Disease Markers

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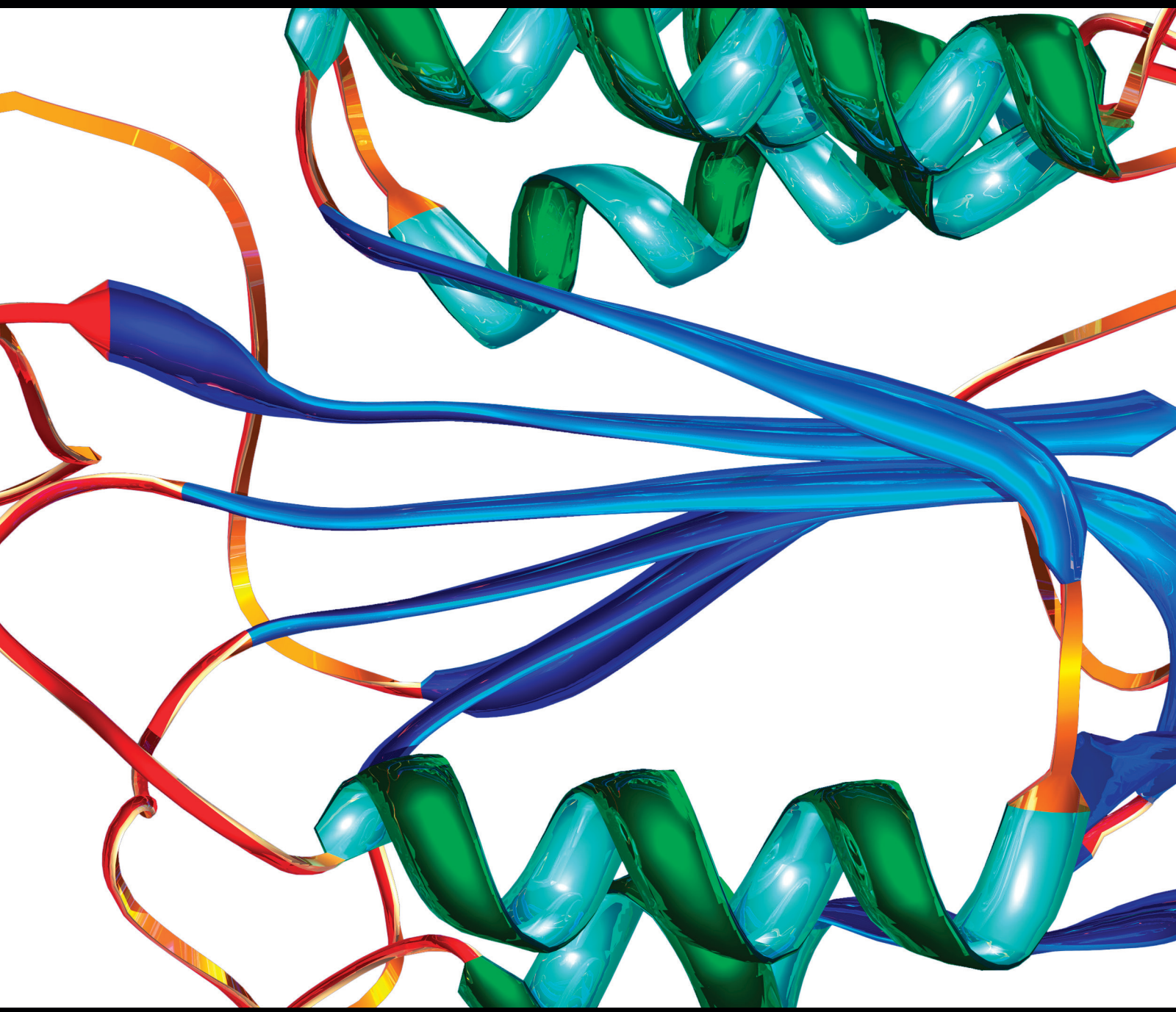

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Editorial Board

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Editorial

Adipokines as Biomarkers in Health and Disease

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Adipokines are important circulating factors mediating intertissue crosstalk throughout the body and thus playing a key role in maintaining endocrine homeostasis. So far, multiple associations of adipokines with widespread non-communicable diseases, including cardiometabolic diseases, cancer, neurodegenerative diseases, and fertility problems, have been identified. However, the precise mechanisms underlying empirically observed associations are still rather poorly understood, and more research must be carried out in order to make sense of and deepen the already published data.

A better understanding of associations of adipokine expression and common diseases seems warranted, and the journal set out to publish this special issue. The preparation of this special issue resulted in a series of 5 articles, submitted by researchers from Kuwait, Czech Republic, Germany, and China. All the published studies were approved by the local Ethical Committees, and the statements are included in the Method section of the respective papers.

First, T. Pavlova et al. (Czech Republic), in their work titled "Irisin Maternal Plasma and Cord Blood Levels in Mothers with Spontaneous Preterm and Term Delivery," focused on the possible association of irisin levels—a protective exercise-induced myokine—with preterm birth and concluded that irisin levels in cord blood were significantly different between preterm and term pregnancies. To the best of our knowledge, this is the first study ever to report the association of irisin cord blood levels with preterm birth.

Second, A. Koch et al. (Germany) in their work titled "Visfatin Serum Levels Predict Mortality in Critically Ill Patients" focused on the potential of visfatin to predict mortality of critically ill patients in the intensive care unit (ICU). Visfatin is an adipokine also called nicotinamide phosphoribosyltransferase (NAMPT), which displays insulin-mimicking effects. The authors report that the visfatin serum concentrations were strongly associated with disease severity and organ failure, irrespectively of the possible presence of type 2 diabetes mellitus (T2DM). Moreover, the authors observed that visfatin levels correlated with biomarkers of renal failure, liver dysfunction, and other adipokines (e.g., resistin, leptin, and adiponectin) in their cohort. Last but not least, high visfatin levels at ICU admission were predictive of an increased mortality, both at the ICU and during a long-term follow-up of approximately two years. Such findings emphasize strongly the need for a replication study of the potential of visfatin for the prediction of organ failure and/or overall survival in ICU patients.

Lastly, three papers focused on the possible associations of specific adipokines with insulin sensitivity or T2DM with or without concomitant metabolic syndrome: (i) F. Wang et al. from China (manuscript titled "Circulating PGRN Levels Are Increased but Not Associated with Insulin Sensitivity or β-Cell Function in Chinese Obese Children") investigated the association of progranulin with insulin sensitivity or β-cell function in a Chinese paediatric cohort, (ii) N. A. Abdella and O. A. Mojiminiyi from Kuwait (manuscript titled "Clinical Applications of Adiponectin Measurements in Type 2 Diabetes Mellitus: Screening, Diagnosis, and Marker of Diabetes Control") focused on adiponectin as a potential diagnostic marker for T2DM or insulin resistance, and (iii) P. Yan et al. from China (manuscript titled "Plasma Neuregulin 4 Levels Are Associated with Metabolic Syndrome in Patients Newly Diagnosed with Type 2 Diabetes Mellitus") investigated the link between neuregulin 4 and metabolic syndrome in newly diagnosed T2DM patients. Each of these works provides unique insights into the field, suggesting, respectively, that (i) progranulin is associated with higher BMI and multiple parameters of obesity in obese children, (ii) adiponectin may be useful as possible diagnostic/therapeutic markers in T2DM, and (iii) neuregulin 4 is associated with parameters of metabolic syndrome in newly diagnosed T2DM patients.

Taken together, the presented papers further emphasize the complexity of the adipokine network that likely serves as master relays for integrating the influences of environmental factors with the genetic and epigenetic makeup of every individual. All the included papers further highlight the importance of larger prospective studies as well as validation studies on different populations of patients and different ethnicities. To conclude, the presented special issue provides a collection of novel findings in the field, contributing possibly to better decision-making in the clinical practice. It should be kept in mind, however, that adipokines are part of large neuroendocrine-immune-metabolic networks, and they exert their effects through complex endocrine, paracrine, autocrine, or juxtacrine crosstalk mechanisms, which should be further investigated with system medicine approaches.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Marek Buzga
Francesc Villaroya

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Research Article

Visfatin Serum Levels Predict Mortality in Critically Ill Patients

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The adipokine visfatin, also termed pre-B-cell colony-enhancing factor (PBEF), is mainly derived from adipose tissue but has been implicated in the regulation of innate immune responses. We hypothesized that visfatin could be a potential circulating biomarker in critical illness and sepsis. We therefore measured serum levels of visfatin in a cohort of 229 critically ill medical patients upon admission to the intensive care unit (ICU). In comparison to 53 healthy controls, visfatin levels were significantly elevated in medical ICU patients, especially in patients with sepsis. Visfatin serum concentrations were strongly associated with disease severity and organ failure but did not differ between patients with or without obesity or type 2 diabetes. Visfatin levels correlated with biomarkers of renal failure, liver dysfunction, and other adipokines (e.g., resistin, leptin, and adiponectin) in critically ill patients. High visfatin levels at ICU admission indicated an increased mortality, both at the ICU and during long-term follow-up of approximately two years. Our data therefore demonstrate that circulating visfatin is a valuable biomarker for risk and prognosis assessment in critically ill patients. Furthermore, visfatin seems to be involved in the pathogenesis of excessive systemic inflammation, supporting further research on visfatin as a therapeutic target.

1. Introduction

Besides their important roles in metabolism, adipocytokines or adipokines, i.e., hormones released from adipose tissue, are increasingly recognized as important regulators of immunity [1]. It has been suggested that adipokines contribute to the excessive systemic inflammatory reaction commonly observed in critical illness. We and others have previously shown that serum levels of the adipokines resistin and adiponectin are significantly elevated in critically ill patients and are associated with patients' mortality [2–6]. Relatively few data exist on visfatin in the setting of critical illness. The adipokine visfatin was initially identified in lymphocytes and is therefore also called pre-B-cell colony-enhancing factor (PBEF) [7]. Leukocytes have been identified as a major source of circulating visfatin [8]. Moreover, visfatin is also involved in activation and attraction of inflammatory cells. Experimental data obtained from human cells and mouse models revealed that visfatin is a chemoattractant for neutrophils [9], promotes neutrophil survival [10], and induces the dose-dependent release of cytokines in monocytes [11]. Interesting findings obtained from smaller trials demonstrated elevated visfatin serum levels in patients with respiratory diseases [12–14] and neonatal sepsis [15] as well as in patients with severe trauma or with critical neurological diseases [2]. Based on these findings, we analyzed circulating visfatin levels in a large cohort of 229 prospectively enrolled critically ill patients at our medical intensive care unit (ICU)

Table 1: Baseline patient characteristics and visfatin serum measurements.

Parameter	All patients	Nonsepsis	Sepsis
Number	229	87	142
Sex (male/female)	133/96	51/36	82/60
Age median (range) (years)	63 (18–90)	61 (18–85)	64 (20–90)
APACHE II score median (range)	16 (2–43)	14.5 (2–33)	18 (3–43)
ICU days median (range)	7 (1–137)	5 (1–45)	9.5 (1–137)
Death during ICU *n* (%)	60 (26%)	15 (17%)	45 (32%)
Death during follow-up (total) *n* (%)	107 (47%)	31 (36%)	76 (54%)
Mechanical ventilation *n* (%)	157 (69%)	53 (61%)	104 (73%)
Preexisting diabetes *n* (%)	73 (32%)	27 (31%)	46 (32%)
Preexisting cirrhosis *n* (%)	23 (10%)	16 (18%)	7 (5%)
BMI median (range) (m^2/kg)	25.9 (15.9–86.5)	25.5 (15.9–53.9)	26.0 (17.1–86.5)
WBC median (range) ($\times 10^3/\mu$l)	12.8 (0–149)	12.0 (1.8–29.6)	14.0 (0–149)
CRP median (range) (mg/dl)	92 (5–230)	17 (5–230)	153 (5–230)
Procalcitonin median (range) (μg/l)	0.7 (0.03–207.5)	0.2 (0.03–100)	2.3 (0.10–207.5)
Creatinine median (range) (mg/dl)	1.35 (0.1–21.6)	1.0 (0.2–15.0)	1.7 (0.1–21.6)
INR median (range)	1.18 (0.9–13)	1.17 (0.9–6.7)	1.18 (0.9–13)
Log visfatin median (range) (ng/ml)	2.61 (0.78–4.25)	2.51 (0.78–3.89)	2.70 (1.08–4.25)

For quantitative variables, median and range (in parentheses) are given. APACHE: acute physiology and chronic health evaluation; BMI: body mass index; CRP: C-reactive protein; ICU: intensive care unit; INR: international normalized ration; WBC: white blood cell.

in order to define the potential pathogenic role of visfatin in critical illness and its utility as a clinical biomarker in the ICU setting.

2. Materials and Methods

2.1. Study Design and Patient Characteristics. Critically ill patients were included at admission to the medical ICU at the University Hospital Aachen, Germany. Patients, who were admitted for postinterventional observational stay or underwent an elective procedure, were excluded [16]. The local ethics committee approved our study in accordance to the ethical standards laid down in the Declaration of Helsinki (reference number EK 150/06). The patients were categorized as sepsis and nonsepsis according to the "Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3)" [17] and were treated following the current guidelines for treatment of sepsis (Surviving Sepsis Campaign) [18]. As a healthy control group, we analyzed blood donors (36 male, 17 female, median age 37 years, range 25–67 years, BMI median 25 kg/m^2, range 19–34 kg/m^2) with normal blood counts, normal values of liver enzymes, and a negative serology for viral hepatitis and HIV [19].

In order to determine long-term outcome, we contacted the patients, their relatives, and/or the general practitioner in approximately 6-month intervals after discharge from hospital for two years [19].

2.2. Measurements of Visfatin and Adipokines. Blood samples were collected at the time of admission (before specific therapeutic measures had been started at the ICU) and centrifuged, and serum was stored at −80°C. Visfatin was analyzed with a commercial ELISA kit (USCN Life Science, #E90638Hu, BIOZOL Diagnostica, Eching, Germany). Measurements of the other adipocytokines and related proteins resistin, adiponectin, leptin, and leptin receptor were included as previously reported [3, 4, 20].

2.3. Statistical Analysis. Due to the high range of visfatin values, especially comparing healthy controls and critically ill patients, all visfatin serum concentrations are presented as logarithmic values. The Mann-Whitney *U*-test was used to test differences between the two groups; correlations were tested according to Spearman's rank correlation method. All values, including outside values as well as far out values, were included. p values less than 0.05 were considered as statistically significant.

The prognostic value of visfatin on the outcome was evaluated by Cox regression models. Survival curves were generated by Kaplan-Meier analyses with a visfatin cutoff level calculated via the Youden Index [21]. All analyses were performed with IBM SPSS Statistics (SPSS; Chicago, Illinois).

3. Results

3.1. Visfatin Serum Levels Are Significantly Elevated in Critically Ill Patients as Compared with Healthy Controls. Visfatin serum levels were measured in a prospectively recruited cohort of 229 critically ill medical patients. Visfatin serum concentrations were approximately one log-fold higher in critically ill patients (median visfatin log 2.61 ng/ml, range 0.78–4.25, Table 1) compared to healthy controls ($n = 53$, median visfatin log 1.66 ng/ml, range 0.30–3.21, $p < 0.001$; Figure 1(a)). Visfatin levels did not correlate with the age, neither in patients ($r = 0.24$, $p = 0.723$) nor in healthy controls ($r = 0.101$, $p = 0.474$). Of the 229 ICU patients, 142 were admitted due to sepsis, while 87 patients had a critical illness due to other origin such as cardiopulmonary,

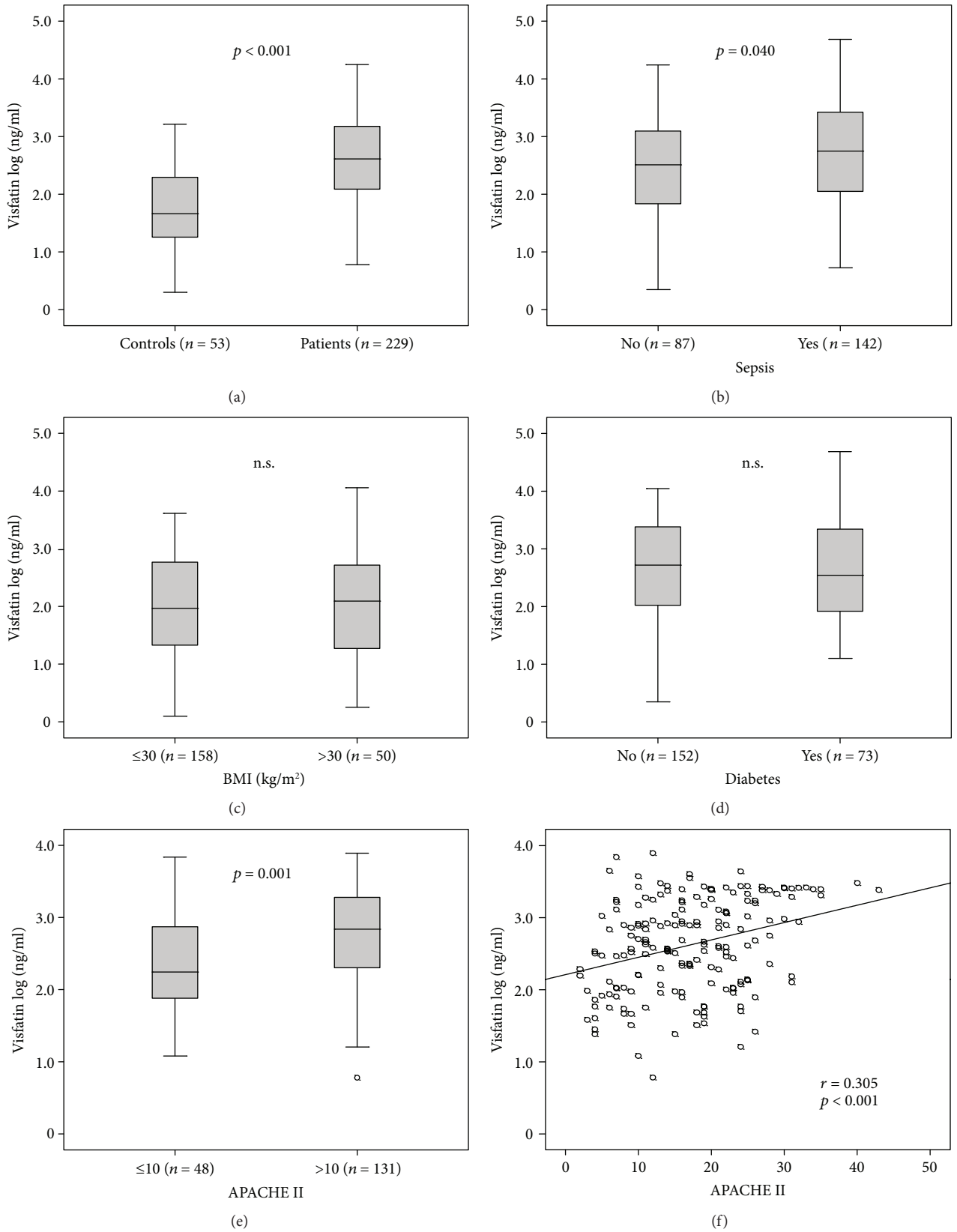

Figure 1: Visfatin levels in critically ill patients. (a) Visfatin serum concentrations (displayed as log visfatin) are significantly elevated in critically ill patients compared with controls. (b–e) Subgroup analyses of visfatin levels in critically ill patients, according to sepsis (b), obesity (c) (defined by body mass index (BMI) above 30 kg/m^2), diabetes (d), or disease severity (APACHE II score above 10). (f) Visfatin levels correlate with APACHE II score in critically ill patients.

Table 2: Disease etiology of the study population leading to ICU admission.

	Sepsis 142	Nonsepsis 87
Etiology of sepsis critical illness		
Site of infection *n* (%)		
Pulmonary	82 (58%)	
Abdominal	26 (18%)	
Urogenital	4 (3%)	
Other	30 (21%)	
Etiology of nonsepsis critical illness *n* (%)		
Cardiopulmonary disorder		29 (33%)
Acute pancreatitis		11 (13%)
Acute liver failure		4 (5%)
Decompensated liver cirrhosis		15 (17%)
Severe gastrointestinal hemorrhage		6 (7%)
Nonsepsis other		22 (25%)

gastrointestinal, or hepatic disorders (Table 2). Patients with sepsis had further elevated visfatin levels compared to nonsepsis ICU patients (visfatin log 2.70 ng/ml versus 2.51 ng/ml, $p = 0.04$; Figure 1(b)). Within the sepsis patients, the site of infection (e.g., pneumonia, bloodstream, abdominal, and urogenital) did not affect visfatin concentrations.

