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Research Article

Relationship between Hyponatremia and Peripheral Neuropathy in Patients with Diabetes

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Objectives. Hyponatremia is a common complication of diabetes. However, the relationship between serum sodium level and diabetic peripheral neuropathy (DPN) is unknown. This study was aimed at investigating the relationship between low serum sodium level and DPN in Chinese patients with type 2 diabetes mellitus. *Methods*. A retrospective study was performed on 1928 patients with type 2 diabetes between 2010 and 2018. The multivariate test was used to analyze the relationship between the serum sodium level and the nerve conduction function. A restricted cubic spline was used to flexibly model and visualize the relationship between the serum sodium level and DPN, followed by logistic regression with adjustment. *Results*. As the serum sodium level increased, the prevalence of DPN had a reverse J-curve distribution with the serum sodium levels (69.6%, 53.7%, 49.6%, 43.9%, and 49.7%; $P = 0.001$). Significant differences existed between the serum sodium level and the motor nerve conduction velocity, sensory nerve conduction velocity, part of compound muscle action potential, and sensory nerve action potential of the participants. Compared with hyponatremia, the higher serum sodium level was a relative lower risk factor for DPN after adjusting for several potential confounders (OR = 0.430, 95%CI = 0.220 – 0.841; OR = 0.386, 95%CI = 0.198 – 0.755; OR = 0.297, 95%CI = 0.152 – 0.580; OR = 0.376, 95%CI = 0.190 – 0.743; all $P < 0.05$). Compared with low-normal serum sodium groups, the high-normal serum sodium level was also a risk factor for DPN (OR = 0.690, 95%CI = 0.526 – 0.905, $P = 0.007$). This relationship was particularly apparent in male participants, those aged <65 years, those with a duration of diabetes of <10 years, and those with a urinary albumin – to – creatinine ratio (UACR) < 30 mg/g. *Conclusions*. Low serum sodium levels were independently associated with DPN, even within the normal range of the serum sodium. We should pay more attention to avoid the low serum sodium level in patients with type 2 diabetes mellitus.

1. Introduction

Diabetic peripheral neuropathy (DPN), one of the most common chronic complications of diabetes, occurs in as many as 50% of patients with diabetes [1]. The most common form of DPN is distal symmetric polyneuropathy. Currently, very few drugs can alter the progression of peripheral neuropathy. Even with frequent visits to medical professionals and use of prescription medications, it turns out that the clinical treatment of DPN is often unsatisfactory. Therefore, early diagnosis and prevention are considered to be far more effective than treatment. Nerve conduction velocity (NCV)

studies are used to diagnose and determine the distribution and severity of DPN, as well as identify possible subclinical lesions [2]. In the late stage, diabetic neuropathy is characterized by axonal degeneration, demyelination, and fiber loss [3]. In early diabetes, a modest decrease in NCV is seen.

Additionally, the potential risk factors for DPN need to be elucidated, although it is complex and difficult. Several common risk factors, including elevated blood glucose and glycosylated hemoglobin levels, age, extended disease duration, reduced estimated glomerular filtration rate (eGFR), obesity, hyperlipidemia [4, 5], elevated urinary albumin-to-creatinine ratio (UACR) [6], low serum albumin level, and hyperuricemia [7, 8], have been postulated. However, the more comprehensive cause of DPN remains to be elucidated. Patients with diabetes, especially elderly patients, often experience electrolyte disorders, such as hyponatremia [9]. Sodium is a vital component of the human body. The external sodium ion concentration is approximately nine times that of the inside of the neuron. The constant gradient of membrane concentration maintained by the sodium-potassium pump plays a crucial role in physiological permeation, potential transfer, and neurotransmission [10]. Early studies with small sample populations showed a highly significant relationship between serum sodium and NCV [11]. Low extracellular sodium had an adverse effect on nerve cells, such as osmotic demyelination [12], and was associated with dyskinesia in patients with Parkinson's [13]. In addition, excessive sodium intake highly correlated with macular edema in patients with type 1 diabetes [14]. However, reports on the relationship between DPN and the serum sodium level are limited. Therefore, this study was aimed at investigating the relationship between the serum sodium level and DPN to provide clues for the early screening of DPN.

2. Materials and Methods

2.1. Study Population. In this study, 1928 patients with type 2 diabetes and an average age of 60.10 years were recruited from the Endocrinology and Neurology Department at the First Affiliated Hospital of Fujian Medical University (FMU) from November 1, 2010, to January 1, 2018; the population included 1053 male and 875 female patients. No patients enrolled in the study had used neurotrophic drugs such as mecobalamin, lipoic acid, and epalrestat earlier. Patients with the following conditions were excluded:

(i) Type 1 diabetes, gestational diabetes, and other specific types of diabetes

(ii) Severe and acute complications, including diabetic ketoacidosis; hyperosmolar nonketotic syndrome; gastrointestinal disturbances, such as severe vomiting and diarrhea; infection; fever with diaphoresis; eating disorders; anorexia; acute or chronic heart failure; and an eGFR of $<60\,\text{mL/(min}\cdot 1.73\,\text{m}^2)$

(iii) Other neurological lesions, such as chronic inflammatory response neuropathy, single neuropathy, demyelinating neuropathy, and neuropathy caused by hypothyroidism

(iv) Taking drugs that can cause neurotoxicity, such as hormones and chemotherapy drugs

(v) Accompanying diseases that affect serum and/or urine sodium levels, such as primary aldosteronism, Cushing syndrome, Addison's disease, syndrome of inappropriate antidiuretic hormone secretion, and cerebral infarction

(vi) Patients with a recent history of sodium supplementation

(vii) Patients with corrected blood sodium less than 130.00 mmol/L or more than 150.00 mmol/L (Figure 1). The study was approved by the ethics committee of the First Affiliated Hospital of FMU, MRCTA, and ECFAH of FMU [2017]131, and written informed consent was obtained from the patients

2.2. Diagnostic Criteria. The participants were diagnosed with diabetes mellitus according to the criteria provided by the World Health Organization in 1999 [8]. The corrected blood sodium level of each patient was also calculated according to the blood glucose level: $\text{corrected serum sodium} = \text{serum sodium} + 0.024(\text{blood glucose} \times 18 - 100)$ [15]. The corrected serum sodium level was used instead of the directly measured blood sodium level. The normal blood sodium level was defined as 135.00–145.00 mmol/L, while hyponatremia and hypernatremia were defined as the level of <135.00 mmol/L and >145.00 mmol/L, respectively [16, 17]. Hypertension was defined as a systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg. Patients actively taking antihypertensive drugs were also classified as hypertensive [18]. Atherosclerosis was defined as the protrusion of plaques into the lumen of the echo structure, protrusion of plaques into the lumen of a vessel with abnormal blood flow, or an intimal – medial thickness ≥ 1.3 mm [19]. The ischemic cardiovascular disease (ICVD)% was estimated based on the 10-year ICVD risk assessment method for Chinese people [20].

2.3. Clinical Measurements. Information regarding patient demographic characteristics, disease duration, lifestyle, medical history, and drug use history was obtained from medical records. All patients underwent a physical examination that included height, weight, blood pressure, and a neurological examination. Blood pressure was measured after 15 min rest. Body weight and height were measured with the patient barefoot and wearing light clothes. The body mass index of each patient was also calculated as $\text{BMI} = \text{weight (kg)}/\text{height}^2\ (\text{m}^2)$.

