compared to control. For urinary biomarkers in diabetic nephropathy, proteomic analyses identified some potential protein biomarkers.

List of urinary proteins that are identified in diabetic patients with or without nephropathy are listed in Table 2.

In 2007, Rao et al. have observed that there were seven proteins which were upregulated with increasing albuminuria and four proteins were downregulated.

After some years, the proteomic analysis was done for the identification of human salivary biomarkers for type-2 diabetes [24]. Recently, in 2009, Jiang and his coworkers [40] studied the identification of urinary soluble E-cadherin as a novel biomarker for diabetic nephropathy. Shinton et al. [41] observed that haptoglobins are a group of serum proteins; originally identified and diagnostic values were determined.

The goals of present research work were the identification and characterization of protein biomarkers for early diagnosis and prognosis of pathological states of diabetes mellitus type 2 and effects of high dose thiamine on the levels of marker proteins. These investigations shall be helpful in assessing the biochemical alterations in the Pakistani diabetic population, which should contribute to the development of treatment plans for this disease which is one of the most widely occurring and debilitating diseases. Results from this research will also contribute to the identification of protein markers of diabetes mellitus type 2 and thus development of novel diagnostic procedures for early detection of this complication in potential diabetics in our population. The findings from present research work will assist in planning preventive and effective treatment strategies for diabetic patients by identification of protein biomarkers and high dose thiamine as nutritional supplement.

Conflict of Interests

The author declares that there is no conflict of interests regarding the publication of this paper.

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Hindawi Publishing Corporation
Journal of Diabetes Research
Volume 2015, Article ID 972073, 7 pages
http://dx.doi.org/10.1155/2015/972073

Research Article

Inflammatory Cytokine Profile Associated with Metabolic Syndrome in Adult Patients with Type 1 Diabetes

Aldo Ferreira-Hermosillo,[1] Mario Molina-Ayala,[1] Claudia Ramírez-Rentería,[2] Guadalupe Vargas,[1] Baldomero Gonzalez,[1] Armando Isibasi,[3] Irma Archundia-Riveros,[3] and Victoria Mendoza[1]

[1]*Endocrinology Department, Hospital de Especialidades Centro Médico Nacional Siglo XXI, Instituto Mexicano del Seguro Social (IMSS), Cuauhtémoc 330, Colonia Doctores, 06720 Mexico City, MEX, Mexico*
[2]*Experimental Endocrinology Investigation Unit, Hospital de Especialidades Centro Médico Nacional Siglo XXI, Instituto Mexicano del Seguro Social (IMSS), Cuauhtémoc 330, Colonia Doctores, 06720 Mexico City, MEX, Mexico*
[3]*Immunochemistry Medical Investigation Unit, Hospital de Especialidades Centro Médico Nacional Siglo XXI, Instituto Mexicano del Seguro Social (IMSS), Cuauhtémoc 330, Colonia Doctores, 06720 Mexico City, MEX, Mexico*

Correspondence should be addressed to Victoria Mendoza; vmendozazu@yahoo.com

Received 15 December 2014; Revised 6 February 2015; Accepted 23 February 2015

Academic Editor: Alfredo Vannacci

Objective. To compare the serum concentration of IL-6, IL-10, TNF, IL-8, resistin, and adiponectin in type 1 diabetic patients with and without metabolic syndrome and to determine the cut-off point of the estimated glucose disposal rate that accurately differentiated these groups. *Design*. We conducted a cross-sectional evaluation of all patients in our type 1 diabetes clinic from January 2012 to January 2013. Patients were considered to have metabolic syndrome when they fulfilled the joint statement criteria and were evaluated for clinical, biochemical, and immunological features. *Methods*. We determined serum IL-6, IL-8, IL-10, and TNF with flow cytometry and adiponectin and resistin concentrations with enzyme linked immunosorbent assay in patients with and without metabolic syndrome. We also compared estimated glucose disposal rate between groups. *Results*. We tested 140 patients. Forty-four percent fulfilled the metabolic syndrome criteria ($n = 61$), 54% had central obesity, 30% had hypertriglyceridemia, 29% had hypoalphalipoproteinemia, and 19% had hypertension. We observed that resistin concentrations were higher in patients with MS. *Conclusion*. We found a high prevalence of MS in Mexican patients with T1D. The increased level of resistin may be related to the increased fat mass and could be involved in the development of insulin resistance.

1. Introduction

Type 1 diabetes mellitus (T1D) is an autoimmune disease characterized by beta cell destruction [1]. Its incidence has increased by ~3% per year in children under the age of 5 and represents 10% of all the cases of diabetes [2]. In Mexico, the incidence of T1D among children under 19 years of age is 6.2 per 100,000 insured cases in the Instituto Mexicano del Seguro Social (IMSS), the largest social health care provider throughout the country [3]. Our country has a very high prevalence of obesity worldwide in all age groups, and it also affects patients with T1D [4]. Metabolic comorbidities associated with increased weight, such as hypertension or dyslipidemia, are also very common among the Mexican population [5]. Some cohorts have previously described T1D patients with features of insulin resistance and metabolic syndrome [6]. This association, sometimes called "double diabetes" [7], is being actively studied on the basis that each entity may increase cardiovascular morbidity on its own and theoretically generate a synergic and detrimental effect on mortality [8] due to cardiovascular diseases, despite intensive glycemic control [9].

Depending on the population studied and the criteria used, metabolic syndrome (MS) has been reported in 8 to 43% of the T1D patients [6, 10, 11]. The MS components, usually associated with an increased cardiovascular risk, were highly

prevalent in patients with T1D and vascular comorbidities [8]. An additional source of controversy is the difficulty to define metabolic syndrome in patients with T1D; since all patients are diabetic, this may represent a selection bias and possibly overdiagnosis; also, the most important feature of MS (insulin resistance) is not part of the pathogenesis of T1D and, finally, the use of exogenous insulin in T1D makes it impossible to predict insulin resistance with simple methods such as the HOMA-IR index calculation. In order to solve these problems, different authors have proposed methods to assess insulin sensitivity such as eGDR (estimated glucose disposal rate), but its utility in patients with T1D and MS has not been determined and since it is based on the measurements of waist-to-hip ratio, it has been suggested that cut-off points for each population need to be validated. In groups with predominantly Hispanic and overweight populations, assessing these cut-offs is even more necessary.

The pathophysiology of the increased cardiovascular risk associated with MS in T1D is complex and not yet completely understood. A wide range of cardiac diseases have been associated with inflammation and cytokine modulation. Type 1 diabetic patients consistently report higher levels of fibrinogen, interleukin-6 (IL-6), C-reactive protein, and tumoral necrosis factor-alpha (TNF-α) compared to nondiabetic patients [12, 13]; however these studies were not designed to prove differences between patients with and without MS. On the other hand, additional studies have demonstrated that patients with type 2 diabetes have similar inflammatory profiles, but the general assumption was that their elevated cytokines were generated by insulin resistance and increased adiposity, since some of them decreased significantly once the patients lost weight [14]. Resistin is a proinflammatory cytokine previously considered to be responsible for the obesity-mediated insulin resistance [15, 16]. This cytokine has been found to be elevated in lean T1D patients, but the clinical significance of this elevation has yet to be elucidated. Given these controversial reports and the lack of information regarding patients with double diabetes, especially in high risk populations such as ours, we aimed to compare the inflammatory profile of patients with T1D with and without MS.

2. Patients and Methods

We performed a cross-sectional evaluation of all patients in the type 1 diabetes clinic from January 2012 to January 2013 (Hospital de Especialidades Centro Médico Nacional Siglo XXI, a tertiary care referral center). We included patients that were 18 years of age or older at the time of the study and had at least 3 visits per year to the clinic, no infections recorded in the 3 months prior to the study, a normal complete blood count and urinary analysis, and no change in insulin dose in the last 3 months. Patients with incomplete records, poor treatment adherence, clinical or biochemical data of infection, end-stage renal disease, evidence of autoimmune diseases (except for treated primary hypothyroidism), and primary dyslipidemias were excluded. The study completed all the requirements by local ethics committee and was conducted in accordance with the Declaration of Helsinki. The protocol's nature and the aim of the study were fully explained to the subjects, who gave their written consent.

2.1. Diagnostic Criteria for MS. Patients were considered to have MS when they presented 3 or more of the joint statement criteria from the American Heart Association/National Heart Lung and Blood Institute (AHA/NHLBI) and the International Diabetes Federation (IDF) [17]: serum triglycerides >150 mg/dL (1.7 mmol/l) or patients receiving treatment for hypertriglyceridemia, serum high-density lipoprotein cholesterol (c-HDL) <40 mg/dL (1.03 mmol/l) in men or <50 mg/dL (1.29 mmol/l) in women or a previously treated dyslipidemia, arterial blood pressure >130/85 mmHg in two different determinations or if the patients were receiving treatment with antihypertensive drugs, and waist circumference (WC) >90 cm in men and >80 in women. Since all the patients were under treatment for type 1 diabetes, they all had fasting plasma glucose >100 mg/dL (5.6 mmol/l) at least once.

2.2. Anthropometric Measurements. At initial evaluation we registered weight (kg) and height (meters), as well as WC (cm). Using these parameters we evaluated waist-to-height ratio (WHtR) and waist-to-hip ratio (WHR). A single investigator, using the same calibrated instruments, performed all the anthropometric measurements. WC was determined at the middle point between the inferior rim of the last costal arch and the superior rim of the anterosuperior iliac spine. Body mass index (BMI) was calculated with the formula that divides weight by height to the square. We used BMI determination to define weight groups, according to the World Health Organization (WHO) [18] classification. Blood pressure was determined in the left arm, after 10 minutes in a resting position, during a fasting state, without coffee or tobacco ingestion in the last week. The sphygmomanometer was calibrated and values were averaged after 2 different measurements with a 5-minute difference between them.

2.3. Biochemical Determinations. Laboratory results were obtained with a 6 mL sample in BD Vacutainer (BD, Franklin Lakes, NJ, USA) and centrifuged at 3150 ×g for 15 minutes, and serum was divided into two aliquots. We analyzed glucose, cholesterol, c-HDL, and triglycerides with a commercially available kit (COBAS 2010 Roche Diagnostics, Indianapolis, USA) using photocolorimetry with spectrophotometer Roche Modular P800 (2010 Roche Diagnostics, Indianapolis, USA). c-HDL samples were treated with enzymes modified with polyethylene glycol and dextran sulphate, analyzed with the same photocolorimetric technique. Glycated hemoglobin (HbA1c) was evaluated by turbidimetric immunoanalysis (COBAS 2010 Roche Diagnostics, Indianapolis, USA). Low-density lipoprotein cholesterol (c-LDL) was calculated with Friedewald formula c-LDL (mg/dL) = CT mg/dL − (c-HDL mg/dL + triglycerides mg/dL/5) if triglycerides were <400 mg/dL [19].

2.4. Cytokine Determination. Adiponectin and resistin were analyzed with a commercial kit (Human Adiponectin and

Resistin Platinum ELISA tests) using enzyme linked immunosorbent assay (ELISA). Interassay coefficients of variation (%CV) for adiponectin and resistin ranged from 5.8 to 6.9% and from 7.8 to 9.2%, respectively. Interleukin-8 (IL-8), interleukin-6 (IL-6), interleukin-10 (IL-10), and total tumor necrosis factor (TNF) were analyzed with BD Cytometric Bead Array (CBA) Human Inflammation Kit using flow cytometry (BD FACSAria, BD Biosciences, USA). The %CV of IL-8 was 4–7%, %CV of IL-6 was 8–10%, %CV of IL-10 was 8–11%, and %CV of TNF was 8–15%. Detection limits for each assay were 1.5 pg/mL for IL-6, 2.7 pg/mL for TNF, 2.3 pg/mL for IL-10, 2.6 pg/mL for IL-8, 0.24 ng/mL for adiponectin, and 0.26 ng/mL for resistin.

2.5. Insulin Resistance Quantification. Insulin resistance was calculated using the estimated glucose disposal rate (eGDR) according to the following formula: 24.31 − (12.22 × waist-to-hip ratio [WHR]) − (3.29 × hypertension [defined as 0 = no, 1 = yes]) − (0.57 × HbA1c). Using this formula, a lower eGDR level indicates greater insulin resistance [20].

2.6. Statistical Analysis. Data was analyzed with STATA v.11. Kolmogorov-Smirnov test was used to determine normality. Results are expressed accordingly with means and standard deviations (SD) or medians and interquartile ranges (IQR). To establish associations between quantitative variables, Student's t-test or Mann-Whitney U test was used. Qualitative variables were associated with χ^2 or Fisher's test. Additionally, correlations were performed using a Spearman test. Receiver operating characteristic (ROC) curves were used to identify the best cut-off point of eGDR and resistin with area under curve (AUC) and 95% confidence intervals. To evaluate the factors associated with the presence of the MS, a multiple logistic regression model was performed. A $p < 0.05$ was considered to be significant.

3. Results

We tested 140 patients during the study period with a median age of 28 years (22–37 years); 70% of them were female. Median time from diagnosis was 17 years (11–25 years). In the whole group, 54% had a WC larger than the recommended for their sex (central obesity), 30% had hypertriglyceridemia, 29% had hypoalphalipoproteinemia, and 19% were hypertensive. According to the WHO classification [18] only 58% of the patients were considered to be in the normal weight range, 33% were overweight ($n = 46$), and 8% were obese ($n = 11$). Using joint statement criteria, 44% of the patients were considered to have MS. Table 1 compares the baseline characteristics of the groups with and without MS.

Patients with MS are significantly older and have longer evolution of the disease. As it was expected, they also have higher BMI, WC, WHtR, and WHR; their total cholesterol, triglycerides, and c-LDL concentrations were higher while c-HDL was lower; however insulin dose per body weight, glomerular filtration rate, and HbA1c concentration were not different between groups.

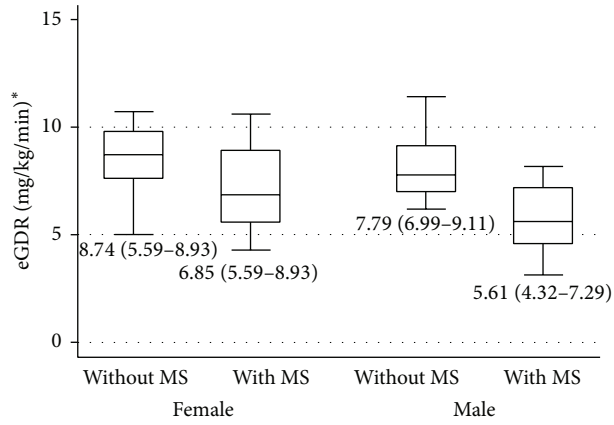

Figure 1: Estimated glucose disposal rate (eGDR) levels in male and female patients with and without MS. *Data is presented as median (IR).

3.1. Insulin Resistance Quantification. Although there were no significant differences in the insulin requirements between the groups, eGDR levels were lower in patients with MS (6.63 mg/kg/min, IQR 4.79–8.59), when compared to the group without MS (8.42 mg/kg/min, IQR 7.49–9.67) ($p < 0.001$). The lowest eGDR was registered in the male patients with MS; however it was not statistically different from their female counterparts (Figure 1).

We performed a receiver operating characteristic (ROC) curve and Youden index for assessing optimal eGDR cut-off point for detecting MS in T1D patients. An eGDR level below 7.32 mg/kg/min showed 80% sensitivity and 66% specificity for MS diagnosis, with an area under curve (AUC) of 0.743 (IC 95% 0.648–0.839) ($p < 0.001$).

3.2. Inflammatory Profile. Table 2 shows the inflammatory profile assessed in all T1D patients. We observed that IL-8 and resistin concentrations were higher in patients with MS; however IL-8 levels were not statistically different between groups. We did not find any difference in the cytokine levels when comparing groups according to sex, HbA1c groups (<7%, 7-8%, or >8%), or obesity grades (BMI rate normal, overweight, or obesity) (data not shown). Resistin was the only cytokine that showed a significant difference between the groups with and without MS. A ROC curve identified that a cut-off point of 1108 pg/mL detects MS with a sensitivity of 71% and specificity of 56%, with an AUC of 0.68 (0.55–0.81). We also quantified correlation coefficients between these cytokines and observed a positive correlation between IL-8, IL-6, IL-10, and resistin in patients with and without MS (Table 2). As it was expected, the resistin concentrations also correlated with IL-6 ($r = 0.398$, $p = 0.001$) and with TNF ($r = 0.533$, $p < 0.001$). Only the group with MS showed a positive correlation in these cytokines: resistin versus IL-6 ($r = 0.532$, $p = 0.001$) and versus TNF ($r = 0.582$, $p = 0.001$). When we correlated the different cytokines, we found a positive correlation between IL-10 and IL-6 ($r = 0.676$, $p \leq 0.001$) and between IL-10 and TNF ($r = 492$, $p = 0.001$); other cytokines did not show any significant correlation.

Table 1: Baseline characteristics of the population comparing patients with and without MS.

Parameter	With MS ($n = 61$)	Without MS ($n = 79$)	p
Age, years (median, IR)	34 (24–42)	26 (20–32)	0.001*
Evolution, years (median, IR)	19 (12.5–27.5)	16 (10–22)	0.021*
WC, cm (mean ± SD)	90.3 ± 10	80 ± 9.2	<0.001†
Male	92.3 ± 11.31	85.9 ± 7.61	0.05†
Female	89.5 ± 9.56	77.2 ± 8.65	<0.001†
BMI, kg/m^2 (mean ± SD)	26.1 ± 3.83	23.5 ± 3.09	<0.001†
Male	25.7 ± 4.1	23.8 ± 3.3	0.124†
Female	26.3 ± 3.7	23.3 ± 3	<0.001†
WHR (mean ± SD)	0.90 ± 0.06	0.87 ± 0.07	0.001†
Male	0.95 ± 0.04	0.90 ± 0.06	0.016†
Female	0.90 ± 0.07	0.86 ± 0.06	0.009†
WHtR (median, IR)	0.54 (0.5–0.6)	0.48 (0.45–0.52)	<0.001*
Systolic blood pressure, mmHg (median, IR)	110 (100–120)	110 (100–120)	NS
Diastolic blood pressure, mmHg (mean ± SD)	68.7 ± 8.43	67 ± 8.34	NS
Insulin dose, units (median, IR)	54 (43–72)	52 (42–60)	NS
U/body weight (kg) (median, IR)	0.67 (0.54–0.93)	0.83 (0.63–1.04)	
Glomerular filtration rate, mL/min (median, IR)	77.9 (30.5–97)	76 (46.9–98)	NS
Fasting glucose, mg/dL (median, IR)	150 (88–220)	140 (94–238)	NS
HbA1c, % (median, IR)	9 (8–10)	8 (8–10)	NS
Cholesterol, mg/dL (median, IR)	198 (163–240)	175 (153–206)	0.005*
Triglycerides, mg/dL (median, IR)	164 (113–241)	89 (65–119)	<0.001*
c-HDL, mg/dL (median, IR)	46 (37–59)	57 (47–67)	<0.001*
Male	44 (35.7–52.7)	46.5 (42.2–54)	0.23
Female	51 (37–63)	61 (53–71)	<0.001*
c-LDL, mg/dL (median, IR)	112 (94.5–137.5)	103 (80–116)	0.018*

IR: interquartile range, WC: waist circumference, BMI: body mass index, WHR: waist-to-hip ratio, WHtR: waist-to-height ratio, HbA1c: glycated hemoglobin, c-HDL: cholesterol associated with high-density lipoprotein, c-LDL: cholesterol associated with low-density lipoprotein.
*Mann-Whitney U test; †Student's t-test.

Table 2: Inflammatory profile in total T1D population and in patients with and without MS.

Parameter	T1D population ($n = 140$)	Patients with MS ($n = 61$)	Patients without MS ($n = 79$)	p^*
IL-8, pg/mL (median, IR)	18.2 (8.68–41.22)	24.6 (12.65–46.5)	13.4 (7.0–34.4)	0.064
IL-6, pg/mL (median, IR)	1.5 (1.5–3.2)	1.5 (1.5–4.7)	1.5	NS
IL-10, pg/mL (median, IR)	2.3 (2.3–3)	2.3 (2.3–4.4)	2.3	NS
TNF, pg/mL (median, IR)	2.7 (2.7–4.8)	2.7 (2.7–7.8)	2.7	NS
Adiponectin, pg/mL (median, IR)	9.4 (5.7–15.5)	8.9 (5.4–14.9)	9.4 (6.6–15.6)	NS
Resistin, pg/mL (median, IR)	1180.3 (775.8–1896.8)	1627.4 (838.8–2233.8)	1055 (631.8–1459.2)	0.010
%Patients with resistin >1108 pg/mL	55%	71%	44%	0.021

IR: interquartile range, T1D: type 1 diabetes, MS: metabolic syndrome, NS: not significant, IL-8: interleukin-8, IL-6: interleukin-6, IL-10: interleukin-10, and TNF: tumor necrosis factor. *Comparing patients with and without MS, using Mann-Whitney U test.

Many patients' results were found in the lowest detection limit for each cytokine. When we consider these patients as "undetectable" and compare them with the patients that reported higher cytokines (detectable), there are significant differences between the percentages of patients in these categories when dividing according to resistin levels. In the group with high resistin level (>1108 pg/mL) we found that IL-6 was detectable (>1.5 pg/mL) in 39.4% versus only 11.5% of detectable IL-6 in the group of patients with low resistin ($p = 0.03$). Furthermore TNF was detectable (levels higher than 2.7 pg/mL) in 47% of patients with high resistin versus 4% in patients with low resistin ($p < 0.001$).

3.3. Risk Factors for MS Development. We assessed factors associated with the presence of MS using a multiple logistic regression analysis. Only familiar history of obesity showed an OR 2.06 (CI 95% 1.02–4.14). Neither familial history of diabetes, dyslipidemia, or hypertension nor adherence to diet, regular exercise, or smoking was related to the presence of MS.

4. Discussion

The presence of features associated with MS in patients with T1D has been associated with an increased risk for macro- and microvascular complications [21]. However, the etiopathogeny and the long-term clinical significance of this association need yet to be established. The prevalence of MS in patients with T1D varies widely depending on the studied population and criteria used for diagnosis [22]. Despite the controversy generated by the difficulties of diagnosing MS in patients with T1D, the joint statement criteria seem to be the most adequate and widely used in these patients. The inclusion of a WC cut-off helps detecting MS in different ethnic and body fat distribution groups, such as ours where traditional anthropometric and biochemical data is not enough to correctly define high risk patterns. In our study, using these criteria, we found that more than 40% of our T1D patients show additional metabolic disturbances that are associated with higher cardiovascular risk. It is known that age plays an important role in the development of MS and our patients with MS were significantly older than the ones without MS; however, this difference was not associated with any significant changes in the biochemical or inflammatory profile.

We report the highest frequency of MS in a group of patients with T1D to our knowledge. Although it reflects the worrying epidemiologic tendencies of the general population in our country, we should also consider that, in addition to the MS diagnosis, the presence of a long-standing autoimmune/inflammatory disorder in adult patients with poorly controlled diabetes could mean a significant increase in cardiovascular mortality and microvascular complications in the near future. Interestingly, the tendency to report higher frequencies of obesity or other components of the MS in patients with T1D is not exclusive of the Mexican population, since recent reports mention an increased frequency of central obesity and dyslipidemia in different cohorts, despite their attempts to strictly control diabetes [23, 24].

Additional studies are required in order to find markers and cut-off points that may unequivocally classify T1D patients with MS and high cardiovascular risk. Cytokines are some of the biomarkers that have been actively studied in the last few years, since some patterns of increased and decreased cytokine levels correlate with specific inflammatory states and morbidities. Obesity, cardiovascular disease, and even type 2 diabetes seem to generate different cytokine profiles that could help to explain the physiopathology of these diseases and even serve as prognostic factors or controls for therapeutic interventions in the future [25–27]. Cytokine levels have not been previously evaluated in adult patients with T1D with an intention to establish differences between patients with and without MS. Our results show that most of the traditional cytokines measured in other studies are similar in both groups, for proinflammatory and anti-inflammatory interleukins. This may reflect a similar state of inflammation in both groups. Additionally, we found a positive correlation between IL-10 (an anti-inflammatory cytokine) with TNF and IL-6 (usually proinflammatory cytokines). This correlation has been found in certain pathologies such as thyroid cancer [28], stroke [29], lupus, and Sjögren syndrome [30], where the inflammatory profile may be altered from their basal state. Adiponectin showed no significant correlations with any other cytokine. Based on the apparently paradoxical correlations between pro- and anti-inflammatory cytokines, we support previous authors' ideas that IL-10 may have differential actions in different stages of the inflammatory response; however only longitudinal studies may be able to solve these questions in vivo. In our study, neither age nor time from diagnosis of T1D translated into differences in cytokine levels.

We consider that resistin may be an important inflammatory cytokine in these patients. It is synthesized mainly by adipose tissue and seems to generate insulin resistance through an inhibitory effect on CD36, fatty acid transport protein 1 (FATP1), acetyl-coA carboxylase, and AMP-activated protein kinase α. Furthermore, resistin has been shown to regulate gene expression of TNF-α and IL-6 via nuclear factor-kβ (NF-kβ) [31]. These characteristics make it a unique marker that may be strongly related to the effect of excess weight and weight changes on the inflammatory profile. Our study shows that resistin is positively correlated to other cytokines in the whole patient group, reflecting the inflammatory state generated by T1D; however, when we divided the group, only the patients with MS consistently showed this positive correlation, while the patients without MS showed a small and nonsignificant correlation. Previous studies by Fehmann and Heyn reported no significant differences in the resistin levels between patients with or without MS; however we should note that their population was not so markedly obese [32].