3.2. Diabetes or Obesity Did Not Impact Visfatin Levels at Admission to the ICU. As high visfatin levels have been consistently associated with obesity, type 2 diabetes, and the metabolic syndrome [7, 22, 23], we tested whether obesity or type 2 diabetes as a comorbidity at ICU admission impacted visfatin levels. Unexpectedly, neither obesity as defined by a body mass index (BMI) above 30 kg/m^2 (Figure 1(c)) nor preexisting type 2 diabetes (Figure 1(d)) was associated with visfatin serum concentrations. Moreover, serum glucose at ICU admission or glycosylated haemoglobin A1 (HbA1c) did not correlate with visfatin levels in critically ill patients (data not shown). In addition, $n = 23$ patients admitted to the ICU had preexisting liver cirrhosis. Their visfatin levels (median log visfatin 2.88, range 1.82–3.74) did not differ significantly from ICU patients without liver cirrhosis (median log visfatin 2.57, range 0.78–4.25, $p = 0.151$).

3.3. Visfatin Serum Concentrations Are Strongly Associated with Disease Severity. Based on our finding of high levels of visfatin in ICU patients, we next tested the potential association of visfatin with the severity of critical illness. In fact, patients with an acute physiology and chronic health II [APACHE II] score above 10 displayed significantly higher visfatin serum levels than patients with APACHE II values below or equal to 10 (Figure 1(e)). Moreover, visfatin levels directly correlated with APACHE II scores ($r = 0.305$, $p < 0.001$; Figure 1(f)), sequential organ failure assessment (SOFA), or simplified acute physiology score 2 (SAPS2) scores (Table 3).

Table 3: Correlations with visfatin (log) serum concentrations at ICU admission (Spearman rank correlation test, only significant results are shown).

Parameters	ICU patients	
	r	*p*
Disease severity		
APACHE II score	0.305	<0.001
SOFA score	0.494	<0.001
SAPS2 score	0.406	<0.001
Inflammation		
C-reactive protein	0.256	<0.001
Procalcitonin	0.379	<0.001
suPAR	0.418	<0.001
White blood cell count	0.131	0.048
Interleukin-6	0.291	<0.001
TNF	0.331	0.003
Interleukin-10	0.423	<0.001
Renal function		
Creatinine	0.421	<0.001
GFR (creatinine)	−0.427	<0.001
Cystatin C	0.383	<0.001
GFR (cystatin C)	−0.372	<0.001
Urea	0.377	<0.001
Uric acid	0.231	<0.001
Liver function		
Protein	−0.352	<0.001
Albumin	−0.365	<0.001
Pseudocholinesterase	−0.316	<0.001
Bilirubin	0.167	0.012
Bilirubin (conjugated)	0.212	0.009
Alkaline phosphatase	0.218	0.001
AST	0.196	0.004
INR	0.315	<0.001
Prothrombin time	−0.336	<0.001
aPTT	0.283	<0.001
D-dimers	0.380	<0.001
Antithrombin III	−0.456	<0.001
Fibrinogen	−0.385	<0.001
Metabolism		
Leptin	−0.340	0.001
Leptin receptor	0.318	0.002
Adiponectin	0.235	0.02
Resistin	0.313	0.002

APACHE: acute physiology and chronic health evaluation; aPTT: activated prothrombin time; AST, aspartate aminotransferase; GFR: glomerular filtration rate; INR: international normalized ratio; SAPS: simplified acute physiology score; SOFA: sequential organ failure assessment; suPAR: soluble urokinase plasminogen activator receptor; TNF: tumor necrosis factor.

3.4. Visfatin Levels Are Correlated with Biomarkers of Renal Failure, Liver Failure, and Metabolic Disturbances in Critically Ill Patients. Due to the well-established role of

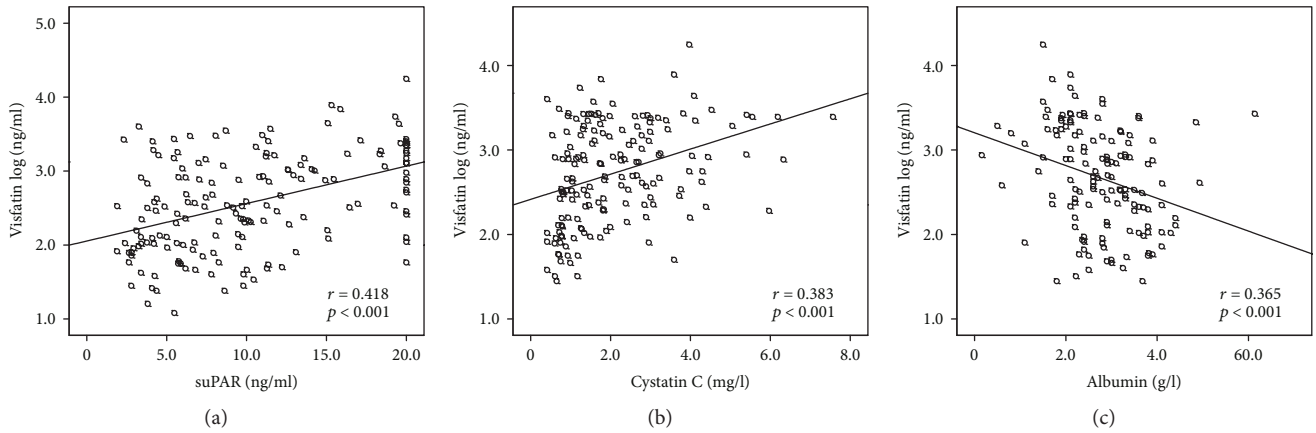

Figure 2: Visfatin levels correlate with inflammation and organ failure. (a–c) Correlation analyses revealed associations between serum visfatin and biomarkers of systemic inflammation (e.g., soluble urokinase plasminogen activator receptor (suPAR)) (a), renal failure (e.g., cystatin) (c, b), or hepatic dysfunction (e.g., albumin) (c).

circulating visfatin in systemic inflammation and cytokine release [24], we analyzed correlations of visfatin in ICU patients with various biomarkers of inflammation, organ dysfunction, and metabolism (Table 3). Visfatin concentrations correlated closely with markers of inflammation including C-reactive protein, procalcitonin, interleukin-6 (IL-6), and other cytokines (Table 3), confirming observations obtained in neonatal sepsis [15]. Visfatin also correlated with soluble urokinase plasminogen activator receptor (suPAR, Figure 2(a)), a prognostic biomarker of inflammation in the ICU setting [25]. Circulating visfatin displayed a close association with renal dysfunction, as indicated by several markers including creatinine, cystatin C (Figure 2(b)), and their glomerular filtration rates (Table 3). Similar results were noted for markers reflecting liver function like albumin (Figure 2(c)), bilirubin, and coagulation factors (Table 3). Visfatin levels correlated with the other adipocytokines and related proteins assessed in our cohort, namely, leptin, leptin receptor, adiponectin, and resistin (Table 3).

3.5. High Visfatin Serum Concentrations at ICU Admission Are Associated with Adverse Prognosis. In critically patients, who subsequently died during the ICU treatment ($n = 60$), we found significantly elevated visfatin levels at admission to the ICU (Figure 3(a)), suggesting that visfatin might serve as a prognostic biomarker in critical diseases. In fact, Cox regression analysis revealed that visfatin was a robust predictor of ICU mortality ($p < 0.001$). Kaplan-Meier curves were calculated with a cutoff value of log visfatin 2.89 ng/ml that showed the optimal ratio of sensitivity and specificity for mortality using the Youden Index. Here, visfatin levels clearly discriminated between survivors and nonsurvivors (Figure 3(b)).

Even patients that are successfully discharged from the ICU have a tremendous risk of mortality during the first years of follow-up [26]. We were able to assess long-term survival in 220 out of the 229 patients. Visfatin levels at ICU admission were significantly higher in patients that died during the follow-up period of approximately two years compared with survivors (Figure 3(c)). Cox regression analysis confirmed the prognostic value of visfatin as a predictor of long-term mortality ($p = 0.001$). Using the calculated optimal cutoff (log visfatin 3.01), patients with high visfatin demonstrated an unfavourable outcome, as depicted by Kaplan-Meier survival curve analysis (Figure 3(d)). The validity and performance of visfatin as a biomarker for the prediction of ICU or overall survival in critically ill patients are summarized in Table 4.

Notably, visfatin levels appeared more suited to predict outcome in comparison to other adipocytokines. By receiver operating characteristics (ROC) curve analyses, visfatin levels reached an area under the curve (AUC) to predict ICU mortality of 0.687, while resistin (0.562), adiponectin (0.623), leptin (0.404), and leptin receptor (0.580) demonstrated lower values. For overall mortality, visfatin reached a higher AUC of 0.686 compared to resistin (0.563), adiponectin (0.638), leptin (0.407), and leptin receptor (0.609).

4. Discussion

The dysregulation of adipocytokines has been widely noted in critical illness and linked to systemic inflammation. Among interesting candidates of adipokines as biomarkers, leptin, adiponectin, and resistin have been thoroughly investigated [1–4, 20]. In this study, we focused on visfatin, an adipocytokine with several metabolic but also inflammation-orchestrating functions [24]. In a large cohort of prospectively enrolled critically ill medical patients, we demonstrate that visfatin serum levels are highly elevated compared to controls, associated with sepsis and disease severity, correlated to organ dysfunction, and, most importantly, serve as a reliable predictor of mortality. Our findings are well in agreement with smaller trials reporting elevated visfatin and the association with poor outcome in patients with respiratory diseases [12–14] and neonatal sepsis [15]. Similar findings have also been reported from patients with severe trauma or with critical neurological diseases [2].

The close association between high visfatin levels and increased short- or long-term mortality in our study may be well explained by the strong correlations between visfatin and inflammatory mediators and cytokines, disease severity

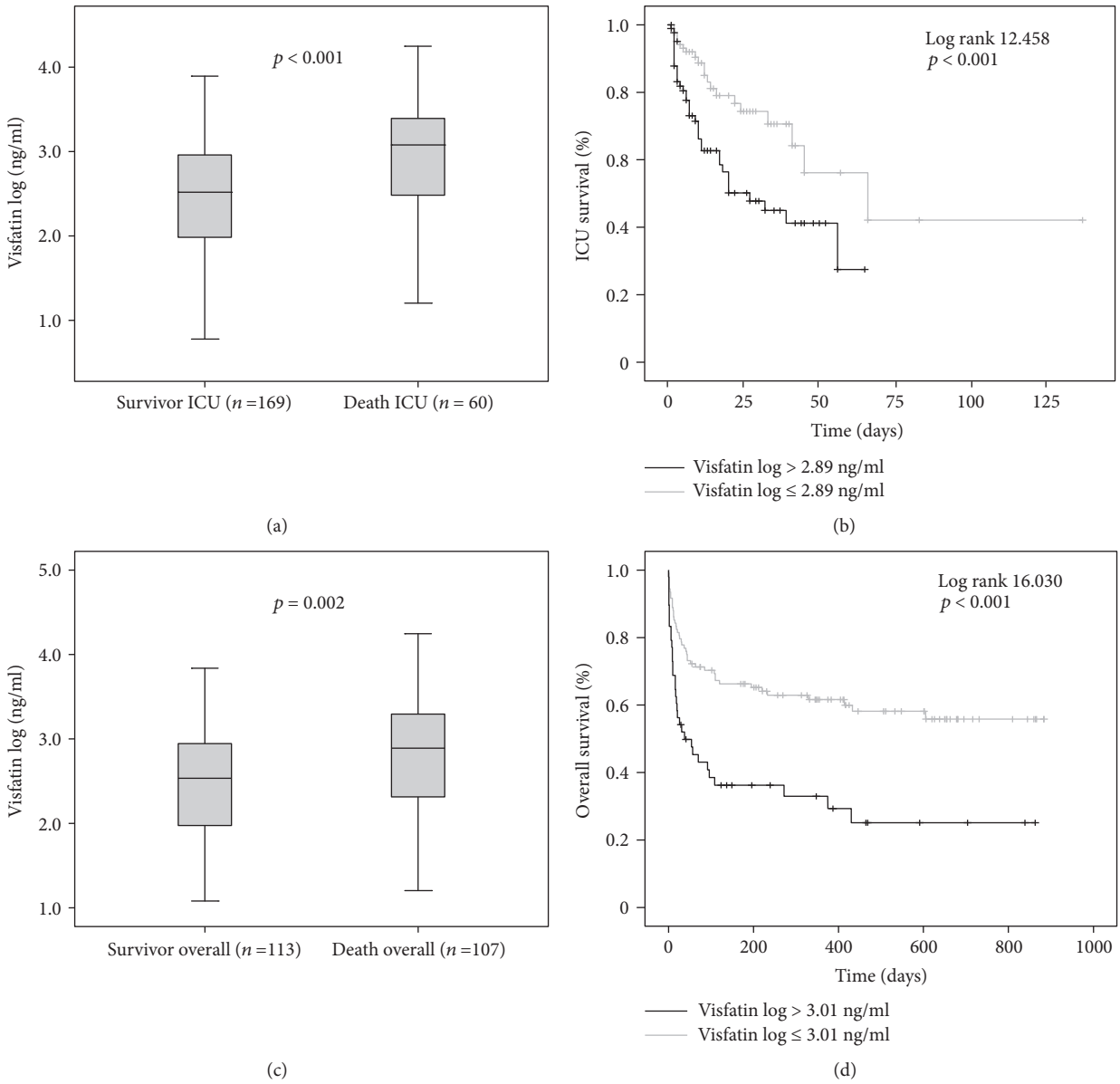

Figure 3: Visfatin is a biomarker for mortality in critically ill patients. (a) At the time of ICU admission, patients that died during the course of ICU treatment had significantly higher serum visfatin levels than survivors ($p < 0.001$). (b) Patients with high or low visfatin levels displayed different ICU mortalities by Kaplan-Meier survival curve analysis. (c) A similar observation was obtained when visfatin levels at ICU admission were compared between patients that died during the total observation period and survivors ($p = 0.002$). (d) High visfatin levels at ICU admission predicted the overall mortality during long-term follow-up in critically ill patients (Kaplan-Meier survival curve analysis for the optimal visfatin cutoff is displayed).

(e.g., clinical scores), and biomarkers reflecting organ failure. However, there is increasing evidence emerging that visfatin is directly involved in the pathogenesis of critical illness and systemic inflammation. Visfatin was found to be a chemoattractant for neutrophils [9] and has direct effects on neutrophil survival [10], which could jointly promote excessive release of cytokines [24], production of oxidative stress factors, and subsequently result in tissue damage and organ failure [2]. In support of this hypothesis, the experimental inhibition of visfatin in mouse models of ventilator-associated lung injury reduced neutrophil infiltration, organ injury, and mortality [9]. Moreover, distinct single-nucleotide polymorphisms (SNPs) in the visfatin gene have been identified in humans [27, 28], of which the SNP −1543T was linked to a reduced risk of mortality, while the SNP −1001G was associated with a higher risk of mortality in patients with acute respiratory distress syndrome [29].

In our cohort, 24% of the critically ill medical patients were obese or morbidly obese, as defined by a BMI above 30 kg/m^2. This is in line with observations in the United States, where at least 25% of adult ICU patients are overweight, obese, or morbidly obese [30, 31]. Interestingly, we did not find dysregulated visfatin levels between ICU patients with or without obesity, supporting that circulating visfatin

Table 4: Serum visfatin (log) performance as a biomarker to predict ICU or overall mortality.

	ICU mortality	Overall mortality
Visfatin (log) optimal cutoff	2.8882	3.0094
Sensitivity	0.63	0.45
Specificity	0.69	0.80
Positive predictive value	0.42	0.68
Negative predictive value	0.84	0.60
Youden Index	0.32	0.25
LHR+	2.02	2.20
LHR−	0.53	0.69
Diagnostic odds ratio	3.77	3.18

LHR: likelihood ratio.

levels in critical illness are primarily attributable to the extent of inflammation and not adiposity itself. Nonetheless, visfatin levels were closely correlated with adiponectin, resistin, and (inversely) leptin, indicating a concerted yet rectified activation of adipose tissue inflammation [1].

As outcome prediction is of major interest in the ICU setting, there is a high medical need to complement current prognostic models (e.g., APACHE II, SAPS, and SOFA) by additional biomarkers that could indicate the long-term prognosis beyond the acute critical illness [32]. Visfatin demonstrated in our study an exceptional value to predict the overall mortality during a two-year follow-up period. Thus, our data indicated that visfatin could be possibly used, either alone or in combination with other adipokines, for a more accurate prognostication in critical illness.

5. Conclusions

We demonstrate in our study comprising 229 critically ill medical patients that circulating levels of the adipokine visfatin were significantly elevated at admission to the ICU, as compared with healthy controls. Visfatin serum concentrations were strongly associated with disease severity, organ failure, and sepsis, but not with obesity or type 2 diabetes. High visfatin levels at ICU admission indicated an increased mortality, both at the ICU and during long-term follow-up. Further research should aim at implementing visfatin as a prognostic biomarker in a comprehensive risk assessment algorithm at the ICU. Moreover, the close association between visfatin and prognosis as well as experimental data on visfatin neutralization in animal models supports to explore visfatin as a therapeutic target in excessive systemic inflammation and sepsis.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare no conflict of interest.

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Research Article

Circulating PGRN Levels Are Increased but Not Associated with Insulin Sensitivity or β-Cell Function in Chinese Obese Children

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Progranulin (PGRN), a novel peptide that has recently emerged as an important regulatory adipokine, is relevant to energy homeostasis and obesity in animals and adult humans. Little is known about its roles in children. The aim of the current study was to determine the potential role of PGRN and explore its relationship to various obesity-related markers in obese children. This was a cross-sectional study composed of 77 children (43 obese and 34 healthy, age 8.68 ± 0.28 and 8.46 ± 0.45 years, resp.). The PGRN levels were significantly higher in obese children (102.44 ± 4.18 ng/mL) comparing to controls (69.32 ± 5.49 ng/mL) ($P < 0.05$). Moreover, the PGRN levels were positively correlated with triglyceride (TG), total cholesterol (TC), IL-6, systolic blood pressure (SBP), and diastolic blood pressure (DBP) in obese children after adjusted for BMI and age. However, there was no correlation of serum PGRN levels with OGTT-derived dynamic parameters, HOMA-IR, or HOMA-β in obese children. The results suggest that serum PGRN levels are significantly higher in obese children in China and correlate significantly with obesity-related markers. Increased PGRN levels may be involved in the pathological mechanism of childhood obesity.

1. Introduction

Childhood obesity has become a global public health issue. The prevalence of obesity has tripled in the last three decades. Among Chinese children, the combined prevalence of overweight and obesity has increased rapidly over the past decades, from less than 3% in 1985 to 19.2% in 2010 [1]. Childhood obesity is associated with a number of adverse health consequences including type 2 diabetes (T2DM), dyslipidemia, and hypertension, all of which will lead to premature cardiovascular diseases [2, 3].

As is well known, obesity is defined as excess fat mass accumulation. Adipose tissue, in addition to energy storage, has been found to have a variety of endocrine functions. It can secrete all kinds of adipokines [4, 5], including leptin, adiponectin, and resistin, all of which play important roles in metabolism and energy homeostasis. Progranulin (PGRN), also known as proepithelin, is a pluripotent growth factor that mediates cell growth, wound healing, tumorigenesis, and neurodegenerative disease [6, 7]. More recently, PGRN has emerged as an important regulatory adipokine of glucose metabolism and insulin sensitivity [8, 9]. For instance, diet-induced obese mice with PGRN deficiency exhibited lower body weight and ameliorated insulin sensitivity, whereas administration of recombinant PGRN induced obesity and glucose intolerance in wild-type mice with standard diet [10]. Consistently, PGRN affects insulin signaling and suppresses insulin-stimulated glucose uptake

in 3T3-L1 adipocytes [10]. Moreover, several clinical investigations also demonstrated that serum PGRN was associated with parameters of adiposity, glucose tolerance, and inflammatory factors [11, 12]. In patients with T2DM, circulating PGRN is significantly higher comparing to normal controls and positively correlates with high-sensitivity C-reactive protein, IL-6, and macrophage infiltration in omental adipose tissue (AT) [13]. In particular, PGRN expression in visceral AT is higher than in subcutaneous AT of insulin-resistant patients [14].