2.4. Biochemical Indices. Blood was obtained from the antecubital vein in all participants between 8.30 and 10.00 a.m. Laboratory tests were done to evaluate the concentration of electrolytes, including sodium, potassium (selective electrode method, Roche), and other biochemical parameters of each patient. All patients completed the electrolyte detection in 10 h of fasting to reduce the impacts of medications and foods. The interassay coefficient of variation for serum sodium was 1%. The estimated glomerular

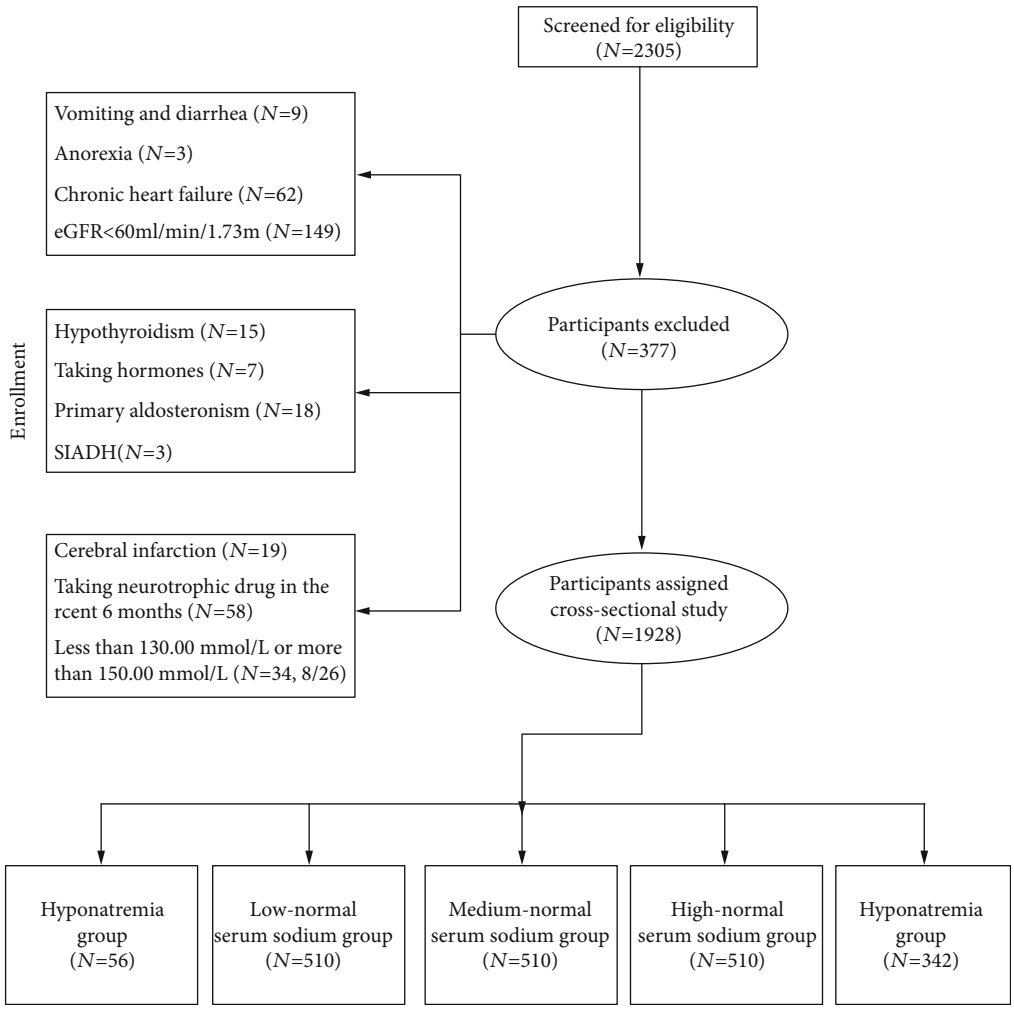

Figure 1: Details of excluded patients.

filtration rate (eGFR) = 186 serum creatinine$^{-1.154}$ × age$^{-0.203}$ (× 0.742 female) [21] of each patient was also calculated. Furthermore, the UACR of each patient was determined in mg/g [22].

2.5. Evaluation of Neuropathy

2.5.1. Neurological Examination. Symptoms of somatic neuropathy were documented, including numbness, burning, deep aching, and unsteadiness in walking. Neurological examinations were completed by the same experienced doctor using age-related evaluation criteria according to standard operations. During the neurological examination, touch sensation was tested using a 10 g monofilament, pain sensation was tested using a pin, reflexes were tested using a tendon hammer, and vibration sensation was tested using a standard 128 Hz tuning fork. Neurological score, neurological reflex score, and sensory function score were recorded using a 2002 Toronto Clinical Scoring System (TCSS) [23].

2.5.2. Nerve Conduction Study. The nerve conduction study of each participant was determined using EMG (Danish Weidi Company, Keypoint). The body temperature of the participants ranged from 30 to 32°C. The patients were subjected to unilateral limb nerve conduction function tests. Then, the median-nerve and ulnar-nerve motor nerve conduction velocity (MCV), sensory nerve conduction velocity (SCV), tibial-nerve and peroneal-nerve MCV, and superficial peroneal-nerve and sural-nerve SCV of each patient were recorded. The corresponding amplitudes of these variables (compound muscle action potential (CMAP)/sensory nerve action potential (SNAP)) were also determined. According to the reference provided by Tang et al. [2, 24] in 1984, nerve conduction slowing was defined as nerve conduction 20% slower than that of the normal average reference value or the occurrence of two or more nerve conduction abnormalities.

2.5.3. DPN Diagnosis. The diagnosis of DPN was based on the criteria proposed by an International European and North American Expert Committee. DPN was defined as patients with diabetes (having or not having clinical symptoms and signs) who had abnormal NCV, including both diagnosis and subclinical DPN [25].

2.5.4. Statistical Analysis. Continuous variables were expressed as mean ± standard deviation (SD) or median

(interquartile range). A chi-square test for comparing distribution was performed. Analysis of variance and Kruskal-Wallis tests were performed to determine the differences in Gaussian variables and non-Gaussian variables. *Post hoc* least significant difference test and Nemenyi test were alternative methods for further pairwise multiple comparisons to locate the source of significance. A multivariate test was used to analyze the relationship between the serum sodium level and the nerve conduction function. Furthermore, logistic regression and forest maps were used to analyze the relationship between DPN and the serum sodium level. Receiver operating characteristic (ROC) curves were configured to establish the cutoff points of the serum sodium level that optimally predicted DPN. Restricted cubic spline was used to flexibly model and visualize the relationship between the serum sodium level and DPN, and an average serum sodium level of 140 mmol/L [26] served as a reference without adjustment. Statistical significance was determined with $P < 0.05$. Statistical analyses were performed using the R software, version 4.0.4.