Our data also differs from the findings of Timar et al. [33]. They found that all proinflammatory cytokines were high and the anti-inflammatory cytokines were low. However, the basal characteristics of their groups also seem radically different and their patients with MS have higher HbA1c and insulin dose/kg than the group without MS. Considering the possible differential effect of cytokines according to age and disease severity, these differences may account for the results in the cytokine levels. Also the methods used to measure cytokines are different and resistin was not considered in their analysis.

We suggest that adding resistin to the cytokine panel in the study of MS will continue proving to be relevant in future reports, especially in populations like ours, where a large proportion of the patients show an increased fat mass.

Traditionally, the definition of insulin resistance in T1D has been proven difficult. Insulin clamps are the gold standard for these studies; however these procedures are invasive and not useful in the everyday clinical setting. The use of eGDR has been recently advocated as a useful way to assess insulin resistance in this group [34]. Its advantages as a validated tool are limited by the fact that race and ethnicity play a significant role in insulin sensitivity. Previous papers published by different authors show that lower eGDR scores correlate with larger insulin resistance, but no specific cut-off point has been established so far. We observed that a cut-off point of 7.32 $mg/kg^{-1}/min^{-1}$ predicts MS development with a sensitivity of 80% and specificity of 66%, in a group consisting entirely of Mexican mestizos. Despite the fact that

this cut-off may not be adequate for other ethnicities, our results show clearly that the patients with MS have the lowest eGDR scores and therefore, they present with more insulin resistance, despite the apparently similar requirements of insulin. Cytokines, and specially resistin, may be some of the factors modulating insulin resistance in T1D and therefore warrant additional studies.

Our study has some limitations. The cross-sectional study design prevents us from identifying the effect of time on MS. Also, we consider that the low (often undetectable) cytokine levels observed could be related to a dilution effect or a low detection power in our methods. We believe that future studies determining cytokines mRNAs from mononuclear cells or adipose tissue may prove more useful; however, the study of mRNA may also be limited, since one must consider the possible effect of posttranscriptional regulation of RNA-binding proteins (i.e., tristetraprolin, adenine- and uridine-rich element (ARE)/poly(U) binding degradation factor 1, ZCCHC11 and regnase-1) or certain microRNAs [35]. Additionally, although we assessed the main pro- and anti-inflammatory cytokines with detectable differences only in resistin, other pathways may be important in the regulation of cytokines, for example, IL-17, pathways related to transforming growth factor-beta (TGF-β) and endothelial activity through E-selectin, vascular cell adhesion molecule (VCAM-1), or von Willebrand factor (vWF).

Prospective, long-term studies with highly sensitive determinations of multiple cytokines may help understand the patterns of inflammation present in patients with double diabetes and may also help us differentiate them from other inflammatory states and stages of the various diseases related to cardiovascular morbidity. Identifying the patients with the highest risk of cardiovascular disease and the mechanisms underlying their etiopathogeny may be some of the final goals of these lines of investigation.

5. Conclusions

We found a high prevalence of MS in Mexican patients with T1D. This condition could be related to the high prevalence of obesity and dyslipidemia already seen in our population, but it may represent an additional risk factor for cardiovascular disease in T1D patients. The increased level of resistin level and its correlation to other inflammatory cytokines may be related to the increased fat mass and could be involved in the development of insulin resistance observed in our patients. Although we did not observe any differences in other cytokines, we consider that more studies are required to fully understand the inflammatory profile in patients with double diabetes. Finally, we determine an optimal cut-off point for diagnosis of MS in our group with a high sensitivity and middle specificity. To our knowledge this seems to be the first study that determines insulin resistance through eGDR in Mexican population.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

This work was supported by institutional grants from the Fondo de Investigación en Salud del Instituto Mexicano del Seguro Social (R-2012-3601-129 to Victoria Mendoza). The authors thank Ismael Mancilla-Herrera, Ph.D., and Esteban Cerezo-Domínguez, M.S., from the Immunochemistry Medical Unit for their technical assistance.

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Hindawi Publishing Corporation
Journal of Diabetes Research
Volume 2015, Article ID 905042, 6 pages
http://dx.doi.org/10.1155/2015/905042

Research Article

Adipose Tissues Characteristics of Normal, Obesity, and Type 2 Diabetes in Uygurs Population

Jun Zhang,[1] Zhiwei Zhang,[1] Yulei Ding,[1] Peng Xu,[1] Tingting Wang,[1] Wenjing Xu,[1] Huan Lu,[1] Jun Li,[2] Yan Wang,[3] Siyuan Li,[1] Zongzhi Liu,[1] Na An,[4] Li Yang,[1] and Jianxin Xie[1]

[1]*School of Medicine, Shihezi University, Shihezi, Xinjiang 832000, China*
[2]*Department of Endocrinology and Metabolism, First Affiliated Hospital, Shihezi University School of Medicine, Shihezi, Xinjiang 832002, China*
[3]*Endocrinology Department of Xinjiang Uygur Autonomous Region People's Hospital, Urumqi, Xinjiang 830001, China*
[4]*Department of Endocrinology, Fifth Affiliated Hospital, Xinjiang Medical University, Urumqi, Xinjiang 830011, China*

Correspondence should be addressed to Jianxin Xie; xiejianxin9017@sina.com

Received 5 December 2014; Revised 2 February 2015; Accepted 5 February 2015

Academic Editor: Sharad Rastogi

Our results showed that, at the same BMI level, Uygurs have greater WHR values, abdominal visceral fat content, and diabetes risks than Kazaks. In addition, values of HDL-C in Uygur subjects were lower than those in Kazak subjects, and values of creatinine, uric acid, diastolic blood pressure, blood glucose, and fructosamine in Uygur male subjects were lower than those in Kazak male subjects. In contrast, systolic blood pressure values in Uygur subjects were greater than those in Kazak subjects, and blood glucose values were greater in Uygur female subjects than in Kazak female subjects. Additionally, in Uygurs, visceral adipose tissue expression levels of *TBX1* and *TCF21* were greater in obesity group than in normal and T2DM groups and lower in T2DM group than in normal group ($P < 0.01$). The visceral adipose tissue expression levels of *APN* in normal group was greater than those in obesity and T2DM groups, and visceral adipose tissue expression levels of *TNF-α* and *MCP-1* in normal group were lower than those in obesity and T2DM groups ($P < 0.01$). In conclusion, T2DM in Uygurs was mainly associated with not only distribution of adipose tissue in body, but also change in metabolic activity and adipocytokines secretion of adipose tissue.

1. Introduction

Diabetes mellitus (DM), also known as simply diabetes, is a chronic and heterogeneous metabolic disorder that affects millions of people worldwide. The previous study showed that the increasing global prevalence of type 2 diabetes mellitus (T2DM) is associated with the rising obesity rates [1], and the obesity is a key factor in the development of T2DM [2]. Yan et al. reported that, for the Uygur and Kazak ethnic groups (i.e., two major ethnic minorities in Xinjiang) at the equal body mass index (BMI) level, more Kazak people developed hypertension, whereas more Uygur people developed diabetes [3]. In addition, the Uygur subjects had significantly greater waist-hip ratio (WHR) than the Kazak subjects, and the Uygur subjects had increased fat distribution in the abdominal viscera, whereas the Kazak subjects had more subcutaneous fat [3]. Additionally, Ibrahim reported that abdominal obesity imparts a greater risk of developing diabetes and future cardiovascular events than peripheral or gluteofemoral obesity, and visceral adipose tissue has a higher rate of insulin-stimulated glucose uptake compared with subcutaneous obesity [4]. Fat depots contribute differently to disease and function [5]. Furthermore, Perrini et al. reported that cytokine release profiles were distinct in the subcutaneous and visceral adipose tissue [6].

Although Uygurs and Kazaks have essentially the same eating habits and living environments, their body fat distributions are different. In the current study, to further explore the correlation of obesity position and T2DM, we examined the characteristic of fat tissues traits and gene expression in visceral adipose tissues of the normal, obesity, and T2DM individuals in Uygurs population.

2. Subjects and Methods

2.1. Subjects. Our study consisted of 980 Uygur participants (580 males and 400 females) and 1122 Kazak participants (415 males and 707 females) from Yili and Kashi in Xinjiang province of China, and the subjects used in our study are new and different individuals from those in [3]. All patients completed a series of conventional questionnaires, including disease history and daily living and eating habits. In addition, all patients underwent the following measurements: blood biochemical analysis and blood pressure, height, weight, waist, and hip circumference measurements; and for the blood pressure measurement the systolic pressure and diastolic pressure were detected and recorded by measuring 3 tests.

Subsequently, 18 Uygur subjects (9 males and 9 females) and 18 Kazak subjects (9 males and 9 females) aged from 40 to 60 years were randomly selected from the 980 Uygur participants and 1122 Kazak participants, respectively, to have body composition analysis, including determining their overall fat content using the underwater weighing test and MRC measurements of subcutaneous abdominal fat weight and visceral fat. We label-grouped all examined samples into 3 groups (normal, obesity, and diabetes, resp.). Our definition of obesity was determined using the 1999 diagnostic criteria from the World Health Organization and the International Obesity Task Force Asian adult standard from 2000. Specifically, a BMI $\geq 25\,kg/m^2$ was defined as obese or overweight, and a BMI $< 25\,kg/m^2$ was normal. Additionally, all patients with T2DM (2-hour postprandial glucose $\geq$ 11.1 mmol/L, fasting glucose $\geq$ 7.0 mmol/L; World Health Organization in 1999) were confirmed by the Xinjiang Uygur Autonomous Region People's Hospital Department.

The intra-abdominal adipose tissues of another 124 Uygur participants, which were contained in the 980 Uygur participants, were collected from Kashi city in Xinjiang province of China: 50 samples for normal control (normal) group, 48 samples for obesity group, and 26 samples for T2DM group. The biopsy of abdominal adipose tissues undergoing surgery was on the protocol to perform pathological analysis, and the mRNA sample taken had been informed before surgery, and every subject accepted to participate by signing a written informed consent.

2.2. Methods

2.2.1. Measurement of Biochemical Indexes. One day before elective abdominal operation, the weight, waist, hip, and glycemic index were detected and BMI and WHR were calculated. T2DM was diagnosed according to T2DM diagnostic criteria (2-hour postprandial glucose $\geq$ 11.1 mmol/L, fasting glucose $\geq$ 7.0 mmol/L; World Health Organization in 1999). The patients suffering from cancer, acute inflammation, liver and kidney disease, patients with type 1 diabetes, and patients recently taking drugs that may interfere with glucose and lipid metabolism were not included in the present study. The fasting plasma glucose (FPG) was detected using the glucose oxidase method, and total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low density lipoprotein cholesterol (LDL-C) were all detected using automatic biochemistry analyzer.

2.2.2. Body Composition Analysis. Height and weight were measured using standard procedures. Waist and hip circumferences were measured using a flexible tape with tension calipers at the extremity (Gulick-Creative Health Product, Inc., Plymouth, MI), midway between the xiphoid and umbilicus during the midexpiratory phase and at the maximum circumference in the hip area, respectively. Skinfold thickness was measured at 5 different anatomical sites (i.e., subscapular diagonal and vertical, chest, midaxillary, abdominal horizontal and vertical, and suprailiac diagonal and vertical) using Lange Skinfold callipers (Cambridge Scientific Instruments Inc., Cambridge, MD), as previously reported [7]. Truncal skin folds were computed as the sum of the skin folds at these 5 anatomic sites. Body composition was determined using underwater weighing, as previously reported [8]. MRI was used to measure intra-abdominal (visceral) and abdominal subcutaneous adipose tissue volume, as previously described [9]. In brief, MRI studies were performed using a 1.5 T imaging device (Philips Gyroscan Intera, Holland). The entire abdominal region was scanned using contiguous axial 10 mm slices. Fat volume was measured in each slide by mapping subcutaneous and intra-abdominal adipose tissue compartments using computerized images. Volume was converted into adipose tissue mass, assuming an adipose tissue density of 0.9196 kg/L [10].

2.2.3. Tissues Sample. The abdominal omental adipose tissues were collected on the day of surgery, and about 3 cm $\times$ 3 cm tissues were collected from each individual, immediately washed with 0.75% NaCl solution, and snap-frozen and stored in liquid nitrogen until RNA extraction.

2.2.4. RNA Isolation and Real-Time PCR. Total RNA was isolated from the visceral adipose tissue (abdominal omental adipose tissue) in the 124 Uygur subjects (50 samples for normal group; 48 samples for obesity group; and 26 samples for T2DM group) by using TRIZOL reagent (Cat#15596-026, Life technologies, Carlsbad, CA, USA) and purified by using an RNeasy minikit (Cat#74106, QIAGEN, GmBH, Germany). RNA integrity was checked on an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Reverse transcription conditions for each cDNA amplification were 25°C for 5 min, 42°C for 60 min, and 70°C for 15 min.

Real-time PCR was used to detect gene expression using the SYBR Premix Ex Taq (Takara) on a 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA) with the primers shown in Table 1. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as internal reference. Part (1 μL) of each RT reaction product was amplified in a 20 μL PCR reaction system. Reaction mixtures were incubated in an ABI Prism 7500 sequence detection system (Applied Biosystems), programmed to conduct 1 cycle at 95°C for 30 s and 40 cycles at 95°C for 5 s and 60°C for 34 s. Moreover, dissociation curves were analyzed using the Dissociation Curve 1.0 software (Applied Biosystems) for each PCR reaction to detect and eliminate possible primer-dimer artifacts. All reactions

Table 1: Primers used in RT-qPCR.

Gene	Sequence ID	Primer (5′-3′)	Product (bp)
APN	NM_004797.3	F: ATGGCCCCTGCACTACTCTA R: CAGGGATGAGTTCGGCACTT	104
TNF-α	NM_000594.3	F: GTGACAAGCCTGTAGCCCAT R: TATCTCTCAGCTCCACGCCA	111
MCP-1	NM_002982.3	F: GATCTCAGTGCAGAGGCTCG R: TTTGCTTGTCCAGGTGGTCC	105
TCF21	NM_003206.3	F: GCAGATCCTGGCTAACGACA R: TGGTTCCACATAAGCGGCTC	134
TBX1	XM_005261271.1	F: AACCTACTGGACGACAACGG R: CTGCGTGATCCGATGGTTCT	189
GAPDH	NM_001256799.1	F: TGTTGCCATCAATGACCCCTT R: CTCCACGACGTACTCAGCG	202

were performed in triplicate. The relative amounts of target gene transcripts were calculated using the comparative cycle-time method.

2.2.5. Patient Consent and Ethics Statement. All participants were informed of the usage of their basic and clinical information prior to the sample collection, and all participants provided written informed consent for study participation. The consent form and ethical approval were provided by the Medical Ethics Committee at First Affiliated Hospital, Shihezi University School of Medicine (reference number 2014LL22).

2.2.6. Statistical Analysis. The SPSS statistical package (version 11.5, SPSS Inc., Chicago, IL, USA) was used for the data analyses. All data are presented as mean plus standard deviation (mean ± SD). The Shapiro-Wilk test was used to test the normality of data. If the data follow the normality role, differences between groups were analyzed using unpaired Student's t-test; else the differences between groups were analyzed using rank sum test. Values of P of <0.05 were considered significant unless otherwise specified.

3. Results

3.1. Clinical Characteristics. The data in Table 2 shows that at similar BMI values, compared with the Kazak subjects, the Uygur subjects had increased values in weight, waist circumference, hip circumference, and WHR. In addition, the values of HDL-C in Uygur male and female subjects were significantly lower than those in Kazak male and female subjects, and the values of creatinine, uric acid, diastolic blood pressure, blood glucose, and fructosamine in Uygur male subjects were significantly lower than those in Kazak male subjects. In contrast, the systolic blood pressure values in Uygur male and female subjects were significantly greater than those in Kazak male and female subjects, and blood glucose values were significantly greater in Uygur female subjects than in Kazak female subjects. Additionally, consistent with the previous report [3], our results also indicated that the Uygurs had a significantly greater risk of diabetes than the Kazaks for both males and females. No significant difference was detected in the values of triglycerides, cholesterol, and LDL-C between both male and female Uygur sbujects and Kazak subjects.

Both 18 individuals from the Uygur and Kazak ethnic subjects (980 and 1122, resp.) and 6 individuals (3 male and 3 female) aged from 40 to 60 in each group (including normal, obesity, and T2DM) were randomly selcected for body composition analysis, and the baseline characteristics for 36 subjects are shown in Table 3. There were no significant differences observed in the ages of the Uygur and Kazak participants. In both Uygur and Kazak ethnic groups, the body weight, BMI, and WHR values of the normal control group were significantly lower than those of the obesity and T2DM groups ($P < 0.05$). Notably, although there was no significant difference in total lipid content between subjects from the two ethnic groups, the ratio of VF/SAF in Uygurs was significantly higher than that of the Kazaks in both obesity and T2DM groups ($P < 0.05$). However, there was no difference between the two ethnic groups regarding fasting blood glucose levels.

3.2. Candidate Gene Expression in the Visceral Adipose Tissue of Normal, Obesity, and T2DM Individuals in Uygurs Population. To reveal the function change of the adipose tissues among normal, obesity, and T2DM individuals, five candidate genes' expressions in visceral adipose tissue of normal, obesity, and T2DM individual in Uygur population were analyzed using real-time PCR. The result showed that five candidate genes, including T-box protein 1 (*TBX1*), transcription factor 21 (*TCF21*), adiponectin (*APN*), tumor necrosis factor-alpha (*TNF-α*), and monocyte chemotactic protein 1 (*MCP-1*), were all expressed in the visceral adipose tissue of normal, obesity, and T2DM individuals in Uygur population. The *TBX1* and *TCF21* expression levels in the obesity group were significantly higher than those of normal and T2DM groups ($P < 0.01$); and the *TBX1* and *TCF21* expression levels in the T2DM group were significantly lower than those of normal and obesity groups (Figure 1, $P < 0.01$). In addition, statistical analysis showed that the ratio of expression level of *TBX1* to *TCF21* (*TBX1*/*TCF21*) in obesity group was significantly greater than those in normal and

Table 2: Basic indices and biochemical analyses.

	Uygur		Kazak	
	Males (580)	Females (400)	Males (415)	Females (707)
Age (years)	44.09 ± 15.99	39.5 ± 14.05	41.25 ± 15.06**	38.31 ± 14.2
Diabetes prevalence rate (%)	6.7	7.9	1.2**	0.7**
Height (cm)	168.46 ± 7.3	157.09 ± 6.15	170.54 ± 7.53**	159.49 ± 6.43
Weight (kg)	74.19 ± 14.23	63 ± 11.79	69.13 ± 12.64**	59.78 ± 10.75**
BMI (kg/m^2)	26.22 ± 5.24	25.55 ± 4.65	23.77 ± 4.13	23.52 ± 4.15
Waist circumference (cm)	93.84 ± 12.01	90.06 ± 18.45	89.52 ± 12.36**	83.91 ± 14.27**
Hip circumference (cm)	101.55 ± 8.77	101.23 ± 15.02	100 ± 9.01*	97.3 ± 13.07*
WHR	0.92 ± 0.08	0.89 ± 0.09	0.89 ± 0.07**	0.86 ± 0.08*
Systolic blood pressure (mmHg)	129.74 ± 20.95	125.16 ± 24.32	128.98 ± 23.97**	120.52 ± 22.61**
Diastolic blood pressure (mmHg)	82.82 ± 14.17	80.88 ± 15.63	83.6 ± 14.29**	80.28 ± 15.20
Creatinine	59.45 ± 7.81	57.17 ± 6.13	64.10 ± 12.75**	53.2 ± 9.26
Uric acid	255.0 ± 44.55	230.08 ± 65.10	271.83 ± 39.89**	221.03 ± 53.62
Glucose (mmol/L)	4.83 ± 0.73	5.14 ± 0.72	4.91 ± 0.51**	4.74 ± 0.63*
Fructosamine	2.32 ± 0.08	2.37 ± 0.15	2.39 ± 0.16*	2.38 ± 0.2
Triglycerides (mmol/L)	1.85 ± 1.52	1.51 ± 1.41	1.09 ± 0.56	1.05 ± 0.56
Cholesterol (mmol/L)	4.13 ± 1.62	3.80 ± 1.92	4.38 ± 1.37	4.58 ± 1.32
LDL-C (mmol/L)	2.13 ± 1.04	2.18 ± 1.02	2.54 ± 1.08	2.56 ± 1.01
HDL-C (mmol/L)	1.54 ± 0.33	1.54 ± 0.36	1.61 ± 0.36*	1.84 ± 0.46**

BMI as covariance, all indices were executed as t-tests between two ethnic subjects both male and female. HDL: high density lipoproteins; LDL: low density lipoproteins. Values are given as the mean ± SD. $^{**}P < 0.01$; $^{*}P < 0.05$.

Table 3: Baseline characteristics of the subjects.

	Uygur			Kazak		
	Normal (6$^{\#}$)	Obesity (6)	T2DM (6)	Normal (6)	Obesity (6)	T2DM (6)
Age (y)	41 ± 6	40 ± 2	50 ± 6	46 ± 6	39 ± 5	42 ± 6
Body weight (kg)	59.0 ± 6.33*	75.10 ± 14.01	80.17 ± 6.56	63.40 ± 10.32*	79.67 ± 13.11	82.43 ± 15.23
BMI (kg/m^2)	23.46 ± 1.55*	38.06 ± 1.922	36.27 ± 2.97	23.77 ± 3.52*	31.74 ± 1.73	33.43 ± 1.83
WHR	0.886 ± 0.490*	0.948 ± 0.325	0.953 ± 0.451	0.867 ± 0.470*	0.951 ± 0.324	0.944 ± 0.534
Total lipid contents (%)	0.214 ± 0.123	0.301 ± 0.118	0.292 ± 0.145	0.226 ± 0.098	0.318 ± 0.157	0.298 ± 0.088
VF/SAF	0.46 ± 0.22	0.56 ± 0.18$^{\&}$	0.54 ± 0.13$^{\&}$	0.42 ± 0.19	0.40 ± 0.22	0.39 ± 0.10
Fasting blood glucose (mg/dL)	4.58 ± 0.24	5.67 ± 0.15	9.89 ± 2.44	4.29 ± 0.18	6.24 ± 0.48	9.44 ± 2.86

T2DM, type 2 diabetes mellitus; VF, visceral fat; SAF, subcutaneous abdominal fat; all such values were expressed as mean ± SD ($n = 6$; 3 males and 3 females, resp.); $^{\#}$the number of subjects in one group; $^{*}P < 0.05$ in the same ethnic group; $^{\&}P < 0.05$ in different ethnic group.

T2DM groups (Figure 1, $P < 0.01$). Additionally, the expression of *APN* in the normal individual was significantly higher than those in obesity and T2DM groups, the expression levels of *TNF-α* and *MCP-1* in normal group were significantly lower than those in obesity and T2DM groups (Table 4, $P < 0.05$), and no significant differences of *APN*, *TNF-α*, or *MCP-1* expression were detected between obesity and T2DM groups (Table 4, $P > 0.05$).

4. Discussion

The previous report showed that visceral and subcutaneous adipocytes may exhibit different properties in the production of bioactive molecules [11]. In the current study, the results showed that, at the same BMI level, both male and female Uygur subjects have significantly greater values in WHR, abdominal visceral fat content, and diabetes risks, compared with Kazak subjects. This result is consitent with the previouse study [3].

In addition, the values of HDL-C in Uygur male and female subjects were significantly lower than those in Kazak male and female subjects, and the values of creatinine, uric acid, diastolic blood pressure, blood glucose, and fructosamine in Uygur male subjects were significantly lower than those in Kazak male subjects. In contrast, the systolic blood pressure values in Uygur male and female subjects were significantly greater than those in Kazak male and female subjects, and blood glucose values were significantly greater in Uygur female subjects than in Kazak female subjects. These results showed that the metabolic difference result from obesity exists between the Uygurs and Kazaks populations.

Toyoda et al. [12] reported that visceral adipose tissue is a better predictor for mortality than subcutaneous tissue. Our results showed that gene expression in the visceral adipose

Table 4: Comparison of adipose cytokines mRNA copy data in the visceral adipose tissue of Uygur population (rank sum test).

	APN	*TNF-α*	*MCP-1*
Normal (50[#])	0.7162[*] (0.5668–0.9564)	0.0250[*] (0.0195–0.0672)	0.1588[*] (0.0872–0.2663)
Obesity (48)	0.4244 (0.2209–0.6004)	0.1096 (0.0637–0.1592)	0.1937 (0.0915–0.3346)
T2DM (26)	0.4120 (0.1967–0.5560)	0.0798 (0.0569–0.1428)	0.1983 (0.1315–0.4083)

[*] $P < 0.05$; [#] the number of subjects in one group.