Up to now, the clinical data have revealed a relationship between PGRN levels and obesity. However, few studies have explored the PGRN levels in obese children. Therefore, the purpose of the present study was to investigate possible correlations between PGRN levels and obesity in Chinese children, and to identify associations between PGRN levels and obesity-related disorders.

2. Materials and Methods

2.1. Study Design. The study was initiated upon approval of the local ethics committee of the Faculty of Medicine of Soochow University, in light of the Helsinki Declaration. A written informed consent of the parent(s) of each subject was obtained before the study.

This study recruited 43 obese children, 13 girls and 30 boys, with BMI above the 95th percentile. Another 34 healthy subjects with BMI below the 85th percentile with similar age and gender distribution were enrolled as controls.

Before the outset of the study, all the patients and control subjects had under taken general physical examination and laboratory evaluation to exclude other illnesses. Those with chronic diseases (cardiovascular, gastrointestinal, or respiratory), history of drug use (steroids or antipsychotics), endocrine disorders (Cushing syndrome or hypothyroidism), or suspected syndromes associated with obesity (Prader-Willi or Laurence-Moon-Biedl syndromes) were excluded from the study. Pubertal development of subjects was evaluated according to Tanner staging [15]. Boys with testicular volume larger than 4 mL and girls with breast development more than Tanner stage II were also excluded to avoid the effect of sex hormones on obesity and relative parameters.

2.2. Assays and Calculations. Height was measured in the standing position, without shoes, using a stadiometer (sensitivity, 0.1 cm), and weight was measured using a portable scale (sensitivity, 0.1 kg) with the patients dressed in light clothing. BMI was calculated by dividing weight (kg) by squared height (m^2).

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured twice at the right arm after a 10 min rest in the supine position using a calibrated sphygmomanometer. For oral glucose tolerance test (OGTT), a 180 min OGTT (1.75 g/kg glucose, maximum 75 g) was performed in the morning after 10 to 12 hours overnight fasting. Blood samples were obtained by an antecubital venous catheter at 0, 30, 60, 120, and 180 min for determination of glucose, insulin, and C-peptide levels as described previously [16].

The homeostasis model assessment for insulin resistance (HOMA-IR) was calculated using the following formula: HOMA-IR = Insulin (mU/mL) × Glucose (mmol/L)/22.5, as previously described [17]. HOMA-IR > 2.5 was used as a cut-off value to differentiate insulin resistant from nonresistant obese subjects [18].

To assess the β-cell function, the homeostasis model assessment for β-cell function (HOMA-β) was calculated as follows: 20 × Insulin (mU/mL)/[Glucose (mmol/L)-3.5] [19]. Moreover, the insulinogenic index and comparable C-peptide index (ΔI30/ΔG30, ΔC30/ΔG30) were calculated as the ratio of the incremental change of insulin or C-peptide to glucose, respectively, from 0 to 30 min of the OGTT as previously reported [20].

2.3. Laboratory Analysis. Blood samples for glucose, insulin, lipid profiles, and PGRN levels were taken after 10–12 h night fasting. Blood was obtained from an antecubital venous catheter and placed on ice. Serum was separated within 20 min and stored at −80°C until analysis.

Fasting glucose was assayed by glucose oxidase method. HbAC1 was measured by isoelectric focusing. Serum insulin levels were measured by RIA using human insulin as standard (Millipore, Catalog number: EZHIASF-14K). Triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), liver function, and high-sensitive CRP concentrations were detected by autoanalyzer (Beckman CX-7 Biochemical Autoanalyser, Brea, CA, USA).

Serum PGRN, IL-6, and TNFα analysis was all performed with the ELISA kits. PGRN concentrations were determined with ELISA kit (R&D, Catalog number: DPGRN0). The ELISA kit has a dynamic range between 1.6 and 100 ng/mL and a detection limit of 0.54 ng/mL. Intra-assay and interassay coefficient of variations (CV) were <4.4% and 7.4%, respectively. IL-6 and TNFα concentrations were determined with ELISA Kit (eBioscience company, Catalog number: BMS213-2TEN and BMS2034, resp.). The IL-6 ELISA kit has a dynamic range between 15 pg/mL and 1540 pg/mL and a detection limit of 2 pg/mL. Intra-assay and interassay CVs were 5.6% and 7.5%, respectively. The assay range for TNFα ELISA kit was 7.8–500 pg/mL, sensitivity was 2.3 pg/mL, and intra-assay and interassay CVs were 6.0% and 7.4%, respectively.

2.4. Statistical Analyses. Statistical analyses of the data were conducted by SPSS 19.0.1 (SPSS Inc., Chicago, IL, USA). All values are presented as mean ± S.E.M. Distribution of data was evaluated with the Kolmogorov-Smirnov test. For numerical comparisons, Student's *t*-test (between obese and control groups and for insulin-resistant and nonresistant subgroups) was used. Categorical variables were compared using chi-squared test. The correlation between the PGRN levels with demographics and clinical characteristics was investigated with Pearson's correlation analysis and partial correlation analysis after adjusting for age and BMI. $P < 0.05$ was considered statistically significant.

Table 1: The clinical and laboratory characteristics of obese and nonobese groups.

Variable	Obese subject	Nonobese subject	P value
Age(years)	8.68 ± 0.28	8.46 ± 0.45	N.S
Boys/girls	13/30	22/12	N.S
BMI (kg/m^2)	25.85 ± 0.38	15.40 ± 0.20	<0.01
SBP (mmHg)	111.63 ± 1.84	97.15 ± 0.81	<0.01
DBP (mmHg)	71.14 ± 1.48	65.76 ± 1.45	<0.01
Glucose (mmol/L)	4.39 ± 0.09	4.56 ± 0.13	N.S
HbAC1 (%)	4.47 ± 0.07	4.32 ± 0.05	<0.01
Insulin (μU/mL)	14.80 ± 1.28	4.31 ± 0.40	<0.01
HOMA-IR	2.86 ± 0.25	0.88 ± 0.09	<0.01
HOMA-β	402.02 ± 53.61	83.12 ± 8.48	<0.01
Insulinogenic index, ΔI30/ΔG30 (μU/mL per mmol/L)	42.15 ± 4.56	62.13 ± 4.12	<0.01
C-peptide index, ΔC30/ΔG30 (ng/mL per mmol/L)	2.99 ± 1.09	4.05 ± 0.76	<0.01
TG (mmol/L)	1.57 ± 0.15	0.86 ± 0.08	<0.01
TC (mmol/L)	4.17 ± 0.10	4.12 ± 0.13	N.S
LDL (mmol/L)	2.94 ± 0.10	2.70 ± 0.18	<0.05
HDL (mmol/L)	1.3 ± 0.03	1.56 ± 0.05	<0.01
GPT (U/L)	33.37 ± 5.18	17.17 ± 1.62	<0.01
GOT (U/L)	29.88 ± 2.11	26.89 ± 1.47	N.S
IL-6 (pg/mL)	8.79 ± 0.21	7.38 ± 0.18	<0.01
TNF-α (ng/L)	15.52 ± 0.56	11.34 ± 1.02	<0.05
hsCRP (mg/dl)	1.89 ± 0.30	0.38 ± 0.08	<0.01

Data are presented as means ± S.E.M. BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; HOMA-IR: homeostasis model assessment of insulin resistance; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; TC: total cholesterol; TG: triglycerides.

3. Results

3.1. The Clinical Characteristics. A total of 77 subjects were enrolled in this study, including 43 subjects (13 girls and 30 boys) in an obesity group and 34 subjects (12 girls and 22 boys) in a control group. Average age was 8.68 ± 0.28 years and 8.46 ± 0.45 years in the obesity group and control group, respectively. Table 1 summarizes the demographics and clinical characteristics of both groups. Among all subjects, those in the obesity group had significantly higher BMI, SBP, DBP, TG, LDL-C, insulin, HbAC1, GPT, HOMA-IR, HOMA-β, insulinogenic index, C-peptide index, hsCRP, IL-6, and TNF-α (all $P < 0.05$) levels than the control group, whereas HDL-C levels were lower in obesity group compared with control group. Moreover, the levels of insulin, HOMA-β, and insulinogenic index were higher in insulin-resistant obese subjects compared to noninsulin-resistant obese subjects (Table 2).

3.2. The Changes in Serum PGRN Concentrations in Obese Children. Compared to control group, obesity group displayed a significant increase in the PGRN concentrations (Figure 1(a)). However, there were no significant differences in serum PGRN concentrations between boys and girls (Figure 1(b)), either in the obesity group or in the control group. Moreover, no significant differences were detected in serum PGRN levels between the noninsulin-resistant obese subjects ($n = 26$) and the insulin-resistant obese subjects ($n = 17$) (Figure 1(c)).

3.3. Association of Serum PGRN Concentrations with Metabolic Parameters. In obese subjects, the serum PGRN concentrations correlated positively and significantly with BMI, TG, TCs, SBP, DBP, and IL-6 (Table 3) levels. After adjusting for age and BMI, PGRN still correlated positively and significantly with TGs, TCs, SBP, DBP, and IL-6, respectively (Figure 1(d)–1(h)). However, there were no correlations between serum PGRN levels and insulinogenic index, HOMA-IR, or HOMA-β in obese children.

4. Discussion

Progranulin is a 68–88 kDa multifunctional protein, which was originally discovered by Anakwe and Gerton in 1990 [21], and has been implicated in cell growth, wound repair, tumor genesis, neurodevelopment, neurodegeneration, and more recently, energy metabolism regulation [8, 9]. The present study analyzed the data of obese Chinese children, aiming to investigate whether correlations could be found between PGRN levels and obesity in this population.

To the best of our knowledge, this is the first study on PGRN and obesity-related markers in Chinese children. We found that serum PGRN concentrations were 1.5-fold higher in obese children, comparing to controls with normal weight. We also found that PGRN levels were positively correlated

Table 2: The clinical and laboratory characteristics of insulin resistant and nonresistant obese subjects.

Variable	IR ($n = 17$)	Non-IR ($n = 26$)	P value
Age (years)	8.79 ± 0.45	8.57 ± 0.34	N.S
Boys/girls	11/6	19/7	N.S
BMI (kg/m2)	25.70 ± 0.05	25.94 ± 0.05	N.S
SBP (mmHg)	111.65 ± 3.10	103.73 ± 4.48	N.S
DBP (mmHg)	74.47 ± 2.48	68.96 ± 1.75	N.S
Glucose (mmol/L)	4.39 ± 0.15	4.38 ± 0.12	N.S
HbAC1 (%)	4.59 ± 0.09	4.35 ± 0.09	N.S
Insulin (μU/mL)	23.14 ± 1.48	9.35 ± 0.80	<0.01
HOMA-IR	4.48 ± 0.28	1.79 ± 0.14	<0.01
HOMA-β	470.15 ± 70.15	367.95 ± 67.95	<0.01
Insulinogenic index, ΔI30/ΔG30 (μU/mL per mmol/L)	51.74 ± 7.62	34.74 ± 5.15	<0.05
C-peptide index, ΔC30/ΔG30 (ng/mL per mmol/L)	2.36 ± 0.27	3.39 ± 1.81	N.S
TG (mmol/L)	1.71 ± 0.27	1.59 ± 0.17	N.S
TC (mmol/L)	4.09 ± 0.18	4.22 ± 0.11	N.S
LDL (mmol/L)	2.82 ± 0.18	3.01 ± 0.11	N.S
HDL (mmol/L)	1.31 ± 0.06	1.30 ± 0.04	N.S
GPT (U/L)	42.89 ± 10.48	27.14 ± 4.98	N.S
GOT (U/L)	34.90 ± 4.36	26.59 ± 1.81	N.S
IL-6 (pg/mL)	8.32 ± 0.18	8.78 ± 0.22	N.S
TNF-α (ng/L)	62.82 ± 1.11	60.67 ± 0.05	N.S
hsCRP (mg/dl)	1.82 ± 0.27	1.93 ± 0.48	N.S

Data are presented as means ± S.E.M. IR: insulin resistant; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; HOMA-IR: homeostasis model assessment of insulin resistance; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; TC: total cholesterol; TG: triglycerides.

with BMI, TG, and TC in obese children. Our results were consistent with previous studies by Alissa and colleagues, who classified Saudi Arabia children into four groups based on quartiles of serum PGRN levels and found that children within the upper quartile of serum PGRN concentration were heavier and had higher concentrations of serum TC and TGs comparing to those in the lower quartile [22]. Moreover, in the previous study conducted by Qu et al., it was found that circulating PGRN concentrations were higher in obese group than healthy subjects and correlated positively with BMI [8]. Consistently, Li et al. [23] proved that serum PGRN concentrations were significantly higher in patients with metabolic syndrome (MS) than in subjects without MS and correlated positively with BMI and waist circumference. Results of these studies all indicate that increased circulating PGRN concentrations were closely related to measures of obesity, both in adults and children. The reasons behind elevated serum PGRN in obese subjects are still a matter of discussion. Our findings shed some light, implying that enhanced synthesis of this adipokine may result from augmented adipose tissue in obese subject, since adipose tissue matrix expresses PGRN gene [10], and it is the important source for circulating PGRN [24]. To verify this hypothesis, further studies are needed to analyze the expression of PGRN expression in adipose tissue in obese subjects.

We also demonstrated that children presenting elevated levels of IL-6, TNF-α, hsCRP, and IL-6 correlated strongly with serum PGRN concentrations. Previous studies in obese adults have also showed a positive correlation between PGRN and inflammatory markers, especially hsCRP [13] and IL-6 [8]. However, it is unclear if the increasing serum PGRN is a consequence of obesity-associated inflammation, or rather the latter is triggered due to the overproduction of PGRN. Nevertheless, the fact that elevated serum concentrations of PGRN were also previously observed in other chronic inflammation diseases, such as asthma [25], systemic lupus erythematosus [26], arthritis [27], and neurodegenerative disease [28], suggests this adipokine as a marker of ongoing inflammation, rather than a triggering factor of it. This hypothesis is also supported by the fact that IL-6 could stimulate PGRN expression in vitro [29]. Thus, it can be speculated that the expression of PGRN in obese children may be stimulated by low-grade inflammation caused by obesity.

The present study showed that the HOMA-IR was higher in obese children comparing to nonobese children, suggesting that obese children had impaired insulin sensitivity, which is consistent with previous studies [2, 17, 18]. Moreover, the OGTT-derived dynamic parameters (insulinogenic index, ΔI30/ΔG30; C-peptide index, ΔC30/ΔG30) and HOMA-β in obese children were higher than control groups in the present study, implying that their islet secretion function was still enough to compensate their rising demand for insulin [30]. We failed to find a correlation between PGRN and HOMA-IR in obese children. This result was inconsistent with several previous studies which showed positive correlations between PGRN and HOMA-IR [8, 31].

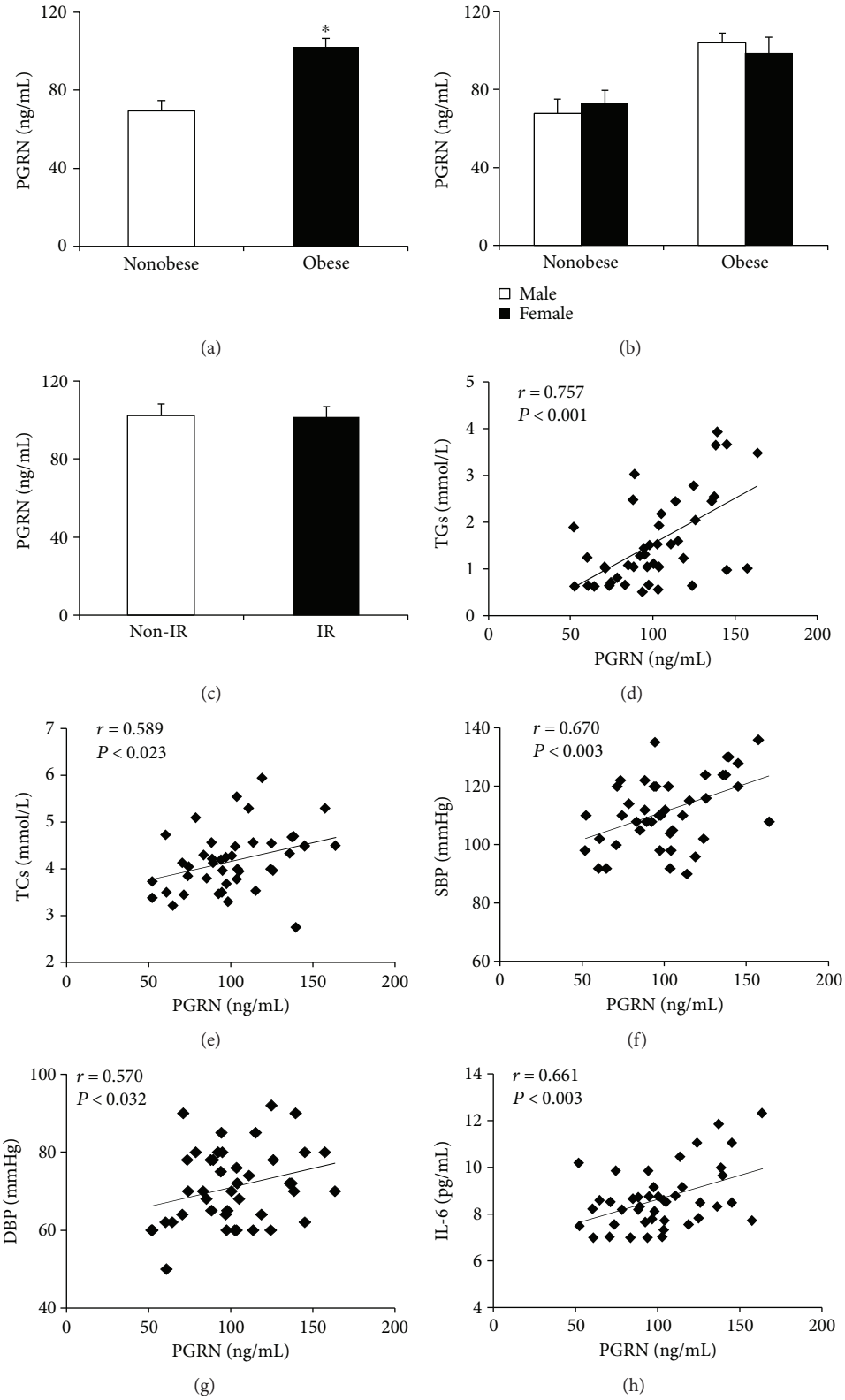

Figure 1: Serum PGRN levels and its correlation with clinical characteristics. (a) PGRN serum levels in nonobese and obese children, (b) PGRN serum levels between boys and girls in nonobese and obese group, (c) PGRN serum levels in noninsulin-resistant (non-IR) obese subject and insulin-resistant (IR) obese subject, and (d–h) scatter plots showing the correlation of serum PGRN levels with TG (d), TC (e), SBP (f), DBP (g), and IL-6 (h) in obese subjects. Data are expressed as mean ± SEM. $^*P < 0.05$ for nonobese versus obese children.

Table 3: Correlation of PGRN levels with clinical characteristics in obese groups.

Variable	r	P value
BMI (kg·m^2)	0.742	0.001
Age (years)	−0.182	0.850
SBP (mmHg)	0.670	0.003
DBP (mmHg)	0.570	0.032
Insulin (μU/mL)	0.250	0.690
HbAC1 (%)	0.219	0.832
Glucose (mmol/L)	0.080	0.968
HOMA-IR	0.260	0.668
HOMA-β	−0.222	0.750
Insulinogenic index, ΔI30/ΔG30 (μU/mL per mmol/L)	0.261	0.692
C-peptide index, ΔC30/ΔG30 (ng/mL per mmol/L)	0.412	0.318
TGs (mmol/L)	0.757	0.001
TCs (mmol/L)	0.589	0.023
HDL (mmol/L)	0.451	0.192
LDL (mmol/L)	0.489	0.122
GPT (U/L)	0.324	0.503
GOT (U/L)	0.222	0.753
hsCRP (mg/dl)	0.434	0.227
TNF (ng/L)	0.258	0.672
IL6 (pg/mL)	0.661	0.003

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; FPG: fasting plasma glucose; HOMA-IR: homeostasis model assessment of insulin resistance; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; TC: total cholesterol; TG: triglycerides.

Moreover, correlations between PGRN and OGTT-derived dynamic parameters (insulinogenic index, ΔI30/ΔG30; C-peptide index, ΔC30/ΔG30) were also not found, which suggests that PGRN does not correlate to islet secretion function. This finding is different from a previous study which showed negative correlation between PGRN and HOMA-β [8]. This discrepancy may be due to relatively mild obesity and insulin resistance in the subjects of our study, comparing to those subjects with T2DM [8] and metabolic syndrome [23] in previous studies. Above all, PGRN may not be a good indicator of insulin resistance and insulin secretion function in mild obese children.