3. Results

3.1. Study Population Characteristics. Of the 1928 participants with type 2 diabetes mellitus, 56 presented with hyponatremia, 1530 presented with normal serum sodium levels, and 342 presented with hypernatremia. In addition, 960 of the 1928 participants were diagnosed with DPN. The patients were divided into three groups based on the diagnostic criteria, including hyponatremia, hypernatremia, and normal serum sodium groups. The normal serum sodium group was further divided into three groups (tertile 1: 135.0–141.5 ($n = 510$); tertile 2: 141.5–143.0 ($n = 510$); tertile 3: 143.0–145.00 ($n = 510$)) (Table 1). Significant changes were observed in sex, BMI, smoking, drinking history, diuretic use, oral hypoglycemic drug use, FPG, eGFR, Cr level, UACR, and TCSS. Within the normal serum sodium group, the low-normal subgroup exhibited a relatively high DPN detection rate (low-normal subgroup: 53.7%; medium-normal subgroup: 49.6%; and high-normal subgroup: 43.9%; $P < 0.01$). In addition, the hyponatremia group exhibited a higher DPN detection rate compared with the low-normal group ($P < 0.05$). Details of pairwise multiple comparisons are shown in Table 1.

3.2. Changes in Nerve Conduction Function by Varying the Serum Sodium Level. As shown in Table 2, the motor and sensory nerve CV and ulnar and sural-nerve SNAP increased with the increase in the serum sodium level, with some statistically significant differences among different groups.

Tibial and peroneal-nerve CMAP and superficial peroneal-nerve SNAP increased and then decreased. In the normal serum sodium group, the low-normal group exhibited a lower MCV, ulnar-nerve SCV, and superficial peroneal-nerve SCV compared with the high-normal group ($P < 0.05$). However, no significant differences were observed in CMAP and SNAP of the normal serum sodium groups. The NCV, peroneal-nerve CMAP, tibial-nerve CMAP, ulnar-nerve SNAP, superficial peroneal-nerve SNAP, and sural-nerve SNAP were lower in the hyponatremia group than in the low-normal group ($P < 0.05$). Meanwhile, a trend analysis was performed, as shown in Table 2.

3.3. Relationship between Serum Sodium Level and DPN. As shown in Figure 2, we used restricted cubic splines to flexibly model and visualize the relationship between the serum sodium level and DPN. The risk of DPN was relatively flat until around 140 mmol/L of the serum sodium level and then started to increase rapidly forward and afterward (P for nonlinearity <0.05) in all serum sodium groups, especially in male participants, those aged <65 years, and those with UACR < 30 mg/g. However, a nonlinear trend was not observed in the normal serum sodium group and its subgroups. It suggested that both hyponatremia and hypernatremia might increase the risk of DPN. A reverse J-curve distribution was observed between the risk of DPN and the serum sodium concentration.

In the whole-group analysis, patients were divided into five groups according to the serum sodium level. Multiple logistic regression analyses showed that, compared with other higher serum sodium levels, hyponatremia was associated with DPN after adjusting for age, sex, duration of diabetes, BMI, systolic blood pressure, diastolic blood pressure, HbA1c, eGFR, serum kalemia, hypotensive drugs (β-blocker, CCB, ACEI, and ARB), statins, hypoglycemic drugs, insulin use, smoking, drinking, and hypertension (OR = 0.430, 95% CI = 0.220 – 0.841, $P = 0.014$; OR = 0.386, 95%CI = 0.198 – 0.755, $P = 0.005$; OR = 0.297, 95%CI = 0.152 – 0.580, $P < 0.001$; OR = 0.376, 95%CI = 0.190 – 0.743, $P = 0.005$, respectively) (Figure 3(a)). In all serum sodium groups, no significant relationship was detected between the subgroups of patients with diabetes aged ≥65 years or those with UACR ≥ 30 mg/g.

In the normal serum sodium group analysis, a fully adjusted logistic regression demonstrated that compared with the low-normal serum sodium level, the high-normal serum sodium level was a relatively lower risk factor of DPN (OR = 0.690, 95%CI = 0.526 – 0.905, $P = 0.007$) (Figure 3(a)). This relationship was particularly apparent in male participants (OR = 0.609, $P = 0.004$), those aged <65 years (OR = 0.599, $P = 0.002$), those with the duration of diabetes < 10 years (OR = 0.632, $P = 0.008$), and those with UACR < 30 mg/g (OR = 0.689, $P = 0.023$) (Figure 3(b)). The optimal serum sodium cutoff points (142.6 mmol/L) were obtained from the ROC curves.

4. Discussion

The present study demonstrated that patients with hyponatremia and those with low-normal serum sodium levels exhibited relatively high rates of DPN detection and relatively low NCV and amplitude. In addition, the serum sodium level was independently associated with the DPN detection rate after adjusting for several potential confounders. This relationship was particularly apparent in patients with the duration of diabetes < 10 years and UACR < 30 mg/g. This trend was also apparent within the normal serum sodium groups.

Table 1: Demographic and clinical characteristics of study participants.