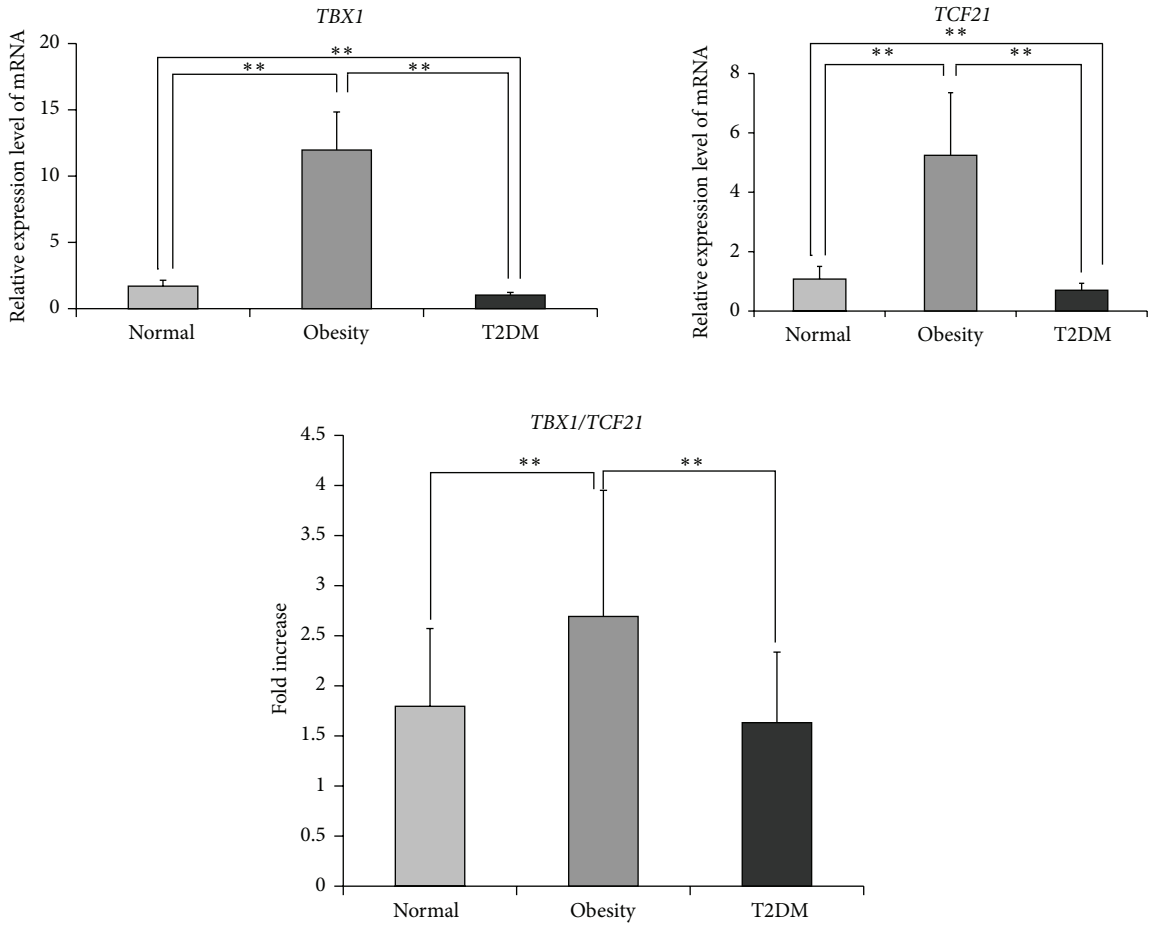

Figure 1: The mRNA expression levels of T-box protein 1 (*TBX1*) and transcription factor 21 (*TCF21*) in the visceral adipose tissue of Uygur population. Comparison between groups by *t*-test, $^{**}P < 0.01$; n = all subjects in each group (50, 48, and 26 for the normal, obesity, and T2DM groups, resp.); T2DM, type 2 diabetes mellitus.

tissue of normal, obesity, and T2DM subjects in the Uygurs was significantly different, and the expression levels of *TBX1* and *TCF21*, the marker of white adipocytes and brown-like white adipocytes, respectively [13], were significantly greater in the obesity group than in normal and T2DM groups and significantly lower in the T2DM group than in normal group ($P < 0.01$). These results suggested that the lipid metabolic activity of visceral adipose among normal, obesity, and T2DM subjects was different in the Uygurs population. The ratio of expression level of *TBX1* to *TCF21* (*TBX1*/*TCF21*) in obesity group was significantly greater than those in normal and T2DM groups ($P < 0.01$), suggesting that the lipid metabolic activity of visceral adipose tissue in obesity individuals was lower than those in normal and T2DM individuals.

APN is an adipocytokine produced by adipose tissue and plays a protective role in vascular injury and insulin resistance (IR). The previous study in other ethnic groups showed that the subjects with diabetes mellitus (DM) had greater insulin and lower APN level [14], and Asian participants, who suffered from higher chronic disease risk for obesity, had lower serum levels of APN than Caucasian participants across all levels of BMI [15]. Our result showed that the visceral adipose tissue expression levels of *APN* were lower in obesity and T2DM groups than in normal group; this is consistent with the previous study [14] and suggested that

downregulation of *APN* might be a reason for the obesity and T2DM.

TNF-α and MCP-1 are two proinflammatory cytokines that can be released by adipose tissue. In the current study, the results showed that the visceral adipose tissue expression levels of *TNF-α* and *MCP-1* in normal group were significantly lower than those in obesity and T2DM groups ($P < 0.01$), which were consistent with the previous study where TNF-α and MCP-1 were elevated in poor glycemic control and good glycemic control overweight and obese patients [16], suggesting that the immune response of visceral adipose tissue was changed during the occurrence of obesity and T2DM.

Collectively, our result indicated that the T2DM in the Uygurs population was mainly associated with not only distribution of adipose tissue in the body, but also the change in metabolic activity and adipocytokines secretion of adipose tissue.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Jun Zhang and Zhiwei Zhang contributed equally to the paper.

Acknowledgments

This work is supported by the Natural Science Foundation of China (no. 30960127), the Program for High-Level Personnel Start at Shihezi University (nos. RCZX201334, RCZX201230), and the National Basic 973 Research Program of China (no. 2009CB526505).

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Hindawi Publishing Corporation
Journal of Diabetes Research
Volume 2015, Article ID 362375, 9 pages
http://dx.doi.org/10.1155/2015/362375

Review Article

Population-Based Studies on the Epidemiology of Insulin Resistance in Children

M. P. van der Aa,[1] S. Fazeli Farsani,[2] C. A. J. Knibbe,[3] A. de Boer,[2] and M. M. J. van der Vorst[1]

[1]*Department of Pediatrics, St. Antonius Hospital, P.O. Box 2500, 3430 EM Nieuwegein, Netherlands*
[2]*Division of Pharmacoepidemiology and Clinical Pharmacology, Utrecht Institute for Pharmaceutical Sciences (UIPS), Utrecht University, P.O. Box 80082, 3508 TB Utrecht, Netherlands*
[3]*Department of Clinical Pharmacy, St. Antonius Hospital, P.O. Box 2500, 3430 EM Nieuwegein, Netherlands*

Correspondence should be addressed to M. M. J. van der Vorst; m.van.der.vorst@antoniusziekenhuis.nl

Received 6 February 2015; Revised 5 May 2015; Accepted 5 May 2015

Academic Editor: Joseph Fomusi Ndisang

Background. In view of the alarming incidence of obesity in children, insight into the epidemiology of the prediabetic state insulin resistance (IR) seems important. Therefore, the aim of this systematic review was to give an overview of all population-based studies reporting on the prevalence and incidence rates of IR in childhood. *Methods*. PubMed, Embase, and Cochrane library were searched in order to find all available population-based studies describing the epidemiology of IR in pediatric populations. Prevalence rates together with methods and cut-off values used to determine IR were extracted and summarized with weight and sex specific prevalence rates of IR if available. *Results*. Eighteen population-based studies were identified, describing prevalence rates varying between 3.1 and 44%, partly explained by different definitions for IR. Overweight and obese children had higher prevalence rates than normal weight children. In seven out of thirteen studies reporting sex specific results, girls seemed to be more affected than boys. *Conclusion*. Prevalence rates of IR reported in children vary widely which is partly due to the variety of definitions used. Overweight and obese children had higher prevalence and girls were more insulin resistant than boys. Consensus on the definition for IR in children is needed to allow for comparisons between different studies.

1. Introduction

Nowadays, the body mass index (BMI) is increasing in many populations and childhood obesity is an emerging problem [1–3]. In the United States the prevalence rates of obesity between 1971 and 1974 in 6–11-year-old white/black children were 4%. Between 1999 and 2002, these prevalence rates increased to 13% and 20% in white and black children, respectively [4]. In 2012 the overall prevalence rate of obesity in 2–19-year-old American children was 17.3% [1]. In developing countries the prevalence rate of overweight and obesity in preschool children (<5 years old) in 2010 was estimated to be 6.1% and 11.7%, respectively [5]. Moreover, the prevalence of overweight in children <5 years of age raised in the African continent between 2000 and 2013 from 5.1 to 6.2% (+1.1%), while in the American Continents, the prevalence increased with 0.5% (6.9 to 7.4%). (http://apps.who.int/gho/data/view.main.NUTWHOOVERWEIGHTv?lang=en).

The rising prevalence of obesity will cause an increase in obesity related complications such as insulin resistance (IR), hypertension, dyslipidemia, and type 2 diabetes mellitus (T2DM) [6, 7]. The energy excess in obesity may result in hyperplasia and hypertrophy of adipocytes, leading to oxidative stress. This oxidative stress of adipocytes induces a chronic low-level inflammation in adipose tissue and production of adipokines, free fatty acids, and inflammatory mediators. This inflammation is related to peripheral IR, IR of hepatocytes, and impaired insulin secretion by the pancreatic beta cells. Finally, this process causes dysregulation of glucose homeostasis and development of T2DM [8]. Although obesity plays a key role in the pathophysiology of IR, IR is

an independent risk factor for cardiovascular and metabolic diseases [9–12]. Therefore, it is important to know the extent of IR in pediatric populations. Knowledge on the prevalence rates of IR and its clinical consequences during childhood will increase the awareness of physicians and other health care professionals. Despite the reported association between IR and increased cardiovascular risk in pediatric populations [13], there is no overview of data on the epidemiology of IR in this population. Many studies focus on the extent of IR in overweight and obese populations, but limited studies have a population-based study design.

The aim of this study is to systematically review all available population-based studies on the epidemiology of IR in pediatric populations. We will describe the weight and sex specific prevalence and incidence rates of IR in the included studies, together with the study-specific definition used to define IR.

2. Methods

2.1. Systematic Search and Study Selection. This review follows the guidelines of "Meta-analysis of Observational Studies in Epidemiology" (MOOSE) [32]. A systematic search was conducted in PubMed, Embase, and the Cochrane library, using the search strategies as displayed in Table 1. The search was performed in December 2014 and covered all publications in the time period between the inception of each database and the search date. All articles in English, French, German, Spanish, and Dutch languages were included and their title and abstract were screened to find the relevant studies. All results were imported into a RefWorks file (http://www.refworks.com/) and duplicate articles were removed. Subsequently, the title and abstract of all unique results were screened using the exclusion criteria. Articles were excluded if they were review articles, studied a population older than 19 years, or did not report prevalence and/or incidence rates of IR in the abstract. Furthermore, all conference abstracts without a full text publication were excluded. All available full text articles were retrieved and their design was scrutinized to select population-based studies. The reference lists of all included population-based studies were investigated to find relevant articles not included in the original search.

2.2. Data Extraction and Analysis. Data were extracted on the study design, sample size, calendar time of data collection, mean age of participants, ethnicity, criteria used to determine IR (method and cut-off value), prevalence, and incidence rates of IR in the complete study population, and if available in subpopulations based on weight category (normal weight, overweight, and obesity), and sex. Data were entered in an excel file. Pooling of data was not possible because of the large variability in study design, population, and definitions used to determine IR. Data are presented in a descriptive manner.

3. Results

3.1. Systematic Search and Study Selection. With the search strategy presented in Table 1, in PubMed, Embase, and Cochrane 6,788 articles (with 4,596 unique studies) were retrieved. Screening of titles and abstracts led to the exclusion of 4,448 articles (Figure 1). The full text of the 148 remaining articles was checked and 76 articles were excluded based on our exclusion criteria. Critical appraisal of the 72 remaining articles resulted in the final inclusion of 18 population-based studies. All included studies reported prevalence rates of IR and none of them reported incidence rates. An overview of the included studies and extracted data is presented in Supplemental Table 1 (see Supplemental Table 1 in the Supplementary Material available online at http://dx.doi.org/10.1155/2015/362375).

3.2. Study Characteristics. The 18 included studies were performed in 13 countries. Except for the African continent, all continents are represented. The studies were performed between 1999 and 2011. Sample sizes varied from 80 to 3,373 children [14, 28]. Most studies recruited their study population at selected schools [14–25, 29–31]. The New Zealand study population were volunteer adolescents who were recruited by Pacific Island community workers, even though it was not reported where they recruited the participants [28].

In the majority of the studies ($n = 14$), the age of the study participants was above 10 years [15, 17–21, 23–26, 28–31]. Four studies included also children younger than 10 years, with ranges that varied between 6 and 19 years [14, 16, 22, 27]. Ethnicity was not reported in 50% of the studies. All study characteristics are presented in Supplemental Table 1.

3.3. Methods and Cut-Off Values to Define IR. In the studies, six different methods were used to determine IR (Table 2). These methods were Homeostasis Model Assessment Insulin Resistance (HOMA-IR), fasted plasma insulin (FPI), Quantitative Insulin Sensitivity Check Index (QUICKI), fasted glucose/insulin ratio (FGIR), HOMA2, and the McAuley index. All these indices are based on FPI; for HOMA-IR, QUICKI, FGIR, and HOMA2 fasted plasma glucose (FPG) values are also needed (Table 2). The McAuley index is the only index for which fasted triglycerides are required besides FPG and FPI. None of the above-mentioned equations use anthropometric measurements or values derived from an oral glucose tolerance test.

HOMA-IR, FPI, and QUICKI were the most frequently used methods to determine IR (HOMA-IR: $n = 14$ [14–27]; FPI: $n = 7$ [17, 19, 20, 28–31]; QUICKI $n = 2$ [17, 23], Table 2).

The cut-off values used to define IR for HOMA-IR ranged from 2.1 to 5.56, while for FPI cut-off values varied between 9.85 and 23.7 μU/mL (corresponding with 68.4 and 164.8 pmol/L, resp.) (Table 2). The study of Budak et al. used a cut-off value different from the other studies, as their definition for IR was a HOMA-IR <3.16 which was in contrast with other studies that defined IR as HOMA-IR greater than a specific value [18]. We did not succeed to contact Budak et al. to verify this cut-off value.

Age and sex specific cut-off values were reported in, respectively, one [30] and three studies [20, 24, 30]. Girls had higher cut-off values for FPI and HOMA-IR compared with

Table 1: Search strategies.

Database	Search strategy
PubMed	("Insulin Resistance"[Mesh] OR insulin resistan* [tiab] OR insulin sensitivity[tiab] OR (resistan* [tiab] AND insulin* [tiab]) OR metabolic syndr* [tiab]) AND ("Prevalence"[Mesh] OR prevalence* [tiab] OR "Incidence"[Mesh] OR incidence* [tiab]) AND ("Child"[Mesh:noexp] OR "Adolescent"[Mesh] OR "Puberty"[Mesh:noexp] OR "Minors"[Mesh] OR Pediatrics[MeSH:noexp] OR child[tiab] OR children[tiab] OR child care[tiab] OR childhood[tiab] OR child* [tiab] OR childc* [tiab] or childr* [tiab] OR childh* [tiab] OR adoles* [tiab] OR boy[tiab] OR boys[tiab] OR boyhood[tiab] OR girl[tiab] OR girls[tiab] OR girlhood[tiab] OR junior* [tiab] OR juvenile* [tiab] OR kid[tiab] OR kids[tiab] OR minors* [tiab] OR paediatr* [tiab] OR pediatr* [tiab] OR prepubert* [tiab] OR pre-pubert* [tiab] OR prepubesc* [tiab] OR pubert* [tiab] OR pubesc* [tiab] OR school age* [tiab] OR schoolchild* [tiab] OR teen[tiab] OR teens[tiab] OR teenage* [tiab] OR youngster* [tiab] OR youth[tiab] OR youths* OR Primary school* [tiab] OR Secondary school* [tiab] OR Elementary school* [tiab] OR High school* [tiab] OR Highschool* [tiab])
Embase	(prevalence/ or incidence/ or (prevalence* or incidence*).ti,ab.) AND (insulin resistance/ or insulin sensitivity/ or metabolic syndrome X/ or (resistan* and insulin*).ti,ab. or insulin sensitivity.ti,ab. or metabolic syndr*.ti,ab.) AND (child/ or boy/ or girl/ or hospitalized child/ or school child/ or exp adolescent/ or adolescence/ or puberty/ or pediatrics/ or (child or children or child care or childhood or child* or childc* or childr* or childh* or adoles* or boy or boys or boyhood or girl or girls or girlhood or junior* or juvenile* or kid or kids or minors* or paediatr* or pediatr* or prepubert* or pre-pubert* or prepubesc* or pubert* or pubesc* or school age* or schoolchild* or teen or teens or teenage* or youngster* or youth).ti,ab. or youths*.ti,ab. or Primary school*.ti,ab. or Secondary school*.ti,ab. or Elementary school*.ti,ab. or High school*.ti,ab. or Highschool*.ti,ab.)
Cochrane	((prevalence* or incidence*) and ((resistan* and insulin*) or insulin sensitivity or metabolic syndr*) and (child or children or child care or childhood or child* or childc* or childr* or childh* or adoles* or boy or boys or boyhood or girl or girls or girlhood or junior* or juvenile* or kid or kids or minors* or paediatr* or pediatr* or prepubert* or pre-pubert* or prepubesc* or pubert* or pubesc* or school age* or schoolchild* or teen or teens or teenage* or youngster* or youth or youths* or Primary school* or Secondary school* or Elementary school* or High school* or Highschool*)).ti,ab.

boys. For both sexes, adolescents aged 14-15 years had the highest cut-off values for FPI [30].

3.4. Prevalence of IR. The overall prevalence rates of IR in 17 out of 18 population-based studies are presented in Figure 2. The study of Ranjani et al. only reported sex specific prevalence rates [27]. The lowest prevalence rate of IR was reported from Greece with 3.1% in children aged 10–12 years (using the cut-off value of HOMA-IR > 3.16 for IR, Figure 2) [23]. In the same study population, three other definitions of IR (HOMA-IR > 2.1, QUICKI < 0.35, and FGIR < 7) were applied resulting in prevalence rates of 9.2, 12.8, and 17.4%, respectively.

The highest prevalence rate of IR was reported by Grant et al. for the 15–18-year-old Pacific Island adolescents in New Zealand [28]. They reported a prevalence rate of 44% with IR defined as FPI > 12 μU/mL. This definition of IR has been used in another study by Bonneau et al. which resulted in a prevalence rate of 11.7% for the 12–18-year-old Argentinian adolescents [17].

3.5. Sex and Weight Specific Prevalence of IR. Thirteen studies reported separate prevalence rates for boys and girls (Figure 3(a)). In 7 out of 13 studies, IR was more prevalent in girls [16, 18, 19, 27–29, 31]. Three studies reported higher prevalence rates for boys [14, 15, 17]. In one study the prevalence rate of IR was similar for boys and girls [20]. In two studies it depended on the criteria used to determine IR whether boys or girls were having the highest prevalence rates [17, 23].

Figure 3(b) shows the influence of weight (normal, overweight, and obesity) on the prevalence of IR. A major difference was observed between normal weight and obese populations. Normal weight populations had substantial lower prevalence rates of IR, irrespective of the used definition for IR. The maximum difference in weight specific prevalence rates of 61.3% was reported in Australian boys, with prevalence rates in normal weight and obese boys of 7.1% and 68.4%, respectively [29].

4. Discussion

To the best of our knowledge, this is the first systematic review summarizing all available population-based studies on the epidemiology of IR during childhood. While we could not find any population-based study reporting the incidence

Table 2: Methods used to calculate insulin resistance.

Method	Parameters	Formula	Cut-off values (range)	Studies using the method
HOMA-IR	FPG, FPI	(FPG (mmol/L) ∗ FPI (mU/L))/22.5	2.1–4.0	[14–27]
FPI	FPI	NA	9.85–23.7 μU/mL	[17, 19, 20, 28–31]
QUICKI	FPG, FPI	1/[log (FPI (mU/L)) + log (FPG (mg/dL))]	0.33–0.35	[17, 23]
FGIR	FPG, FPI	(FPG [mg/dL]/FPI [mU/L])	7	[23]
HOMA2	FPG, FPI	Computer model: HOMA2-calculator: http://www.dtu.ox.ac.uk/homa	2	[28]
McAuley index	FPI, triglycerides	(2.63 − 0.28 ln [FPI] − 0.31 ln [fasting triglycerides])	6.3	[28]

FPG: fasted plasma glucose; FPI: fasted plasma insulin; FGIR: fasted glucose insulin ratio; HOMA-IR: homeostasis model assessment (for insulin resistance); QUICKI: Quantitative Insulin Sensitivity Check Index.

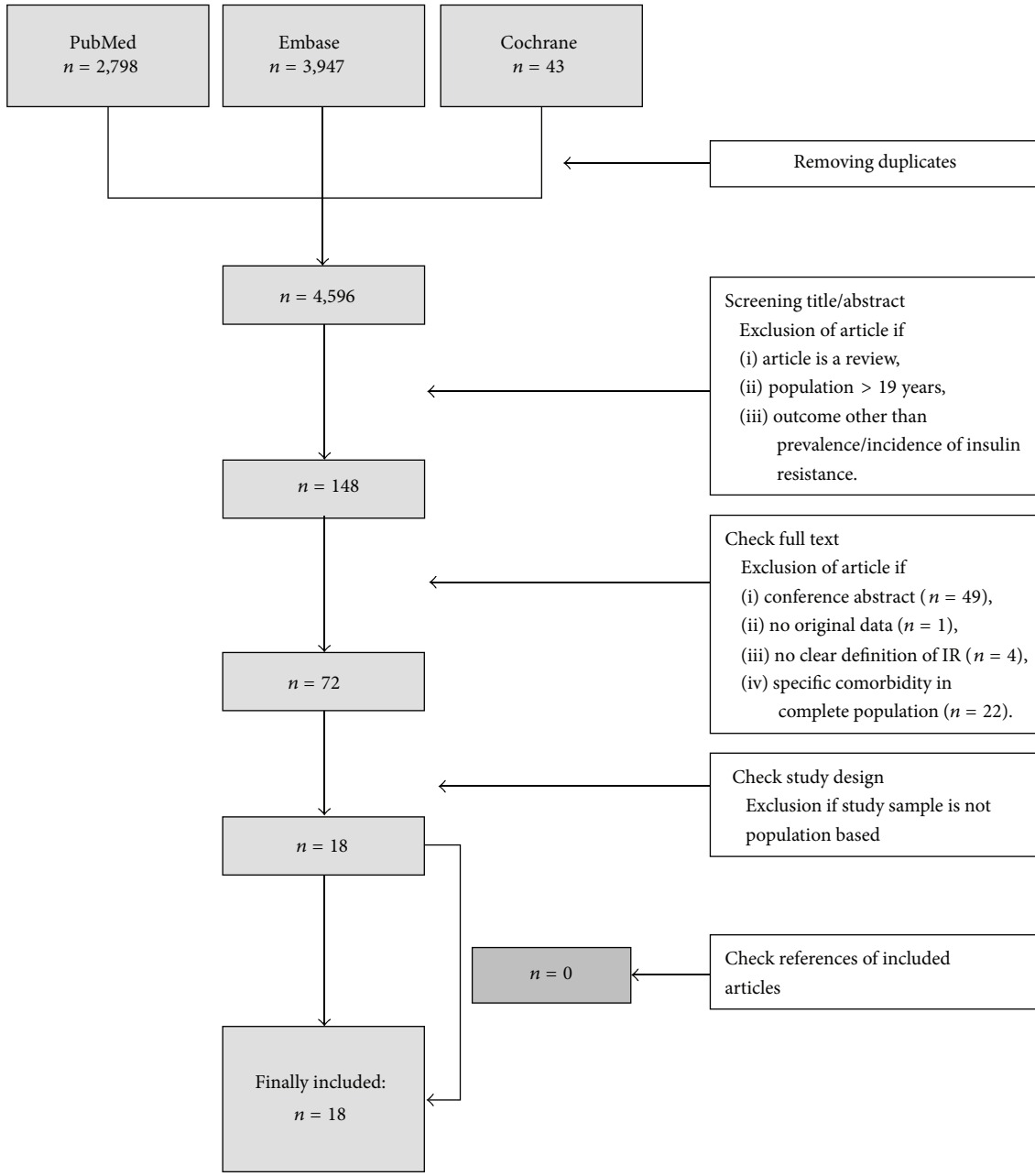


Figure 1: Flowchart of search and included studies.

rate of IR in children, the reported prevalence rates varied between 3.1% in Greek children and 44% in Pacific Island teenagers living in New Zealand. There was not only variation in the prevalence rates of IR, but we also observed that these 18 included studies used 6 different methods combined with diverse cut-off values to determine IR. For instance, the FPI cut-off values varied between 9.85 and 23.7 μU/mL (corresponding with 68.4 and 164.8 pmol/L, resp.) [19, 30] and the HOMA-IR cut-off values ranged between 2.1 and 5.56 [16, 23]. The lack of a uniform definition and cut-off value to determine IR impedes pooling of data, therefore reporting on overall prevalence rates.