Few studies in the literature investigated the relationship between blood pressure and PGRN, especially in children. Qu et al. and Xu et al. have reported positive correlation between PGRN and blood pressure in adults [8, 32]. In the present study, children with excess body weight manifested with significantly higher blood pressure levels than the controls. More importantly, serum PGRN levels positively correlated with SBP and DBP levels after adjusted for BMI, which suggested the elevation of PGRN might act as an independent risk factor for hypertension. As is well known, PGRN may induce inflammation, chronic inflammation may alter endothelial function and reduce the arterial stiffness [33], thereby affecting the blood pressure regulation. However, the role of PGRN in etiopathogenesis of hypertension is still not fully understood.

One potential limitation of this study stems from a relatively small sample size. Furthermore, owing to lack of biological materials, we could not determine the expression of PGRN gene in adipose tissue of the study subjects.

In conclusion, this study showed that serum levels of PGRN were elevated in obese children, and may serve as a marker of ongoing obesity-related inflammation. Furthermore, our study also suggested that the elevation of PGRN levels in obese children may be an early marker and a potential therapeutic target for management of obesity-related disorders.

Data Availability

The authors declare that the data supporting the findings of this study are available within the article or are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Fengyun Wang and Ting Chen contributed equally to this study.

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Research Article

Clinical Applications of Adiponectin Measurements in Type 2 Diabetes Mellitus: Screening, Diagnosis, and Marker of Diabetes Control

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Background. Adipose tissue-derived adiponectin has pleiotropic protective effects with suppression of inflammatory and metabolic derangements that may result in insulin resistance, metabolic syndrome, type 2 diabetes mellitus (T2DM), and cardiovascular disease. The aim of this study was to evaluate adiponectin as a diagnostic marker of T2DM and diabetes control. *Methods*. Fasting adiponectin, insulin, glucose, and HbA1c were determined in 376 patients with known T2DM and 575 subjects with undiagnosed diabetes but with family history of T2DM. Clinical and anthropometric data were recorded. Subjects were classified on the basis of degree of adiposity, insulin resistance (IR), and achievement of target HbA1c levels. Receiver operating characteristic (ROC) curve analysis was used to examine the diagnostic performance for undiagnosed DM. *Results*. In undiagnosed subjects, adiponectin was significantly lower in subjects with IR and diabetic subjects compared with those without. The area under the adiponectin ROC curve for diagnosis of DM was 0.740. In known T2DM subjects, those with good control had significantly higher adiponectin (8.6 versus 7.4 μg/mL) compared to subjects with poor control. *Conclusions*. Adiponectin levels are associated with better glycemic control and could be a useful adjunct for screening for IR and T2DM. Therapeutic measures that increase adiponectin levels might be valuable targets for improving diabetes control and decreasing complications.

1. Introduction

Type 2 diabetes mellitus (T2DM) is highly prevalent and is one of the leading causes of mortality and morbidity worldwide. T2DM is characterized by insulin resistance or impaired insulin secretion often in association with obesity which causes insulin resistance through secretion of various adipocyte-derived proteins. Adiponectin, a bioactive adipocytokine exclusively secreted by mature adipocytes in adipose tissue possesses anti-inflammatory, antiatherogenic, and insulin-sensitizing properties. It is the most abundant adipocytokine synthesized by adipocytes and the only adipose-specific protein that is negatively regulated in obesity [1–3]. Various cross-sectional studies have documented the association of low adiponectin levels with obesity, insulin resistance, metabolic syndrome (MetS), and progression from prediabetes to T2DM.

The pleiotropic protective effects of the adiponectin occurs via several postulated mechanisms that could, potentially, reduce the risk of T2DM and its associated complications. Some of the multiple anti-inflammatory and antiatherogenic effects of adiponectin involve vascular endothelial cell survival and activation through inhibition of TNF-α signaling [4] and regulation of endothelial nitric oxide synthase (eNOS). Adiponectin exerts potent insulin-sensitizing action through fatty acid oxidation, increased energy consumption, and stimulation of insulin secretion [2, 5]. There is strong accumulating evidence from several prospective studies that showed low adiponectin levels as a predictor of the incidence of T2DM [6, 7]. Adiponectin has been shown to significantly correlate

inversely with obesity, hypertension, dyslipidemia, fasting plasma glucose levels, and insulin resistance [8–11], which are known risk factors for subsequent development of T2DM. Given the prominent biological functions and associations of adiponectin as a protective adipocytokine against T2DM, this study assesses the potential roles of adiponectin as a useful clinical diagnostic indicator of incident diabetes among individuals at increased risk as well as diabetes control among T2DM patients.

2. Materials and Methods

2.1. Subjects and Clinical Features. The study subjects were recruited from polyclinics, specialized diabetes clinics, and hospitals in Kuwait where subjects with T2DM were advised to invite their first-degree relatives (FDR) for screening to assess their risks of developing T2DM as described previously [12]. 575 apparently healthy FDR of patients with T2DM were studied. To evaluate adiponectin as a marker of DM control, we studied 376 patients with known T2DM duration of 12.4 ± 8.1 years. Prediabetes and T2DM were confirmed according to the criteria of the American Diabetes Association [13]. For the first-degree relatives, criteria for inclusion in the study were both parents, one parent, and/or a sibling with T2DM and absence of any hematologic, genetic (especially presence of hemoglobin variants), and illness-related factors that could affect or interfere with the estimation of HbA1c. None of the study subjects was taking any medication at the time of the study. None of the study subjects had a history of surgery on reproductive organs (e.g., oophorectomy), and female subjects were specifically asked if their menstruation had stopped. None of the female study subjects was menopausal at the time of the study. The study was approved by the Ethics Committees of the Faculty of Medicine, Kuwait University, and the Ministry of Health, Kuwait, and performed in accordance with the Declaration of Helsinki. All subjects gave informed voluntary consent to participate in the study.

Height was measured to the nearest 0.1 cm and body weight to the nearest 0.1 kg using a stadiometer, and the body mass index (BMI) (kg/m^2) was calculated [12]. The body mass index (BMI) was calculated according to the following formula: weight in kilograms divided by the square of the height in meters. Patients with BMI $> 30\ kg/m^2$ are classified as obese and those with BMI < 25 are classified as normal. Those with BMI > 24.9 to <30 are classified as overweight. Waist circumference (WC) was measured half way between the xiphisternum and the umbilicus at the point corresponding to the maximal abdominal protuberance. Two consecutive measurements of systolic blood pressure (SBP) and diastolic blood pressure (DBP) were taken from each subject after at least 10 min rest.

2.2. Laboratory Methods

2.2.1. Assays. As described previously [12], fasting plasma adiponectin was measured using a commercially available enzyme-linked immunoassay (ELISA) kit (Linco Research, Missouri, USA) with a sensitivity of 0.39 μg/mL. The intra- and interassay coefficients of variation on pooled plasma specimen with adiponectin concentration of 8.2 μg/mL were 4.7% and 6.8%, respectively.

Fasting serum insulin was determined on an automated analyzer Beckman DXI 800 (Beckman Corporation) using the paramagnetic particle chemiluminescence immunoassay method [12]. Insulin resistance was calculated using the homeostasis model assessment (HOMA-IR) using the HOMA2 calculator (version 2.2.2) downloaded from https://www.dtu.ox.ac.uk/homacalculator/download.php (Diabetes Trials Unit, Oxford). HOMA-IR > 2 was used as the cut-off point for determination of insulin resistance [14]. The HOMA2 calculator also gives estimates of steady-state beta cell function (%B) and insulin sensitivity (%S).

HbA1c levels were measured using high-performance liquid chromatography on a TOSOH G8 analyzer (Tosoh Corporation, Tokyo, Japan) [12]. HbA1c values or fasting plasma glucose (FPG) based on ADA diagnostic criteria [13] was used to categorize the subjects as follows: normal subjects—HbA1c $< 5.7\%$ (<39 mmol/mol) or FPG < 5.6 mmol/L; subjects with prediabetes—HbA1c 5.7%–6.4% (39–46 mmol/mol) or FPG 5.6–6.9 mmol/L; subjects with diabetes—HbA1c $\geq 6.5\%$ (≥ 48 mmol/mol) or FPG ≥ 7.0 mmol/L.

FPG, alanine aminotransferase (ALT), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), apolipoprotein A1 (Apo-A1), and apolipoprotein B (Apo B) were analyzed on an automated analyzer (Beckman DXC 800, Beckman Corporation) [12]. Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula [15]. The formula is valid as long as TG ≤ 4.5 mmol/L.

2.3. Statistical Methods. IBM SPSS 19.0 software (IBM) was used for statistical analysis. Data are presented as mean $\pm$ standard deviation (SD) unless otherwise specified. The Kolmogorov–Smirnov goodness-of-fit test was to test for normality of the data. Several variables (insulin, HOMA-IR, B%, and TG) that diverged significantly from normal distribution were log transformed when parametric tests were used. Comparisons between two groups were performed with the Mann–Whitney U test, and the Kruskal-Wallis analysis of variance was used to compare between more than two groups. The chi-square test was used to compare categorical variables. To determine the predictors of adiponectin, we performed linear and multivariate regression analysis with adiponectin as the dependent variable. All cardiometabolic variables were included in the multivariate stepwise regression model. Binary logistic regression analysis was performed to estimate the odds ratios (ORs) and 95% confidence intervals (CIs) for the association between adiponectin and T2DM. We performed receiver operating characteristic (ROC) analysis on the usefulness of adiponectin for the detection of diabetes. $p < 0.05$ was considered statistically significant for all analyses.

3. Results

3.1. General Results. 73 of 575 study subjects were found to have previously undiagnosed T2DM. Tables 1 and 2

Table 1: Clinical and anthropometric characteristics of study subjects screened for glycemic status grouped by degree of adiposity.

BMI (kg/m^2)	Normal (BMI < 25)	Overweight (24.9–30)	Obese (>30)	p (Kruskal-Wallis)
Age (yrs)	25.7 ± 8.2	28.9 ± 8.9	31.8 ± 8.8	<0.0001
WC (cms)	83.8 ± 9.2	95 ± 9	108.7 ± 12.6	<0.0001
SBP (mm/Hg)	109 ± 10	115 ± 15	116 ± 13	0.001
DBP (mm/Hg)	69 ± 8	72 ± 8	74 ± 7	<0.0001
TC (mmol/L)	4.48 ± 0.82	4.93 ± 0.98	5.15 ± 1.13	<0.0001
TG (mmol/L)	0.81 ± 0.54	1.09 ± 0.72	1.32 ± 0.95	<0.0001
HDL-C (mmol/L)	1.25 ± 0.36	1.18 ± 0.33	1.16 ± 0.31	0.23
LDL-C (mmol/L)	2.79 ± 0.71	3.04 ± 0.83	3.34 ± 0.92	<0.0001
ALT (IU/L)	22.52 ± 17.05	24.63 ± 15.34	27.37 ± 16.57	<0.0001
Insulin (μU/mL)	5.38 ± 4.68	6.8 ± 6.07	10.86 ± 9.47	<0.0001
S%	143.19 ± 59.44	122.24 ± 54.92	94.4 ± 51.81	<0.0001
B%	87.55 ± 36.83	100.43 ± 67.71	111.05 ± 54.5	<0.0001
HOMA-IR	0.9 ± 0.65	1.07 ± 0.74	1.46 ± 1.04	<0.0001
Glucose (mmol/L)	5 ± 0.99	5.05 ± 0.66	5.22 ± 1.03	0.03
HbA1c (%)	5.88 ± 0.83	5.87 ± 0.62	8.75 ± 26.39	0.001
Adiponectin (μg/mL)	8.95 ± 3.82	8.57 ± 5.12	6.82 ± 2.97	<0.0001

Abbreviations: WC: waist circumference; SBP: systolic blood pressure; DBP: diastolic blood pressure; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein; LDL-C: low-density lipoprotein; ALT: alanine aminotransferase; S%: insulin sensitivity; B%: beta cell function; HOMA-IR: homeostasis model assessment of insulin resistance; HbA1c: hemoglobin A1c. Data are presented as mean ± standard deviation

summarize clinical and anthropometric parameters of study subjects screened for glycemic status grouped by degree of adiposity and T2DM, respectively. Subjects who were obese had significantly higher blood pressure, lipid profile, ALT, insulin, B%, HOMA-IR, fasting glucose, and HbA1c levels and lower S% compared to normal individuals. Adiponectin levels were significantly lower with increasing degree of adiposity (Table 1). In Figure 1, FDR screened for undiagnosed diabetes were categorized according to their state of IR. Subjects who were insulin resistant were found to have significantly reduced adiponectin concentrations (5.7 versus 8.1 μg/mL) compared to subjects without IR. In Table 2, FDR diagnosed with T2DM had significantly higher WC, SBP, ALT, insulin, B%, and HOMA-IR but significantly lower S% and adiponectin levels (6.9 versus 8.6 μg/mL) compared to those subjects who did not develop T2DM.

Table 3 shows the clinical, anthropometric, and metabolic characteristics of known T2DM patients ($n = 376$) grouped according to their achievement of target HbA1c levels. T2DM patients with poor glycemic control (>53 mmol/mol) had higher ALT, B%, and HOMA-IR but significantly lower adiponectin levels (7.4 versus 8.6 μg/mL) compared to subjects with good control (<53 mmol/mol).

3.2. Predictors of Circulating Adiponectin. The associations of adiponectin with cardiometabolic variables are shown in Table 4. With the exception of HbA1c, adiponectin was significantly associated with cardiometabolic variables. However, when all the cardiometabolic variables were included in the full model of regression analysis, the strongest significant predictors of circulating adiponectin were waist circumference, HDL-cholesterol, apolipoprotein A1, apolipoprotein B, and HOMA-IR.

3.3. Regression Analysis. Binary logistic regression analysis showed that adiponectin was significantly associated with T2DM with an odds ratio of 0.88 [95% confidence interval (CI) 0.80–0.96; $p = 0.007$].

3.3.1. Performance Characteristics for Detection of T2DM. Using the ADA glucose and HbA1c diagnostic criteria as reference, ROC curve (Figure 2) analyses for the use of adiponectin to detect T2DM showed that the area under the adiponectin curve was 0.740 (95% CI 0.570–0.910). At the cut-off point of 7.5 μg/mL, the diagnostic sensitivity and specificity of adiponectin for T2DM were 88% and 51%, respectively.

4. Discussion

This study demonstrates the potential clinical significance of adiponectin measurements in T2DM. Our results confirm and extend those of several studies that demonstrated the protective role of high adiponectin levels with lower risk of T2DM and association of low adiponectin levels with risk factors for T2DM [16, 17] and subsequent cardiovascular complications associated with the disease [18, 19].

The significant progressive decrease in adiponectin (Table 1) in line with increasing degree of insulin resistance (Table 1 and Figure 1) with increasing degrees of obesity indicate the utility of adiponectin as a screening tool. Obesity is an important determinant of insulin resistance and a known risk factor for development of T2DM and cardiovascular disease. The significant associations of adiponectin with cardiometabolic risk factors (Table 4) show the usefulness for the identification of high-risk first-degree relatives of T2DM patients who tend to exhibit a higher propensity to be insulin resistant [20]. Hypoadiponectinemia

Table 2: Clinical and anthropometric characteristics of subjects screened for type 2 diabetes mellitus.

HbA1c	Normal (<5.7%)	Undiagnosed diabetes (≥6.5%)	p (Mann–Whitney U)
Age (yrs)	28.2 ± 8.6	32 ± 9.7	0.019
WC (cms)	93.6 ± 16.0	104.6 ± 14.7	<0.0001
SBP (mm/Hg)	113 ± 13	120 ± 17	0.005
DBP (mm/Hg)	73 ± 9	73 ± 10	0.37
TC (mmol/L)	4.65 ± 0.91	5.02 ± 0.9	0.001
TG (mmol/L)	1.0 ± 0.71	1.28 ± 0.69	<0.0001
HDL-C (mmol/L)	1.20 ± 0.34	1.07 ± 0.26	0.007
LDL-C (mmol/L)	2.96 ± 0.76	3.32 ± 0.81	<0.0001
ALT (IU/L)	23.42 ± 16.57	30.91 ± 19.24	<0.0001
Insulin (μU/mL)	7.46 ± 6.95	11.87 ± 11.33	<0.0001
S%	119.31 ± 56.71	91.43 ± 54.42	0.002
B%	101.52 ± 54.32	110.15 ± 70.08	0.1
HOMA-IR	1.12 ± 0.79	1.66 ± 1.42	0.002
Glucose (mmol/L)	4.97 ± 0.64	5.91 ± 1.76	<0.0001
HbA1c (%)	5.63 ± 0.40	7.58 ± 1.14	<0.0001
Adiponectin (μg/mL)	8.57 ± 4.42	6.92 ± 2.86	<0.0001

Abbreviations: WC: waist circumference; SBP: systolic blood pressure; DBP: diastolic blood pressure; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein; LDL-C: low-density lipoprotein; ALT: alanine aminotransferase; S%: insulin sensitivity; B%: beta cell function; HOMA-IR: homeostasis model assessment of insulin resistance; HbA1c: hemoglobin A1c. Data are presented as mean ± standard deviation.

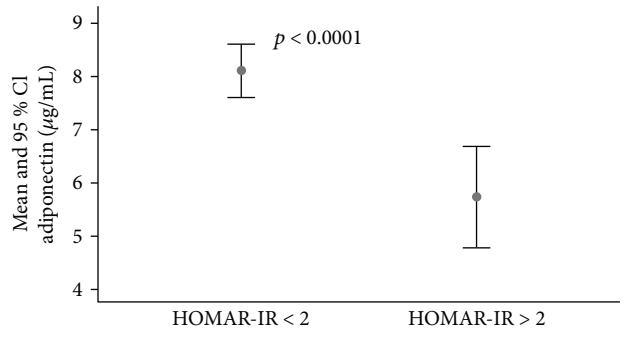

Figure 1: Adiponectin levels in subjects with undiagnosed diabetes grouped by homeostasis model assessment of insulin resistance. CI = confidence interval.

has been shown to precede a decrease in insulin sensitivity [5] as well as predict progression from normoglycemia to prediabetes [21]. These studies and our findings highlight the potential of adiponectin as a screening tool that could be used to monitor progression from prediabetes to diabetes and associated complications.

Table 3: Clinical and anthropometric characteristics of type 2 diabetic subjects grouped by achievement of target HbA1c.

HbA1c	<53 mmol/mol	>53 mmol/mol	p Mann–Whitney U
Age	55.4 ± 13.5	57.8 ± 9.4	NS
WC (cms)	102.6 ± 13.2	107 ± 12.9	NS
BMI (kg/m^2)	30.3 ± 7.7	32.8 ± 6.0	0.05
SBP (mm/Hg)	132 ± 28	132 ± 20	NS
DBP (mm/Hg)	82 ± 13	82 ± 12	NS
TC (mmol/L)	4.88 ± 1.11	5.02 ± 1.18	NS
TG (mmol/L)	1.54 ± 1.06	1.75 ± 1.19	NS
HDL-C (mmol/L)	1.21 ± 0.34	1.11 ± 0.29	0.04
LDL-C (mmol/L)	3.07 ± 0.95	3.04 ± 0.98	NS
ALT (IU/L)	21.91 ± 12.72	34.15 ± 177.75	0.02
Insulin (μU/mL)	11.51 ± 14.74	17.15 ± 25.48	NS
S%	88.05 ± 41.30	73.46 ± 50.21	0.03
B%	77.08 ± 53.19	43.40 ± 37.69	<0.0001
HOMA-IR	1.51 ± 1.04	2.31 ± 2.0	0.03
Glucose (mmol/L)	6.73 ± 2.03	11.23 ± 4.08	<0.0001
HbA1c (%)	6.34 ± 0.47	10.69 ± 2.53	<0.0001
Adiponectin (μg/mL)	8.58 ± 4.5	7.37 ± 4.35	0.04

Abbreviations: WC: waist circumference; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein; LDL-C: low-density lipoprotein; ALT: alanine aminotransferase; S%: insulin sensitivity; B%: beta cell function; HOMA-IR: homeostasis model assessment of insulin resistance; HbA1c: hemoglobin A1c; NS: not significant ($p > 0.05$). Data are presented as mean ± standard deviation

The usefulness of adiponectin for the detection of the metabolic syndrome had been shown in a previous study [14]. The present study on subjects at high risk of T2DM has shown that adiponectin has good performance characteristics for the detection of previously undiagnosed T2DM. The diagnostic sensitivity, specificity and area under the ROC curve (Figure 2) show that adiponectin could be a useful adjunct for the diagnosis of T2DM in a population screen for T2DM. Even after adjustment for confounding risk factors, the binary logistic regression association of adiponectin with T2DM with an odds ratio of 0.88 further highlights low adiponectin as a strong predictor of incident T2DM [19].