	Corrected serum sodium (mmol/L)					
	Hyponatremia 130.0–135.0 ($n = 56$)	Low-normal sodium 135.0–141.5 ($n = 510$)	Medium-normal sodium 141.5–143.0 ($n = 510$)	High-normal sodium 143.0–145.00 ($n = 510$)	Hypernatremia 145.0–150.0 ($n = 342$)	P
Corrected serum sodium (mmol/L)	133.7 ± 1.5	140 ± 1.2	142.3 ± 0.4	143.9 ± 0.5	146.3 ± 1.3	<0.001
Serum sodium (mmol/L)	132.6 ± 1.5	138.6 ± 1.9	140.9 ± 1.6	142.5 ± 1.6	144.6 ± 2.0	<0.001
Age (year)	61.7 ± 12.7	59.6 ± 12.7	59.3 ± 11.7	60.5 ± 11.6	60.9 ± 11.2	0.186
Male, *n* (%)	35 (62.5)	303 (59.4)	292 (57.3)	273 (53.5)	150 (43.9)[d]	<0.001
Duration of diabetes (year)	9 (3.3–11.8)	7 (2–10)	7 (3–10)	8 (3–12)	8 (3–12)	0.105
BMI (kg/m^2)	22.7 ± 4.8	24.6 ± 3.7[a]	24.7 ± 3.6	24.5 ± 3.5	24.3 ± 3.7	0.004
Smoking, *n* (%)	18 (32.1)	141 (27.6)	139 (27.3)	124 (24.3)	63 (18.4)[d]	0.013
Drinking, *n* (%)	6 (10.7)	59 (11.6)	70 (13.7)	49 (9.6)[c]	23 (6.7)	0.021
Hypertension, *n* (%)	28 (50)	255 (50)	244 (47.8)	259 (50.8)	185 (54.1)	0.514
RASS-blocker, *n* (%)	16 (28.6)	135 (26.5)	105 (20.6)	115 (22.5)	71 (20.8)	0.128
β-Blocker, *n* (%)	3 (5.4)	34 (6.7)	34 (6.7)	41 (8)	21 (6.1)	0.803
CCB, *n* (%)	13 (23.2)	128 (25.1)	114 (22.4)	137 (26.9)	97 (28.4)	0.305
Diuretic, *n* (%)	8 (14.3)	35 (6.9)[a]	25 (4.9)	23 (4.5)	17 (5)	0.022
Statins, *n* (%)	7 (12.5)	45 (8.8)	40 (7.8)	58 (11.4)	38 (11.1)	0.255
OAD, *n* (%)	47 (83.9)	384 (75.3)	419 (82.2)[b]	420 (82.4) [b]	280 (81.9)	0.021
Insulin use, *n* (%)	25 (44.6)	187 (36.7)	202 (39.6)	191 (37.5)	136 (39.8)	0.672
SBP (mmHg)	138.5 ± 22.2	136.8 ± 20.5	136.9 ± 19.1	136.7 ± 20.6	139.9 ± 20.6	0.140
DBP (mmHg)	75.9 ± 9.4	78.9 ± 11.3	78.5 ± 11.1	78.8 ± 11.9	77.9 ± 10.4	0.275
HbAlc (%)	9 ± 2.9	9.3 ± 2.6	9.1 ± 2.3	9.2 ± 2.4	9.1 ± 2.4	0.443
FPG (mmol/L)	8.1 ± 3.6	8.8 ± 3.5	8.8 ± 3.6	8.7 ± 3.5	9.4 ± 4.2[d]	0.045
TCH (mmol/L)	4.33 ± 1.56	4.75 ± 1.54	4.63 ± 1.26	4.67 ± 1.11	4.67 ± 1.3	0.206
TG (mmol/L)	1.2 (0.8–1.6)	1.4 (0.9–2.1)	1.4 (1–2.1)	1.4 (1–2.1)	1.4 (1–2.1)	0.050
HDL-C (mmol/L)	1.17 ± 0.44	1.11 ± 0.36	1.1 ± 0.33	1.12 ± 0.31	1.15 ± 0.35	0.270
LDL-C (mmol/L)	2.64 ± 1.42	2.88 ± 1.14	2.89 ± 1.06	2.87 ± 0.93	2.91 ± 1.03	0.538
Serum kalium (mmol/L)	4.01 ± 0.48	4.04 ± 0.46	4.02 ± 0.44	4.01 ± 0.46	3.98 ± 0.52	0.420
eGFR (mL/(min·1.73m^2))	96.6 ± 33.3	105 ± 32[a]	109.1 ± 35.7	105.4 ± 28.7	103.2 ± 28.4	0.034
Cr (mmol/L)	71.6 ± 28.8	64.4 ± 27[a]	61.7 ± 20.2	62 ± 19.6	64.1 ± 26.8	0.017
UACR (mg/g)	34.8 (8.5–187)	12.8 (6.2–63.2)[a]	11.7 (6.2–72.6)	12.7 (6.2–38.5)	14.5 (7.3–58)[d]	0.017
Atherosclerosis, *n* (%)	23 (41.1)	137 (26.9)	149 (29.2)	151 (29.6)	102 (29.8)	0.260
Left ABI	1.06 ± 0.16	1.05 ± 0.13	1.07 ± 0.13	1.06 ± 0.11	1.09 ± 0.42	0.302
Right ABI	1.05 ± 0.14	1.09 ± 0.43	1.11 ± 0.6	1.07 ± 0.1	1.09 ± 0.27	0.577
ICVD %	10 (3.3–19)	10 (3–16)	8 (2–16)	8 (3–16)	8 (3–14)	0.222
Toronto Clinical Scoring System Score (TCSS)	2.5 (0–7)	1.0 (0–5.5)[a]	2.0 (0–6)	1.0 (0–5.0)[c]	2 (0–6)[d]	0.025
DPN, *n* (%)	39 (69.6)	274 (53.7)[a]	253 (49.6)[a]	224 (43.9)[ab]	170 (49.7)[a]	0.001

ABI: ankle brachial index; BMI: body mass index; CCB: calcium channel blockers; Cr: serum creatinine; eGFR: estimated glomerular filtration rate; FPG: fasting plasma glucose; HbAlc: glycosylated hemoglobin; HDL-C: high-density lipoprotein cholesterol; ICVD: 10-year risk of ischemic cardiovascular disease; LDL-C: low-density lipoprotein cholesterol; OAD: oral antidiabetic agents; TCH: cholesterol; TG: triglyceride; UACR: urinary albumin-to-creatinine ratio. *Post hoc* analysis: [a]compared with the hyponatremia group, $P < 0.05$; [b]compared with the low-normal sodium group, $P < 0.05$; [c]compared with the medium-normal sodium group, $P < 0.05$; [d]compared with the high-normal sodium group, $P < 0.05$.

Table 2: Nerve conduction velocity and nerve conduction amplitude of the different serum sodium groups.

	Serum sodium (mmol/L)					*P* value for trend analysis	
	Hyponatremia 130.0–135.0 ($n = 56$)	Low-normal sodium 135.0–141.5 ($n = 510$)	Medium-normal sodium 141.5–143.0 ($n = 510$)	High-normal sodium 143.0–145.00 ($n = 510$)	Hypernatremia 145.0–150.0 ($n = 342$)	*P* value	Linear term	Quadratic term
Median-nerve MCV (m/s)	49.73 ± 5.79	52.12 ± 5.33[a]	52.33 ± 6.35[a]	52.97 ± 5.97[ab]	52.80 ± 5.71	0.001	<0.001	NC
Ulnar-nerve MCV (m/s)	49.49 ± 7.40	52.45 ± 7.12[a]	53.70 ± 6.49[ab]	53.66 ± 6.13[ab]	53.70 ± 6.21[ab]	<0.001	<0.001	NC
Tibial-nerve MCV (m/s)	39.13 ± 7.90	42.86 ± 5.38[a]	43.67 ± 5.23[ab]	43.91 ± 5.15[ab]	43.64 ± 5.08[b]	<0.001	<0.001	NC
Peroneal-nerve MCV (m/s)	39.15 ± 7.81	41.84 ± 6.75[a]	42.88 ± 5.51[b]	43.06 ± 5.08[ab]	43.13 ± 5.34[ab]	<0.001	<0.001	NC
Median-nerve SCV (m/s)	45.21 ± 10.06	49.35 ± 8.80[a]	49.50 ± 8.62[a]	49.71 ± 9.05[a]	50.08 ± 8.01[a]	0.002	<0.001	NC
Ulnar-nerve SCV (m/s)	46.87 ± 8.63	51.13 ± 8.39[a]	52.07 ± 7.02[a]	52.00 ± 8.34[ab]	52.63 ± 6.73[ab]	<0.001	<0.001	NC
Superficial-nerve SCV (m/s)	45.22 ± 10.65	48.62 ± 13.06[a]	49.24 ± 11.92[a]	49.57 ± 13.09[ab]	49.31 ± 13.11[a]	<0.001	0.027	NC
Sural-nerve SCV (m/s)	41.30 ± 13.36	48.38 ± 10.57[a]	49.05 ± 10.46[a]	48.56 ± 12.00[a]	49.18 ± 10.22[a]	<0.001	<0.001	NC
Median-nerve CMAP (mv)	11.99 ± 4.50	12.68 ± 4.19	12.73 ± 4.12	12.49 ± 3.62	12.28 ± 4.03	0.339	NC	NC
Ulnar-nerve CMAP (mv)	11.45 ± 3.18	11.77 ± 3.21	11.73 ± 3.04	11.97 ± 2.96	12.02 ± 3.10	0.450	NC	NC
Tibial-nerve CMAP (mv)	8.30 ± 5.26	10.40 ± 5.32[a]	10.56 ± 5.66[a]	10.66 ± 5.05[a]	10.22 ± 5.02[a]	0.020	NC	0.002
Peroneal-nerve CMAP (mv)	4.46 ± 3.24	6.34 ± 3.77[a]	6.41 ± 3.65[a]	6.71 ± 3.86[a]	6.19 ± 3.57[a]	<0.001	NC	<0.001
Median-nerve SNAP (μv)	14.42 ± 8.99	17.85 ± 10.75	17.92 ± 10.69	17.99 ± 9.94	17.72 ± 10.22	0.121	NC	NC
Ulnar-nerve SNAP (μv)	8.93 ± 6.43	10.36 ± 5.55[a]	10.73 ± 6.24[a]	10.99 ± 5.65[a]	11.81 ± 7.98[ab]	0.001	0.001	NC
Superficial-nerve SNAP (μv)	13.04 ± 14.07	16.09 ± 12.13[a]	16.68 ± 12.34[a]	16.13 ± 11.32[a]	15.74 ± 10.90[a]	0.034	NC	0.040
Sural-nerve SNAP (μv)	9.13 ± 7.61	11.40 ± 7.71[a]	11.82 ± 7.81[a]	12.20 ± 7.83[a]	12.21 ± 8.37[a]	0.013	0.005	NC