Although substantial variation in the prevalence rates of IR could be partly explained by differences in the study population characteristics (e.g., age, weight, ethnicity, pubertal status, etc.), the use of different methods and cut-off values to determine IR may play an important role as well. As an example, in the study by Manios et al. in 481 Greek school children, different methods resulted in various prevalence rates (i.e., 3.1 versus 12.8 and 17.4% for HOMA-IR, QUICKI, and FGIR, resp., Figure 2) [23]. Even if studies use the same method to measure IR, different cut-off values impede comparison between studies. Again, in the study by Manios et al., the use of different cut-off values for HOMA-IR method

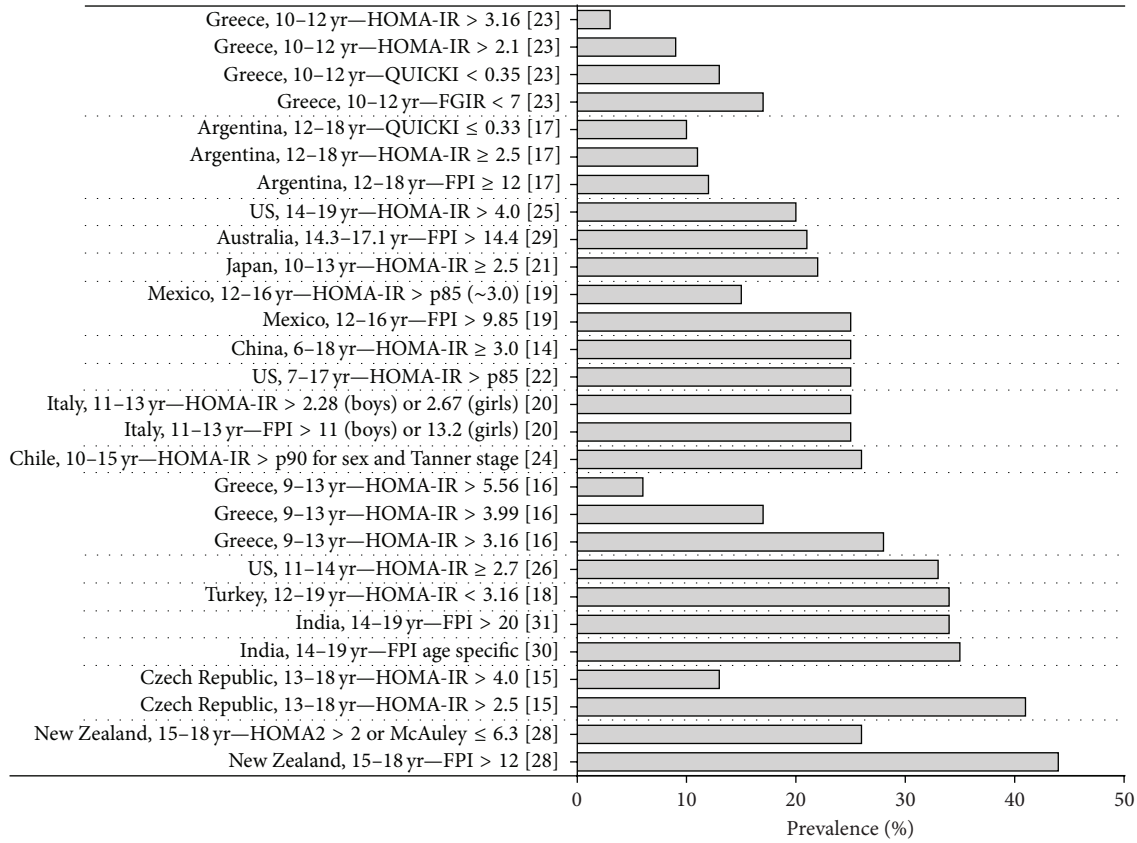


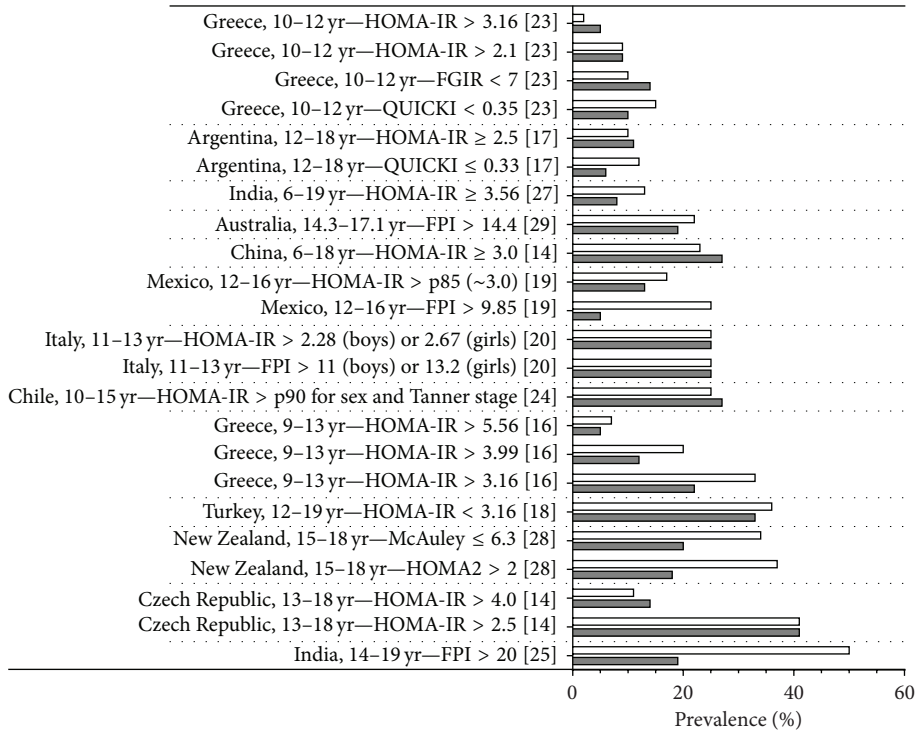
Figure 2: The overall prevalence rates (%) of IR in the included studies.

(>3.16 and >2.1) in the same study population resulted in prevalence rates of 3.1 and 9.2%, respectively [23]. A lower cut-off value results in a higher prevalence rate of IR and vice versa.

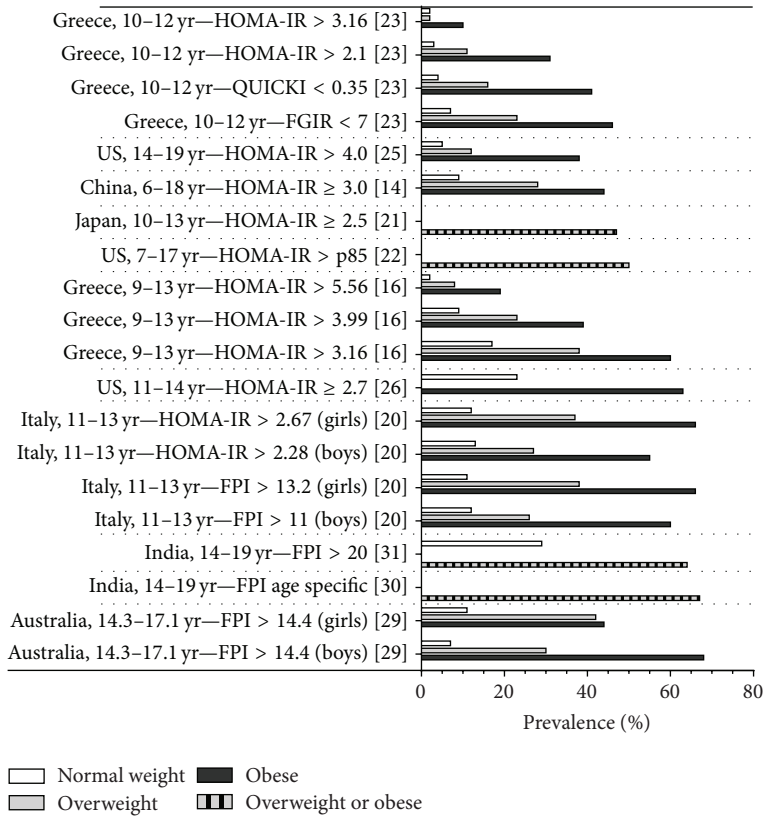
The highest reported prevalence rate for IR was 44% in Pacific Island teenagers (New Zealand) [28]. In that study IR was defined as FPI > 12 μU/mL, which is a relatively low cut-off value that might contribute to the high reported prevalence rate. In another study in Mexico, which used the lowest cut-off value for FPI (FPI > 9.85 mU/L) a prevalence rate of 24.8% was reported [19]. When the same cut-off values would have been used in these two studies, the difference in prevalence rates would even have been larger. Even though the difference between these two populations cannot be quantified precisely, not only because of different cut-off values, but also because other factors such as age, weight, and pubertal stage were not taken into account, this analysis shows that prevalence rates of IR are variable in different populations, which was also observed in other studies.

Overweight or obesity is an important factor influencing the prevalence of IR. The effect of overweight or obesity on IR is clearly observed in all presented studies as prevalence rates in overweight or obese children and adolescents were reported to be higher than in normal weight children and adolescents (Figure 3(b)). Most studies (7 out 11 studies presenting weight specific prevalence rates) not only differentiated between normal weight and overweight/obesity, but also stratified into normal weight, overweight, and obese children and adolescents [14, 16, 20, 23, 25, 26, 29]. These studies show an increased prevalence in obese children compared to overweight children. In the study by Caserta et al., odds ratios for IR were calculated for obese and overweight boys and girls comparing to their normal weight peers. The odds ratios of 9.1 (95% confidence interval 4.0–20.4) and 13.2 (4.7–36.9) were reported for obese boys and girls and lower odds ratios of 2.4 (1.2–4.9) and 6.0 (3.1–11.9) were reported for overweight boys and girls, respectively [20]. These results show that with normal weight increasing to obesity the prevalence of IR is rising.

A higher prevalence rate of IR has been observed in girls compared with boys in 7 out of 13 studies reporting sex specific prevalence rates (Figure 3(a)) [16, 18, 19, 27–29, 31]. This is in line with the prevalence of T2DM, of which IR is a precursor, as population-based studies on the prevalence of T2DM in children and adolescents also show higher prevalence rates in girls [33]. Hirschler et al. found no significant sex-related differences in IR. In their study, IR was associated with BMI and pubertal stage only, and not with gender. Their findings suggested that higher values in IR in girls compared to boys could be due to differences in pubertal development [34]. A study by Moran et al. measured IR using the euglycemic insulin clamp in children at all Tanner stages. At all Tanner stages, girls were more insulin resistant compared to boys. According to Moran et al., this difference in IR between boys and girls could partially be explained



(a) Sex specific prevalence



(b) Weight category specific prevalence

Figure 3: Prevalence of IR by sex (a) and weight category (b).

by higher levels of adipose tissue in girls compared to boys. However, in an obese subpopulation no difference in IR levels was observed between boys and girls [35]. It is known that pubertal development starts earlier in girls compared to boys (Tanner stage 2 at 11.4–11.9 years versus 11.9–12.3 years, resp.) [36]. Therefore, boys and girls between 10 and 14 years of age might be at another Tanner stage. Since IR is related to pubertal stage [34, 37], a comparison between pubertal girls and boys of the same age might result in a higher prevalence rate for IR in girls, because of a higher Tanner stage. The best comparison between boys and girls in pubertal age would be based on Tanner stages instead of age. Unfortunately, prevalence rates related to Tanner stages were not reported in any of the studies, so we were not able to check the effect of puberty on the prevalence of IR.

Our review has some limitations that should be addressed. At first, we could not compare results and pool the data of different studies, because of the heterogeneity in definition of IR in the presented studies. However, we were able to present an overview of the currently available population-based studies, showing higher prevalence rates in girls compared to boys, and in overweight and obese children compared to normal weight children. Another limitation is that all included studies were conducted in recent years. All studies were published between 2004 and 2014 and the data were collected between 2000 and 2011. However, in eight of eighteen studies, the exact period of data collection was not mentioned [14, 15, 18, 19, 26–28, 30]. Therefore, we could not evaluate whether the prevalence of IR is rising along with the increasing prevalence of obesity and T2DM. Finally, as already discussed above, the influence of Tanner stage on prevalence of IR could not be studied because of a lack of data.

5. Conclusion

In conclusion, the overall prevalence rates of IR in population-based studies of children and adolescents ranged between 3.1 and 44%, which could be partly explained by the use of different methods and cut-off values to determine IR. The prevalence rate of IR was up to 68.4% in obese boys. Girls seemed to have higher prevalence rates of IR than boys, which may however be related to their earlier pubertal development. Consensus on the definition for IR in children is needed to allow for comparisons between different studies, and to assess the value of IR as a screening measure for children and adolescents with an increased risk of cardiometabolic diseases.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Hindawi Publishing Corporation
Journal of Diabetes Research
Volume 2015, Article ID 485132, 6 pages
http://dx.doi.org/10.1155/2015/485132

Research Article

The Genetic Profile from HLA and Non-HLA Loci Allows Identification of Atypical Type 2 Diabetes Patients

Matias Fabregat,[1] Mariana Fernandez,[1] Gerardo Javiel,[2,3] Graciela Vitarella,[3] and Adriana Mimbacas[1]

[1]*Biodiversity and Genetic Department, Instituto de Investigaciones Biológicas Clemente Estable, 11600 Montevideo, Uruguay*
[2]*Teaching Care Unit, Unit of Diabetes "Hospital Pasteur", ASSE, Ministry of Public Health, 11600 Montevideo, Uruguay*
[3]*Diabetology Service of "Centro de Asistencia del Sindicato Médico del Uruguay", 11800 Montevideo, Uruguay*

Correspondence should be addressed to Adriana Mimbacas; amimbacas@iibce.edu.uy

Received 29 January 2015; Accepted 23 March 2015

Academic Editor: Joseph Fomusi Ndisang

The complex diagnosis and treatment of diabetes highlight the need for markers to define how to monitor patients correctly during the course of their disease. Different studies demonstrate the existence of patients who cannot be clearly classified. We have previously shown that it is possible to differentiate "atypical diabetic patients" based on genotyping the HLA. In this work we show that the analysis of non-HLA related to type 1 diabetes in the *INS-VNTR*, SNP rs689, and rs3842753 improves the identification of these patients. We genotyped 913 individuals comprising controls from the general population and "classic" and "atypical" diabetic patients. We compared the distribution of these loci and analyzed linkage disequilibrium. The haplotype was in LD for all the SNPs that were evaluated. Regarding their association with the disease, the haplotype IAC was associated with type 1 (odds 2.60, 1.82–3.72, CI 95%) and "atypical diabetes" (odds 1.50, 1.01–2.23, CI 95%), whereas we did not observe an association with type 2 diabetes. Therefore, our results confirm that atypical diabetes is a different entity of the disease where the patient presents with a genetic background of T1D and a T2D phenotype, findings that are likely to be relevant for patient diagnosis and management in the clinic.

1. Introduction

Uruguay is a little country located in South America with a population of 3.286.314 inhabitants according to the last census [1]. The population has been characterized as of European descent, with a small contribution of African descendants and native amerindian popultion [2]. Diabetes mellitus is a worldwide public health problem and Uruguay is not an exception showing a high prevalence of the disease, 8%, and an equal percent that are likely undiagnosed despite the small size of its population [3]. Diabetes is a multifactorial disease where genetic and environmental factors interact. This complexity could be an important influence in the actual classification and treatment of this pathology. Previously, in an effort to link the current classification with advances at molecular genetics we found that in several studies a significant proportion of diabetic patients did not present a correlation between genotype and phenotype [4–6]. Type 2 diabetes (T2D) diagnosed patients using international criteria [7] presented Human Leukocyte Antigen (HLA) alleles associated with type 1 diabetes (T1D) and positive antibodies in an intermediate value between both types, thus suggesting the coexistence of both types of diabetes. Several authors have published about this kind of diabetes [8–14] and these "atypical patients" cannot be grouped into any of the established groups by international guidelines. More importantly, these atypical patients do not have an adequate response when undergoing treatment [15].

Recently Maruthur and colleagues found evidence that in T2D the pharmacogenetic interactions for some antihyperglycemic drugs are consistent with their pharmacokinetics and pharmacodynamics, showing the importance of genetics variants [16]. In line with these observations we hypothesized that in "atypical patients" non-HLA genes, in addition to the HLA susceptibility alleles, could be influencing the lack of

genotype-phenotype correlation and the course and development of the disease.

Here we perform an in depth analysis of the presence of other non-HLA loci related to T1D in this atypical diabetic population, particularly focusing on the insulin gene (Insulin-Varaibale number tandem repeat, SNP rs689, and rs3842753). We show that in the Uruguayan population these variants are in complete linkage disequilibrium [17]. This region is located on chromosome 11p15.5, in the promoter region of the insulin gene, and affects the level of transcription of *INS* and *IGF2* genes [10, 18]. Mathematical models estimate that, adding the *HLA* and *VNTR* contribution, up to 60% of the total genetic component of T1D is obtained [19]. *VNTR* polymorphisms located in the insulin promoter, 596 bp upstream of the insulin start codon, consist of a highly polymorphic tandem repeat sequence ACAGGGGTGTGGGG (14 pb) whose variants are grouped into three classes of alleles according to their length [20, 21]. Regarding our population, *VNTR* is in linkage disequilibrium with rs689 where class I *VNTR* allele is associated with autoimmunity and inherited linked to the Adenine SNP allele. Furthermore the class III *VNTR* alleles are associated with autoimmunity protection and inherited linked to the Timina SNP allele. Population studies have shown that class III *VNTR* alleles have a dominant protective effect and are associated with a 60–70% reduction in the risk of developing T1D [19–23]. Even in the presence of autoantibodies and a high-risk HLA genotype, individuals carrying class III *VNTR* alleles present a significant reduction in the probability of developing the disease [24, 25]. Based on this association, major studies have used 23HphI (rs689) as an associated marker to study the *VNTR* [21, 26, 27].

Another SNP in linkage disequilibrium with the variants described above is rs3842753, a transversion at position +1140 relative to the initial translation codon of the *INS* gene. This polymorphism is located in the 3′UTR region of the *INS* mRNA and the cytosine-containing variant may cause instability of the mRNA [28]. In European and Uruguayan populations, the cytosine-containing allele is associated with T1D and is in complete linkage disequilibrium with the *VNTR* allele of class I and rs698 [17, 25]. Given the likely coexistence of both types of diabetes in our population and the presence of linkage disequilibrium as described in previous papers, we propose the in depth analysis of the genetic profile of these "atypical patients." The analysis should include patients that present a phenotype characteristic of T2D with HLA susceptibility alleles associated with T1D compared to (a) the general population and (b) type 1 diabetes and type 2 diabetes patients without a HLA association.

2. Materials and Methods

In this unmatched case-control study, a total of 913 individuals, including diabetes patients (413) and controls (500), were enrolled between 2004 and 2012. Recruitment of patients was done by the outpatient health center of Montevideo city.

2.1. Control Samples. We selected 500 unrelated individuals from the DNA bank of the Department of Biodiversity and Genetics, IIBCE. This collection is a representative sample of Montevideo's general population and was randomly selected from 15 different medical institutions, public and private, when individuals assisted to their annual routine control.

Due to local legislation at the time (ref. 1081/1996), the MSP (Public Health Ministry) did not authorize collecting information about the clinical characteristics of patients when the study involved analyses of DNA samples. Therefore, the only characteristic recorded for this population is the age (>18 years old).

2.2. Diabetic Patient Samples. 413 patients from 3rd attention level at Clinics for Diabetes of Reference Health Centers of Montevideo were analyzed. For the preparation of this study we only considered those patients receiving comprehensive care of their diabetes, following a nutritional plan and presenting a good adherence to physical activity according to their functional ability within the recommendations of the American Diabetes Association (ADA) and "Asociación Latinoamericana de Diabetes" (ALAD) [7, 29]. The diabetes samples were subclassified as type 1 diabetes, type 2 diabetes, and "atypical diabetes."

2.2.1. Type 1 Diabetes Patients (168). Those patients were defined according to the ADA criteria [22], with an age of onset < 15 years and a body mass index < 25 kg/mts^2.

2.2.2. Type 2 Diabetes Patients, Classical and Atypical (153 + 92). The population diagnosed with T2D was divided into two groups based on the presence or absence of T1D HLA susceptibility alleles described in the Uruguayan population [15, 30] according to the following inclusion criteria.

"Atypical diabetes" (92 individuals) (a) patients who had good adherence to the treatment; (b) they fulfilled the objectives of education and nutrition plans according to international guidelines; (c) present doubts on classification of diabetic type and/or not good therapeutic response (two consecutive measurements of glycated hemoglobin within three months not reduced in 1.5% [31]); (d) patients with susceptibility HLA alleles for autoimmune disease. We considered DQB1 ∗ 0201-0302 and DR 3-4 as susceptible ones in the Uruguay, (e) body mass index ≥ 25 kg/mts^2 [32].

2.2.3. Type 2 Diabetes (153 Individuals). Those patients were fulfilling the same requirements a and b of atypical patients but without diagnostic doubts, responded to treatment, and do not present HLA alleles associated with autoimmune disease.

Samples from patients who had other endocrine disorders or tumors were excluded.

All subjects were interviewed by specialist medical doctors and gave a written informed consent to participate in the study. The protocol was approved by the Ethical Committee of the Ministry of Public Health (MSP) and the corresponding ethical committee of each participating institution.

Table 1: Clinical characteristics of diabetes patients.

Variable	Median ± SD			*P* corrected		
	DT1	DT2	AD	DT1 versus DT2	DT1 versus AD	DT2 versus AD
Age (years)	35.64 ± 17.4	65.05 ± 9.9	61.84 ± 13.4	<0.001	<0.01	0.05
BMI (Kg/m^2)	22.96 ± 3.7	31.20 ± 5.7	30.74 ± 5.7	<0.01	<0.01	0.569
HbA1c (%)*	9.85 ± 2.4	8.28 ± 1.7	8.32 ± 1.9	<0.01	<0.01	0.859
Cholesterol	4.88 ± 1.4	5.53 ± 1.1	5.23 ± 1.2	0.002	0.99	0.065
HDL	1.50 ± 0.5	12.67 ± 1.7	1.22 ± 0.3	0.013	0.001	0.041
LDL	2.80 ± 1.0	3.33 ± 1.7	3.01 ± 1.1	0.013	0.207	0.1
TG	1.28 ± 0.7	2.26 ± 1.4	2.21 ± 0.2	<0.001	<0.001	0.838

Units without bracket were expressed in IS, BMI = body mass index, TG = triglycerides, and HbA1c (%)* = glycated hemoglobin value at initial study.

2.3. Molecular Analysis. High molecular weight DNA extraction was performed from peripheral blood by standard phenol chloroform protocol.

The HLA typing was performed by reverse ASO technique (Innogenetics Ltd., Belgium, UE). *INS 5′VNTR*, rs3842753, and HLA were processed in a previous work [4, 5, 15, 17, 31].

The SNP rs689 (−23 HPh1) for controls and T2D were processed in a previous work [16, 33]: Atypical diabetes patients were genotyped by PCR-RFLP with the following primers: forward-5′AGCAGGTCTGTTCCAAGG-3′ and reverse-5′CTTGGGTGTGTAGAAGAAGC-3′ which amplifies a 360 bp fragment. The identification of the genotypes was performed by digesting the DNA fragments with *Bsm*AI. Sequencing was used for confirmation of genotypes (Macrogen, Ltda, Korea).

2.4. Statistical Analysis. (a) Calculations of power sample size were done with the Epi Info 3.4.3 database and the statistics software for public health professionals and the Quanto statistical package (http://biostats.usc.edu/software) considering the prevalence of diabetes in Uruguay (8%) [3]. We assumed 95% of confidential interval and power 80% in an unmatched case-control design.

(b) The statistical analysis of a polymorphism was done with the web tool for SNP analysis SNPStats (http://bioinfo.iconcologia.net/snpstats/start.htm).

(c) Selection of inheritance model: best inheritance mode was selected according to SNPStat program. The statistic follows a chi-square distribution with degrees of freedom equal to the number of additional parameters in the more complex model and Akaike information criterion (AIC) and Bayesian information criterion (BIC). The statistical test *P* values were calculated via an exact test. The reference category used by program was the homozygous form.

(d) Hardy-Weinberg equilibrium (HWE) for allelic and genotype frequencies was tested by chi-square test.

(e) Association between one polymorphism and disease: we make the contingency table and then apply a chi-square test and the estimation of the OR (odds ratio) for each genotype with respect to the reference genotype (Epi Info and SNPStat).