One of the interesting findings in our study is the significant difference in adiponectin concentrations between T2DM subjects with good glycemic control and poor glycemic control (Table 3). The association of higher adiponectin levels with better glycemic control suggests that therapeutic modalities that increase adiponectin levels may be valuable targets for management of T2DM. Methods that enhance or mimic adiponectin levels have been shown to be effective therapeutic strategies for improving diabetes control, treatment of insulin resistance, and other metabolic abnormalities associated with T2DM. Thiazolidinediones are a class of

Table 4: Multivariate regression analysis of the associations of cardiometabolic variables with adiponectin concentration as a dependent variable in subjects screened for type 2 diabetes mellitus.

	β coefficient	95% confidence interval	p	β^* coefficient	95% confidence interval	p
WC (cms)	−0.341	−0.114 to −0.066	<0.0001	−0.314	−0.178 to −0.008	0.023
BMI (kg/m^2)	−0.224	−0.211 to −0.062	<0.0001	0.220	−.028 to 0.348	0.059
SBP (mm/Hg)	−0.151	−0.086 to −0.018	0.003	−0.045	−0.082 to 0.055	0.695
DBP (mm/Hg)	−0.160	−0.138 to −0.033	0.002	−.097	−0.178 to 0.074	0.415
TC (mmol/L)	−0.187	−1.325 to −0.421	<0.0001	−0.145	−2.701 to 1.343	0.507
TG (mmol/L)	−0.291	−2.269 to −1.159	< 0.0001	−0.135	−1.927 to 0.346	0.172
HDL-C (mmol/L)	0.338	3.170 to 5.578	<0.0001	0.309	1.397 to 6.588	0.003
LDL-C (mmol/L)	−0.240	−1.809 to −0.759	<0.0001	−0.161	−2.865 to 1.085	0.376
ApoA1 (g/L)	0.136	0.169 to 3.673	0.032	−.462	−13.608 to −2.371	0.006
Apo B (g/L)	−0.269	−4.647 to −1.995	< 0.0001	−0.271	−1.165 to −0.502	<0.0001
HOMA-IR	−0.252	−1.550 to −0.548	<0.0001	−0.166	−1.377 to −0.012	004
Glucose (mmol/L)	−0.173	−1.247 to −0.352	<0.0001	−0.097	−0.791 to 0.039	0.076
HbA1c (mmol/mol)	−0.061	−0.050 to 0.012	0.227	0.055	−0.672 to 1.286	0.054

Abbreviations: WC: waist circumference; BMI: body mass index; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein; LDL-C: low-density lipoprotein; Apo A1: apolipoprotein A1; Apo B: apolipoprotein B; HbA1c: hemoglobin A1c; SBP: systolic blood pressure; DBP: diastolic blood pressure; HOMA-IR: homeostasis model assessment of insulin resistance; β^*: inclusion of all cardiometabolic variables in the regression model.

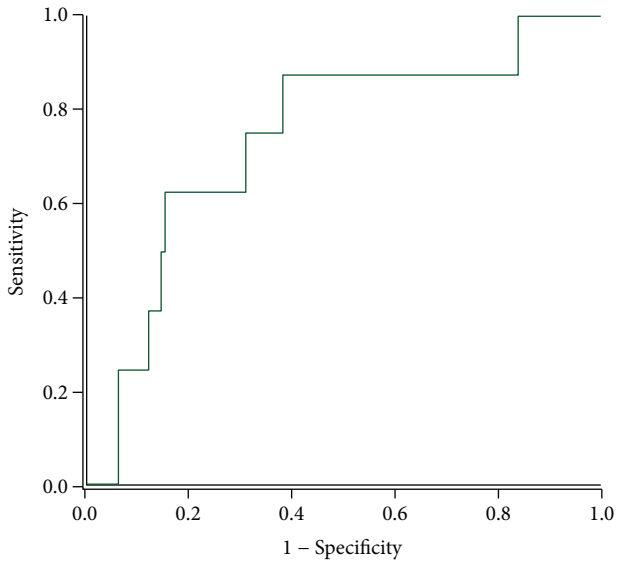

Figure 2: Receiver operating characteristic analysis of the usefulness of adiponectin for the diagnosis of type 2 diabetes mellitus.

antidiabetic drugs, known to exert an insulin-sensitizing effect, mediated partly by upregulating plasma adiponectin levels [2]. Several other drugs like statins, angiotensin-converting enzyme inhibitors, and angiotensin II receptor blockers that target adiponectin synthesis have also been reported to improve glucose tolerance and ameliorate insulin resistance [22]. However, although adiponectin is present at relatively high amounts in circulation and can be easily measured, the efficacy of adiponectin as a potentially useful therapeutic agent needs to be demonstrated experimentally and in clinical trials.

One of the main limitations of this study is that it is a cross-sectional study which does not help establish a causal relation between low adiponectin and T2DM development. Additionally, study participants used in our study were those at high risk for developing T2DM; thus, the associations observed with adiponectin and T2DM risk may not be applicable to those without family history of T2DM. Our study does not provide data on adiponectin multimeric complexes particularly high molecular weight (HMW) adiponectin, which has been shown to be the most biologically active form. However, others have reported similar associations of both total adiponectin and HMW with incident T2DM [23] and cardiometabolic risk factors [24].

5. Conclusion

Adiponectin levels are associated with incident diabetes and glycemic control and could be useful adjuncts for screening for IR and T2DM. The significant associations of adiponectin levels with clinical and cardiometabolic parameters reveal its potential as a biomarker in assessment of prediabetic state and T2DM screening.

Disclosure

An abstract of this manuscript was presented as a poster (A-176) at the 2014 Annual and Scientific Meeting of the American Association of Clinical Chemists in Chicago, IL, USA.

Conflicts of Interest

The authors declare that they have no conflict of interests.

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Research Article

Irisin Maternal Plasma and Cord Blood Levels in Mothers with Spontaneous Preterm and Term Delivery

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Irisin, an adipomyokine identified in 2012, has been investigated in association with common pregnancy complications, including gestational diabetes mellitus, preeclampsia, and intrauterine growth restriction. The objective of this study is to examine the potential role of irisin in preterm birth (PTB) by comparing its level between mothers with term and preterm labor. Maternal peripheral blood and cord blood samples were collected from 30 mothers who delivered prematurely and from 35 mothers who delivered at term. Irisin concentrations were measured in all samples using ELISA, and four common single nucleotide polymorphisms in the irisin gene were determined (rs16835198, rs726344, rs3480, and rs1746661). Univariable and multivariable regression modeling was applied to evaluate maternal and cord blood irisin concentrations in relation to preterm/term labor. Irisin concentration in umbilical cord blood was found to be associated with PTB in the univariable model ($p = 0.046$). On the other hand, no differences in maternal blood irisin levels between mothers with preterm and term deliveries were established. To the best of our knowledge, this is the first study determining irisin levels in term and preterm deliveries in maternal peripheral blood and umbilical cord blood. Our study shows a possible association between cord blood irisin concentration and PTB occurrence.

1. Introduction

1.1. Preterm Birth. Preterm birth (PTB), that is, delivery before 37 weeks of pregnancy, is a leading cause of neonatal morbidity and mortality. Around 15 million babies are currently born preterm every year; moreover, this number continues to rise [1]. The rate of this serious pregnancy complication ranges from 5 to 18% of live births depending on the country [2]. PTB may either be induced, in most cases due to maternal or fetal infection, or spontaneous. Spontaneous PTB occurs either with intact membranes or after preterm premature rupture of membranes (PPROM) [3]. PPROM, defined as the rupture of the amniotic sac before the onset of labor and prior to week 37 of pregnancy, causes approximately one-third of all PTB cases [4].

1.2. Irisin in Pregnancy Complications. Adipokines, that is, secretory proteins released from adipose tissue, typically include cytokines, hormone-like molecules, growth factors, and other inflammatory mediators. The role of adipokines has been investigated in association with both the physiology [5] and pathophysiology [6] of pregnancy. Specifically, adipokines are known to affect uterine contractility [7, 8], pregnancy outcomes [9], and fetal growth [10].

Irisin was identified in 2012 as an exercise-induced myokine which drives the conversion of white adipose tissue (WAT) into brown adipose tissue (BAT) [11]. One year later, Roca-Rivada et al. found that irisin also acts as an adipokine, since it is released especially by subcutaneous adipose tissue [12]. As irisin was suggested to improve obesity and insulin resistance [11, 13], its therapeutic potential in metabolic

Table 1: Baseline characteristics and irisin levels in study groups.

Variable		PTB $n = 30$	Term $n = 35$	All $n = 65$	Test	p Value
Age	Years	28.9 ± 5.3	30.4 ± 4.7	29.7 ± 5.0	t-test	0.215
Height	cm	166.3 ± 6.8	169.7 ± 7.4	168.1 ± 7.3	t-test	0.063
Weight (preconception)	kg	64.4 ± 12.9	63.2 ± 13.1	63.7 ± 12.9	t-test	0.681
Weight (delivery)	kg	74.3 ± 11.6	76.2 ± 12.8	75.3 ± 12.2	t-test	0.513
Weight gain	kg	9.9 ± 4.9	13.0 ± 4.1	11.6 ± 4.7	t-test	**0.007**
BMI (preconception)	kg/m^2	23.3 ± 4.7	21.9 ± 4.4	22.6 ± 4.6	MW	0.188
BMI (delivery)	kg/m^2	26.9 ± 4.5	26.5 ± 4.3	26.7 ± 4.3	t-test	0.688
Infant birth weight	g	1887 ± 580	3357 ± 501	2678 ± 912	Welch	**<0.001**
Infant birth length	cm	43.2 ± 4.6	49.5 ± 1.8	46.6 ± 4.6	Welch	**<0.001**
Gestational age	week	32.3 ± 3.2	39.2 ± 1.0	36.0 ± 4.2	KS	**<0.001**
Maternal irisin	ng/ml	12.0 ± 2.4	11.5 ± 1.5	11.7 ± 2.0	t-test	0.642
Fetal irisin	ng/ml	7.7 ± 2.2	6.8 ± 1.5	7.2 ± 1.9	MW	0.067

Data are expressed as mean ± standard deviation. p values express the difference in variables between the PTB and term delivery group based on a selected test. p values in bold are statistically significant. PTB/term: mothers with preterm/term delivery; MW: Mann–Whitney test; KS: Kolmogorov–Smirnov test.

disease treatment has attracted extensive interest. Furthermore, irisin was investigated in association with many pregnancy complications. Since irisin was also suggested to improve glucose tolerance [11], its involvement in gestational diabetes mellitus (GDM) was initially examined [14–23]. In addition, due to the possible association between irisin and blood pressure [24], its role in preeclampsia (PE) was later also investigated [25, 26]. In a similar manner, the role of irisin in energy homeostasis [27] was investigated in studies focusing on irisin in fetal growth restriction [28–30]. Nevertheless, as far as we know, this is the first study focusing on the role of irisin in PTB and PPROM.

1.3. Aims of the Study. This study thus aims to (i) investigate irisin levels in maternal circulation and in umbilical cord at the time of delivery and (ii) compare these levels between mothers who delivered prematurely and those who delivered at term. In addition, (iii) we examined associations between four selected single nucleotide polymorphisms (SNPs) in the irisin gene and irisin levels in maternal and cord blood.

2. Material and Methods

2.1. Subjects. A total of 65 Central European Caucasian women were recruited for the present study at the Department of Obstetrics and Gynaecology, University Hospital Brno (Czech Republic). Specifically, a total of 30 mothers with preterm and 35 with term deliveries were enrolled. Moreover, 16 mothers from the PTB group had PPROM, while the rest ($n = 49$) delivered with intact membranes. Signed informed consent was obtained from all participants and archived. The study was approved by the Committee for Ethics of Medical Experiments on Human Subjects, Faculty of Medicine, Masaryk University (Czech Republic), in adherence to the Declaration of Helsinki guidelines. Information about maternal anamnesis and anthropometry, current and previous pregnancies, and socioeconomic status was obtained using a standardized questionnaire and summarized in Table 1.

Inclusion criteria for participants of the study were spontaneous conception, singleton pregnancy, spontaneous delivery, and live birth. The presence of bacterial infection was further determined by both vaginal swab and urinalysis.

2.2. Sampling. The peripheral blood samples were collected from each mother at the time of delivery or at least one week before delivery. In the case of preterm deliveries, sampling was performed prior to the initiation of corticosteroid or tocolytic treatment. Umbilical cord blood samples were collected from umbilical cord vessels immediately after childbirth.

The plasma samples were prepared by centrifugation of 5 ml of both maternal peripheral and cord blood samples (2500g, 10 min). Immediately after sampling, the resulting supernatant was collected into a clean tube, aliquoted and stored at −80°C until analysis. DNA was extracted from 5 ml of both maternal peripheral and cord blood samples using the standard method based on proteinase K, subsequently stored at −20°C until analysis. The samples were collected between 2012 and 2014, while the analyses were performed between 2016 and 2017.

2.3. Biochemical Analysis. The irisin plasma levels were determined using a commercially available sandwich enzyme-linked immunosorbent assay (ELISA) kit (Phoenix Pharmaceuticals, EK-067-29) according to the manufacturer's instructions. The minimum detectable concentration of irisin was 1.7 ng/ml, linear range 1.7–25 ng/ml, and intra- and inter-assay variations were below 10 and 15%, respectively. Samples were diluted 2-fold prior performing the assay with assay buffer and were measured in duplicate.

2.4. Genotyping. Four selected SNPs were genotyped using touchdown polymerase chain reaction followed by restriction fragment length polymorphism (PCR-RFLP). Primers were designated using the Primer3Plus program [31] as

follows: GGCCCATTCTGAAAAACTAGG and ACACCT CAGGCAAGTTAAGT for rs16835198, CAGTGACTTCC CCTGAGCTT and CGACAGTTCTGGGAAACAGA for rs726344, GGAAGGAAGGGGCGGTCCTT and CATCTT CCATTGGTGGTCAA for rs3480, and TGGAGAGAGTT ATGTAGGGGACA and CTTCCGCAGGCTTTATTCTG for rs1746661. Utilized restriction enzymes included BsmAI, Hpy188I, StyI, and HhaI, respectively.

2.5. Statistical Analysis. All statistical analyses were carried out using statistical software R (version 3.3.3.). Conventional values of $p < 0.05$ were considered statistically significant. Descriptive characteristics of variables are represented by mean ± standard deviation.

Genotype distributions were tested for Hardy-Weinberg equilibrium by Fisher's exact test.

Statistical independence was tested by Pearson's chi-squared test using contingency tables, and the correlation between two variables was expressed as Cramér's coefficient. The risk of PTB associated with the individual genotypes was evaluated by linear regression model. The adjusted odds ratios for the independent variables with their 95% confidence intervals were calculated. The proper adjustment was performed for maternal age, preconception BMI, smoking status, educational status, infection, and infant gender. As only a few cases of TT and AA homozygotes were identified (in rs726344 and rs1746661, resp.), the analysis was performed as A (GG versus GA + AA) and T (GG versus GT + TT) dominant models and the genotype categories were merged. In the case of rs16835198, T dominant model was used only in infants.

The normal distribution of variables was tested using normality tests (Shapiro–Wilk, Pearson's, Anderson–Darling, etc.). In cases of skewed variables, logarithmic and square root transformation was performed and normal distribution was tested again. Variables or transformed variables with normal distribution were compared between case and control groups using parametric tests (*t*-test, Welch's test). Otherwise, nonparametric tests were used (Mann–Whitney, Kolmogorov–Smirnov).

Univariable and multivariable linear regression models were used to investigate maternal and cord blood irisin concentrations in relation to other variables. In these models, maternal/cord blood irisin levels constituted the dependent variable while all other variables were considered independent. In the case of categorical variables the reference category was selected and compared with the other one or two categories. Multivariable linear regression model utilized the following additional independent variables: PTB status, maternal age, preconception BMI, gestational age, primiparity, smoking status, educational status, infection, genotypes, and infant gender. Missing values were imputed using Multiple Imputation by Chained Equations method, and the models were built using imputed data [32].

3. Results

Baseline anthropometric characteristics of mothers and infants are summarized in Table 1. As expected, maternal weight gain during pregnancy and infant birth weight and length were significantly lower in mothers with preterm compared to term deliveries.

Table 2: Linkage disequilibrium between four irisin single nucleotide polymorphisms.

Mother	rs16835198	rs726344	rs3480	rs1746661
rs16835198	—	0.125	0.269	0.177
rs726344	0.999	—	0.091	0.006
rs3480	0.735	0.601	—	0.356
rs1746661	0.999	0.091	1.000	—
Infant	rs16835198	rs726344	rs3480	rs1746661
rs16835198	—	0.091	0.464	0.139
rs726344	0.999	—	0.115	0.052
rs3480	0.936	0.818	—	0.263
rs1746661	0.999	0.999	0.999	—

Data express the linkage disequilibrium between four irisin polymorphisms in Czech mothers and their infants. D′ values are given below the empty cells, and r^2 values above the empty cells.

3.1. Genetic Analysis of Investigated SNPs. Four common polymorphisms were identified, all in the noncoding region of the irisin gene. Both maternal and fetal genotype frequencies of all selected SNPs were in Hardy-Weinberg equilibrium, except for SNP rs3480 in maternal samples. A linkage disequilibrium (LD) was determined between all SNPs in both maternal and cord blood ($p < 0.001$ for all SNP combinations, except for LD between rs726344 and rs1746661 with $p = 0.014$) (Table 2).

No association between the genotypes of investigated SNPs and their respective maternal or cord blood irisin concentration was observed. While a weak association was observed between rs726344 in mothers and the occurrence of PTB (Cramér's V = 0.27; $p = 0.029$), no association was found between the remaining SNPs and PTB incidence.

Using linear regression model, we found lower risk of PTB for mothers carrying GA + AA genotypes compared to mothers with GG genotype in rs726344 (adjusted OR = 0.06, 95% CI: 0.01; 0.62, $p = 0.018$). With the same model, we found the higher risk of PTB for mothers carrying TT compared to the mothers with GG genotype in rs16835198 (adjusted OR = 24.94, 95% CI: 1.79; 347.92, $p = 0.017$). For rs3480, we found lower risk of PTB for mothers carrying GG compared to AA (adjusted OR = 0.06, 95% CI: 0.01; 0.64), $p = 0.019$). In rs1746661, we found no association between PTB risk and genotype.

3.2. Maternal and Cord Blood Irisin Levels in Term and Preterm Deliveries. No significant differences in maternal or cord blood irisin levels between mothers with preterm and term deliveries were established using either the *t*-test or the Mann–Whitney test, respectively. Nevertheless, a trend towards decreased irisin levels in PTB ($p = 0.067$) was observed. Irisin levels were significantly higher in maternal peripheral blood compared to umbilical cord blood (11.6 ± 2.0 versus 7.2 ± 1.9, $p < 0.001$) (Figure 1). More specifically, maternal irisin was approximately 63% higher compared to

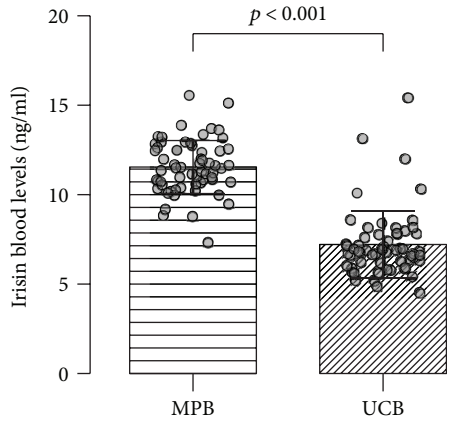

Figure 1: Comparison of irisin levels between maternal peripheral blood and umbilical cord blood. Individual points represent irisin concentrations in samples of maternal peripheral blood (MPB) and umbilical cord blood (UCB). Irisin concentrations are presented as mean ± standard deviation.

cord blood irisin concentration. Maternal irisin levels appeared not to correlate with cord blood irisin. Similarly, no correlation between maternal or cord blood irisin levels and the other variables listed in Table 1 was observed.