CMAP: compound muscle action potential; MCV: motor nerve conduction velocity; SCV: sensory nerve conduction velocity; SNAP: sensory nerve action potential. *Post hoc* analysis: [a]compared with the hyponatremia group, $P < 0.05$; [b]compared with the low-normal sodium group, $P < 0.05$; [c]compared with the medium-normal sodium group, $P < 0.05$; [d]compared with the high-normal sodium group, $P < 0.05$. NC: nonconformance.

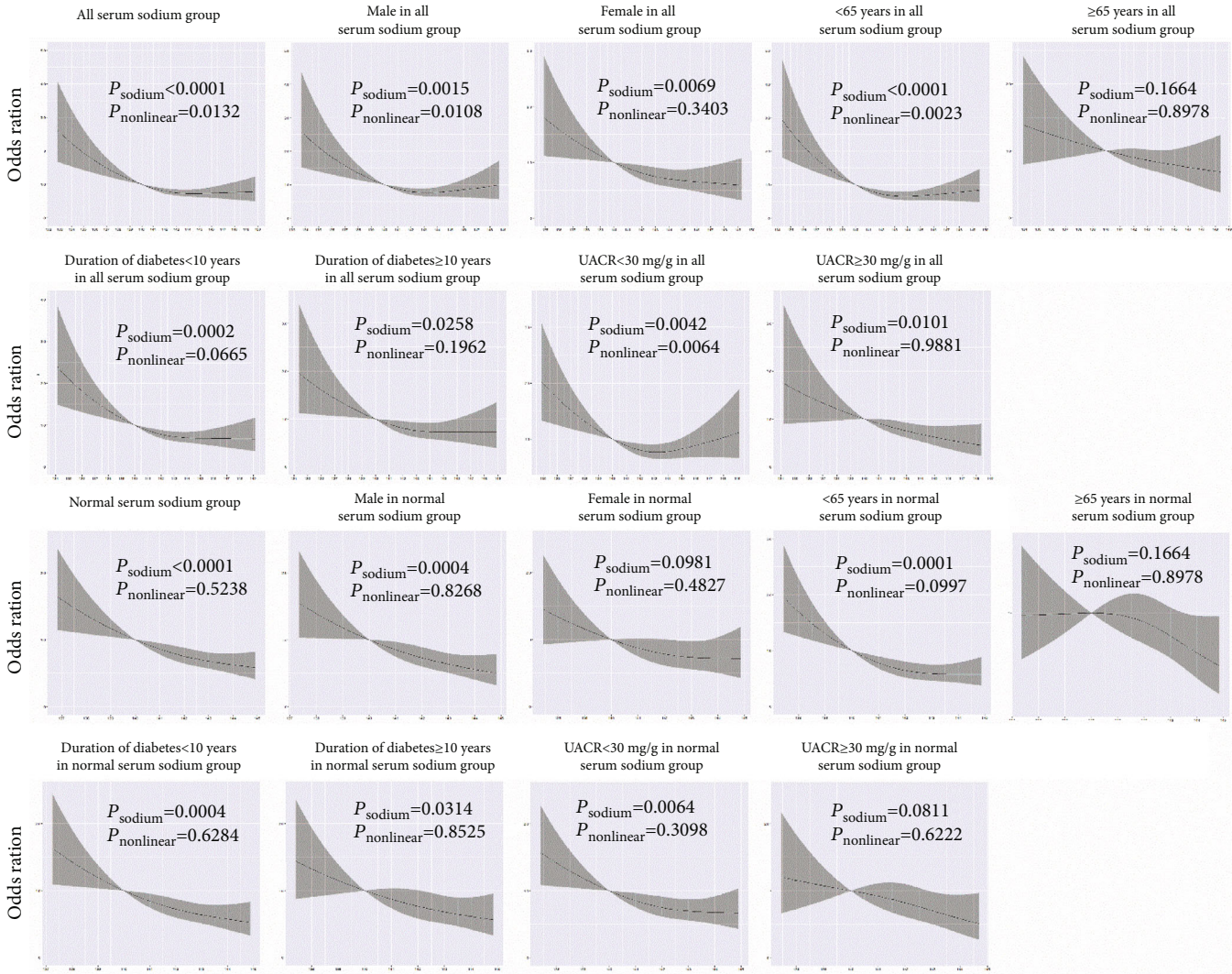

Figure 2: Relationship between serum sodium level and diabetic peripheral neuropathy. Restricted cubic splines were used to flexibly model and visualize the relationship between the serum sodium level and DPN. The risk of DPN was relatively flat until around 140 mmol/L of the serum sodium level and then started to increase rapidly forward and afterward (P for nonlinearity <0.05) in all serum sodium groups, especially in male patients, those aged <65 years, and those with UACR < 30 mg/g. However, a nonlinear trend was not observed in normal serum sodium group and its subgroups. The average serum sodium level of 140 mmol/L serves as a reference.

We demonstrated that patients with lower serum sodium levels were more likely to have DPN. Hospitalized patients often experience electrolyte disorders [27]. Hyponatremia and hypernatremia are the most common electrolyte disorders [28]. Sodium is a vital component of the human body. Serum sodium is closely related to hypertension [29], renal function [30, 31], fractures [32], and insomnia [33]. Hyponatremia is an independent risk factor for diabetes mellitus [28]. Otherwise, low extracellular sodium causes adverse effects in neuronal cells. Osmotic edema can also increase neuronal excitability through the activation of N-methyl-d-aspartate receptors [34], which may accelerate the development of dyskinesia [35]. Another study clarified that the serum sodium level was inversely associated with dyskinesia in patients with Parkinson's disease [13]. Currently, no studies correlating the serum sodium level with DPN in patients with type 2 diabetes mellitus are available. However, considering the important role of sodium in the central nervous system and diabetes mellitus, we do believe that the relationship exists between abnormal serum sodium levels and DPN. An early study with small sample populations demonstrated that serum sodium, but not acute blood glucose, levels had a highly significant relationship with NCV [11]. In our study, we demonstrated that hyponatremia was associated with a higher incidence of DPN and decreased NCV and amplitude. This trend, except for amplitude, was also apparent within the normal serum sodium groups, implying that hyponatremia might be a biomarker, rather than a cofounder. Furthermore, this relationship was particularly apparent in male patients with diabetes, those with duration of diabetes < 10 years, and those with UACR < 30 mg/g. In our opinion, this might be due to fewer effects of other complicated factors in these groups, which made this relationship much more obvious.