(f) Haplotype analysis: D statistic (under the assumption of no association) and correlation coefficient between alleles and the observed frequency were done. Linkage disequilibrium and haplotype were calculated with SNPStat program. Analysis of multiple SNPs and haplotype and analysis of association between haplotypes and disease were done.

3. Results

Nine hundred and thirteen DNAs were analyzed in this study. Five hundred correspond to samples obtained from the general population of Uruguay and four hundred and thirteen correspond to diabetes patients. First we analyzed the clinical characteristics of diabetes patients and performed the comparison between the three groups defined previously (Table 1). We found a significant statistical difference when we compared T1D with any other subgroup in all variables considered. However, the only difference between T2D and "atypical diabetes" was in the level of HDL. These results are in accordance with previous reports [14]. Our genetic analysis showed that all SNPs analyzed in all groups were in Hardy-Weinberg equilibrium (HWE). The best heredity model for each SNP analyzed was the log-additive model, according to the AIC and BIC criteria of the SNPstat program. Allelic frequencies for all samples and association with the disease are shown in Tables 2(a), 2(b), and 2(c).

According to the D' value and the correlation coefficient between alleles and the observed frequency, the evaluated haplotype was in linkage disequilibrium with all SNPs analyzed. The most frequent haplotype was IAC in controls and diabetes patients (Table 3). Haplotype with frequencies minor 1% was considered rare. Regarding the association with the disease, the high protective effect (IIITA) was present in T1D. The contingency table for haplotypes associated with T1D versus no presence of this haplotype revealed an association in T1D and "atypical diabetes" but resulted in no significant association in T2D (Table 4).

4. Discussion

Our study allowed us to analyze the genetic profile of "atypical diabetes" comparing the phenotype of type 2 diabetes with HLA susceptibility alleles in the general population, T1D, and T2D without HLA associated. Based on our results we propose that this marker can aid in the diagnosis and management of patients who present difficulties in control and

Table 2: Allelic frequencies of the SNPs: (a) type 1 diabetes, (b) type 2 diabetes, and (c) atypical diabetes.

(a)

Variant	Allelic frequencies		OR	CI	P corrected
	Type 1 diabetes	Control			
INS-VNTR					
I	0.91	0.72	3.79	2.51–5.74	0.000
III	0.09	0.28			
rs689					
A	0.88	0.72	2.83	1.96–4.09	0.0000
T	0.12	0.28			
rs3842753					
A	0.86	0.72	2.44	1.72–3.47	0.0001
C	0.14	0.28			

(b)

Variant	Allelic frequencies		OR	CI	P corrected
	Type 2 diabetes	Control			
INS-VNTR					
I	0.75	0.72	1.28	0.94–1.72	0.11
III	0.25	0.28			
rs689					
A	0.67	0.72	0.81	0.60–1.07	0.14
T	0.33	0.28			
rs3842753					
A	0.68	0.72	0.84	0.63–1.12	0.26
C	0.32	0.28			

(c)

Variant	Allelic frequencies		OR	CI	P corrected
	Atypical diabetes	Control			
INS-VNTR					
I	0.82	0.72	1.75	1.13–2.71	0.01
III	0.18	0.28			
rs689					
A	0.79	0.72	1.51	1.51–2.25	0.04
T	0.21	0.28			
rs3842753					
A	0.74	0.72	1.23	0.84–1.79	0.31
C	0.24	0.28			

OR: odds ratio; CI: confidence intervals.

Table 3: Haplotype frequencies for all groups.

	INS	rs689	rs3842753	Freq.
Type 1 diabetes	I	A	C	0.7492
	III	T	A	0.2275
	I	T	A	0.012
	Rare	*	*	0.0113*
Type 2 diabetes	I	A	C	0.7035
	III	T	A	0.2703
	I	T	A	0.0199
	Rare	*	*	0.0062
Atypical diabetes	I	A	C	0.7187
	III	T	A	0.2558
	I	T	A	0.0119
	*Rare	*	*	0.0136

*Means other haplotypes different to previous.

Table 4: Haplotype association with the disease.

	Haplotype	OR (95% CI)	P value
Diabetes type 1	IAC	2.60 (1.82–3.72)	<0.0001
	No IAC		
Diabetes type 2	IAC	0.83 (0.62–1.10)	NS
	No IAC		
Atypical diabetes	IAC	1.50 (1.01–2.23)	<0.05
	No IAC		

NS = non significant.

follow-up. In addition, our analysis of the INS VNTR locus, as the second marker of importance for T1D susceptibility, further supports the notion that patients with difficulties in all approach areas (diagnosis, treatment, and evolution) have a strong genetic basis.

The last ADA guidelines classified diabetes in four clinical categories but recognized that some patients cannot be clearly classified as type 1 or 2 diabetes. Importantly, such difficulties in classification may occur at any age and genetic studies could improve the timely diagnosis in these kinds of patients, without having to wait for the disease to progress.

Mathematical models estimate that together the HLA and INS VNTR contribution may account for near 60% of the total genetic basis for developing T1D [19]. Therefore these markers, typically associated with autoimmune diabetes, when present in T2D patients, can modify the expected development of the pathology.

It has been shown that the second susceptibility locus for autoimmune disease, after HLA, corresponds to a mini satellite in the insulin gene (INS-VNTR) and in Caucasians populations this VNTR is in linkage disequilibrium with −23 HphI SNP. Previous studies estimate that 10% of the genetic susceptibility to T1D corresponds to this mini satellite [20].

In previous works, we showed that there are patients in our population who cannot be classified as either type 1 or type 2 diabetes according to the international guidelines. The clinical presentation, evolution, and difficulty in reaching expected therapeutic goals make them "atypical patients" in relation with HLA alleles [14]. Now we have gained insight into understanding the genetic basis of "atypical diabetes" by showing an association with genes different from HLA. It is noteworthy that, in these patients, there are no homozygous individuals for allele III of 5′*VNTR*. The protective allele III in the promoter region of *INS* is associated

with a greater functional reserve of β cells in response to a direct hyperglycemic stimulus [34]. Although we did not analyze the functional reserve of beta cells, it is striking to find a high frequency of susceptibility allele I in "atypical patients" and lower presence of III alleles in their genotypes. This observation could suggest an earlier beta cell failure in these patients and therefore could be one possible explanation to understand the cellular basis of their bad treatment response, a possibility that should be analyzed in future investigations.

In conclusion, our results confirm that atypical diabetes is a different entity of the disease where patients combine a genetic background of T1D with a T2D phenotype, thus highlighting the importance of genetics as a new tool in clinical practice.

Conflict of Interests

There are no financial competing interests.

Authors' Contribution

Matias Fabregat conducted experimental procedures and validations, data acquisition, compilation, analysis, and interpretation and also was involved in retrieving conceptual information and draft paper preparation; Mariana Fernandez contributed to draft paper preparation and performed critical revision to structure the content intellectually. Gerardo Javiel provided substantial contributions to the concept and design of the work. He was in charge of the admission protocol and monitoring of patients. He was involved in retrieving medical conceptual information and draft paper preparation, performed a critical revision to structure the content intellectually, and gave approval for the final version to be published. Graciela Vitarella was in charge of the admission protocol and monitoring of patients. She was involved in retrieving medical conceptual information and draft paper preparation, performed a critical revision to structure the content intellectually, and gave approval for the final version to be published. Adriana Mimbacas provided substantial contributions to the concept and design of the work and controlled experimental procedures, validations of the results, and statistical analysis. She was involved in retrieving conceptual information and draft paper preparation, performed critical revision to structure the content intellectually, and gave approval for the final version to be published.

Acknowledgments

The authors thank all the patients that participated in the study and the health personnel who contributed to the development of this study. They thank Dr. Jose Luis Badano for correcting the English paper.

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Hindawi Publishing Corporation
Journal of Diabetes Research
Volume 2015, Article ID 943659, 8 pages
http://dx.doi.org/10.1155/2015/943659

Research Article

MicroRNA-223 Expression Is Upregulated in Insulin Resistant Human Adipose Tissue

Tung-Yueh Chuang,[1] Hsiao-Li Wu,[1] Chen-Chun Chen,[2] Gloria Mabel Gamboa,[3] Lawrence C. Layman,[1,4,5,6] Michael P. Diamond,[1] Ricardo Azziz,[1,7] and Yen-Hao Chen[1]

[1]*Department of Obstetrics/Gynecology, Georgia Regents University, 1120 15th Street, Augusta, GA 30912, USA*
[2]*Department of Biostatistics, Georgia Regents University, 1120 15th Street, Augusta, GA 30912, USA*
[3]*Department of Surgery, Georgia Regents University, 1120 15th Street, Augusta, GA 30912, USA*
[4]*Section of Reproductive Endocrinology, Infertility & Genetics, Georgia Regents University, 1120 15th Street, Augusta, GA 30912, USA*
[5]*Institute of Molecular Medicine and Genetics, Georgia Regents University, 1120 15th Street, Augusta, GA 30912, USA*
[6]*Neuroscience Program, Georgia Regents University, 1120 15th Street, Augusta, GA 30912, USA*
[7]*Department of Medicine, Georgia Regents University, 1120 15th Street, Augusta, GA 30912, USA*

Correspondence should be addressed to Yen-Hao Chen; yechen@gru.edu

Received 5 November 2014; Revised 26 March 2015; Accepted 26 March 2015

Academic Editor: Joseph Fomusi Ndisang

MicroRNAs (miRNAs) are short noncoding RNAs involved in posttranscriptional regulation of gene expression and influence many cellular functions including glucose and lipid metabolism. We previously reported that adipose tissue (AT) from women with polycystic ovary syndrome (PCOS) or controls with insulin resistance (IR) revealed a differentially expressed microRNA (miRNA) profile, including upregulated miR-93 in PCOS patients and in non-PCOS women with IR. Overexpressed miR-93 directly inhibited glucose transporter isoform 4 (GLUT4) expression, thereby influencing glucose metabolism. We have now studied the role of miR-223, which is also abnormally expressed in the AT of IR subjects. Our data indicates that miR-223 is significantly overexpressed in the AT of IR women, regardless of whether they had PCOS or not. miR-223 expression in AT was positively correlated with HOMA-IR. Unlike what is reported in cardiomyocytes, overexpression of miR-223 in human differentiated adipocytes was associated with a reduction in GLUT4 protein content and insulin-stimulated glucose uptake. In addition, our data suggests miR-223 regulates GLUT4 expression by direct binding to its 3′ untranslated region (3′UTR). In conclusion, in AT miR-223 is an IR-related miRNA that may serve as a potential therapeutic target for the treatment of IR-related disorders.

1. Introduction

MicroRNAs (miRNAs) are short (20–24 nucleotide) noncoding RNAs involved in posttranscriptional regulation of gene expression. miRNA genes can be epigenetically regulated and miRNAs can themselves repress key enzymes that drive epigenetic remodeling and directly modulate gene transcription in the nucleus through recognition of specific target sites in promoter regions [1]. miRNAs influence many cellular functions including glucose and lipid metabolism [2–6]. Insulin resistant adipocytes are known to contain a differentially expressed miRNA profile [7]. In insulin resistant 3T3-L1 adipocytes, approximately 80 miRNAs have been found to be up- or downregulated [8], while miR-320 and miR-29 have been demonstrated to regulate insulin action through the PI3K/AKT pathway [5, 8].

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders, affecting ≥7–9% of reproductive-aged women, even when defined conservatively [9]. About 60–70% of PCOS patients demonstrate insulin resistance (IR) above and beyond that predicted by body mass, race, or age, resulting in compensatory hyperinsulinemia and an increased risk for type 2 diabetes mellitus (T2DM) and metabolic syndrome. The underlying cellular mechanisms leading to IR in PCOS remain to be completely elucidated, as no gross defects in the traditional insulin signaling pathways

have been found, including insulin binding, insulin receptor expression, and the IRS-1/PI3 K/AKT pathway [10, 11].

We previously reported that miR-93 is upregulated in adipose tissue (AT) from PCOS and non-PCOS women who display IR [11]. Overexpressed miR-93 directly inhibits glucose transporter isoform 4 (GLUT4) expression, influencing glucose metabolism. In addition we also observed that miR-223 was abnormally expressed in PCOS women with IR. miR-223 is overexpressed in insulin resistant myocardial cells and, paradoxically, overexpression of miR-223 by transfection has been reported to increase GLUT4 protein expression but not mRNA, thereby improving glucose uptake in cardiomyocytes [12].

It is unclear whether miR-223 may also regulate IR in adipocytes. In the present study, we examined the role of miR-223 in the AT of four groups of women: those without PCOS or IR; those without PCOS, but with IR; those with PCOS, but without IR; and women with PCOS and IR. We hypothesized that abnormal expression of miR-223 plays a role in the metabolic dysfunction of PCOS and IR.

2. Materials and Methods

2.1. Study Subjects. Subcutaneous abdominal AT samples from 33 women (30 White, 1 Black, and 2 Asian) were studied. Subjects were recruited at the Cedars-Sinai Medical Center in Los Angeles. The diagnosis of PCOS was performed as previously described [11]. In brief, the diagnosis of PCOS was made according to the National Institutes of Health (NIH) 1990 criteria [13]: (i) clinical evidence of hyperandrogenism and/or hyperandrogenemia; (ii) oligoovulation; and (iii) the exclusion of related disorders. Specific criteria for defining clinical hyperandrogenism, hyperandrogenemia, oligoovulation, and the exclusion of related disorders have been previously described [13]. All subjects had no significant illness including diabetes, had not received hormonal therapy or medications that could alter the metabolic or hormonal status for at least three months before the study, and were between the ages of 18 and 45 years. The study was approved by the Institutional Review Board, and all subjects gave informed written consent.

2.2. Hormonal Assays. Hormonal assays for total and free testosterone (T), dehydroepiandrosterone sulfate (DHEAS), insulin, and glucose were performed as previously described [14].

2.3. Adipose Tissue Biopsy and Real-Time PCR (qPCR). Approximately 5 g of subcutaneous AT was excised through a small incision in the lower abdomen, as previously described (http://www.youtube.com/watch?v=Gy2pFUjDlDM [15]). Total RNA was extracted using the miRACLE Isolation Kit (Jinfiniti Biosciences, Augusta, GA). First-strand cDNAs of mRNA and miRNA were synthesized using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) and First-Strand cDNA Synthesis Kit for miRNA (OriGene, Rockville, MD). Real-time PCR was performed using an iTaq Universal SYBR Green Supermix (Bio-Rad Laboratories, Inc., Hercules, CA). Primers were purchased from OriGene. Experiments were performed on an Applied Biosystems 7300 Real-Time PCR System. *ACTB* and miR-103 [16] were used as internal controls. Relative fold change of targets genes expression was calculated by using the $2^{-\Delta\Delta Ct}$ method.

2.4. Differentiation of Human Preadipocytes to Adipocytes. To induce differentiation, preadipocytes were cultured to full confluence and then maintained in differentiation medium (Cat# DM-2, Zen-Bio Inc., Research Triangle Park, NC) for one week (day 7 of differentiation) before being cultured in adipocyte medium (Cat# AM-1, Zen-Bio Inc., Research Triangle Park, NC) for an additional week (day 14 of differentiation).

2.5. Western Blot. For western blot analysis, 30 μg protein was utilized. Blots were probed with specific primary antibodies and the appropriate secondary antibodies (Jackson ImmunoResearch Lab. West Grove, PA). β-actin was used as a loading control. GLUT4 antibody was purchased from Abcam, Cambridge, MA.

2.6. Transfection. Transfection was performed as described previously [17]. Briefly, for each well of 6-well plate, 2 μg of plasmid was used for transfection. miR-223 overexpression plasmid (Cat# SC400292), noninsert empty plasmid control (Cat# PCMVMIR), and transfection reagent MegaTran 1.0 (Cat# TT200002) were purchased from OriGene (Rockville, MD). Assays were done at 48 hours after transfection.

2.7. Luciferase Reporter Assay. A luciferase reporter assay was performed as described previously [17]. GLUT4 3′UTR luciferase plasmid was purchased from OriGene (Rockville, MD). The 3′UTR luciferase plasmid (1 μg) with either miR-223 overexpression or empty plasmid (2 μg) was transfected in each well of a 12-well plate. 48 hours after transfection luciferase activity was assayed by a luciferase assay system (Promega, Madison, WI) measured on a fluorescence microplate reader (POLARstar Omega, BMG Labtech, Germany).

2.8. Glucose Uptake Assay. 6-NBDG (6-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-6-deoxyglucose; Life Technologies, Carlsbad, CA) was used to determine insulin-stimulated glucose uptake in human differentiated adipocytes. Briefly, insulin-stimulated glucose uptake was determined by first changing the adipocyte medium to a low-glucose serum free (LGSF) medium (0.1% BSA) for 4 hours. Insulin (100 nM) was added and incubated for another 1 hour. After incubation, 6-NBDG (20 μM) was added and incubated for 40 minutes. After incubation with 6-NBDG, adipocytes were washed three times with PBS, lysed by adding lysis buffer. 6-NBDG in cell lysate was measured on a fluorescence microplate reader (POLARstar Omega, BMG Labtech, Germany; excitation: 485 nm, emission: 535 nm).

Table 1: Clinical characteristics of study subjects.

	Non-PCOS without IR ($n = 7$)	Non-PCOS with IR ($n = 9$)	PCOS ($n = 8$)	PCOS with IR ($n = 11$)
BMI (kg/m^2)	24.58 ± 5.47	34.97 ± 6.76**	24.81 ± 3.16	31.31 ± 5.55
Age (yrs)	33.17 ± 8.47	34.86 ± 7.08	28.29 ± 2.36	27.00 ± 4.76
mFG score	0.17 ± 0.41	0.63 ± 0.92	5.71 ± 5.40*	7.00 ± 3.59**
Free testosterone (pg/mL)	1.73 ± 0.33	2.66 ± 1.29	4.56 ± 2.47*	6.66 ± 2.51**
Total testosterone (ng/mL)	26.50 ± 5.54	23.86 ± 3.76	45.71 ± 25.42	37.90 ± 19.14
DHEAS (μg/dL)	127.8 ± 30.7	252.7 ± 31.7*	286.3 ± 31.2**	244.6 ± 23.1*
Fasting glucose (mcg/dL)	68.50 ± 14.39	87.38 ± 8.85	88.14 ± 5.58*	93.15 ± 17.03**
Fasting insulin (mIU/mL)	5.00 ± 3.56	13.75 ± 3.24*	5.50 ± 2.35	20.43 ± 7.16^{**++}
HOMA-IR	0.88 ± 0.67	2.94 ± 0.66*	1.20 ± 0.58	4.65 ± 1.61^{**++}
Prolactin (ng/mL)	13.3 ± 5.50	10.99 ± 5.54	10.37 ± 5.14	12.83 ± 5.99
TSH (IU/mL)	2.05 ± 0.53	2.94 ± 1.48	1.66 ± 0.70	1.95 ± 0.93
17-HP (ng/dL)	25.29 ± 9.23	21.14 ± 7.24	29.90 ± 20.09	35.86 ± 14.86

Data are expressed as mean ± SD.
$^{**}P < 0.01$ versus control group.
$^{*}P < 0.05$ versus control group.
$^{++}P < 0.01$ versus PCOS group.
mFG score is the modified Ferriman-Gallwey hirsutism score; HOMA-IR is homeostasis model assessment for estimating insulin resistance. DHEAS is dehydroepiandrosterone sulfate, TSH is thyroid stimulating hormone, and 17-HP is 17 alpha-hydroxyprogesterone.

2.9. Statistical Analysis. Insulin resistance at baseline was estimated using the homeostasis model assessment (HOMA-IR); a HOMA-IR value <2.5 was considered normal and a HOMA-IR value ≥2.5 indicated IR [18]. Comparisons of multiple groups were carried out by ANOVA followed by a posttest analysis using the Fisher (among groups) and Dunnett (compared to controls) tests (XLSTAT Software, NY). Logistic regression was used to adjust data for body mass index (BMI). Group comparisons (PCOS versus non-PCOS and IR versus non-IR) were carried out by unpaired Student's t-test (SAS 9.3, SAS Institute Inc., Cary, NC). Significant differences were defined as $P < 0.05$. All values are presented as mean ± SEM.

3. Results

Of the 33 subjects included, 15 (7 without and 8 with PCOS) did not have IR as defined. Among the 18 subjects with IR, 8 did not have PCOS and 10 had PCOS. Table 1 depicts the clinical characteristics of the subjects included. As expected, subjects with PCOS had higher values for terminal body and facial hair growth and free T; and subjects deemed to have IR by HOMA-IR also had higher insulin levels than those without IR. While no subjects had diabetes as measured by fasting glucose, mean glucose in women with PCOS and IR was higher than non-PCOS non-IR women.

The expression of miR-223 was significantly increased among all women with IR ($P = 0.0004$; Figure 1(a)). However, no difference in miR-223 expression was detected (Figure 1(b)) with regard to PCOS status. Comparing all four subgroups (7 subjects without PCOS and without IR, 8 without PCOS but with IR, 8 with PCOS but without IR, and 10 with both PCOS and IR), miR-223 was only significantly overexpressed in the two groups of women with IR, compared to subjects without PCOS and without IR ($P < 0.01$; Figure 1(c)). Next, we examined the association of miR-223 expression with measures of IR, including HOMA-IR. Our results indicated that miR-223 expression was positively correlated with HOMA-IR ($r = 0.64$, $P < 0.01$; Figure 1(d))

Subjects with IR (regardless of the presence of PCOS) tended to have a greater mean body mass index (BMI) than subjects without IR (Table 1), a difference that reached significance only in women without PCOS, between those with IR and those without IR. There were no statistical differences in age. To gauge the possible effects of these differences on miR-223 expression we first determined whether an association existed between miR-223 expression and age or BMI for the entire group combined. Our results indicate that miR-223 expression did not vary according to age ($r = -0.11$, $P = 0.288$) but was positively correlated with BMI ($r = 0.46$, $P = 0.01$). Consequently, we compared miR-223 expression values adjusted for BMI for subjects with and without PCOS and with and without IR. The adjustment did not change our results, with the difference in miR-223 expression between women with and without IR ($P = 0.0193$) and the absence of a difference between women with and without PCOS ($P = 0.1178$) (Table 2) remaining.

To determine the role of and mechanism by which miR-223 induced IR in adipocytes, we overexpressed miR-223 in human differentiated adipocytes *in vitro* to achieve an approximately twofold increase in expression compared to empty plasmid controls ($P < 0.01$; Figure 2(a)), similar to the level of miR-223 overexpression in human AT (Figure 1(a)). We found overexpression of miR-223 inhibited glucose uptake stimulated by insulin in human differentiated adipocytes *in vitro* (Figure 2(b)). In addition, our data indicated that the induced overexpression of miR-223 was associated with a decrease in GLUT4 protein content

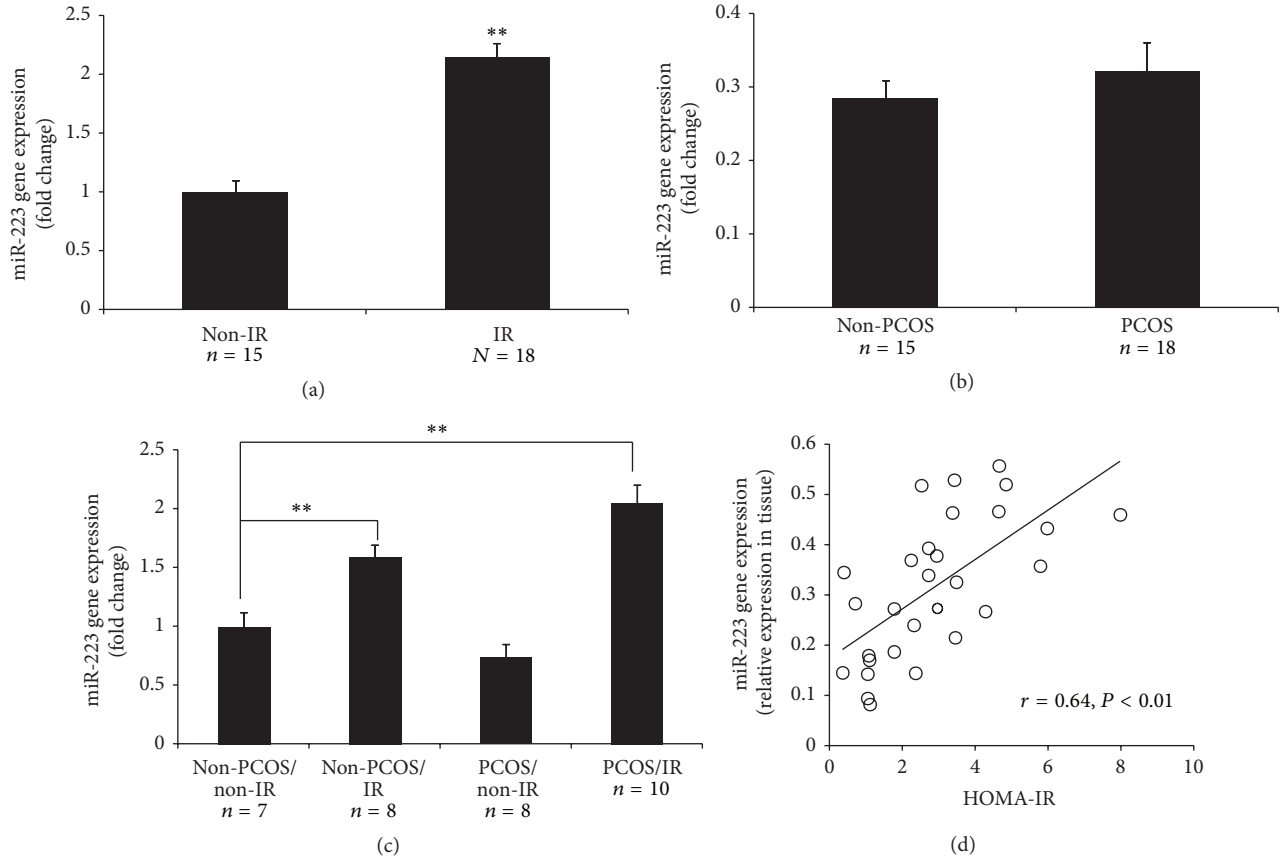

Figure 1: miR-223 expression in human AT. (a) Depicted is comparison of the levels of miR-223 expression in the AT of women with and without IR; miR-223 was significantly overexpressed in the IR group. (b) Depicted is comparison of the levels of miR-223 expression in the AT of women with and without PCOS; the expression of miR-223 did not differ between two groups. (c) Depicted is comparison of the levels of miR-223 expression in the AT of women: (A) without PCOS or IR, (B) without PCOS (non-PCOS), but with IR, (C) with PCOS and IR, and (D) with PCOS, but without IR; the expression of miR-223 was significantly higher in the AT in women with IR. $^{**}P < 0.01$ comparing tissues from women with or without PCOS and with IR versus that of women without PCOS or IR. (d) Depicted is the association between miR-223 expression and HOMA-IR. (Two group comparisons, (a) and (b), were carried out by unpaired Student's t-test; comparison of (d) was carried out by ANOVA followed by a posttest by using the Fisher (among groups) and Dunnett (compared to control group) test.)