The 13 variables listed in Table 3 as potential confounders in association with maternal/cord blood irisin concentrations were analyzed using univariable logistic regression. Variables used in the model included PTB status, maternal age, preconception BMI, gestational age, primiparity, smoking status, educational status, infection, genotypes, and infant gender. While according to this model maternal irisin level was not associated with any of these variables, cord blood irisin concentration was found to be associated with PTB occurrence ($p = 0.046$) (Figure 2) and smoking status ($p = 0.025$) (Table 3). In the multivariable model, maternal and cord blood irisin concentrations were investigated in relation to each of the 13 variables listed in Table 4 (i.e., identical with the above-mentioned list) and those remaining were used for purposes of adjustment. Based on this model, an association was established between maternal irisin concentration and parity ($p = 0.046$) (Table 4). The remaining variables were not significantly associated with maternal/cord blood irisin concentrations.

3.3. Irisin Levels in Deliveries with and without PPROM. No significant differences in maternal (12.0 ± 1.2 versus 11.6 ± 2.2 ng/ml) or cord blood (7.4 ± 1.9 versus 7.2 ± 1.9 ng/ml) irisin concentrations between mothers with and without PPROM were established.

4. Discussion

To the extent of our knowledge, this is the first study to analyze irisin levels in term and preterm deliveries. We demonstrated for the first time that there is no difference in maternal peripheral blood irisin levels between mothers with preterm and term deliveries. Furthermore, our study also showed a possible association between cord blood irisin concentration and PTB occurrence.

4.1. SNP in Irisin Gene. We found no association between the genotypes of the four investigated SNPs in the irisin gene and their respective maternal or cord blood irisin concentration. The finding is in agreement with previous studies, reporting no association between circulating irisin level and rs16835198, rs3480 [33], or rs726344 [34].

However, we observed a significant association between maternal rs726344 genotype and the occurrence of PTB, more specifically the risk of PTB lower by 94% in mothers with GA + AA compared to mothers with GG genotype in rs726344. In a recent study, Salem et al. (2018) report a significant relationship between rs726344 and PTB in an Israeli cohort. The authors reported 2.18 fold higher chance of delivering in term in mothers with GG genotype compared to AG and AA genotypes [35]. The opposite association observed in mothers in our study compared to Salem et al. could be caused by different ethnicity of both populations as well as by local geographical influences on fecundity of populations in given regions. Salem et al. investigated the rs1746661, too, and found no association with PTB as in the present study, either.

4.2. Irisin Association with Body Composition. The results of previous studies investigating the correlation of irisin level with metabolic parameters are controversial. While positive correlations between circulating irisin and BMI, body weight, fat mass, fat free mass, and elevated irisin level in obese patients compared to normal weight patients have been reported [34–36].

Negative correlations with anthropometric parameters (BMI, fat mass percentage, and waist to hip ratio) and decreased irisin level among obese subjects compared to lean participants have also been established [37]. Other studies have also reported an association between irisin level and insulin resistance [38, 39]. In addition, Piya et al. investigated circulating irisin in association with body composition in pregnant women and found a negative correlation of irisin level with BMI and a positive correlation with blood glucose, insulin, insulin resistance index (HOMA-IR), total cholesterol, triglycerides, and low- and high-density lipoproteins [19]. Similarly, Ebert et al. reported a positive correlation between irisin concentration and insulin, HOMA-IR, and total cholesterol in healthy pregnant women [15].

We observed an association between parity and maternal irisin concentration, specifically lower irisin levels in primiparous women after adjustment for the other variables (PTB status, maternal age, preconception BMI, gestational age, smoking status, educational status, infection, genotypes, and infant gender). Since irisin level is associated with body composition during pregnancy [15, 19] and since body composition may simultaneously be associated with parity [40, 41], we suggest that the relationship between parity and irisin concentration observed in the present study could be deduced from the different body compositions of primiparous and multiparous women. By virtue of the fact that circulating irisin is predominantly (approximately 72%) produced

Table 3: Univariable linear regression model with maternal and cord blood irisin levels as dependent variables.

Variable	Reference category	Comparative category	Mother			Infant		
			β	95% CI	p value	β	95% CI	p value
PTB status	Term	Preterm	0.018	(−0.064; 0.099)	0.669	0.113	(0.002; 0.224)	**0.046**
Maternal age			0.002	(−0.007; 0.011)	0.647	0.004	(−0.009; 0.016)	0.557
Preconception BMI			0.000	(−0.009; 0.009)	0.965	−0.001	(−0.013; 0.011)	0.855
Gestational age			−0.002	(−0.012; 0.009)	0.764	−0.013	(−0.027; 0.001)	0.069
Primiparity	No	Yes	−0.060	(−0.145; 0.024)	0.160	−0.023	(−0.144; 0.097)	0.702
Smoking status	Nonsmoker	Smoker + former smoker	−0.037	(−0.156; 0.082)	0.538	−0.185	(−0.346; −0.024)	**0.025**
Education	Basic	Secondary	−0.004	(−0.113; 0.106)	0.948	0.062	(−0.091; 0.214)	0.420
	Basic	University	−0.004	(−0.107; 0.099)	0.942	0.048	(−0.096; 0.191)	0.509
Infection	No	Yes	0.004	(−0.091; 0.100)	0.928	0.071	(−0.060; 0.202)	0.282
Infant gender	Female	Male	−0.041	(−0.123; 0.041)	0.317	−0.055	(−0.170; 0.060)	0.341
rs16835198	GG	GT	−0.012	(−0.101; 0.078)	0.797	0.077	(−0.040; 0.194)	0.192*
	GG	TT	0.096	(−0.035; 0.226)	0.147			
rs726344	GG	GA + AA	0.015	(−0.072; 0.103)	0.726	−0.046	(−0.172; 0.080)	0.467
rs3480	AA	AG	−0.069	(−0.163; 0.025)	0.148	0.042	(−0.111; 0.195)	0.583
	AA	GG	−0.030	(−0.131; 0.070)	0.547	0.013	(−0.164; 0.190)	0.883
rs1746661	GG	GT + TT	−0.001	(−0.083; 0.081)	0.979	0.035	(−0.087; 0.157)	0.569

Univariable logistic regression analysis investigates 13 variables as potential confounders in association with maternal/cord blood irisin concentrations. As only a few cases of TT and AA homozygotes were identified (in rs726344 and rs1746661, resp.), the analysis was performed as A (GG versus GA + AA) and T (GG versus GT + TT) dominant models and the genotype categories were merged. Significant results are in bold. *GG versus GT + TT (T dominant model).

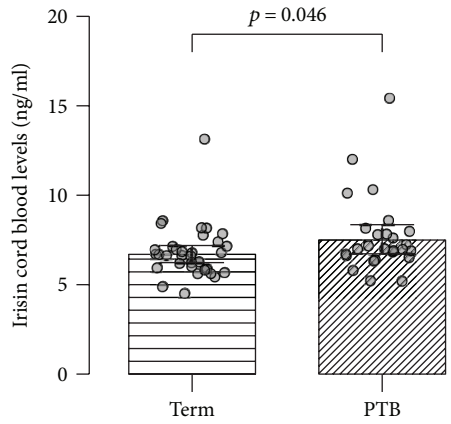

Figure 2: Comparison of irisin levels in umbilical cord blood between mothers with term and preterm delivery. Individual points represent irisin concentrations in cord blood samples of mothers with term and preterm (PTB) deliveries. Irisin concentrations are presented as estimated mean values with 95% confidence intervals.

by muscle tissue [11, 12], and because parity influences body fat distribution and BMI [41, 42], we also assume change in muscle mass and thus an alteration in circulating irisin level.

4.3. Maternal Irisin Level during Pregnancy. It has further been suggested that irisin is involved in the physiology of pregnancy. During all three trimesters, irisin precursor is expressed in the placenta and the irisin serum level is higher in pregnant women compared to nonpregnant ones throughout the entire pregnancy [18, 25]. Increased maternal serum irisin during pregnancy may either be explained by placental production or it may constitute a compensatory response caused by irisin resistance during gestation [25]. With respect to the dynamics of irisin level during pregnancy, Garcés et al. reported a significant increase of approximately 16% occurring between early (weeks 11–13) and middle pregnancy (weeks 24-25) and an increase of approximately 21% between early and late pregnancy (weeks 38–40). No differences between middle and late pregnancy were detected [25]. Therefore, Garcés et al.'s results indicate a growing trend of irisin concentration during normal pregnancy.

Based on the present study, maternal irisin does not seem to be associated with the pathophysiology of PTB. On the other hand, an association between irisin level and different pregnancy complications has been reported before. Lower irisin serum concentrations were reported in mothers with GDM compared to mothers with uncomplicated pregnancies in the first trimester [16], between weeks 24–28 [21] and weeks 26–30 of pregnancy [18] as well as at term [22]. On the other hand, no significant differences between mothers with GDM and uncomplicated pregnancies in irisin levels during the second trimester [16], between weeks 24–28 [15] and at term [21], were reported. Likewise, irisin level was studied in association with PE. While Garcés et al. reported decreased maternal irisin concentration in the third trimester in preeclamptic women compared to physiological pregnancies, no differences within the group of mothers in the first and second trimester were observed [25]. In another study, no significant differences were found between PE mothers and controls before delivery [26]. Therefore, even though most studies suggest that irisin is involved in the

Table 4: Multivariable linear regression model with maternal and cord blood irisin levels as dependent variables.

Variable	Reference category	Comparative category	Mother			Infant		
			β	95% CI	p value	β	95% CI	p value
PTB status	Term	Preterm	−0.026	(−0.227; 0.175)	0.796	0.212	(−0.059; 0.483)	0.122
Maternal age			−0.003	(−0.016; 0.010)	0.649	0.000	(−0.017; 0.016)	0.958
Preconception BMI			−0.001	(−0.011; 0.010)	0.915	0.000	(−0.013; 0.013)	0.955
Gestational age			0.001	(−0.022; 0.024)	0.935	0.008	(−0.022; 0.038)	0.591
Primiparity	No	Yes	−0.130	(−0.257; −0.002)	**0.046**	−0.030	(−0.195; 0.136)	0.718
Smoking status	Nonsmoker	Smoker + former smoker	0.017	(−0.161; 0.194)	0.852	−0.176	(−0.371; 0.019)	0.076
Education status	Primary	Secondary	−0.024	(−0.158; 0.110)	0.719	0.067	(−0.105; 0.238)	0.437
		University	0.045	(−0.101; 0.191)	0.536	0.083	(−0.098; 0.263)	0.361
Infection	No	Yes	0.001	(−0.121; 0.124)	0.982	0.055	(−0.103; 0.214)	0.485
Infant gender	Women	Men	−0.063	(−0.172; 0.046)	0.251	−0.012	(−0.158; 0.135)	0.873
rs16835198	GG	GT	0.032	(−0.095; 0.159)	0.613	0.157	(−0.007; 0.321)	0.061*
		TT	0.178	(−0.040; 0.396)	0.106			
rs726344	GG	GA + AA	0.035	(−0.084; 0.154)	0.555	−0.140	(−0.298; 0.019)	0.082
rs3480	AA	AG	−0.081	(−0.225; 0.064)	0.267	0.082	(−0.109; 0.273)	0.389
		GG	−0.093	(−0.282; 0.096)	0.327	0.181	(−0.104; 0.467)	0.207
rs1746661	GG	GT + TT	0.117	(−0.030; 0.264)	0.116	−0.001	(−0.154; 0.153)	0.992

In the multivariable linear regression model, maternal and cord blood irisin concentrations were investigated in relation to the 13 variables listed in the table. In the case of categorical variables, a reference category was selected and compared with one or two other categories. As only a few cases of TT and AA homozygotes were identified (in rs726344 and rs1746661, resp.), analysis was performed as A (GG versus GA + AA) and T (GG versus GT + TT) dominant models and the genotype categories were merged. Significant results are in bold. *GG versus GT + TT (T dominant model).

pathophysiology of pregnancy, the role of maternal irisin in pregnancy complications remains unclear.

Our findings that irisin level in maternal peripheral blood is not associated with PTB occurrence or the other variables (except for parity) are in agreement with Garcés et al. who observed that maternal irisin level is significantly related only to insulin sensitivity during pregnancy regardless of gestational age and other variables [25]. Maternal irisin levels measured in the present study are in agreement with an existing study by Szumilewicz et al. who reported a mean irisin concentration of 14.78 ng/ml in pregnant women [43], which is consistent with our results (11.7 ± 2.0 ng/ml).

4.4. Cord Blood Irisin Level. Using a univariable model, we discovered a positive association between cord blood irisin concentration and PTB occurrence in the studied cohort, that is, a higher irisin level in preterm infants compared with term deliveries. Onset of labor constitutes a strong stimulus for the release of irisin into maternal and fetal circulations [44] and could increase cord blood irisin level by nearly 40% [45]. It has been suggested that increased irisin release into cord blood may be caused by temporary utero-placental ischemia during vaginal delivery, thus leading to fetal stress [44]. Similarly, fetal stress and increased cord blood irisin level secretion could occur during PTB. This mechanism could explain the positive association between cord blood irisin level and PTB occurrence observed in the present study. From another point of view, irisin improves glucose homeostasis and could compensate for metabolic changes during pregnancy [43]. Pregnancy has also been associated with increased insulin resistance [46]. Therefore, irisin could be part of a pathway maintaining glucose homeostasis during labor. Increased irisin levels detected in PTB infants in the present study suggest the impairment of glucose homeostasis which leads to PTB or, conversely, the impairment of glucose homeostasis due to PTB occurrence.

4.5. Irisin ELISA Kits. It is important to note that many studies based on commercial ELISA kits have evaluated irisin levels in different biological fluids; however, these studies were later called into question by Albrecht et al. (2015), demonstrating that, in addition to irisin, commercial ELISA kits also detect nonspecific cross-reacting proteins. Furthermore, the same group provided evidence against the physiological effect of irisin in the human body [47]. Nevertheless, skepticism regarding irisin was refuted and the reliability of the irisin ELISA assays was confirmed [48, 49]. Jedrychowski et al. detected and quantified irisin using mass spectrometry providing strong evidence that irisin is a true circulating protein [50]. Moreover, they offered a method that could be used as a gold standard to evaluate irisin ELISA kit validity. In terms of irisin ELISA kits, it was reported that the Aviscera irisin ELISA kit (now available from Phoenix Pharmaceuticals) correctly detected spiked irisin at physiological concentrations [34, 49]. Kits using this particular antibody were able to correctly discern both endogenous and exogenous irisin within the physiological range in humans [51, 52]. Also worth mentioning, the ELISA kit used in the present study (Phoenix Pharmaceuticals, EK-067-29) has been further validated by Western blot and verified by MALDI-TOF mass spectrometry [53].

Another limitation of the study could possibly be the partial degradation of irisin prior to analysis as no protease inhibitor was used after blood sampling (as recommended

by the manufacturer of the ELISA kit (EK-067-29)). However, all the samples in our study (from the participants delivering at term as well as PTBs) were sampled using the same sampling scenario in the same facility by the same specialists so the effect observed could be explained theoretically only by huge variability in irisin degradation between the subjects which we do not presume. Moreover, Cavalier et al. (2014) reported stable irisin level during −80°C storage both with and without aprotinin for one month [54]. We therefore presume irisin should be stable after long-term storage at −80°C even without the use of protease inhibitor.

5. Conclusions

To the best of our knowledge, this is the first study to compare irisin levels between mothers with preterm and term deliveries. We investigated maternal and cord blood irisin levels in mothers with preterm and term deliveries and detected significantly higher irisin levels in maternal peripheral blood (11.6 ± 2.0 ng/ml) compared to cord blood (7.2 ± 1.9 ng/ml). When comparing PTB with mothers who delivered at term using a univariable model, we found an association between PTB and cord blood irisin concentration. Finally, we found no correlation between any of the selected SNPs and irisin blood concentration.

Data Availability

The authors declare that the data supporting the findings of this study are available within the article or are available from the corresponding author upon reasonable request.

Conflicts of Interest

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

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Research Article

Plasma Neuregulin 4 Levels Are Associated with Metabolic Syndrome in Patients Newly Diagnosed with Type 2 Diabetes Mellitus

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Neuregulin 4 (Nrg4) has been proposed to play a role in the pathogeneses of obesity, insulin resistance, and dyslipidemia. However, information about the link between Nrg4 and metabolic syndrome (MetS) is scarce, especially in patients with newly diagnosed type 2 diabetes mellitus (nT2DM). This study aimed at investigating whether Nrg4 is associated with MetS in nT2DM patients. A total of 311 patients with nT2DM were recruited. Plasma Nrg4 concentration was determined by ELISA. Plasma Nrg4 concentration was lower in nT2DM patients with MetS than in nT2DM patients without MetS ($P = 0.001$). Nrg4 concentration showed negative correlations with most of the analyzed indicators of MetS. MetS was less prevalent among subjects in the highest quartile of plasma Nrg4 concentration than among those in the lowest quartile ($P < 0.01$). Age- and sex-adjusted plasma Nrg4 concentrations were positively correlated with concentrations of high-density lipoprotein cholesterol (HDL-C) and apolipoprotein A (both $P < 0.05$) and negatively correlated with triglyceride, high-sensitivity C-reactive protein (hs-CRP), and gamma-glutamyltransferase concentrations, neutrophil count, and white blood cell (WBC) count (all $P < 0.05$). In multivariate analysis, Nrg4 was independently associated with hs-CRP level, WBC count, and HDL-C level ($P = 0.001$ or $P < 0.05$). Multiple logistic regression analysis of MetS prediction by Nrg4 revealed an odds ratio of 0.560 (95% CI: 0.374–0.837; $P < 0.01$). Decreased plasma Nrg4 levels, which may be associated with augmented oxidative stress, inflammation, and dyslipidemia, might be involved in the development of MetS in nT2DM patients.

1. Introduction

Metabolic syndrome (MetS) is characterized by a cluster of metabolic disorders, including insulin resistance (IR), hyperglycemia, dyslipidemia, central obesity, and hypertension. These disorders are important risk factors of type 2 diabetes mellitus (T2DM), cardiovascular diseases, and all-cause mortality among adults and children [1, 2]. With the worldwide spread of obesity and T2DM, MetS is increasingly being seen as an important public health problem. Overall prevalence of MetS ranges from 21.9% to 49.4% in the general populations of the United States, Europe, and Asia (Thailand, Mainland China, and Hong Kong) [3]. Although IR is considered to be a key factor in the disease, the precise pathogenic mechanism of MetS is not clear. No specific or effective prevention and therapeutic strategies are available for MetS. Thus, it is imperative to identify novel biomarkers to identify MetS.

Adipose tissue is an active endocrine organ secreting a host of bioactive adipokines, including adiponectin and leptin. Adipokines modulate glucose and lipid metabolism, inflammation, and insulin sensitivity and, thus, might be involved in the pathogeneses of IR, diabetes, and MetS [4–6]. Neuregulin 4 (Nrg4) is a secreted water-soluble protein that has been found in the circulation. Nrg4 is expressed in multiple organs, with the highest expression levels in brown adipose tissue [7, 8]. A member of the

epidermal growth factor (EGF) family of extracellular ligands, Nrg4 binds to and activates receptor tyrosine kinases ErbB3 and ErbB4 and acts as an autocrine, paracrine, or endocrine signal by releasing the EGF-like domain after photolytic cleavage [6–12]. Nrg4 has many biological functions, including the inhibition of apoptosis and inflammation and the promotion of neurite outgrowth [11, 13–17]. Rosell and Kaforou [17] showed that Nrg4 is upregulated in cold-induced beige/brite cells and highly expressed during brown adipocyte differentiation. Nrg4 mRNA expression was downregulated in the adipose tissues of several obese mouse models and negatively correlated with the percentage of body fat mass and liver fat content in humans [6, 8, 18]. These observations suggest that Nrg4 insufficiency may be a common feature of obesity. Furthermore, Nrg4-deficient mice fed a high-fat diet exhibited significant increases in body weight (BW), plasma triacylglyceride and fasting blood glucose levels, and worsening of IR and fatty liver. Nrg4-overexpressing mice displayed the opposite results in liver and adipose tissues [7, 8]. These results demonstrate that Nrg4 may play a crucial role in the regulation of insulin sensitivity, energy balance, and glucose and lipid metabolism.