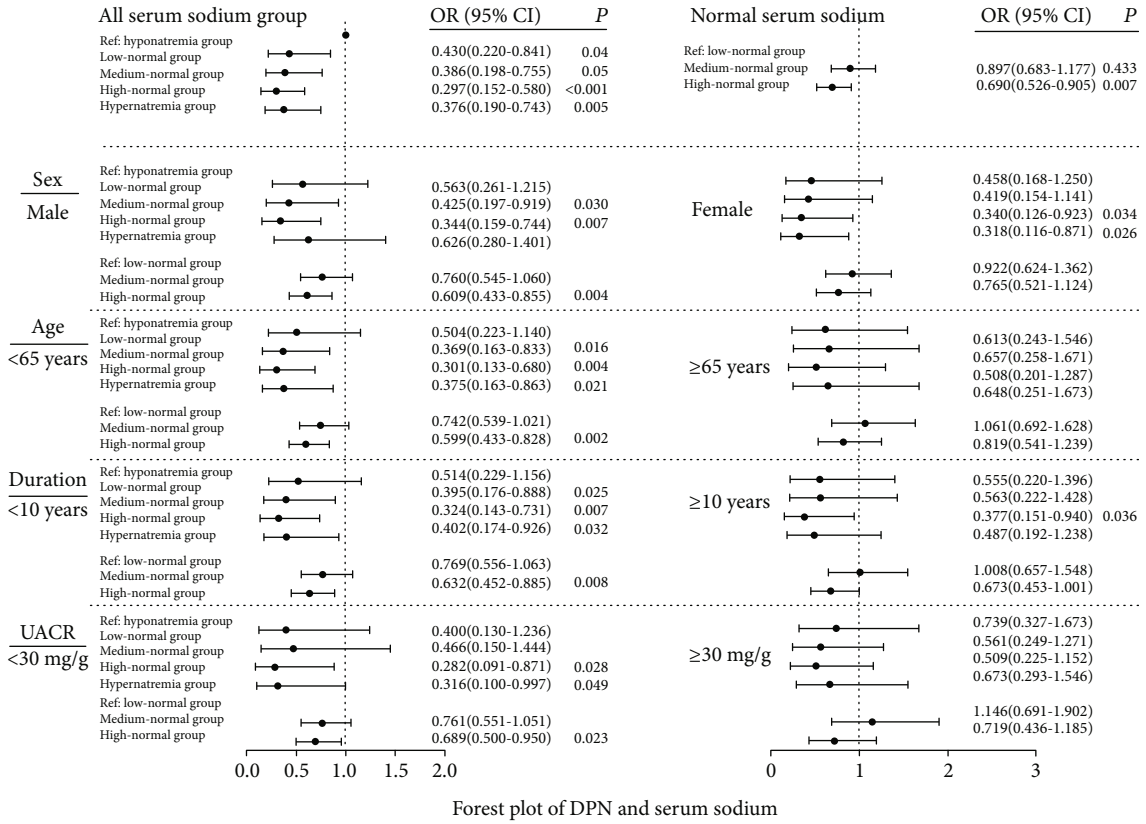

Figure 3: Plot of diabetic peripheral neuropathy and serum sodium level. (a) All serum sodium group and normal serum sodium group. Adjusted for age, sex, duration of diabetes, BMI, systolic blood pressure, diastolic blood pressure, HbA1c, eGFR, serum kalemia, hypotensive drugs (β-blocker, CCB, ACEI, and ARB), statins, hypoglycemic drugs, insulin use, smoking, drinking, and hypertension. (b) Subgroup analysis of all serum sodium group and normal serum sodium group. Adjusted for hypotensive drugs (β-blocker, CCB, ACEI, and ARB), statins, hypoglycemic drugs, insulin using, smoking, and drinking.

Several mechanisms might explain the relationship between DPN and hyponatremia. Sodium is a vital osmotic active solute in the extracellular compartment [36]. The sodium concentration was regulated by many factors, and it was important to maintain permeation and electrochemical gradient across cell membranes. The constant concentration gradient across the membrane played a crucial role in cell volume control, glucose transport and membrane potential, pH regulation, and neurotransmission [37]. Low extracellular sodium had an adverse effect on nerve cells, leading to osmotic demyelinating syndrome (ODS) [12]. Another explanation might be that hyponatremia could lead to a loss of excitatory neurotransmitters and transmission delays in the action potential of motor neurons [38]. The movement of sodium ions into the axon was responsible for generating the action potential [11]. The pathogenesis of DPN significantly correlated with cell electrophysiology changes, Na-K ATP enzyme dysfunction, nerve cell membrane hypoxia, cell swelling and rupture, and neuronal apoptosis [39, 40]. It was not surprising that the changes in extracellular sodium could modify nerve conduction. Otherwise, low serum sodium could lead to the functional decline of islet cells, increase blood glucose [41], and eventually cause neuropathy. We proposed the hypothesis that a relationship existed between DPN and serum sodium levels. Nevertheless, the finding of a relationship between serum sodium and DPN still needs confirmation from further studies.

Both hyponatremia and hypernatremia were associated with increased mortality [42]. Slight increases or decreases in serum sodium levels may be related to impaired neuromotor function [43]. However, the relationship between the serum sodium level and the DPN detection rate in the high-normal serum sodium group did not vary significantly from that in the hypernatremia group. Hence, a reverse J-curve distribution was observed between the risk of DPN and the serum sodium level. Previous studies showed that patients with hypernatremia presented with elevated plasma osmotic pressure, cell dehydration, and vascular endothelial injury, which could induce an inflammatory response and further exacerbate the progression of diabetes [44]. Excessive sodium also led to axonal degeneration in inflammatory demyelinating disease [45]. Patients with severe comorbidity and those with the highest recorded serum sodium and severe hyponatremia (>150 mmol/L or <130 mmol/L) [46] were excluded from this study, limiting the degree of nerve damage exhibited by the participants. We also excluded some complex diseases that might cause hypernatremia and hyponatremia. The abnormal serum sodium level might be attributed to diabetes mellitus and diuretics [28].

This study had several limitations. First, relatively few patients presented with hyponatremia. Also, patients' sodium intake and VitB levels were not recorded, which might affect the development of DPN. Second, our study design did not allow us to evaluate the causes of hyponatremia. In addition, as previously stated, the study was not designed to evaluate factors leading to this relationship. Although a role for hyponatremia in DPN is biologically plausible, we could not determine from our data whether the relationship between hyponatremia and DPN reflected a direct effect of hyponatremia, a surrogate marker for underlying comorbidities or reason for DPN, or both. Moreover, only one single measurement of fasting electrolytes was taken, which might not be an accurate estimation of the serum sodium level. The present study reported that a relative protective effect of higher-normal serum sodium concentrations was observed in patients with diabetes. However, the discrepancy in the DPN detection rate was not observed in the high-normal serum sodium group and the hypernatremia group. No survival advantage was noted once the serum sodium reached 145 mmol/L [47], and hypernatremia might cause other chronic diseases and more serious public health problems [48].