Table 2: Relationship of miR-223 to PCOS and IR status and BMI.

	Odds ratio	Unadjusted 95% CL** (lower, upper)	P value	Odds ratio	Adjusted 95% CL** (lower, upper)	P value
IR versus non-IR						
miR-223*	1.144	(1.037, 1.262)	0.0071	1.134	(1.021, 1.260)	0.0193
BMI	1.135	(0.985, 1.308)	0.0810	1.029	(0.881, 1.202)	0.7158
	Odds ratio	Unadjusted 95% CL** (lower, upper)	P value	Odds ratio	Adjusted 95% CL** (lower, upper)	P value
PCOS versus non-PCOS						
miR-223*	1.027	(0.971, 1.087)	0.3525	1.059	(0.985, 1.139)	0.1178
BMI	0.929	(0.822, 1.049)	0.2350	0.875	(0.749, 1.023)	0.0937

*The miR-223 has been multiplied by 100 for the depiction of the odds ratio and confidence limit; the P value of the comparisons is not affected.
**95% CL is the 95% confidence limit.

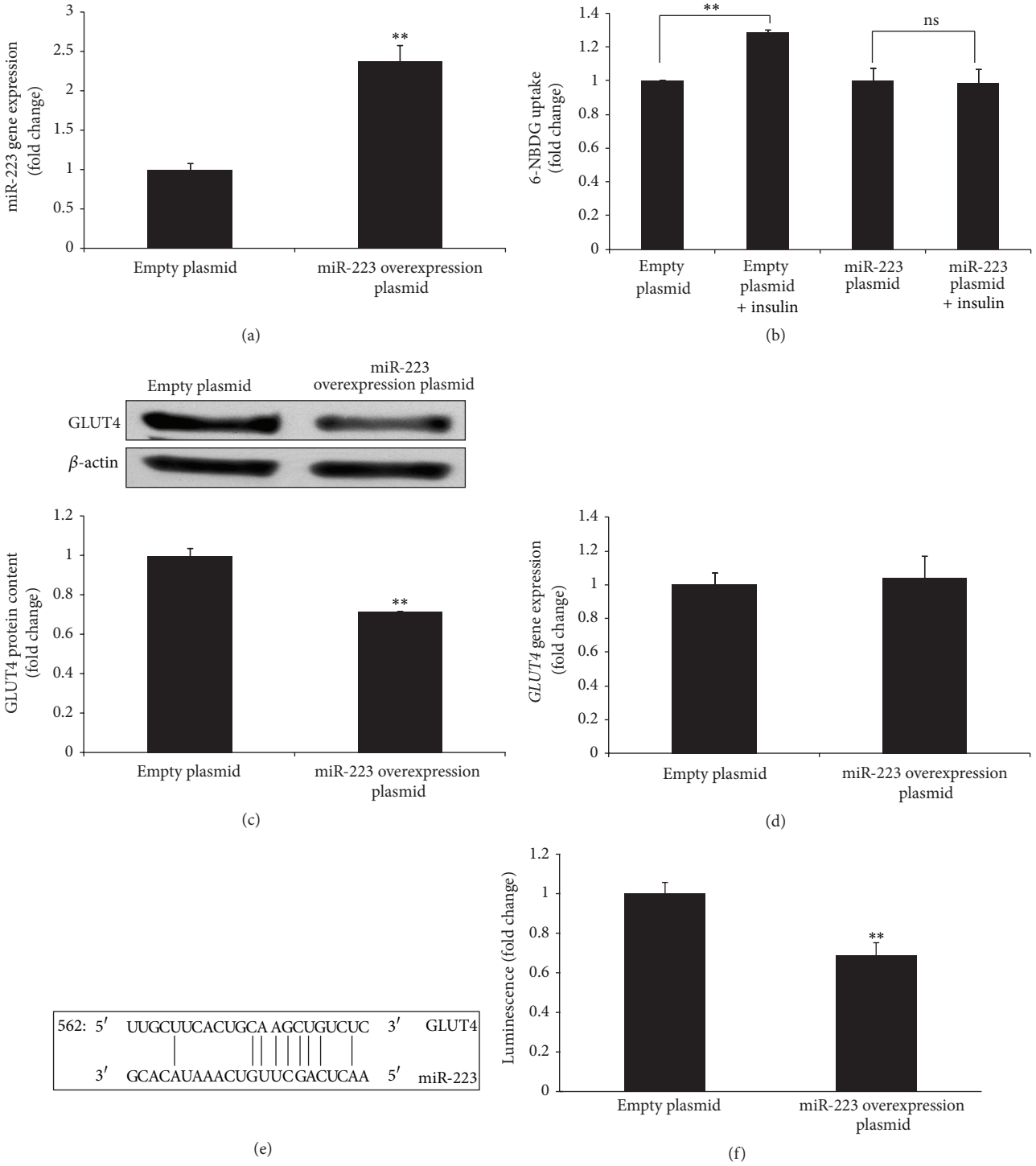

Figure 2: miR-223 regulates GLUT4 expression in human differentiated adipocytes. (a), (c), and (d) Depicted are the miR-223, GLUT4 protein, and *GLUT4* gene expression in human differentiated adipocytes after being transfected with miR-223 overexpression plasmid. (b) Depicted is 6-NBDG uptake stimulated by insulin in human differentiated adipocytes after being transfected with miR-223 overexpression plasmid. (e) Depicted is a GLUT4 3′UTR predicted binding site for miR-223. (f) Depicted is the GLUT4 3′UTR luciferase reporter which was repressed by overexpression of miR-223. $^{**}P < 0.01$ comparing to empty plasmid. (In (a), (c), (d), and (f), the comparisons of significance were carried out by unpaired Student's *t*-test. In (b), the comparison of significance was carried out by ANOVA followed by a posttest by using the Fisher (among groups) and Dunnett (compared to control group) test.)

(Figure 2(c)), but not *GLUT4* gene expression (Figure 2(d)). These data suggest GLUT4 could be a direct target of miR-223. Analysis of GLUT4 3′UTR sequence using the free energy-based miRNA prediction program PITA [18] revealed one potential target site for miR-223 in GLUT4 (Figure 2(e)). To address whether direct binding of miR-223 to the GLUT4 3′UTR is responsible for the observed suppression of GLUT4, we performed a luciferase assay in which direct binding of miR-223 to the vector GLUT4 3′UTR gene transcript would repress a luciferase reporter. Transient cotransfection of miR-223 and luciferase expression plasmids in human differentiated adipocytes demonstrated direct binding of miR-223 to the GLUT4 3′UTR site, resulting in a significant reduction in luciferase (Figure 2(f)).

As tumor necrosis factor-α (TNF-α) induces IR in adipocytes [19], the role of TNF-α in the regulation of miR-223 expression in human differentiated adipocytes was also examined. Consistent with the hypothesis that TNF-α increases IR in adipocytes, at least in part, via the modulation of miR-223 expression, we observed that treatment of human differentiated adipocytes with TNF-α (10 ng/mL) for 24 hours significantly increased miR-223 expression (Figure 3).

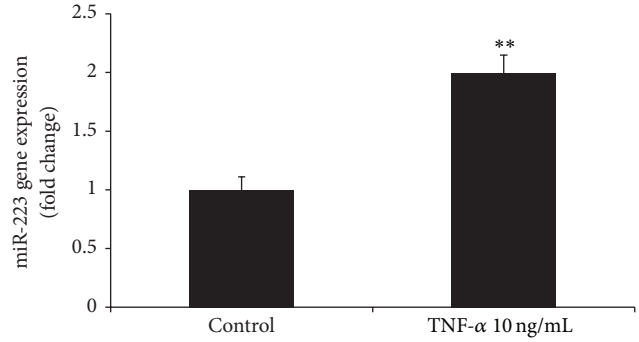

Figure 3: TNF-α induces expression of miR-223 in human differentiated adipocytes. Human differentiated adipocytes were treated with TNF-α (10 ng/mL) for 24 hours. Expression of miR-223 was detected by real-time PCR. $^{**}P < 0.01$ comparing to control; the comparisons of significance were carried out by unpaired Student's t-test.

4. Discussion

In a previous study, we examined the expression of miR-223 in AT from a total of 25 subjects, and our findings indicated that miR-223 tended to be overexpressed in PCOS and non-PCOS women with IR [11]. These trends were confirmed in the present study, analyzing a larger number of subjects, such that miR-223 was significantly overexpressed in women with IR, regardless of PCOS status.

GLUT4 is the major protein responsible for insulin-mediated glucose translocation into adipocytes [20] and plays an important role in the regulation of glucose homeostasis. In adipocytes a 50% decrease in GLUT4 content leads to a 50% decrease in GLUT4 translocation [21]. Moreover, AT-specific GLUT4 impacts glucose tolerance, insulin sensitivity, and glucose metabolism *in vivo* [22, 23]. *GLUT4* gene expression in AT correlated with HOMA-IR [11]. In cardiomyocytes, overexpression of miR-223 stimulates glucose uptake and increases GLUT4 protein content but not the level of mRNA [12].

In the present study, we examined the regulation of GLUT4 expression by miR-223. Similar to cardiomyocytes [12], overexpression of miR-223 in adipocytes did not alter GLUT4 mRNA expression. However, unlike cardiomyocytes, miR-223 overexpression was associated with a decrease in GLUT4 protein content and glucose uptake in AT. The discrepancy between cardiomyocytes and adipocytes could solely reflect differences in tissue specific regulation. However, miR-223 does appear to be overexpressed in AT and the myocardium of IR subjects and suggests that miR-223 may serve as a therapeutic target for IR.

In cardiomyocytes, overexpression of miR-223 enhances insulin-stimulated glucose uptake by increasing GLUT4 but not by altering insulin signaling and AMPK activity (baseline and phosphorylation) [12]. Insulin signaling components and AMPK are not targets of miR-223. This suggests that reduced insulin-stimulated glucose uptake in miR-223 overexpressed human differentiated adipocytes could be due to decreased levels of GLUT4, not by altering GLUT4 translocation.

That miR-223 decreased GLUT4 protein, but not mRNA, in adipocytes which suggests that miR-223 may regulate GLUT4 expression by binding to its 3′UTR. Although *in silico* analysis (algorithms miRanda, PicTar, and TargetScan) indicated that GLUT4 was not a predicted target of miR-223, we found one potential binding site in the 3′UTR sequence of GLUT4 by using the free energy-based miRNA prediction program PITA. Furthermore, by the GLUT4 3′UTR reporter assay, we demonstrated that miR-223 regulates GLUT4 expression by direct binding to its 3′UTR site.

We previously reported that the expression of miR-93 was significantly increased in the subcutaneous abdominal AT of all PCOS patients studied and non-PCOS women with IR [11]. Alternatively, miR-223 was increased in women with IR, regardless of PCOS status. Both miR-93 and miR-223 regulated GLUT4 protein content in adipocytes [11]. Together, these data suggest that miR-93 expression is associated with both IR and PCOS, whereas miR-223 is not involved in PCOS per se but is related to IR. As we previously noted that PCOS women with IR had the lowest expression of GLUT4 [11], it is possible that miR-93 and miR-223 may have additive effects on the regulation of GLUT4 expression.

In addition to our findings that miR-223 and miR-93 regulate IR in AT by targeting GLUT4, these two miRNAs have also been found to suppress proinflammatory activation of macrophages by targeting IRAK4 (for miR-93) [24] and Pknox1 (for miR-223) [25]. Macrophage activation is associated with IR [26]; therefore, these data suggest that miR-223 and miR-93 could also regulate IR by regulating inflammation.

miR-223 and miR-93 have been found to have similar functions yet they may or may not target the same genes. Both miRNAs regulate cancer activity by targeting the same gene E2F1 [27, 28]. However, to promote cancer activity, they also target different genes including C/EBPβ, FOXO1, NFI-A, STAT5A, ARTN, FBXW7, and SEPT6 (for miR-223) [29–33]

and FUS1, RhoC, PTEN, CDKN1A, TGFβR2, and NRF2 (for miR-93) [34–38]. Both miRNAs act as antiangiogenesis regulators, but miR-93 directly targets vascular endothelial growth factor A (VEGF-A) [39], while miR-223 targets β1 Integrin [40]. These data suggest that miR-223 and miR-93 may also have additive effects on these functions.

In conclusion, our data indicates that miR-223 is overexpressed in the subcutaneous AT of subjects with IR, regardless of PCOS status, and that miR-223 expression is positively associated with IR *in vivo*. Overexpression of miR-223 decreased GLUT4 protein content and inhibited insulin-stimulated glucose uptake in cultured human adipocytes. In addition TNF-α induced miR-223 expression *in vitro*, suggesting that TNF-α exerts its negative effect on insulin action at least in part through its modulation of the expression of this miRNA. Together these data suggest the possibility that miR-223 could be a potential therapeutic target for IR.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

The authors gratefully acknowledge the contribution of the PCOS patients and controls who participated in this study. This work was supported by NIH Grants RO1-DK073632 (to Ricardo Azziz) and an endowment from the Helping Hand of Los Angeles, Inc. (to Ricardo Azziz).

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Hindawi Publishing Corporation
Journal of Diabetes Research
Volume 2015, Article ID 328585, 7 pages
http://dx.doi.org/10.1155/2015/328585

Research Article

Association of Metabolic Syndrome with the Cardioankle Vascular Index in Asymptomatic Korean Population

Su-Hyun Nam,[1] Sung-Goo Kang,[1] Yun-Ah Lee,[1] Sang-Wook Song,[1,2] and Jun-Seung Rho[1]

[1] *Department of Family Medicine, St. Vincent's Hospital, College of Medicine, The Catholic University of Korea, Suwon 442-723, Republic of Korea*
[2] *Health Promotion Center, St. Vincent's Hospital, College of Medicine, The Catholic University of Korea, Suwon 442-723, Republic of Korea*

Correspondence should be addressed to Sung-Goo Kang; hippo94@naver.com

Received 3 February 2015; Accepted 24 February 2015

Academic Editor: Sharad Rastogi

Aim. Metabolic syndrome is characterized by a cluster of atherosclerotic cardiovascular risk factors. The cardioankle vascular index (CAVI) reflects arterial stiffness and may be used as an indicator of atherosclerotic cardiovascular disease. In this study, we investigated the association of CAVI with metabolic syndrome. *Methods*. A total of 1,144 adults were included in this study. We measured CAVIs and examined blood samples to identify metabolic syndrome according to WHO Asia Pacific criteria and NCEP-ATPIII criteria. AST, ALT, *r*-GTP, BUN, creatinine, high sensitivity C-reactive protein, and uric acid were also measured. *Results*. CAVI values were significantly higher in subjects with metabolic syndrome than those without metabolic syndrome and increased according to the number of metabolic syndrome components present. Subjects with high fasting blood sugar levels or high blood pressure showed high CAVI values. Multiple regression analysis showed that age, sex, diastolic blood pressure, and uric acid were independent predictors of CAVI. *Conclusion*. Subjects with metabolic syndrome had high CAVIs, which indicated arterial stiffness, and were closely associated with an increase in the number of metabolic risk factors. The individual risk factors for metabolic syndrome have the synergistic effect of elevating arterial stiffness in asymptomatic Korean population.

1. Introduction

Metabolic syndrome is an abnormal metabolic state caused by a combination of arteriosclerotic risk factors such as abdominal obesity, dyslipidemia, glucose intolerance, and hypertension and is related to insulin resistance [1]. The national nutrition survey in Korea from 2007 to 2010 showed that the prevalence of metabolic syndrome in Korean adults aged over 30 years was 31.9% in men and 29% in women. Due to the fact that the lifestyle and food habits of the Korean people have become westernized and that the population has aged, metabolic syndrome continues to increase gradually in Korea [2]. Metabolic syndrome is associated with early vascular alterations, such as increased arterial stiffness and vascular endothelial cell injury, and increases the morbidity rate of type 2 diabetes mellitus and the mortality rate of cardiovascular disease [3, 4].

Pulse wave velocity (PWV) can be measured easily in noninvasive ways and has been considered a predictor of cardiovascular disease, reflecting vascular injury and arterial stiffness [5, 6]. Among these measures of PWV, brachial ankle PWV is seriously affected by hemodynamic variables such as blood pressure and autonomic nerve function, while the cardioankle vascular index (CAVI) is not influenced by blood pressure but reflects the stiffness of all blood vessels, including the aorta, the femoral artery, and the tibial artery [7]. CAVI, when measured with high accuracy, can be used as a more reliable indicator of arterial stiffness and the health of the arteries as they pertain to arteriosclerosis [7].

Nakamura reported that among 109 male and female adults who had undergone coronary angiography there was a positive relationship between the number of blood vessels with coronary artery stenosis and CAVI and a meaningful correlation between the severity of coronary atherosclerosis

and the increase of CAVI [8]. It was suggested that CAVI can be used as a predictor of cardiovascular disorders in people with risk factors for cardiovascular disorders.

There is one study that showed the association between metabolic syndrome and CAVI in Chinese population. But there is a distinct lack of research on the usefulness of CAVI as an indicator of arterial stiffness in Korean metabolic syndrome patients. Research is needed to determine whether CAVI can be used as a good early predictor of cardiovascular disease and whether there is a relationship between CAVI and risk factors for cardiovascular disorders in metabolic syndrome. Thus, in this study, we investigate the relationship between CAVI and metabolic syndrome in Korean men and women.

2. Methods

2.1. Subjects. From January 2011 to January 2012, 1,144 male and female adults who had undergone a complete physical examination at a hospital in Gyeonggi-do were recruited. Eight who had a history of cancer, heart disease, and peripheral vascular disease were excluded.

2.2. Ethics Statement. This study was implemented in accordance with ethical and safety guidelines upon the approval of the Institutional Review Board in The Catholic University of Korea, St. Vincent's Hospital (IRB approval number UC 13RISI 0010). The study was exempted from the written informed consents of participants because we reviewed the health screening data and medical record retrogradely. The IRBs approved this consent procedure.

2.3. Physical Measurements and Blood Test. Weight and height were measured to one decimal place in kilograms and centimeters, respectively. Body mass index was calculated by dividing weight (kg) by the value of the square of height (cm^2). Waist circumference (WC) was measured in centimeters at the midsection from the lower rib to the upper crista iliaca, while subjects were standing with their feet 30 cm apart, exhaling comfortably. Blood pressure was measured with an automatic blood pressure monitor while subjects were seated after having rested for 20 minutes. In the morning after subjects had fasted overnight, blood samples were drawn from the brachial veins. Fasting plasma glucose (FPG), triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), aspartate transaminase (AST), alanine transaminase (ALT), gamma-glutamyl transpeptidase (r-GTP), blood urea nitrogen (BUN), creatinine (Cr), high sensitivity C-reactive protein (hs-CRP), and uric acid (UA) were also measured.

2.4. Measurement of CAVI. CAVI was measured noninvasively using a Vasera VS-1000 system. While subjects were resting in the supine position, cuffs were wrapped around both their upper arms and ankles. Electrocardiographic electrodes were attached to the upper arms, and a microphone was placed on the sternal angle to measure blood pressure and record an electrocardiogram and a phonocardiogram. After around 5 minutes, volume waveforms and the other data were printed from an embedded printer. PWV was calculated by dividing the directly measured length from the heart to the artery of ankle joint by the sum of the arrival time interval of the brachial pulse wave and the pulse wave of the ankle joint and the time from the closing sound of the aortic valve to the notch of the brachial pulse wave. From this value, the values of the brachial systolic blood pressure (SBP) and diastolic blood pressure (DBP) were applied to the formula of the stiffness parameter β. CAVI, based on the formula of stiffness parameter β, reflects the stiffness of the aorta, femoral artery, and tibial artery and is not influenced by blood pressure. CAVI was calculated using the following formula [5]:

$$\text{CAVI} = a\left[\left(\frac{2\rho}{\Delta P}\right) \times \ln\left(\frac{P_s}{P_d}\right) \text{PWV}^2\right] + b \qquad (1)$$

(P_s is SBP; P_d is DBP; $\Delta P = P_s - P_d$; ρ is blood density; and a and b are constants).

2.5. Definition of Metabolic Syndrome. Metabolic syndrome was defined according to the criteria suggested in the report of the NCEP-ATPIII in 2005 and the Asia Pacific criteria. These criteria include the presence of any three of the following five risk factors [9, 10].

Criteria of metabolic syndrome are as follows:

(1) abdominal obesity: WC of 90 cm or greater in men and 85 cm or greater in women;

(2) TG: TG levels of 150 mg/dL (1.69 mmol/L) or greater or being on medicine to treat higher TG levels;

(3) HDL-cholesterol: <40 mg/dL (1.04 mmol/L) in men, <50 mg/dL (1.29 mmol/L) in women, or being on medicine to treat lower HDL-cholesterol;

(4) blood pressure: 130/85 mmHg or greater or being on medicine to treat hypertension;

(5) glucose: 100 mg/dL (6.1 mmol/L) or greater or being on medicine to treat hyperglycemia.

2.6. Statistical Analysis. The data were analyzed using the Statistical Package for Social Sciences (SPSS) version 12.0 (SPSS Inc., Chicago, USA). The continuous variables in each of the two groups were compared using the t-test. We carried out analysis of covariance (ANCOVA) after adjusting for age in the association between CAVI and metabolic syndrome and performed multiple linear regression analysis to evaluate factors which affected CAVI. $P < 0.05$ was regarded as statistically significant.

3. Results

3.1. Characteristics of the Subjects. Of the 1,144 subjects, 372 (32.7%) had metabolic syndrome, 770 (63.7%) did not have metabolic syndrome, and 507 (69%) were men. Subjects in the metabolic syndrome group were older and had greater BMIs, WCs, SBP, DBP, FPG, TG, TC, AST, ALT, Cr, hs-CRP, and UA levels but had lower HDL-C levels than subjects

Table 1: Clinical characteristics of subjects without and with metabolic syndrome.

	Nonmetabolic syndrome	Metabolic syndrome	P value
Age (year)	55.08 ± 8.28	56.43 ± 8.78	0.01
Sex			0.001
Male	507 (64.3%)	282 (35.7%)	
Female	263 (74.1%)	92 (25.9%)	
BMI (kg/m^2)	23.18 ± 2.60	25.88 ± 2.62	<0.001
WC (cm)	82.93 ± 6.59	90.59 ± 6.08	<0.001
SBP (mmHg)	123.34 ± 14.54	131.91 ± 14.95	<0.001
DBP (mmHg)	74.25 ± 9.55	79.79 ± 10.41	<0.001
FBS (mg/dL)	92.06 ± 13.12	101.06 ± 16.52	<0.001
TG (mg/dL)	99.84 ± 52.64	193.17 ± 104.20	<0.001
TC (mg/dL)	202.97 ± 38.22	208.45 ± 35.38	0.02
HDL-C (mg/dL)	52.31 ± 28.48	40.53 ± 7.77	<0.001
AST (IU/L)	21.51 ± 8.91	23.19 ± 10.59	0.008
ALT (IU/L)	22.04 ± 14.02	29.48 ± 20.46	<0.001
Cr (mg/dL)	0.85 ± 0.20	0.87 ± 0.17	0.10
UA (mg/dL)	5.38 ± 1.38	5.91 ± 1.36	<0.001
CRP (mg/dL)	0.16 ± 0.48	0.23 ± 0.35	0.10

P values were calculated by t-test or chi-square test.
Values are presented as mean ± SD or number (%).
BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; AST, aspartate transaminase; ALT, alanine transaminase; Cr, creatinine; CRP, C-reactive protein; UA, uric acid.

in the nonmetabolic syndrome group. The characteristics of subjects are shown in Table 1; $P < 0.001$ was regarded as statistically significant.