Decreased Nrg4 levels may lead to the development of IR, T2DM, and MetS. A recent study demonstrated that Nrg4 mRNA levels in subcutaneous and visceral adipose tissues were significantly lower in patients with impaired glucose tolerance or T2DM than in normal individuals [8]. These findings suggest that Nrg4 may work as a novel adipokine protecting metabolic homeostasis. However, two recent clinical studies found that serum Nrg4 levels were significantly higher in T2DM patients than in healthy controls and were positively correlated with waist circumference (WC), fasting plasma glucose (FPG) and triglyceride (TG) concentrations, blood pressure, and IR [19, 20]. The discrepant findings might be due to differences in study design and methodology.

Recently, Dai et al. [5] indicated that patients with nonalcoholic fatty liver disease (NAFLD) had lower serum levels of Nrg4 than non-NAFLD controls. Decreased serum Nrg4 level was an independent risk factor of NAFLD, which currently is considered to be the hepatic manifestation of IR and MetS [5]. These findings strongly suggest that decreased Nrg4 may contribute to the pathogenesis of MetS. To our knowledge, only one cross-sectional study has investigated the relationship between Nrg4 and MetS in obese adults, showing decreased serum Nrg4 levels in MetS subjects with obesity [6]. No study has analyzed the association between plasma Nrg4 levels and risk of MetS in normal weight, overweight, or obese patients with T2DM. Thus, the purpose of this study was to evaluate the determinants and associations of plasma Nrg4 concentration with MetS characteristics, as defined by the Chinese Medical Association/Chinese Diabetes Society (CDS) diagnostic criteria, in newly diagnosed T2DM (nT2DM) patients.

2. Materials and Methods

2.1. Study Design and Subjects. A total of 311 patients with nT2DM were recruited to the study. Diagnosis of T2DM was made on the basis of oral glucose tolerance tests (OGTTs) and 1998 WHO diagnostic criteria. Subjects with nT2DM were not treated with hypoglycemic agents or insulin. All participants completed a standardized questionnaire regarding their medical history and lifestyle factors (smoking and alcohol) and underwent a comprehensive health examination according to standard procedures. Exclusion criteria were as follows: patients with (1) type 1 diabetes mellitus or other endocrine disorder, (2) acute complications of T2DM, gall bladder, or biliary tract disease, viral hepatitis, alcoholic liver disease, drug-induced liver disease, autoimmune hepatitis, liver and renal dysfunction, acute or chronic inflammatory disease, pregnancy, or cardiovascular or cerebral vascular disease, (3) history of use of lipid-lowering and antihypertensive drugs, and (4) total parenteral nutrition, smoking habit, or alcohol consumption. Hypertension without antihypertensive drug treatment was not an exclusion criterion. All nT2DM patients were categorized into quartiles based on their plasma Nrg4 level: quartile 1, Nrg4 < 1.97 ng/ml; quartile 2, 1.97 ng/ml ≤ Nrg4 < 2.80 ng/ml; quartile 3, 2.80 ng/ml ≤ Nrg4 ≤ 4.10 ng/ml; quartile 4, Nrg4 > 4.10 ng/ml. The study protocol followed the ethical guidelines of the 1964 Declaration of Helsinki and was approved by the human research ethics committee of the Affiliated Hospital of Southwest Medical University. Written informed consent was obtained from all patients.

2.2. Definition of MetS. MetS was defined by the CDS criteria [21]. For a diagnosis of MetS, participants had to meet three or more of the following criteria: (1) BW in the overweight or obese range, defined as a body mass index (BMI) ≥ 25.0 kg/m^2; (2) hyperglycemia, defined as FPG ≥ 6.1 mmol/l and/or 2 h plasma glucose (2hPG) ≥ 7.8 mmol/l, or previously diagnosed T2DM and receiving treatment; (3) hypertension, defined as systolic blood pressure (SBP)/diastolic blood pressure (DBP) ≥ 140/90 mmHg, or previously diagnosed hypertension and receiving treatment; and (4) dyslipidemia, defined as TG ≥ 1.7 mmol/l and/or high-density lipoprotein cholesterol (HDL-C) < 0.9 mmol/l (men) or <1.0 mmol/l (women). Patients with nT2DM were considered to have MetS if they had two or more of the above factors except nT2DM.

2.3. Anthropometric and Biochemical Measurements. Anthropometric measurements were performed in all participants before breakfast. BW (kg), height (m), BMI, SBP, and DBP were measured by standard methods, as described previously [22]. Body fat percentage (BF%) was calculated using the following equation [23]: $\mathrm{BF\%} = 1.20 \times \mathrm{BMI} - 10.8 \times \mathrm{sex}$ (male = 1, female = 2) + 0.23 × age (year) − 5.4.

Blood samples were collected from participants in the morning, either after an overnight fast or 2 h after beginning the 75 g OGTT. Plasma samples were collected by centrifugation at 4°C and stored at −80°C until analytical processing. FPG, 2hPG, and glycated hemoglobin A1C (HbA1c) levels were measured by the glucose oxidase method and anion-exchange HPLC, respectively. Total cholesterol (TC), TG, HDL-C, low-density lipoprotein cholesterol (LDL-C), apolipoprotein A (apoA), and apolipoprotein B (apoB) levels were

analyzed enzymatically by using a 7060 full-automatic biochemical analyzer (Hitachi, Tokyo, Japan). Alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), and alkaline phosphatase (ALP) levels were also analyzed. White blood cell (WBC), neutrophil, and lymphocyte counts were determined by using an automated blood cell counter (Mindray BC-6800, Shenzhen, China), according to the manufacturer's instructions. The neutrophil to lymphocyte ratio (NLR) was calculated as the simple ratio between the absolute neutrophil and lymphocyte counts. High-sensitivity C-reactive protein (hs-CRP) levels were measured by latex-enhanced immunoturbidimetric assay. Fasting plasma insulin (FIns) concentrations were measured with an electrochemiluminescence immunoassay (Roche Elecsys Insulin Test, Roche Diagnostics, Mannheim, Germany). The apoB to apoA ratio (apoB/apoA) was calculated as the simple ratio between apoB and apoA. The triglyceride glucose (TyG) index was calculated as the natural logarithm of [TG (mg/dl) × FPG (mg/dl)/2] [24]. Homeostasis model assessment of insulin resistance (HOMA-IR) and homeostasis model assessment of β-cell insulin secretion (HOMA-IS) were calculated from FIns and FPG levels by the following formulas [4]: HOMA-IR = FIns (μU/ml) × FPG (mmol/l)/22.5 and HOMA-IS = [20 × FIns (μU/ml)]/[FPG (mmol/l) − 3.5].

2.4. Plasma Nrg4 Measurement. Plasma Nrg4 concentrations were determined with an enzyme-linked immunosorbent assay (ELISA) (Aviscera Biosciences, Santa Clara, CA), following the manufacturer's protocol. This assay has been shown to be highly sensitive to human Nrg4, with a sensitivity of 0.125–0.25 ng/ml. The linear range of the standard was 0.25–16.0 ng/ml. Intra- and inter-assay variations were both less than 10%.

2.5. Statistical Analysis. All analyses were performed with the Statistical Package for Social Sciences version 20.0 (SPSS, Chicago, IL). All data distributions were analyzed for normality by the Kolmogorov–Smirnov test. Data are expressed as the mean ± standard deviation (SD) for continuous variables or percentage (%) for categorical variables, unless otherwise specified. Two groups were compared by chi-square (χ^2) tests for categorical variables, Student's t-test for normally distributed continuous variables, or Mann–Whitney U tests for nonparametric distributed continuous variables. More than two groups were compared by one-way analysis of variance (ANOVA) followed by the post hoc least significant difference test for normally distributed continuous variables and Kruskal–Wallis test followed by adjustment for multiple pairwise comparisons for nonparametric distributed covariates. Spearman's correlation coefficients were used to describe associations between plasma Nrg4 concentration and other variables. The partial correlation coefficient was used for sex- and age-adjusted data.

Multiple linear regression was performed to identify variables that were independently associated with plasma Nrg4 concentration. Logistic regression analysis was performed to ascertain the association of Nrg4 concentration with the presence of MetS. In logistic regression, we analyzed all potential confounding variables. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated. All reported P values were two-sided, and a P value of < 0.05 was considered statistically significant.

3. Results

3.1. Clinical and Biochemical Characteristics of nT2DM Patients with or without MetS. Table 1 summarizes the anthropometric and biochemical parameters of the 311 nT2DM patients enrolled in the cross-sectional study. Compared to nT2DM patients without MetS, nT2DM patients with MetS had higher levels of BMI, BF%, SBP, DBP, TC, TG, apoB, FPG, 2hPG, ALT, GGT, WBC count, hs-CRP, FIns, HOMA-IR, apoB/apoA, and TyG index ($P < 0.001$ or $P < 0.01$ or $P < 0.05$) and lower levels of HDL-C, apoA, and lymphocyte count ($P < 0.001$ or $P < 0.05$). Age, LDL-C, HbA1c, ALP, NLR, neutrophil count, and HOMA-IS did not significantly differ between the groups.

3.2. Plasma Nrg4 Levels Depending on MetS Components in nT2DM Patients. Plasma Nrg4 levels were lower in nT2DM patients with MetS than in those without MetS ($P = 0.001$; Figure 1(a)). Furthermore, plasma Nrg4 levels were significantly lower in nT2DM patients with elevated TG levels ($P < 0.01$; Figure 1(c)), decreased HDL-C levels ($P < 0.05$; Figure 1(d)), and overweight or obese patients ($P < 0.01$; Figure 1(e)) compared to their controls. Plasma Nrg4 levels decreased in a stepwise fashion as the number of MetS components increased (P for trend < 0.01; Figure 1(f)). Subjects with four or five components of MetS had lower plasma Nrg4 levels than those with only one component ($P < 0.01$). However, there was no significant difference in plasma Nrg4 levels in the context of hypertension ($P > 0.05$; Figure 1(b)).

3.3. Clinical and Biochemical Characteristics in nT2DM Patients according to Plasma Nrg4 Quartile. Table 2 presents the clinical and biochemical characteristics distinguished by quartile of plasma Nrg4 concentration. TG, HDL-C, apoA, GGT, hs-CRP, and Nrg4 concentrations and WBC count were significantly different between patients in different plasma Nrg4 quartiles ($P < 0.001$ or $P < 0.01$ or $P < 0.05$). Compared to subjects in the lowest quartile of plasma Nrg4 concentration, patients in the highest quartile had lower levels of BMI, TG, GGT, WBC count, and hs-CRP ($P < 0.01$ or $P < 0.05$) and higher levels of plasma Nrg4, HDL-C, and apoA ($P < 0.001$ or $P < 0.01$ or $P < 0.05$). Prevalence of MetS was markedly lower in subjects in the highest plasma Nrg4 quartile than in subjects in the lowest quartile ($P < 0.01$). However, prevalence of overweight or obesity, hypertension, elevated TG levels, and decreased HDL-C levels showed no significant differences across quartiles of plasma Nrg4 concentration.

3.4. Linear Regression Analyses of Variables Associated with Plasma Nrg4 Levels in All nT2DM Patients. We investigated the relationship between plasma Nrg4 concentration and the other parameters. Plasma Nrg4 levels were correlated positively with HDL-C and apoA levels but negatively with TG, hs-CRP, and GGT levels and WBC and

Table 1: Clinical and biochemical characteristics of nT2DM patients with and without MetS.

Covariate	Without MetS ($n = 133$)	With MetS ($n = 178$)	P value
Male/female	65/68	83/95	0.696
Age (years)	53.21 ± 10.36	54.06 ± 9.86	0.464
BMI (kg/m^2)	22.71 ± 2.43	26.96 ± 3.24	<0.001
BF% (%)	26.10 ± 4.87	31.06 ± 4.61	<0.001
SBP (mmHg)	121.84 ± 9.90	129.79 ± 16.34	<0.001
DBP (mmHg)	71.99 ± 8.62	76.42 ± 9.48	<0.001
FPG (mmol/l)	10.11 ± 3.76	11.00 ± 3.59	0.036
2hPG (mmol/l)	17.38 ± 8.32	18.57 ± 5.64	0.017
HbA1c (%)	9.37 ± 2.83	9.38 ± 1.98	0.957
TC (mmol/l)	4.29 ± 0.93	4.77 ± 1.62	0.040
TG (mmol/l)	1.30 ± 0.62	2.85 ± 2.26	<0.001
HDL-C (mmol/l)	1.31 ± 0.29	1.00 ± 0.34	<0.001
LDL-C (mmol/l)	2.63 ± 0.80	2.78 ± 1.02	0.147
apoA (g/l)	1.53 ± 0.33	1.36 ± 0.26	<0.001
apoB (g/l)	0.84 ± 0.21	0.97 ± 0.29	<0.001
ALT (U/l)	22.03 ± 13.45	25.26 ± 13.87	0.006
AST (U/l)	21.72 ± 13.05	22.30 ± 10.53	0.139
GGT (U/l)	35.81 ± 6.21	40.75 ± 2.81	<0.001
ALP (U/l)	88.94 ± 44.47	83.06 ± 38.15	0.389
WBC ($\times 10^9$/l)	6.52 ± 1.77	7.22 ± 2.56	0.004
Neutrophil ($\times 10^9$/l)	4.45 ± 1.59	4.91 ± 2.42	0.148
Lymphocyte ($\times 10^9$/l)	1.51 ± 0.55	1.68 ± 0.58	0.010
NLR	3.51 ± 0.25	3.58 ± 0.29	0.317
hs-CRP (mg/l)	5.98 ± 1.33	6.34 ± 0.96	0.007
FIns (μU/ml)	8.45 ± 4.68	11.04 ± 7.26	0.002
HOMA-IR	3.80 ± 2.61	5.30 ± 3.92	<0.001
HOMA-IS	39.98 ± 37.33	39.63 ± 41.68	0.619
TyG index	4.94 ± 0.30	5.29 ± 0.47	<0.001
apoB/apoA	0.57 ± 0.19	0.73 ± 0.23	<0.001

BMI: body mass index; BF%: body fat percentage; SBP: systolic blood pressure; DBP: diastolic blood pressure; FPG: fasting plasma glucose; 2hPG: 2 h plasma glucose; HbA1c: glycated hemoglobin A1c; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; apoA: apolipoprotein A; apoB: apolipoprotein B; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma-glutamyltransferase; ALP: alkaline phosphatase; WBC: white blood cell; NLR: neutrophil to lymphocyte ratio; hs-CRP: high-sensitivity C-reactive protein; FIns: fasting plasma insulin; HOMA-IR: homeostasis model assessment of insulin resistance; HOMA-IS: homeostasis model assessment of β-cell insulin secretion; apoB/apoA: apolipoprotein A/apolipoprotein B; TyG: triglyceride glucose.

neutrophil counts ($P \leq 0.001$ or $P < 0.01$ or $P < 0.05$). All of these correlations remained statistically significant after adjusting for sex and age (all $P < 0.05$). We performed multiple stepwise regressions to determine which variables were independently associated with plasma Nrg4 concentration. Only hs-CRP level, WBC count, and HDL-C level were independently related to plasma Nrg4 concentration ($P = 0.001$ or $P < 0.05$), with a multiple regression equation of $Y_{\text{Nrg4}} = 3.449 - 0.194X_{\text{hs-CRP}} - 0.131X_{\text{WBC}} + 0.646X_{\text{HDL-C}}$ (Table 3).

Multivariable-adjusted ORs for the association of plasma Nrg4 concentration with increased presence of MetS are shown in Table 4. Risk of presence of MetS decreased by 15% per 1 SD increase in plasma Nrg4 levels. The OR of MetS as predicted by Nrg4 concentration in the presence of all potential confounding variables was 0.560 (95% CI: 0.374–0.837; $P < 0.01$), indicating that there was a 44% decrease in the odds of having MetS for each 1 ng/ml increase in Nrg4 levels.

4. Discussion

Plasma concentrations of Nrg4, a novel adipokine expressed in adipose tissues (especially brown adipose tissue), may be associated with obesity, IR, T2DM, lipid metabolism, inflammation, and atherosclerosis [6, 8, 9, 15]. When fed a high-fat diet, mice overexpressing Nrg4 specifically in the liver and adipose tissues gained significantly less weight than control mice and exhibited improvements in glucose tolerance, lipid metabolism, and insulin sensitivity, and decreased hepatic steatosis [8, 18]. In humans, several studies found that Nrg4 mRNA levels were significantly lower in the subcutaneous

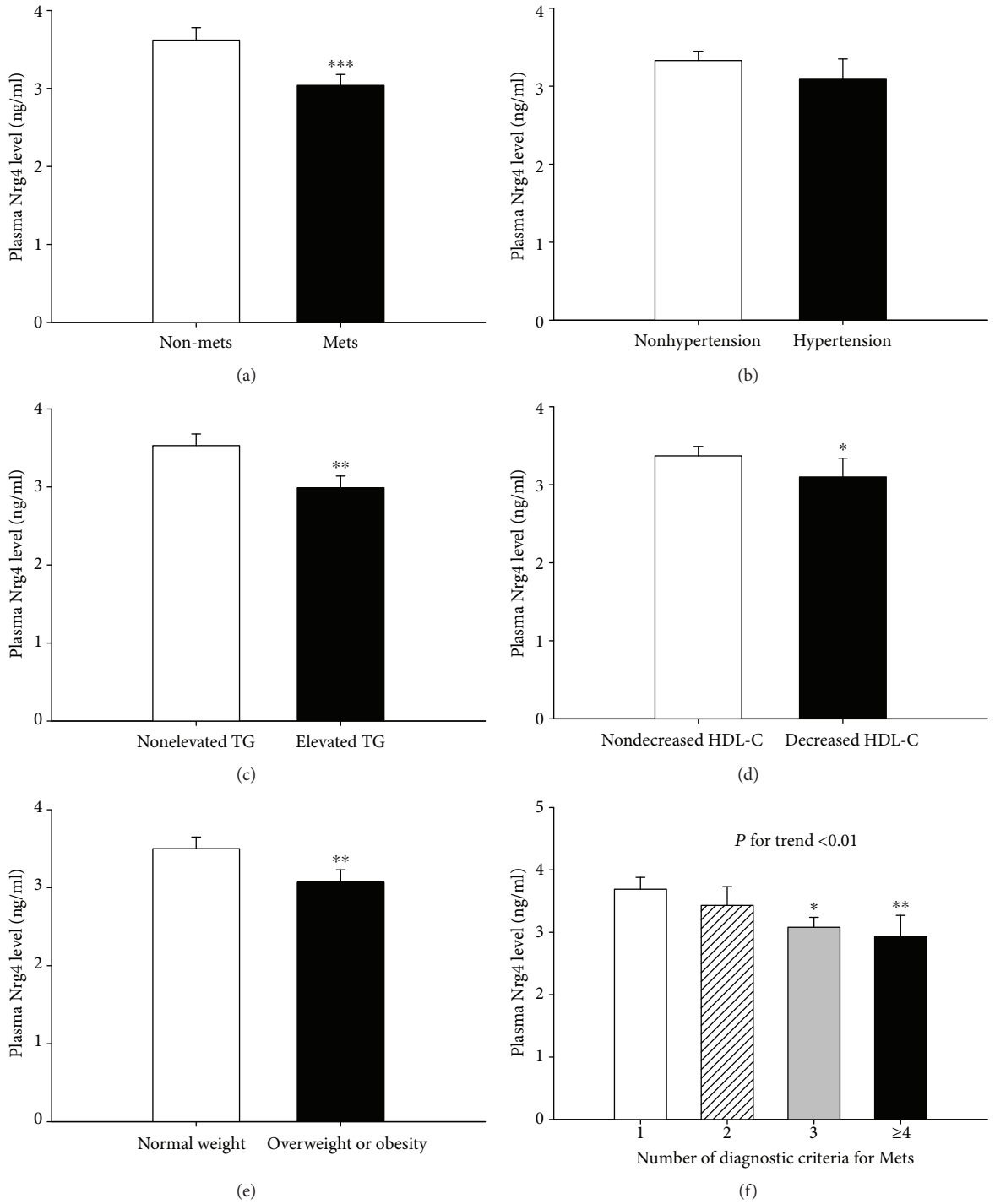

Figure 1: Plasma Nrg4 concentrations for nT2DM patients (a) with ($n = 178$) or without MetS ($n = 133$), (b) with ($n = 52$) or without hypertension ($n = 259$), (c) with ($n = 141$) or without elevated TG levels ($n = 170$), (d) with ($n = 91$) or without decreased HDL-C levels ($n = 220$), (e) with normal weight ($n = 157$) or with overweight or obesity ($n = 154$), (f) with one ($n = 99$), two ($n = 34$), three ($n = 131$), or four or more components of MetS ($n = 47$). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P = 0.001$ versus nT2DM patients without MetS or without elevated TG levels or without decreased HDL-C levels or with normal weight or with one component of MetS.

and visceral adipose tissues of individuals with impaired glucose tolerance and T2DM. Moreover, Nrg4 mRNA or circulating Nrg4 levels were negatively related to the presence of MetS components [6, 8]. Taken together, these previous observations suggest that plasma Nrg4 concentration might be a useful biomarker of obesity-related metabolic and cardiovascular disorders.