5. Conclusions

We must acknowledge that hyponatremia and low-normal serum sodium levels may serve as surrogate markers of DPN, treatment, or case mix. However, we believe that the mild abnormal serum sodium level should not be neglected. Since even minor serum sodium disturbances are associated with mortality, patient outcomes can be significantly improved by frequently monitoring electrolytes and discontinuing drugs with adverse effects when necessary [28]. Patients with lower sodium levels require particular care. Further studies are needed to understand the factors leading to this prognostic relationship and the potential benefit from therapeutic strategies aimed at this metabolic disturbance.

Data Availability

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

Conflicts of Interest

We declare that we have no conflicts of interest.

Authors' Contributions

Yongze Zhang and Chuanchuan Li are co-first authors.

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Research Article

The Predictive Effect of Health Examination in the Incidence of Diabetes Mellitus in Chinese Adults: A Population-Based Cohort Study

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Background. The incidence of diabetes mellitus (DM) was increasing in recent years, and it is important to screen those nondiabetic populations through health examination to detect the potential risk factors for DM. We aimed to find the predictive effect of health examination on DM. *Methods*. We used the public database from Rich Healthcare Group of China to evaluate the potential predictive effect of health examination in the onset of DM. The colinear regression was used for estimating the relationship between the dynamics of the health examination index and the incident year of DM. The time-dependent ROC was used to calculate the best cutoff in predicting DM in the follow-up year. The Kaplan-Meier method and Cox regression were used to evaluate the HR of related health examination. *Results*. A total of 211,833 participant medical records were included in our study, with 4,172 participants diagnosing as DM in the following years (among 2-7 years). All the initial health examination was significantly different in participants' final diagnosing as DM to those without DM. We found a negative correlation between the incidence of years of DM and the average initial FPG ($r = -0.1862$, $P < 0.001$). Moreover, the initial FPG had a strong predictive effect in predicting the future incidence of DM (AUC = 0.961), and the cutoff was 5.21 mmol/L. Participants with a higher initial FPG (>5.21 mmol/L) had a 2.73-fold chance to develop as DM in follow-up (95%CI = 2.65 – 2.81, $P < 0.001$). *Conclusion*. Initial FPG had a good predictive effect for detecting DM. The FPG should be controlled less than 5.21 mmol/L.

1. Introduction

Diabetes mellitus (DM) is caused by various pathogenic factors such as genetic factors, immune dysfunction, microbial infections and their toxins, free radical toxins, and mental factors, leading to hypofunction of pancreatic islets and insulin resistance, which could result in a series of metabolic disorder syndromes, such as electrolytes, and electrolytes are clinically characterized by high blood glucose [1]. In diabetic patients, the proportion of type 2 DM is about 95%, which is more common in middle-aged and elderly people after the age of 30 [2]. In those type 2 DM patients, the secretion of insulin is not low or even higher than the healthy population and the main cause is that the body is not sensitive to insulin, that is, insulin resistance [3].

In recent years, the incidence rate of DM has been increasing. The complications are the biggest cause of death in diabetic patients [4]. Because the cells are incapable to absorb glucose, it remains in the serum. Prolonged high blood glucose can damage the capillaries in the kidneys, heart, eyes, or nervous system, eventually leading to infections, heart diseases, cerebrovascular diseases, renal failure, blindness, lower limb gangrene, and other diseases [5]. The International Diabetes Federation (IDF) estimates that 8.3% of adults (approximately 382 million people) have DM. There are currently 175 million undiagnosed cases, a large amount of whose complications are not noticed [6].

It is not only cost-effective but also a very convenient predicting method to use the health examination indicators of a large population reasonably to provide certain prediction efficiency for potential diabetic patients [7, 8]. Although previous studies reported on the use of health examination indicators to predict DM, most of the models did not analyze the probability of DM during the follow-up period. There was a deviation in estimating the best cutoff [3, 4, 7]. In this study, we aimed to discuss the relationship between the dynamic change of health examination in follow-up years and the incidence of DM. We adopted the time-dependent ROC methods to calculate the best cutoff to discuss the predictive effect of the health examination indicators in the incidence of DM. Therefore, we wished to find the predictive value of health examination in the future incidence of DM.

2. Methods

2.1. Data Resources. This study was designed based on a population cohort in China. The data were downloaded from the public database which was established by Rich Healthcare Group. The data included the health examination and the incidence of DM which was sorted by Chen et al. [7]. The data included the medical records of the Chinese population from 2010 to 2016. All the participants were at least 20 years old. The inclusion and exclusion criteria were referred from the study of Chen et al. [7]. Briefly, this study included patients with available data of body mass index (BMI) and fasting plasma glucose (FPG) value. All the participants were followed up for at least 2 years. Other health examination indexes included total cholesterol, triglyceride, high-density lipoprotein (HDL), low-density lipoprotein (LDL), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), and endogenous creatinine clearance rate (CCR). Finally, all the participants with follow-up FPG were included in the study and a total of 211,833 participants were included.

As the acquisition and analysis standard, FPG was collected with at least 10-hour fasting at each visit. The diagnosis standard of DM was defined as FPG > 7.00 mmol/L.

2.2. Study Design and Statistical Analysis. This study is aimed at analyzing the predictive effect of the health examination index in the future diagnosis of DM. Firstly, we compared the difference in health examination index between DM patients and those undiagnosed participants. Secondly, we compared the dynamics of health examination based on the visit intervals. Subgroups were divided based on the visit intervals (2-3 years, 3-4 years, 4-5 years, and above 5 years). Next, we tried to find a health examination index to predict the future incidence of DM. We used the colinear regression to find the dynamic change of those indexes based on visit intervals. Due to the incidence of DM collected by follow-up year, we used the time-dependent ROC methods to search for the best cutoff of different health examination indexes. The area under the curve (AUC) was used to estimate the accuracy of the index [9]. Finally, we used the Kaplan-Meier methods to calculate the incidence of DM and used the Cox regression to calculate the HR for incidence of DM and described with 95% confidence intervals (95% CIs). All the statistical significance was defined as P value less than 0.05. The data were analyzed by STATA 15.0 (StataCorp, College Station, TX, USA) and R software (version 3.51).

3. Results

3.1. The Comparison of Health Examination Index between DM Patients and Nondiabetic Participants. As previously mentioned, a total of 211,833 participants were included in our study. During the follow-up years, there were 4,172 participants that were diagnosed with DM in the following year. All the health examination indexes were significantly different (Table 1, all $P < 0.001$). Those patients were older (54.7 years) and have a larger BMI (26.17 kg/m^2) compared to the nondiabetic cohort (41.8 years and 23.17 kg/m^2). The initial FPG was higher in the DM group (5.90 mmol/L) compared to nondiabetic participants (4.90 mmol/L). Both cholesterol and triglyceride were greater in DM patients (5.05 mmol/L and 2.09 mmol/L) compared to nondiabetic participants (4.70 mmol/L and 1.32 mmol/L). The trend was also found in AST and ALT (29.1 U/L and 35.2 U/L compared to 23.9 U/L and 23.7 U/L, respectively). Despite the significance shown in statistics, the difference of HDL, LDL, BUN, and CCR between the two groups was not shown. 71.87% of the patients were male, and 4.1% of the diabetic patients had a family history. The percentage of the current smoker was greater in DM cohort patients (35.41%) compared to those without DM (19.74%).