3.2. Relationship between CAVI and Metabolic Syndrome Components. The average value of CAVI was 7.55 ± 0.96 in the metabolic syndrome group, which was significantly higher ($P = 0.04$) than that of the nonmetabolic syndrome group (7.41 ± 0.86, Figure 1). CAVI values were statistically significantly higher in subjects with metabolic syndrome than those without metabolic syndrome after adjustment for sex, age, and the other factors ($\beta = 0.143$, $P = 0.04$). After analyzing the influence of the components of metabolic syndrome on CAVI, we found that subjects in the metabolic syndrome group whose SBP values were 130 mmHg or greater or whose DBP values were 85 mmHg or greater had higher CAVI values than subjects in the nonmetabolic syndrome group ($P < 0.001$, Figure 2). The group in which WCs exceeded 95 cm in men and 85 cm in women had slightly higher CAVI values than the control group, but the difference was not statistically significant ($P = 0.78$, Figure 2). In the group whose TG levels were 150 mg/dL or greater, higher CAVI values were observed than in the control group, but the difference was not statistically significant ($P = 0.09$, Figure 2). Higher CAVI values also were measured in the group whose FPG values were 100 mg/dL or greater, than in the group whose FPG values were 100 mg/dL or less, but the difference was not statistically significant ($P = 0.02$, Figure 2). CAVI values were slightly higher in the group with HDL-C levels less than 40 mg/dL in men and 50 mg/dL in women than in the control group, but the difference was not statistically significant ($P = 0.08$, Figure 2). After having analyzed the correlation between CAVI and the number of components of metabolic syndrome, we discovered that CAVI was significantly greater in the group which had more components ($P = 0.003$, Figure 3), and this relationship was linear ($F(1, 1138) = 18.180$, $P = 0.001$).

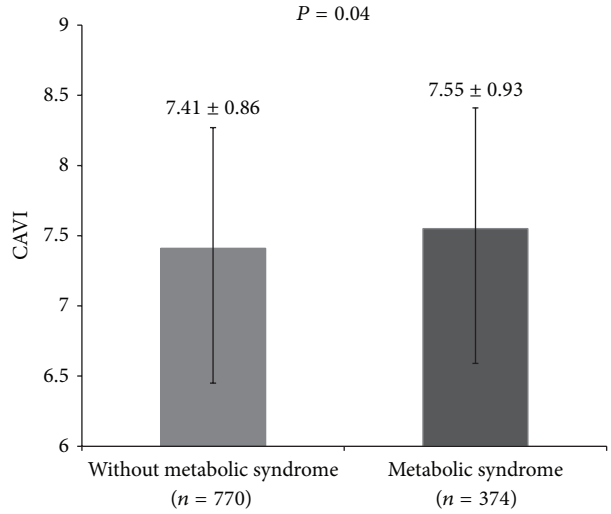

Figure 1: Comparison of cardioankle vascular index (CAVI) between subjects with and without metabolic syndrome. P values were calculated by ANCOVA and age-adjusted CAVI.

3.3. The Factors That Affect CAVI. After performing multiple linear regression analysis, using risk factors for metabolic syndrome which can potentially influence CAVI, we found that age ($P < 0.001$), sex ($P < 0.001$), DBP ($P = 0.04$), and UA ($P = 0.001$) were independent predictors of CAVI (Table 2).

4. Discussion

The risk factors for coronary artery disease include old age, dyslipidemia, hyperglycemia, obesity, and hypertension, among others [4]. Except for age, these factors are consistent with the components of metabolic syndrome. As a result, since metabolic syndrome may increase the risk of coronary artery disease, detecting and managing cardiovascular disease risk factors early in people with metabolic syndrome are very important in preventing cardiovascular disease due to atherosclerosis.

PWV, as an index which reflects arterial stiffness and blood vessel injury, is a strong predictor of cardiovascular disease morbidity and mortality [5, 6]. CAVI is derived from stiffness parameter β theory and can be determined by measuring PWV and blood pressure. CAVI is not affected by blood pressure at the time of measurement and reflects arterial stiffness more precisely than brachial ankle PWV [7].

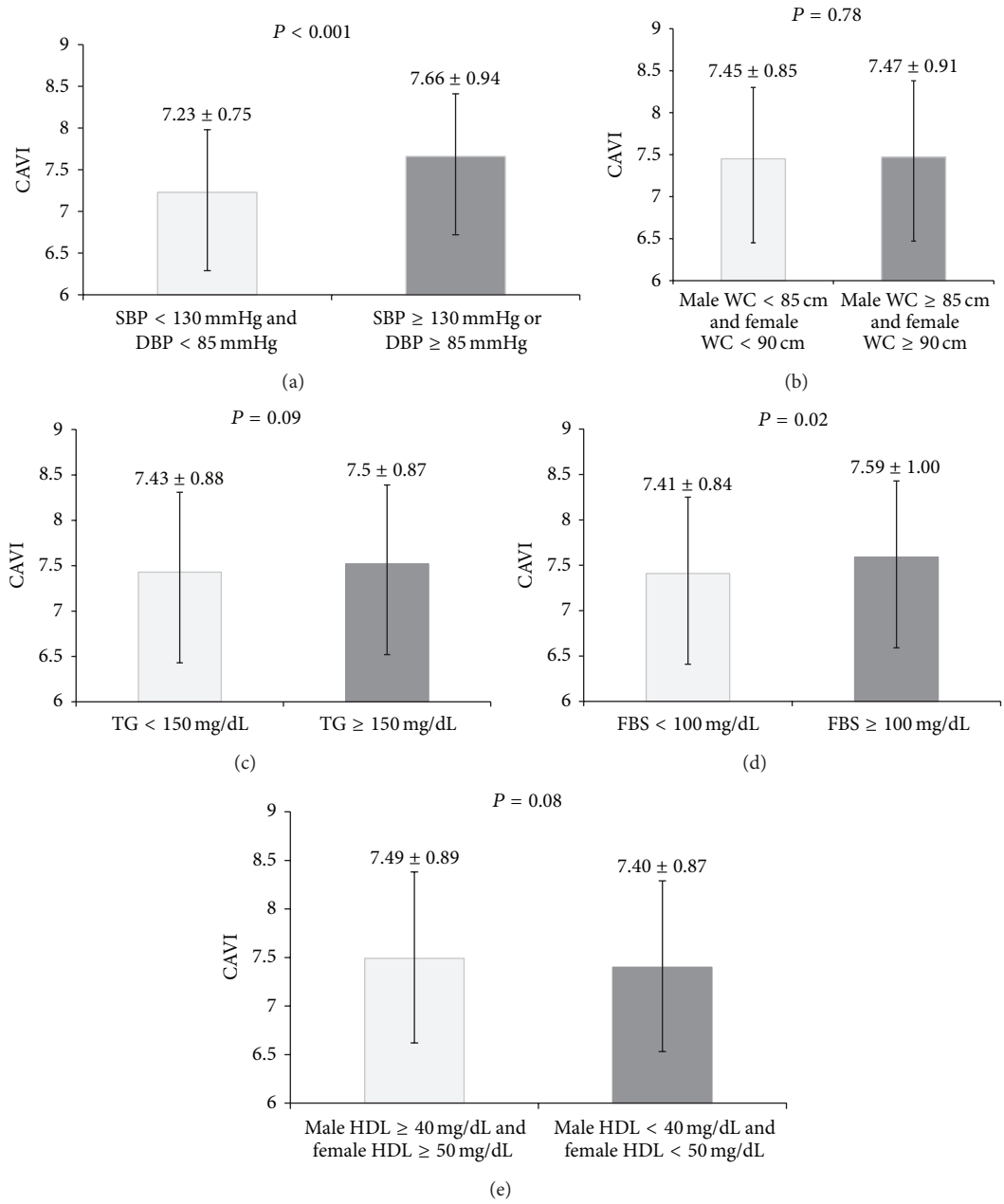

Figure 2: The relation between cardioankle vascular index (CAVI) and the component of metabolic syndrome. *P* values were calculated by ANCOVA and age-adjusted CAVI.

Thus, in this study we examined the relationship between the components of metabolic syndrome and CAVI as an index of arterial stiffness.

The study of Shirai et al. showed that, in adults aged 20 to 70 who received an annual health check and had no coronary risk factors, there was a direct association between CAVI and Age. CAVI increases with age almost linearly from 20 to 70 years in males and females. Also, CAVI of men is higher than that of women in almost all age groups [11]. In this study, after performing multiple linear regression analysis, using risk factors for metabolic syndrome which can potentially influence CAVI, we found that age and sex were independent predictors of CAVI.

In this study, CAVI values were found to be greater in the metabolic syndrome group than in the control group and were highly significant even when adjusted for age and sex. Also, in the group whose subjects had at least one component of metabolic syndrome, compared with the group whose subjects did not have metabolic syndrome, CAVI was statistically significantly higher and increased linearly with the number of metabolic syndrome components. These results are consistent with the results of previous studies which

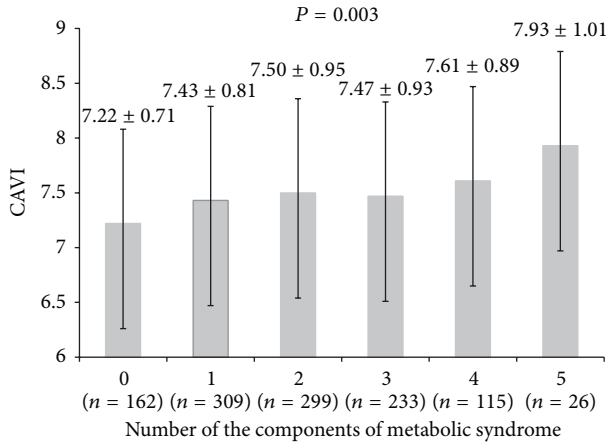

Figure 3: The relation between cardioankle vascular index (CAVI) and the number of components of metabolic syndrome. P values were calculated by ANOVA and age-adjusted CAVI.

Table 2: Multiple linear regression between CAVI and laboratory parameters.

Variables	β	P value
Age (year)	0.015	<0.001
Female	−0.233	<0.001
BMI (kg/m^2)	−0.004	0.87
SBP (mmHg)	0.002	0.41
DBP (mmHg)	0.009	0.04
FBS (mg/dL)	−0.002	0.24
TG (mg/dL)	−0.001	0.09
HDL-C (mg/dL)	−0.001	0.54
UA (mg/dL)	0.064	0.001
WC (cm)	−0.017	0.06
AST (IU/L)	0.002	0.70
ALT (IU/L)	0.002	0.31
Number of the components of metabolic syndrome	0.117	<0.001

CAVI, cardioankle vascular index; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; UA, uric acid; WC, waist circumference; AST, aspartate transaminase; ALT, alanine transaminase.

investigated the relationship between CAVI and metabolic syndrome [12, 13]. Metabolic syndrome components interrelate and cluster in various combinations, and they may promote increases in arterial stiffness and cardiovascular disease risk factors [12, 13]. CAVI values were influenced and elevated by the existence and increase of each metabolic syndrome component, even when the number of metabolic syndrome components was below the diagnostic criteria of metabolic syndrome [3]. Therefore, since metabolic syndrome components interact organically, metabolic syndrome components need to be managed to regulate arterial stiffness and prevent cardiovascular disease.

Among the components of metabolic syndrome, the elevation of SBP or DBP had a significant relationship with the elevation of CAVI. After having examined these relationships through multiple linear regression analysis, we found that DBP was an independent predictor of CAVI. The study of Takaki et al. showed that, in adults aged 30 to 80 who had undergone coronary angiographies, there was no direct association between CAVI and SBP or DBP [14]. The study of Ibata et al. showed that there was no significant relationship between CAVI and SBP in the case of normotensive patients [15]. However, in the study of healthy male and female adults of Kadota et al., simple regression analysis conducted with adjustment for age and gender showed that CAVI was related to SBP and DBP [16]. Furthermore, multiple linear regression analyses conducted with adjustment for several variables indicated that SBP was an independent predictor of CAVI. In the study of hemodialysis patients and type 2 diabetes patients of Shirai et al., CAVI had a minor association with SBP and no association with DBP [7]. In the study of hypertensive patients of Okura et al., CAVI had a minor association with SBP. CAVI was not influenced by blood pressure but was influenced by long-term hypertension status. Okura observed that CAVI could not be used to evaluate the long-term risks of arterial stiffness [17]. Arterial stiffness also is an independent predictor of the progression of hypertension in normotensive adults [18]. The elevation of CAVI resulting from the increased tension in arterial walls reflects the increase of arterial stiffness. Thus, the degree of elevation of CAVI may correspond to the degree of atherosclerotic change. A very high CAVI may indicate that the atherosclerotic process already is well underway [8].

Various hypotheses about the mechanism whereby metabolic syndrome influences arterial stiffness have been suggested. First, metabolic syndrome is associated with sympathetic nerve hyperactivity. The increase of tension in sympathetic nerves inhibits artery distensibility and increases the stiffness of blood vessels [19, 20]. The peptide adiponectin secreted by adipose tissue is associated with insulin sensitivity, is protective to blood vessels, and shows antiatherosclerotic and anti-inflammatory effects [21]. A decrease in adiponectin secretion results in the increase of arterial stiffness by promoting insulin resistance. In metabolic syndrome, the bioavailability of nitric oxide is decreased, endothelial cell dysfunction is promoted, and cytokines associated with various inflammatory processes are secreted and become involved in inflammatory reactions. The molecular components of these abnormal pathological states interact with each other. Hyperplasia of vascular smooth muscle cells, hypertrophy, and collagenesis are promoted, and arterial stiffness increases [22, 23].

In this study, increased FPG was among the metabolic syndrome components associated with a significant increase in CAVI. Ohnishi et al. reported that brachial ankle PWV increases with increasing FPG [24]. Significantly higher CAVI values were measured in type 2 diabetes patients. Some reports have shown that HbA1C is a significant risk factor for the increase of CAVI [15, 16]. These results are consistent with previous reports indicating that diabetes affects the PWVs of central elastic arteries selectively [25]. Diabetic patients are

exposed to hyperglycemia in the long term, and advanced glycation end products (AGEs) are generated in their bodies by the nonenzymatic glycation of matrix proteins. AGEs contribute to cardiovascular complications such as increased atherosclerosis and vascular stiffness [26, 27].

One interesting finding in this study was that UA was significantly elevated in the metabolic syndrome group and was an independent predictor of the elevation of CAVI. The increase of serum UA has a significant association with metabolic syndrome and is a risk factor for cardiovascular disease [28, 29]. UA causes oxidative stress and endothelial cell dysfunction, is associated with blood vessel and systemic inflammatory reactions, and finally can lead to cardiovascular disease [30]. Sun et al. have reported that higher UA levels were detected in metabolic syndrome patients and that the increase of UA correlated with brachial ankle PWV [31]. Liu et al. reported that there was a significant relationship between CAVI and UA among persons with metabolic syndrome [13]. Nagayama et al. also reported that high serum uric acid is associated with increased CAVI in healthy Japanese subjects [32]. Sun et al. explained that the proinflammatory properties of UA could increase PWV by being partially related to the chronic inflammatory status seen at diagnosis of metabolic syndrome [31]. Therefore, this study has provided additional clues about the elevation of UA as a risk factor for cardiovascular disease.

This study has a few limitations. First, since this study was cross-sectional, we could not deduce the temporal order of the onset of metabolic syndrome and the elevation of CAVI. Second, as patients who came to the health promotion center of a general hospital were targeted, there could have been a selection bias. Third, we lacked some information associated with metabolic syndrome and arteriosclerosis, such as that regarding smoking, drinking, exercise, and family history.

Despite these limitations, this study contributes to the early assessment of cardiac disease by showing that metabolic syndrome and its components have a significant association with increased CAVI as a predictor of arterial stiffness in asymptomatic Korean population. In the future, a clinical trial will be needed to investigate whether CAVI decreases when metabolic syndrome components are improved, and a cohort study will be needed to investigate the relationship between CAVI and the onset of metabolic syndrome, to establish the risk of elevated CAVI in metabolic syndrome more clearly.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Sung-Goo Kang and Jun-Seung Rho conceived and designed the experiments. Sung-Goo Kang and Jun-Seung Rho performed the experiments. Su-Hyun Nam, Sung-Goo Kang, and Yun-Ah Lee analyzed the data. Sang-Wook Song and Yun-Ah Lee contributed reagents/materials/analysis tools. Su-Hyun Nam and Sung-Goo Kang wrote the paper.

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Hindawi Publishing Corporation
Journal of Diabetes Research
Volume 2015, Article ID 340838, 11 pages
http://dx.doi.org/10.1155/2015/340838

Review Article

Recent Trends in Therapeutic Approaches for Diabetes Management: A Comprehensive Update

Pragya Tiwari[1,2]

[1]*Department of Metabolic and Structural Biology, Central Institute of Medicinal and Aromatic Plants (CSIR-CIMAP), P.O. Box CIMAP, Lucknow, Uttar Pradesh 226015, India*
[2]*Molecular Biology and Biotechnology Division, ICAR-National Bureau of Fish Genetic Resources, Canal Ring Road, P.O. Dilkusha, Lucknow, Uttar Pradesh 226002, India*

Correspondence should be addressed to Pragya Tiwari; priyatiwari9452@gmail.com

Received 24 January 2015; Revised 30 April 2015; Accepted 1 May 2015

Academic Editor: Joseph Fomusi Ndisang

Diabetes highlights a growing epidemic imposing serious social economic crisis to the countries around the globe. Despite scientific breakthroughs, better healthcare facilities, and improved literacy rate, the disease continues to burden several sections, especially middle and low income countries. The present trends indicate the rise in premature death, posing a major threat to global development. Scientific and technological advances have witnessed the development of newer generation of drugs like sulphonylureas, biguanides, alpha glucosidase inhibitors, and thiazolidinediones with significant efficacy in reducing hyperglycemia. Recent approaches in drug discovery have contributed to the development of new class of therapeutics like Incretin mimetics, Amylin analogues, GIP analogs, Peroxisome proliferator activated receptors, and dipeptidyl peptidase-4 inhibitor as targets for potential drugs in diabetes treatment. Subsequently, the identification and clinical investigation of bioactive substances from plants have revolutionized the research on drug discovery and lead identification for diabetes management. With a focus on the emerging trends, the review article explores the current statistical prevalence of the disease, discussing the benefits and limitations of the commercially available drugs. Additionally, the critical areas in clinical diabetology are discussed, with respect to prospects of statins, nanotechnology, and stem cell technology as next generation therapeutics and why the herbal formulations are consistently popular choice for diabetes medication and management.

1. Introduction

Diabetes is a major killer worldwide and its unprecedented rise poses a serious threat to mankind. According to recent estimation, 387 million people worldwide are affected from the disease with a prevalence rate of 8.3% and 46.3% still remains undiagnosed [1]. Furthermore, maximum percentage of 387 million people lives in low and middle income countries and comprise of 40–59 age group in the population. Population survey by the Indian Council of Medical Research [2] suggested that China leads the survey with an estimation of 98.4 million cases and India coming next with 65.1 million diabetes patients [3]. It has been seen that certain features in Asian Indians make them more prone to diabetes and coronary artery disease [4, 5] which include increased insulin resistance [6] and greater abdominal adiposity as reported [7]. Figure 1 depicts a schematic representation of general occurrence and approaches in diabetes management.

The selection and application of a glucose lowering therapy are dependent on a number of considerations like the severity of hyperglycemia, hepatic and renal associated functions, risks of hypoglycemia, body mass index, ability to self monitor the blood glucose level, and also the cost of the medication. The therapeutics for type 1 diabetes includes stimulation of insulin secretion through GLP analogues like Exenatide and Liraglutide [8, 9], insulin injections to compensate for β cell defects, dipeptidyl peptidase-4 (DPP-4) inhibition by Sitagliptin, and increased islet survival [10, 11] and islet cell regeneration through islet neogenesis associated protein (INGAP) peptide therapy aiming at islet cell regeneration among others [12].

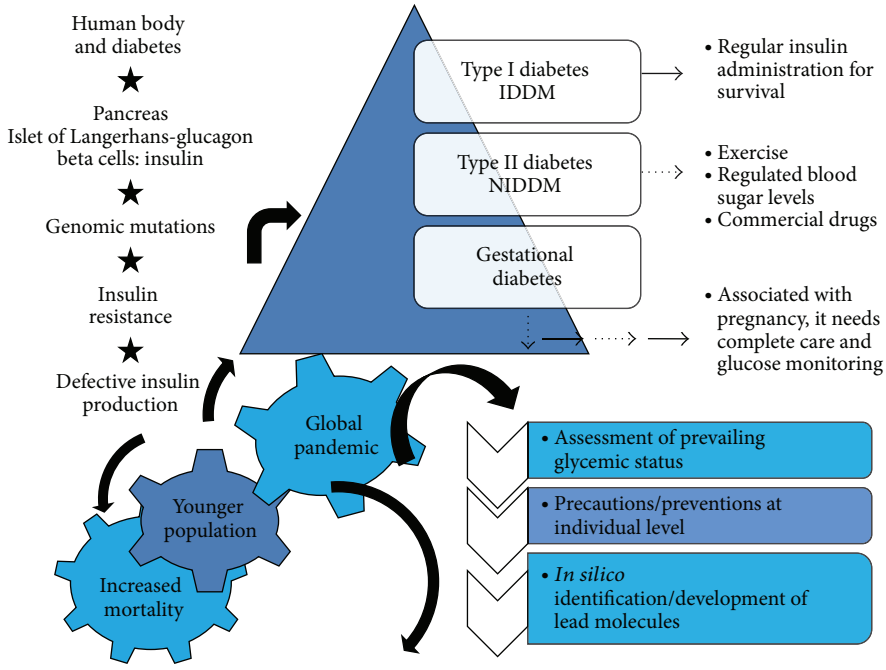

Figure 1: A schematic representation of general occurrence and approaches in diabetes management.

The treatment approach for type 2 diabetes includes several conventional therapeutics, namely, sulfonylureas and repaglinide enhance insulin secretion, troglitazone increases insulin action in fat and muscle, metformin promotes insulin mechanism in liver tissue, and miglitol and acarbose enact delayed carbohydrate absorption from food intake, respectively [13]. The drugs used for the treatment of type 2 diabetes poses limitations in the sense that they have significant side effects. The other major medications strategies constitutes combinational therapy of insulin with sulfonylureas which reduced the daily requirement of insulin [14] and insulin and metformin combination therapy (approved by FDA); minimizing weight gain due to insulin therapy [15] and troglitazone-insulin in combination efficiently reduced insulin requirement and improved glycemic control [16].

Secondary Forms of Diabetes. Subsequently, secondary forms of the disease may arise due to defects/mutations in genome of the organism: gene mutations in MODY1-hepatocyte nuclear factor-4-alpha (HNF4α) in chromosome 20q12-q13.1; MODY2-glucokinase (GCK) gene mutation in chromosome 7p15-p13; MODY3-hepatocyte nuclear factor-1-alpha (HNF1A) gene mutation in chromosome 12q24.2; MODY4-insulin promoter factor-1 (IPF1) gene mutation in chromosome 13q12.1; MODY5-hepatic transcription factor-2 (TCF2) gene mutation, chromosome 17cen-q21.3; MODY6- neurogenic differentiation 1 (NEUROD1) gene mutation in chromosome 2q32; MODY7-Kruppel-like factor 11 (KLF11) gene mutation in chromosome 2p25; MODY8 (or diabetes-pancreatic exocrine dysfunction syndrome)-carboxylester lipase (CEL) gene mutation, chromosome 9q34; MODY9-paired box gene 4 (PAX4) mutation, chromosome 7q32:

(i) *Mutations occurring in mitochondrial genome,* referred to as mitochondrial diabetes MELAS syndrome (mitochondrial myopathy, stroke-like syndrome encephalopathy, and lactic acidosis).

(ii) *Genetic syndromes,* Klinefelter's syndrome, Turner's syndrome, Down syndrome, Prader-Willi, Laurence-Moon-Biedl, Friedreich's ataxia, Huntington's chorea, myotonic dystrophy, and porphyria.

(iii) *Drug/chemical induced diabetes,* Thiazides, DPH, α-interferon, L-asparaginase, vacor, nicotinic acid, pentamidine, steroids, levothyroxine, and diazoxide [17].

However, the disease together with its multiple complications puts forward the immediate requirement to act with a well defined strategy. The underlying platform is aimed at achievement of complete glycemic regulation, possible through assessment of present glycemic status, and analysis of the associated disorder would aim at allocating the healthcare facilities to the affected people [18]. The new generation of drugs like sulphonylureas or insulin can induce hypoglycemia as well as weight gain [19] while the biguanide like metformin can cause gastrointestinal effects such as diarrhoea and nausea and, rarely, lactic acidosis. Thiazolidinedione use is also associated with weight gain, which is an issue of concern since type 2 diabetic patients are already obese [20]. Recent generation of drugs like the incretin mimetics may produce nausea, vomiting, and diarrhoea [21]. The drugs showing potential for the cure

Table 1: Representation of the multiple primary and secondary forms of diabetes, the defective metabolism, and the adverse effects on the body organs.