In this report, we describe the first clinical study in a Chinese nT2DM population. Consistent with previous findings [6], we observed that plasma Nrg4 levels were

Table 2: Clinical and biochemical characteristics by quartile of plasma Nrg4 concentration in all nT2DM patients.

Covariate Sample size	Quartile 1 77	Quartile 2 78	Quartile 3 79	Quartile 4 77	P value
Nrg4 (ng/ml)	1.49 ± 0.31	2.30 ± 0.24***	3.38 ± 0.38***	5.99 ± 1.69***	<0.001
Male/female	41/36	37/41	33/46	37/40	0.560
Age (years)	53.45 ± 9.61	52.56 ± 10.75	52.85 ± 10.52	55.95 ± 9.11	0.142
BMI (kg/m^2)	26.09 ± 3.73	24.89 ± 3.70*	24.98 ± 3.59	24.61 ± 3.23*	0.054
BF% (%)	29.91 ± 5.39	28.61 ± 5.92	28.57 ± 5.11	28.68 ± 4.76	0.333
SBP (mmHg)	126.03 ± 15.36	127.37 ± 14.88	125.66 ± 14.56	126.52 ± 13.25	0.894
DBP (mmHg)	75.09 ± 10.89	75.09 ± 9.01	73.85 ± 8.87	74.08 ± 8.64	0.766
FPG (mmol/l)	10.48 ± 3.85	11.18 ± 3.95	10.38 ± 3.57	10.44 ± 3.37	0.496
2hPG (mmol/l)	18.29 ± 8.37	18.81 ± 7.40	17.41 ± 5.98	17.75 ± 5.69	0.605
HbA1c (%)	8.90 ± 2.24	9.67 ± 2.53	9.24 ± 2.13	9.61 ± 2.56	0.244
TC (mmol/l)	4.51 ± 1.37	4.43 ± 1.29	4.81 ± 1.76	4.52 ± 1.01	0.503
TG (mmol/l)	2.56 ± 2.48	2.47 ± 2.07	1.95 ± 1.49	1.76 ± 1.27*	0.006
HDL-C (mmol/l)	1.02 ± 0.31	1.12 ± 0.38	1.17 ± 0.36*	1.22 ± 0.34**	0.005
LDL-C (mmol/l)	2.70 ± 0.86	2.50 ± 0.88	2.89 ± 1.08	1.22 ± 0.34	0.124
apoA (g/l)	1.36 ± 0.28	1.38 ± 0.25	1.48 ± 0.34	1.51 ± 0.31	0.020
apoB (g/l)	0.92 ± 0.26	0.91 ± 0.27	0.93 ± 0.31	0.91 ± 0.24	0.979
ALT (U/l)	24.50 ± 16.06	24.54 ± 13.07	24.35 ± 13.93	22.09 ± 11.77	0.551
AST (U/l)	23.26 ± 14.92	21.42 ± 8.91	22.93 ± 12.61	20.60 ± 9.16	0.555
GGT (U/l)	42.82 ± 9.55	45.13 ± 5.83	39.72 ± 4.55	26.80 ± 3.12*	0.013
ALP (U/l)	86.55 ± 44.65	85.85 ± 47.29	88.42 ± 38.42	81.43 ± 32.54	0.563
WBC (×10^9/l)	7.46 ± 2.17	7.19 ± 3.07	6.47 ± 1.74**	6.59 ± 1.78*	0.018
Neutrophil (×10^9/l)	5.04 ± 1.96	5.03 ± 2.98	4.30 ± 1.60	4.50 ± 1.54	0.065
Lymphocyte (×10^9/l)	1.69 ± 0.61	1.53 ± 0.57	1.65 ± 0.53	1.55 ± 0.57	0.240
NLR	3.81 ± 0.44	4.04 ± 0.57	3.06 ± 0.23	3.29 ± 0.20	0.096
hs-CRP (mg/l)	12.64 ± 1.98	6.68 ± 2.07**	3.55 ± 0.88***	1.94 ± 0.54***	<0.001
FIns (μU/ml)	9.75 ± 5.69	11.46 ± 7.94	8.89 ± 5.83	9.64 ± 5.74	0.175
HOMA-IR	4.44 ± 3.06	5.71 ± 4.54	4.15 ± 3.24	4.33 ± 2.68	0.113
HOMA-IS	42.64 ± 5.50	38.50 ± 3.89	34.47 ± 4.27	38.49 ± 4.32	0.755
TyG index	5.17 ± 0.47	5.21 ± 0.43	5.12 ± 0.48	5.05 ± 0.38	0.124
apoB/apoA	0.70 ± 0.24	0.68 ± 0.22	0.66 ± 0.24	0.63 ± 0.20	0.273
MetS, n (%)	55 (71.43)	46 (58.97)	43 (43.04)**	34 (44.16)**	0.007
Components of MetS					
Overweight or obesity, n (%)	43 (55.84)	35 (44.87)	40 (36.71)	29 (37.66)	0.130
Hypertension, n (%)	16 (20.78)	14 (17.95)	11 (13.92)	11 (14.29)	0.623
Elevated TG, n (%)	41 (53.25)	39 (50)	34 (34.18)	27 (35.06)	0.109
Decreased HDL-C, n (%)	31 (40.26)	24 (30.77)	20 (20.25)	16 (20.78)	0.050

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ versus quartile 1 group.

significantly lower in nT2DM patients with MetS than in nT2DM patients without MetS. Plasma Nrg4 levels progressively decreased as the number of components of MetS increased. Prevalence of MetS was significantly lower in subjects in the highest quartile of plasma Nrg4 concentration than in those in the lowest quartile. Plasma Nrg4 levels were positively correlated with a favorable lipid profile (high levels of HDL-C and apoA) and negatively correlated with an unfavorable metabolic profile (high levels of TG). Most importantly, we found that plasma Nrg4 concentration was independently associated with MetS, even after adjusting for all potential confounders. Collectively, these data demonstrate that Nrg4 is an independent protective factor for MetS and that decreased Nrg4 concentration may play an important role in the pathophysiology of MetS. However, the underlying mechanism remains unclear.

IR has been considered to be a key factor for development of MetS. HOMA-IR is a validated and widely used method to evaluate IR from FPG and FIns in large-scale and

Table 3: Linear regression analysis of variables associated with plasma Nrg4 concentration in all nT2DM patients.

Variable	Simple Estimate	P value	Adjusted P value*	Multiple Estimate	P value
Sex	0.040	0.487			
Age	0.099	0.081			
BF%	−0.049	0.392	0.272		
BMI	−0.127	0.025	0.263		
SBP	0.016	0.772	0.674		
DBP	−0.084	0.139	0.685		
TC	0.049	0.386	0.998		
TG	−0.208	<0.001	0.046		
HDL-C	0.194	0.001	0.016	0.119	0.035
LDL-C	0.030	0.600	0.785		
apoA	0.159	0.005	0.028		
apoB	−0.035	0.547	0.750		
apoB/apoA	−0.121	0.034	0.127		
ALT	−0.010	0.864	0.471		
AST	−0.023	0.688	0.534		
GGT	−0.119	0.037	0.049		
ALP	−0.052	0.360	0.333		
WBC	−0.156	0.006	0.012	−0.131	0.020
Neutrophil	−0.118	0.037	0.041		
Lymphocyte	−0.066	0.244	0.377		
NLR	−0.043	0.454	0.150		
hs-CRP	−0.447	<0.001	<0.001	−0.194	0.001
FPG	−0.005	0.935	0.857		
2hPG	−0.046	0.423	0.637		
HbA1c	0.043	0.449	0.103		
TyG index	−0.097	0.087	0.215		
FIns	−0.051	0.366	0.477		
HOMA-IR	−0.039	0.493	0.800		
HOMA-IS	−0.035	0.538	0.637		

*Adjusted for sex and age.

Table 4: Odds ratios for the presence of MetS according to plasma Nrg4 concentration in all nT2DM patients.

	Increased MetS OR (95% CI)	P value
Model 1	0.85 (0.752–0.961)	0.009
Model 2	0.833 (0.717–0.967)	0.016
Model 3	0.750 (0.574–0.980)	0.035
Model 4	0.741 (0.555–0.990)	0.042
Model 5	0.683 (0.492–0.947)	0.022
Model 6	0.560 (0.374–0.837)	0.005

OR: odds ratio; CI: confidence interval. Model 1 unadjusted; model 2 adjusted for sex, age, and parameters of adiposity (BMI and BF%); model 3 adjusted for factors listed in model 2 plus parameters of glycolipid metabolism (SBP, DBP, FPG, 2hPG, HbA1c, TC, TG, HDL-C, LDL-C, apoA, and apoB); model 4 adjusted for factors listed in model 3 plus measures of insulin resistance (apoB/apoA, TyG index, FIns, HOMA-IR, and HOMA-IS); model 5 adjusted for factors listed in model 4 plus markers of inflammation (WBC count, neutrophil count, lymphocyte count, NLR, and hs-CRP); model 6 adjusted for factors listed in model 4 plus biomarkers of liver function (ALT, AST, GGT, and ALP).

epidemiological studies [24, 25]. The TyG index was shown to be more sensitive and specific for IR than the euglycemic-hyperinsulinemic clamp [25]. The apoB/apoA ratio was significantly associated with IR in nondiabetic subjects in the United States, independent of traditional risk factors, MetS components, and inflammatory risk factors [26]. As expected, we found that compared to nT2DM patients without MetS, those with MetS had elevations of indicators of IR (FIns, HOMA-IR, apoB/apoA, and TyG index), suggesting that IR plays an important role in the development of MetS. Unfortunately, we did not find any difference in IR among patients in the four plasma Nrg4 quartiles, nor were plasma Nrg4 concentrations correlated with any index of IR. These data suggest that Nrg4 may not have a direct role in IR. Several previous studies demonstrated that Nrg4 overexpression could attenuate obesity-induced IR in animal models [7–9, 18]. In contrast, two recent clinical studies found that there were no differences in HOMA-IR among obese adults with different quartiles of serum Nrg4 concentration [9] and that circulating Nrg4 concentration was positively correlated with IR [19]. The discrepant findings between our study and those of the previous clinical trials might be due to methodological differences (e.g., in MetS definition, sample size, smoking habit, selected subgroups, and inclusion/exclusion criteria). In the present study, the OR for the association of plasma Nrg4 concentration with increased presence of MetS remained significant even after we adjusted for potential confounders, including all measures of IR. Hence, low plasma Nrg4 concentration seemed to add to the risk of MetS independently of IR, suggesting that plasma Nrg4 may protect against MetS via IR-independent mechanisms.

Accumulating experimental evidence suggests that all of the components of MetS, including IR, T2DM, hypertension, dyslipidemia, and visceral obesity, may increase oxidative stress and reduce antioxidant defenses [27]. Oxidative stress is thought to mediate the development of MetS [28]. GGT, a biomarker of hepatobiliary disease and alcohol consumption/abuse, is the principal enzyme responsible for extracellular catabolism of the antioxidant glutathione [29–31]. Elevated levels of GGT are involved in oxidative stress, lipid peroxidation, and mitochondrial dysfunction [31], and serum GGT activity changes in response to oxidative stress [29]. GGT levels were correlated positively with a marker of oxidative stress (F2-isoprostanes) and negatively with antioxidant levels [30]. Recent epidemiological and experimental data revealed that elevations in serum GGT concentration were associated with the presence of MetS and its components, even after adjustment for alcohol consumption and established risk factors [29, 31]. Consistent with these observations, we found that plasma GGT levels were lower in nT2DM patients with MetS than in those without MetS, suggesting that plasma GGT levels play an important role in the development of MetS. Surprisingly, we also found that plasma GGT levels were significantly different among patients in the four quartiles of plasma Nrg4 concentration, with nT2DM patients in

the highest quartile exhibiting significantly lower levels of GGT than patients in the lowest quartile. Thus, there seems to be cross talk between plasma Nrg4 and GGT levels. Plasma Nrg4 levels remained significantly and negatively correlated with GGT after controlling for sex and age in a partial correlation analysis. Slattery et al. [32] recently reported that *Nrg4* gene expression was upregulated among patients with high oxidative balance scores (lower oxidative stress) compared to patients with lower scores. These results suggest that Nrg4 could potentially be used as a biomarker of oxidative stress and that decreased Nrg4 levels are likely a consequence of the augmented oxidative stress in MetS. Further study of this novel relationship between Nrg4 and GGT is warranted.

Chronic low-grade inflammation has been associated strongly with obesity and IR and, therefore, MetS [33]. A sensitive biomarker of low-grade systemic inflammation, hs-CRP is produced and released by the liver under the stimulation of proinflammatory cytokines [34]. Several studies support the concept that hs-CRP is a predictor of cardiovascular disease and T2DM [33, 35]. High concentrations of hs-CRP have been associated with MetS and its individual components in different populations [35]. WBC count is a routinely used marker of systemic inflammation that recently was reported to be associated with MetS and its individual components [36]. In nondiabetic individuals, lymphocyte count was associated with insulin sensitivity and adiposity [37, 38]. Neutrophil count correlates with hs-CRP concentration better than any other major white cell type [39]. Finally, numerous studies linked elevated levels of WBC subfractions (neutrophil and lymphocyte counts) and NLR to MetS [38]. We found that nT2DM patients with MetS had higher levels of several inflammatory markers (WBC, NLR, and hs-CRP) and lower lymphocyte counts than patients without MetS. These findings confirm the well-known association of these inflammatory markers with MetS in adults. In addition, nT2DM patients in the highest quartile of plasma Nrg4 levels had significantly lower WBC counts and hs-CRP levels than subjects in the lowest quartile. Thus, there seems to be a potential relationship between plasma Nrg4 levels and inflammation. Nrg4 may regulate the expression of proinflammatory cytokines in adipose tissue and influence circulating levels of WBCs and hs-CRP. Partial correlation analysis confirmed that the plasma Nrg4 levels were significantly and negatively correlated with hs-CRP level, WBC count, and neutrophil count after controlling for sex and age. Therefore, Nrg4 may play a key regulatory and anti-inflammatory role in development of MetS. Most importantly, hs-CRP and WBC were independently related to plasma Nrg4 levels. Taken together, these results demonstrate that proinflammatory cytokines may suppress Nrg4 expression in adipocytes. Thus, the reduction of plasma Nrg4 concentration is likely a consequence of augmented proinflammatory cytokine signaling in obesity [8]. Consistent with this hypothesis, recent studies demonstrated the interaction of Nrg4 with inflammation. For example, Bernard et al. [14] showed that Nrg4 expression was decreased in human inflammatory bowel diseases (Crohn disease and ulcerative colitis) and in mouse models of colitis, which may allow for macrophage persistence and ongoing inflammation. Similarly, Feng and Teitelbaum [40] reported reduced levels of Nrg4 in a mouse model of total parenteral nutrition-induced small intestinal inflammation. McElroy et al. [13] found that Nrg4 can protect against experimental necrotizing enterocolitis. Ma et al. [18] observed that Nrg4 overexpression reduced expression of genes encoding macrophage markers and the macrophage chemokine Mcp1 in liver and adipose tissues. Most recently, Schumacher et al. [15] suggested that Nrg4 expression in C57Bl/6 mice was suppressed by active inflammation during experimental colitis and rebounded during the recovery phase. Administration of exogenous Nrg4 reduced colonic macrophage numbers and ameliorated inflammation. Taken together, these previous and our present findings consistently raise the possibility that Nrg4 may have an important anti-inflammatory role through stimulating proinflammatory macrophage apoptosis and reducing gene expression of macrophage markers. The augmentation of inflammation due to Nrg4 downregulation may be important for the development of MetS. Future study is required to confirm our findings.

Obesity and dyslipidemia are the components of MetS that are most often seen in patients with T2DM. Obesity per se is an important risk factor of T2DM development and is associated with low-grade systemic inflammation and dyslipidemia. BMI is commonly used as a standard measurement of overall adiposity in adults [41]. Using both BMI and BF% provided more accurate measurements of the degree of obesity, especially for muscular athletes [42]. Elevated TG and reduced HDL-C levels reflect impaired lipid metabolism and are the main phenotypic features of dyslipidemia in diabetes [43]. Consistent with these findings, we found that compared to nT2DM patients without MetS, patients with MetS had higher levels of parameters of adiposity (BMI and BF%), TC, TG, and apoB, and lower levels of HDL-C and apoA, suggesting that obesity and dyslipidemia play key roles in development of MetS. In addition, patients in the highest quartile of Nrg4 concentration showed significantly lower BMIs and TG levels and significantly higher HDL-C and apoA levels than patients in the lowest quartile. Plasma Nrg4 levels were correlated negatively with TG and positively with HDL-C and apoA levels, even after adjusting for sex and age. These correlations reflect the role of Nrg4 in the modulation of weight and lipid metabolism. Our findings are inconsistent with those of two recent studies [19, 20], which showed that serum Nrg4 levels were or were not positively correlated with parameters of adiposity (BMI, WC, hip circumference, and neck circumference) and TG and were negatively correlated with HDL-C levels. The discrepant findings might be due to methodological differences. Meanwhile, Wang et al. [8] reported that Nrg4 mRNA expression was downregulated in the adipose tissues of several mouse models of obesity and inversely correlated with the percentage of body fat mass and liver fat content in humans. Nrg4 overexpression protected mice from diet-induced weight gain and adiposity and inhibited lipogenesis through liver X receptor (LXR) and sterol-regulatory element binding protein 1c (SREBP1c) and its target genes [8]. Similarly, Ma and colleagues [18] demonstrated that

Nrg4 overexpression increased the expression of adipose triglyceride lipase (*Atgl*) gene and blocked hepatic lipid storage by inhibiting expression of peroxisome proliferator-activated receptor gamma (PPARγ) and its target genes. Consistent with these findings, we found that plasma Nrg4 levels were significantly lower in patients with overweight or obesity or decreased HDL-C or elevated TG levels compared to their controls. Our multiple stepwise regression analyses showed that HDL-C level was independently correlated with plasma Nrg4 level. However, the physiological mechanisms underlying the relationship between Nrg4 and HDL-C remain obscure.

Our study has some limitations. Firstly, our findings must be interpreted with caution because the cross-sectional study design makes it hard to infer causality between plasma Nrg4 levels and MetS. Large prospective studies are needed to verify this potential causal relationship. Secondly, our findings may not be generalizable to other populations because the study was based on Chinese nT2DM patients at a single center. Further studies are warranted to determine the role of plasma Nrg4 in the development of MetS in other populations. Thirdly, the current gold standard to recognize IR, euglycemic-hyperinsulinemic clamp, is less frequently used and difficult to apply in large studies. As Nrg4 is thought to be related to IR, this study failed to examine the relationship between plasma Nrg4 levels and IR, as measured by HOMA-IR, apoB/apoA, and TyG index. Fourthly, our analyses were based on a single determination of plasma Nrg4 level, which is subject to random measurement error and may have underestimated the strength of the associations. Fifthly, newly diagnosed hypertension subjects were not excluded because of their small numbers. Their inclusion or exclusion would not have materially affected the results. Finally, BMI and BF% were used instead of WC to represent abdominal obesity. Although BMI might not represent fat accumulation accurately, BMI $\geq 25\,\text{kg/m}^2$ was equivalent to WC = 85.5 cm (a value similar to the Japanese-specific cutoff point for abdominal obesity) as a predictor of the 5-year incidence of diabetes in Japanese–American men [44]. We believe that this CDS MetS criterion would not change the main implications of the present study.

Our study also has several strengths. This study included a relatively large number of patients. Furthermore, we tentatively defined MetS using CDS criteria appropriate for the Chinese population. Most importantly, our study is, to our knowledge, the first to evaluate the association between plasma Nrg4 level and MetS in Chinese nT2DM patients.

5. Conclusion

Our study examines a possible mechanistic association between the plasma Nrg4 level and MetS in nT2DM subjects. Plasma Nrg4 level could be a potential biomarker for the development of MetS. Further prospective studies are required to confirm the contribution of Nrg4 to the development of MetS. If this correlation were to be confirmed, increasing the levels of circulating Nrg4 might be crucial for the prevention or management of MetS and its related diseases.

Conflicts of Interest

The authors have no relevant conflicts of interest to disclose.

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