Classification of diabetes	Effects on the body	Symptoms
	Primary diabetes	
Insulin dependent diabetes mellitus	Destruction of β cells	Deficiency of insulin
Non insulin dependent diabetes mellitus	Insulin resistance	Loss of insulin secretion
	Secondary diabetes/symptoms due to primary diabetes	
Hormonal imbalance	Acromegaly Pheochromocytoma	
Pancreatic dysfunction	Pancreatitis Pancreatectomy Cushing's syndrome Glucagonoma	
Drugs or chemical induced reactions, for example, anticancer agents Thiazide Psychoactive agents like glucocorticoids, streptozotocin, or diazoxide	Drug induced reactions	Hypersensitivity reactions
Insulin receptor abnormalities	Genetic syndromes Hyperlipidemia Muscular dystrophy	
Malnutrition	Hyperglycemia	Enhanced sugar levels
Glycosuria	Glycosuria Loss of weight	Excessive secretion of sugar in urine
Ketonuria	Ketosis and elimination in urine Dehydration	
Lipemia	—	Increased levels of lipid, fatty acids, and cholesterol in blood
Acidosis	—	Lowering of pH of blood
Cataract and lesions of blood vessels (atheromatous, and artherosclerotic)		

of diabetes have been used singly and also in combination of multiple oral agents and with addition of insulin but achieving the complete glycemic control is a challenging task.

The present trends in diabetes therapeutics and management have highlighted an urgent requirement for extensive investigations aiming at identification and clinical trials of natural products and their analogues in drug discovery studies. In an attempt to address the global issue, the paper provides a comprehensive update highlighting the global scenario and statistical prevalence of diabetes. Furthermore, the emerging trends in clinical diabetology were discussed, exploring the advantages as well as the limitations of the commercially available therapeutics. The present era has witnessed the development of next generation therapeutics, statins, nanotechnology, and stem cell technology and the reasons why the natural products and analogues define a prospective field in diabetes medication and management.

2. Classification of Diabetes

On the basis of insulin deficiency, diabetes can be classified into the following types as follows.

2.1. Insulin Dependent Diabetes Mellitus (IDDM). It is also known as juvenile onset diabetes or type 1 diabetes, which accounts for 5–10% of the patients, resulting from cellular-mediated autoimmune destruction of the pancreatic cells. The disease can affect people of all ages but usually occurs in children or young adults. Regular supply of insulin injections is essential for the control of glucose level in blood. The rate of β cell destruction varies showing fast deterioration in infants and children while the degeneration of β cells is slower in adults. Symptoms like ketoacidosis occur in children and young individuals while others exhibit modest fasting hyperglycemia that can change to severe hyperglycemia or ketoacidosis in response to stress or infection [22]. These patients have higher risk for developing other autoimmune disorders such as Grave's disease, vitiligo, celiac sprue, autoimmune hepatitis, myasthenia gravis, Hashimoto's thyroiditis, Addison's disease, and pernicious anemia [22]. This form of diabetes follows a hereditary pattern and is common in people of African and Asian descent [23]. Table 1 discusses the classification of the disease and the associated multiple complications.

2.2. Idiopathic Diabetes. A minor number of patients having type 1 diabetes, mostly of Asian and African ancestry, have no etiologies. These are prone to ketoacidosis and have permanent insulinopenia. The occurrence of ketoacidosis is in episodes and the level of insulin deficiency between episodes fluctuates. Idiopathic diabetes has genetic predisposition and an absolute need for insulin replacement therapy depends on the condition of the patient [22].

2.3. Noninsulin Dependent Diabetes Mellitus (NIDDM). It is also referred to as adult onset diabetes, which accounts for 90–95% of all diabetes. Major metabolic syndromes like obesity, insulin resistance, and dyslipidaemia have led to an epidemic of type 2 diabetes [24]. The treatment of this type of diabetes is through oral hypoglycemic drugs, dietary in nature. Insulin resistance as well as loss of insulin secretion contributes to the onset of disease. Type 2 diabetes mellitus is the most common form of diabetes and is the fourth leading cause of death in developed countries with a twofold excess mortality and two- to fourfold increased risk of coronary heart disease and stroke [25].

2.4. Gestational Diabetes Mellitus (GDM). It is defined as any degree of glucose intolerance resulting in hyperglycaemia of variable severity that is diagnosed during pregnancy [26]. GDM, or impaired glucose intolerance which is first diagnosed during pregnancy [27], is a major type affecting 14% women during pregnancy or 135,000 women a year in the United States and is a risk factor for type 2 diabetes in mothers [28]. The magnitude of the reported risk varies due to variations in ethnicity, selection criteria, and tests for GDM and type 2 diabetes [29]. Gestational diabetes can lead to respiratory distress syndrome, neonatal hypoglycemia, and fetal macrosomia. More infants have increased rates of birth trauma, shoulder dystocia, and cesarean delivery. Recent guidelines recommend adequate glycemic control as a strategy to decrease these maternal and fetal complications. Most women who have gestational diabetes can successfully control their blood sugar with diet and exercise, while some will require oral diabetes medication or insulin.

2.5. Catamenial Hyperglycaemia. Diabetic ketoacidosis (DKA) is a condition, arising due to infection, inadequate insulin or poor insulin compliance, acute pancreatitis, stroke, drugs, metabolic disturbances within the body, or negligence with the treatment [30]. The uncontrolled hyperglycaemia with DKA occurring before the menstrual cycle in females is known as catamenial diabetic ketoacidosis or catamenial hyperglycaemia. The uncontrolled hyperglycemia resulted in increased insulin requirement, up to 4 times. The condition is aggravated even after continuous insulin infusion, resulting in vomiting, and leading to significant acidosis, ketonuria, and hyperglycaemia. The strange fact was that even several tests like inflammatory markers, blood count renal function, electrocardiogram and chest radiograph, thyroid function, and urine and blood cultures were all found to be normal. The conditions leading to catamenial hyperglycaemia remain undiagnosed [31]. Hormonal changes occurring during menstrual cycle together with changes in diet and exercise levels may play a role [32]. An effective diet and exercise plans [33] including an increased insulin infusion dosage [32] will be the right medication strategy for the treatment of catamenial diabetic ketoacidosis as well as for avoiding any diabetic emergencies.

3. Nanotechnology and Diabetes

The interface of nanotechnology in the treatment of diabetes has introduced novel strategies for glucose measurement and insulin delivery. Researchers have demonstrated the advantages of glucose sensors and closed-loop insulin delivery approaches in facilitating the diabetes treatment to make it [34] beneficial in both type 1 and type 2 diabetes.

A nanomedical device is a microcapsule containing pores which has been a promising tool in the drug delivery approach. These pores are considerably large to allow the passage of small molecules such as oxygen, glucose, and insulin but are small enough to allow the movement of larger immune system molecules such as immunoglobulins and graft-borne virus particles. Microcapsules containing replacement islets of Langerhans cells, mostly derived from pigs, could be implanted beneath the skin of diabetes patients. This could temporarily restore the body's delicate glucose control feedback loop without the need for powerful immunosuppressants that can leave the patient at serious risk for infection [35] Table 2 describes the critical problems associated with diabetes and the role of nanomedicine in the treatment.

The nanoparticle targeted drug delivery approach has enormous benefits which include the improved bioavailability of drugs by targeting specific tissues, organs, and tumors thereby providing the highest dose of drug directly at the targeted site. One of the biggest technological challenges is the scalability of a nanoparticle. Manufacturing three-dimensional nanostructures as compared to stand-alone or two-dimensional layer-shaped nanosurfaces is a complex task since manufacturing techniques are yet to be standardized. Another apprehension is that the exposure to nanoparticles might be toxic or hazardous. Concerns about the potential ill effects of engineered nanomaterials such as carbon buckyballs and nanotubes through inhalation, ingestion, or absorption through the skin are increasing [35].

Insulin forms an essential requirement for type 1 and type 2 advanced diabetes and the traditional systems of insulin delivery included infections, painful administration, and poor compliance of patients. However, recent micro- and nanotechnologies have facilitated the insulin administration process through regulation of insulin delivery constituting pulmonary, nasal, transdermal, and closed-loop delivery [36].

4. Statin Therapy: A New Perspective

Statins are defined as inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A and inhibit the crucial process of LDL cholesterol in liver, thereby decreasing its level in the blood

Table 2: Description of some problems associated with diabetes and possible nanomedicine solutions.

Measurement problems	Nanometrology solutions
Continuous blood glucose monitoring	Biocompatible nanofilms
Stable implanted enzyme electrodes	"Smart tattoo" of glucose
Noninvasive monitoring	Nanosensors
Improved diagnosis	NIR QDs, gold nanoparticles
Targeted molecular imaging	Single-molecule detection
Understanding mechanisms	
Therapy problems	Nanotherapeutic solutions
Improved insulin delivery	Islet nanoencapsulation
Islet cell transplantation	Insulin nanoparticles
Oral insulin	
Closed-loop insulin delivery	Artificial nanopancreas

besides increasing healthy blood vessel lining [37]. Since the long term effect of diabetes include the high risk of cardiovascular diseases, statins (HMG-CoA reductase inhibitor) are a main line of therapy in reducing cardiovascular risk in the patients suffering from type 2 diabetes [38, 39]. The lipid lowering agents, popularly known as statins, cause inhibition of HMG-CoA reductase specifically and reversibly. The enzyme catalyzes the conversion of HMG-CoA to mevalonic acid, the rate-limiting step in the formation of cholesterol. These compounds are highly effective in reducing cholesterol levels as compared to dietary supplements [40].

Statin therapy reduces low density lipoprotein (LDL) cholesterol to a significant level thereby greatly decreasing the chances of developing a coronary artery disease [41]. National Institute for Health and Clinical Excellence (NICE) and Scottish Intercollegiate Guidelines Network (SIGN) diabetes guidelines showed lipid lowering therapy as primary prevention (when used regularly) for patients with type 2 diabetes, aged over 40 (Grade A recommendation), as well as its consideration for patients aged over 40 with type 1 diabetes (Grade B recommendation) [41]. A recent information published at the meeting of the European association for the study of diabetes in Stockholm suggests that statin treatment is being less explored and applied in patients with type 2 diabetes among a large American group of over 100,000 subjects [42].

Statins have good efficacy and are effective in lowering cardiovascular events in people with modest levels of cholesterol and without cardiovascular disease. However, the HMG-CoA reductase inhibitors or statin therapy also has some disadvantages. The therapy has some side effects like renal dysfunction and muscle disorders from myositis to frank rhabdomyolysis and hepatic dysfunction which is rare and can be tolerated by the patient [41]. The trial conducted with 6422 patients showed that young individual and those showing absence of disease showed ineffective or poor compliance with statin therapy [38]. However, the therapy should be focused on older patients since in younger patients the poor compliance was seen. Also, the patients with high risk factors and symptoms of heart problems should be administered with statins [41]. However, reports have suggested that statins may raise the blood sugar levels moderately and lead to diabetes mellitus [37]. Despite exhibiting good toleration and less adverse effects, statins may cause side effects like myopathies and increase in levels of liver enzymes in type 2 diabetes [43].

5. Stem Cell Technology: A Novel Therapeutic Approach

The interest to find a possible therapeutic for diabetes has eventually explored various new scientific areas of research, with the stem cell technology being one of them. It is known that both type 1 and type 2 diabetes result from the β cell deficiency of the pancreatic cells, resulting in insufficient insulin secretion. The strategies should aim at either removing the defects in pancreatic β cell or enhancing the sensitivity of the body cells to the action of insulin. β cell replacement strategies offer a novel source while current strategies aiming at islet cells and pancreas transplantation are limited due to shortage of donor organs [44].

In contrast to type 1 diabetes, which is caused by autoimmune destruction of pancreatic β cells, type 2 diabetes results from irregularities in β cells function together with insulin resistance in peripheral organs [45]. Mesenchymal stem cell (MSC) therapy has emerged as a promising therapy in the treatment of type 1 diabetes due to its immunosuppressive nature. MSCs have been found to display immunomodulatory effects both in *in vitro* and *in vivo* conditions due to direct contact and production of soluble markers [46–49]. MSCs have the potential to differentiate into a number of mesenchymal cell lineages. The hematopoietic stem cells are the multipotent stem cells that can give rise to all the cell type in blood and also possess immunomodulatory effect. Hence, the transplantation of hematopoietic stem cell has proved to be a promising therapeutic, resulting in improvement in β cell function in newly diagnosed type 1 diabetic patients [50]. Further studies have demonstrated that the induced pluripotent stem (iPS) cells can be generated from type 1 diabetic patients by reprogramming their adult fibroblasts with three transcription factors (OCT4, SOX2, and KLF4). The cells known as diabetes induced pluripotent stem cells; (DiPS) are pluripotent and have the ability to differentiate into insulin producing cells. This is beneficial in type 1 disease modeling and cell replacement therapies [51].

Some studies have shown that bone marrow derived MSCs have the ability to differentiate into insulin producing cells both *in vitro* and *in vivo* [52–54]. The significance of human embryonic stem cells (ESCs) in the treatment of diabetes has attracted great attention due to their pluripotent nature and large scale production of different cell lineages in cultures. The research has various limitations since there is absence of reliable methods for generating specific cell types, immunological rejection of the transplanted cells, and difficulty in purification of specific lineages [55]. Further concerns include the uncontrolled proliferation of the transplanted embryonic stem cells into a specific type, once they are transplanted [56]. Still, despite of its manifold limitations both scientific and ethical, the application of stem cell technology holds immense prospects in treatment of diabetes.

6. Gene Therapy in Diabetes

The series of experiments leading to cloning and expression of insulin in the cultures cells in the 1970s was a tremendous revolution in the field of medicine and application of gene therapy in the treatment of diabetes was suggested as a possible cure. Regulating the sugar levels is the most important aspect in the treatment which also reduces the complications associated with the disease. Somatic gene therapy involving the somatic cells of the body includes two methods of gene delivery. The first one known as *ex vivo* gene therapy is described as the one in which the tissues are removed from the body; the therapeutic gene is inserted *in vitro* and then reimplanted back in the body while the *in vivo* therapy involves the insertion of gene therapy vectors directly to the patients by subcutaneous, intravenous, or intrabronchial routes, or by local injection [57]. The application of *ex vivo* therapy aims at the generation of cells which possess the properties of β cells, for example, insulin producing cells [58]. This therapy has also been used to generate β cells for transplantation. However, the concern lies in the aspect of surgically removing the tissue from the patient and reimplantation of the genetically modified tissues back into the body of the patients [57]. Furthermore, type 1 diabetes results from autoimmune destruction of insulin synthesizing pancreatic β cells and islet transplantation has been explored as a possible solution for the treatment. The invention of insulin gene therapy substitutes β cell function by generating insulin secretory non-β cells, not vulnerable to autoimmune reactions, offering a prospective therapeutic approach for type 1 diabetes [59].

The *in vivo* gene therapy is the method of choice as a therapeutic strategy because it is simpler and the vector containing the desired gene is directly inserted into the patient, but the development of safe (not toxic to host) and effective vectors remains as a challenging task for gene therapist. Presently, the strategies for *in vivo* therapy involve three methods: genetic transfer of glucose lowering genes which are noninsulin in nature. Presently, the strategies for *in vivo* therapy include genetic transfer of glucose lowering genes which are non-insulin in nature and application of blood sugar lowering genes: an enhancer of glucose utilization by liver or skeletal muscles and an inhibitor of glucose production by the liver [57]. For example, glucokinase as a transgene is found to have glucose lowering effect in the liver [60]. It was a possibility that the gene *Gck* enhances glucose utilization by the body [61]. The genetic transfer of glucokinase had been used as an adjuvant therapy in the treatment of diabetes [62]. In another strategy which was carried out to regulate the glucose production in liver, a gene known as "protein targeting to glycogen" (PTG) was used to convert glucose to glycogen [63, 64]. The PTG protein belongs to the family of glycogen targeting subunits of protein phosphatase-1 which regulated the metabolism of glycogen. Experiments performed in rats have indicated that adenoviral mediated PTG transfer stimulates glycogen synthesis in the liver and decreases blood glucose levels in rats. This has been considered as a therapeutic approach for diabetes [63].

Other areas of genetic engineering include transfer of genes which show response to glucose and the use of gene therapy to induce β cells production in the liver [57]. The glucose responsive genes that have been manipulated to enhance conversion of proinsulin to insulin and those which after modification exhibit expression show responses to blood glucose level [65, 66]. The liver cells do not produce hormones which convert proinsulin to insulin; therefore, new proteolytic cleavage sites have been incorporated into the proinsulin molecule, recognized by a protease, furin that is present in many tissue systems, including liver [67–69]. The insulin gene can be modified to encode insulin which has single-chain [70] having 20–40% activity of normal mature insulin [71].

Research has also been carried out to induce the synthesis of β cells formation in the liver. Kojima et al. reported that it is possible to induce the formation of β cells by the endocrine cells by delivering islets specific transcription factors [72, 73]. The regulation of insulin production and its control remains as a difficult task since the knowledge about insulin metabolism is much less [74]. The strategy aiming at induced β cells neogenesis seems to be a promising approach as a therapeutic for diabetes, since it can offer a solution for the autoimmunity in type 1 diabetes [57].

7. Medical Nutrition Therapy

Medical nutrition therapy in prevention and management of diabetes puts forth numerous advances in clinical research, aiming to use nutrition therapy for the treatment of disorders and illnesses. American Diabetes Association in 1994 coined the term "medical nutrition therapy" constituting 2 phases, namely, adjudging the nutritional requirement of a person and treatment through counseling and nutrition therapy, respectively [75]. The objectives of nutritional therapy in diabetes is to regulate optimal level of lipids in blood, ideal body weight, and blood glucose level in normal range. Nutrition therapy as a therapy for diabetes depends on certain factors such as patient's age-based nutritive requirements and food preferences as well as other medical conditions together with an exercise regime and recommended nutritional requirement depending upon the patient's abilities and health conditions [76, 77]. Calorie requirement to maintain

ideal body weight for moderately active individual is 30–35 kcal/kg/day; for obese people it is 20–30 kcal/kg/day. It is estimated that gradual weight loss of 1 lb per week should occur, if the calorie intake is reduced by 500 calories/day [76, 77]. According to recent recommendations, the percent of carbohydrate intake is based on the patient's intake of protein and fat. Low carbohydrate/high protein diet is popular and may be associated with initial weight loss and improved glycemic control but is difficult to maintain for longer time periods. Protein intake is maintained at 10–20% of all calories; total fat intake should be restricted to <30% of total calories; high fibre diet (20–35 g/day of soluble and insoluble fiber), sodium restriction to 2400–3000 mg/day, alcohol intake (≤2 drinks/day in men, ≤1 drink/day in women), and multivitamins should be taken in the diet [76, 77].

8. Natural Products and Diabetes

Literature has suggested the utilization of herbal medications for the treatment of insulin dependent and noninsulin dependent diabetes since time immemorial. Plants possessing antidiabetic properties may be suitable as adjunct to the existing therapies or as a prospective source of new hypoglycemic compounds. Since time immemorial, naturopathic therapies have been applied for a number of health ailments and continue to gain popularity in the present arena as well. Ancient literature revealed that diabetes was a known disease since Brahmic period and finds a mention in Ayurvedic literature, Sushruta samhita written in fourth and fifth centuries BC [78]. Two forms of diabetes were described: one genetic in nature and the other due to dietary indiscretion [78]. Herbal medicines are becoming immensely popular among the masses for being cost effective and with relatively few side effects. Although plant based medicines have been used traditionally in treating diseases throughout the world, the mechanism of most of the herbs is still to be defined and standardized [79]. Many new bioactive drugs isolated from plants having hypoglycaemic effects demonstrate antidiabetic activity equal to and sometimes even more potent than known oral hypoglycaemic agents such as daonil, tolbutamide, and chlorpropamide. However, many other active agents obtained from plants have not been well characterized [80]. Grover et al. [81] postulated that plants possessing antidiabetic activities are of significant interest for ethnobotanical community as they are recognized to contain valuable medicinal properties in different parts and a number of them have shown varying degree of hypoglycemic and antihyperglycemic activity. The bioactive constituents found in many plant species are isolated for direct use as drugs lead compounds, or pharmacological agents. These traditional approaches might offer a natural key to unlock diabetic complications [82]. The chemical structures of a phytomolecule play a critical role in its antidiabetic activity. Several plant species being a major source of terpenoids, flavonoids, phenolics, coumarins, and other bioactive constituents have shown reduction in blood glucose levels as demonstrated by Jung et al. [83]. Several plants like *Allium sativum* Linn. (Liliaceae), *Gymnema sylvestre* (Retz.) Schult (Asclepiadaceae), *Murraya koenigii* (L.) Spreng. (Rutaceae), *Allium cepa* (Liliaceae), *Withania somnifera* Dunal (Solanaceae), and *Ferula foetida* Linn. (Umbelliferae) have been found to possess antidiabetic properties when assessed in experimental models of diabetes. The antidiabetic properties of *G. sylvestre* had been discussed in detail [84, 85] owing to its significance in diabetes treatment and management.

9. Future Perspectives

Diabetes has remained as one of the most challenging health problems in the 21st century accounting for a global presence. Diabetes is a serious public health problem, but the good news is that important advances are being made in prevention, detection, and treatment of diabetes. For the management of type 1 diabetes, patients require insulin administration 3-4 times a day throughout their lives and their blood sugar levels should be regularly monitored to avoid complications like retinopathy and risks of cardiovascular diseases. It has been estimated that around 1300 patients with type 1 diabetes receive whole organ (pancreas) transplant and do not require insulin infusion but the demand for organs transplantation is higher than supply. Another risk factor is the rejection of transplanted organ; therefore, patient is given strong immunosuppressive drugs which can lead to other serious diseases [86].

For the management of type 2 diabetes, a well monitored glycemic control is required. The need to control the progressive deterioration of β cell function is essential since it can lead to a loss of glycemic control. Conventional drugs and insulin are effective but cannot repair the associated metabolic and glucoregulatory dysfunctions. The menace of diabetes is increasing day by day and aggressive and targeted combinational therapy is the need of the hour particularly incretin based therapy and peptide analogs. This may restore and preserve β cell function and halt the progression of type 2 diabetes [87]. In the present era, the effectiveness and the success of the new drug will depend on its ability to treat/relieve one or more of the metabolic disturbances whether increased production of insulin or enhancement in glucose uptake and utilization by the peripheral tissues particularly skeletal muscle. Besides new generations of therapeutics, several other classes have also been reported as alternative strategies alone or in combinations to provide an effective treatment for diabetes.

The prospects of leptin therapy are one of the emerging trends in the treatment of diabetes. It is a hormone secreted by adipocytes, which acts on the neurons within the central nervous system. The multiple actions of this hormone include control of excessive increase in weight, by suppressing the intake of food and increasing the expenditure of energy [88]. Leptins also regulate glucose homeostasis through the activation of leptin receptors (LEPRs) [89–92]. It has been shown that the central nervous system regulates the sugar lowering effect of leptins; it was assumed that the antidiabetic action of leptins could have been influenced by neurons in the brain with reference to type 1 diabetes. Leptin therapy

improves insulin-deficient type 1 diabetes by CNS-dependent mechanisms in mice [93].

Another area of drug research includes designing and use of mucoadhesive microcapsules of various drugs like glipizide to achieve controlled release of the drug and its effective targeting. Mucoadhesion has been a novel approach in drug delivery designing because it causes the slow release of the drug at the action or absorption site thereby enhancing the interaction of the drug with the underlying tissue forms, enhancing the bioavailability of the drugs [94]. There is no end to the drug delivery approaches which have been followed as a possible cure for diabetes. The transdermal insulin administration approach (which has been developed as a consequence of painful and complicated insulin therapy) maintains constant levels of insulin without the deposits of insulin in the skin frequent with subcutaneous insulin injections (http://www.ondrugdelivery.com/, 2006). A research by Odegaard and colleagues revealed that activated macrophages display a beneficial role in the regulation of nutritional homeostasis and suggests that polarization of the macrophages towards the alternative state might be a useful possibility in the treatment of type 2 diabetes [95].

Great strides have been made clinically in the prevention, development, and treatment of the disease but no therapeutic method have been completely successful till date. With new technologies revolutionizing the treatment possibilities, the search for an effective medication is not far ahead. The extensive research leading to the discovery of the pathway genes contributing to the development of the disease and the sequencing of complete genomes have revolutionized the diabetes research. The development of the techniques like the PCRs, DNA microarray, and gene knockouts with silencing has opened up a new area in the identification of the defective genes/mutations in the genome of the organism. The increasing prevalence of diabetes globally is creating a financial burden on the economy of the respective country. Unlike some other diseases, treatment exists for diabetes, and if managed correctly, it is very effective in reducing complications such as heart attacks, amputations, blindness, and kidney failure. With the ongoing research, a right therapeutic for the treatment of diabetes is not unachievable.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgment

The author Pragya Tiwari is thankful to the director of CSIR-CIMAP and ICAR-NBFGR, Lucknow, for encouragement and support. The motivation and guidance provided by Dr. N. S. Nagpure, HOD, MBB Division, NBFGR, Lucknow, are greatly acknowledged. The author is solely responsible for literature collection, writing, and analysis of the paper.

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