Table 1: The comparison of physical examination index between diabetes patients and nondiabetic participants.

Variables	Diabetes ($N = 4,172$)	Nondiabetes ($N = 207,659$)	P
Age (year)	54.7 (13.20)	41.8 (12.50)	<0.001
Male (%)	3,000 (71.87)	113,123 (54.48)	0.002
Current smoker (%)	415 (35.41)*	11,660 (19.74)*	<0.001
Current drinker (%)	49 (4.18)*	1,302 (2.20)*	<0.001
Family history (%)	171 (4.10)	4173 (2.01)	<0.001
BMI (kg/m^2)	26.17 (3.48)	23.17 (3.31)	<0.001
Initial FPG (mmol/L)	5.90 (0.71)	4.90 (0.59)	<0.001
Cholesterol (mmol/L)	5.05 (0.94)	4.70 (0.90)	<0.001
Triglyceride (mmol/L)	2.09 (1.50)	1.32 (1.01)	<0.001
HDL (mmol/L)	1.29 (0.34)	1.37 (0.31)	<0.001
LDL (mmol/L)	2.90 (0.70)	2.76 (0.68)	<0.001
ALT (U/L)	26 (18-41)	18 (13-27)	<0.001
AST (U/L)	25 (21-32)	22 (18-26)	<0.001
BUN (mmol/L)	5.01 (1.28)	4.65 (1.18)	<0.001
CCR (μmol/L)	72.7 (15.2)	70.0 (15.8)	<0.001
Final FPG (mmol/L)	7.84 (1.90)	5.08 (0.51)	<0.001

*There were missing data. Age, BMI, FPG, cholesterol, triglyceride, HDL, LDL, BUN, and CCR were described as mean and standard deviation; ALT and AST were described as medians (interquartile ranges (IQR)). Male, current smoker, current drinker, and family history were described as number and percentage. Abbreviation: BMI: body mass index; FPG: fasting plasma glucose; HDL: high-density lipoprotein; LDL: low-density lipoprotein; ALT: alanine aminotransferase; AST: aspartate aminotransferase; BUN: blood urea nitrogen; CCR: endogenous creatinine clearance rate.

3.2. The Comparison of Health Examination Indexes between DM Patients and Nondiabetic Participants in terms of Different Visit Intervals. Next, we compared the health examination indexes according to different visit intervals (Table 2). The difference was similar to the total cohort. However, we found that the gap of difference was changed dynamically. Also, we found a negative correlation between the incidence of years of DM and the average initial FPG (Figure 1, $r = -0.1862$, $P < 0.001$).

3.3. The Best Cutoff and AUC of Health Examination in Predicting Future DM. The best cutoff and AUC were analyzed by time-dependent ROC which had counted the time into the incidence of DM. All the indexes were calculated for the 5-year incidence of DM. The AUCs are shown in Figure 2, Supplement Figure 1, and Table 3. Among all the continuous data, age, BMI, initial FPG, and triglyceride had a good predictive effect in the future incidence of DM (AUC > 0.700). Among these, the initial FPG had a strong predictive effect in predicting the future incidence of DM (AUC = 0.961) with a cutoff of 5.21 mmol/L. For further understanding the predictive effect of initial FPG, we used the index to predict 3-year and 4-year incidence of DM. Both AUCs were larger than 0.94 with a cutoff of 5.49 mmol/L and 5.3 mmol/L, respectively, which means the higher cutoff may have an accurate predictive effect for the shorter period of the incidence of DM (Supplement Figure 2).

3.4. The Hazard Ratio for the Incidence of DM. According to the accurate predictive effect of the health examination indexes reported previously, we used age, BMI, initial FPG, and triglyceride as the factors to calculate the HR for the incidence of DM (Table 4). Due to the better control of the FPG in the population, we adopted the 5.21 mmol/L of FPG as the cutoff for calculating HR. In terms of FPG, we found that the incidence rate of DM was 0.21% in 3 years, 0.67% in 4 years, and 2% in 5 years, if initial FPG was less than 5.21 mmol/L, compared to 3.88%, 10.22%, and 24.35% if FPG was larger than 5.21 mmol/L (Figure 3).

In terms of HR of the DM incidence, participants older than 48 years old had a 1.699-fold chance to have DM (95%CI = 1.635 – 1.765, $P < 0.001$) compared to younger participants. Participants who have a larger BMI (>24.49 kg/m^2) may have a higher chance to have DM (HR = 1.499, 95%CI = 1.432 – 1.566, $P < 0.001$). Similarly, participants who have a higher triglyceride (>1.09 mmol/L) had a higher chance to have DM (HR = 1.48, 95%CI = 1.41 – 1.56, $P < 0.001$). Most importantly, participants who have a higher initial FPG (>5.21 mmol/L) had a 2.73-fold chance to have DM (95%CI = 2.65 – 2.81, $P < 0.001$).

4. Discussion

In our study, we found that the health examination indexes were significantly different between those patients who would have DM in the follow-up year and those who are nondiabetic participants. We found that the initial FPG in the health examination of healthy participants could have a certain predictive effect on the future incidence of DM. We used the colinear regression method to suggest that the greater initial FPG could predict a shorter incidence of DM and those participants who have an initial FPG of more than 5.2 mmol/L would have a 2.73-fold risk to be diagnosed as DM in the follow-up years.

In the difference of the initial health examination between DM patients and nondiabetic participants, we found that greater age, BMI, initial FPG, cholesterol and triglyceride, AST, and ALT were described in the DM cohort. In terms of BMI and age, Chen et al. had discussed previously [7]. They suggested that young age itself is a remarkable protective factor for developing DM since the prevalence of DM was more common in the middle-aged and elderly population. Several studies showed that BMI was a strong risk associated with the development of metabolic disorders, which includes type 2 DM and cardiovascular diseases [10, 11]. In terms of AST and ALT, which might be related to the liver function, they showed the potential relationship between liver function and the incidence of DM [12]. The liver is the metabolism center of the three major materials of sugar,

Table 2: The comparison of physical examination index between diabetes patients and nondiabetic participants in terms of different follow-up years.

Variables	Follow-up 2 to 3 years			Follow-up 3 to 4 years			Follow-up 4 to 5 years			Follow-up above 5 years		
	Diabetes ($N = 1,764$)	Nondiabetes ($N = 106,055$)	P	Diabetes ($N = 1,259$)	Nondiabetes ($N = 57,622$)	P	Diabetes ($N = 905$)	Nondiabetes ($N = 35,316$)	P	Diabetes ($N = 246$)	Nondiabetes ($N = 8,666$)	P
Age (year)	54.60 (13.51)	41.71 (12.91)	<0.001	55.00 (13.20)	41.93 (12.56)	<0.001	54.10 (12.60)	42.03 (11.43)	<0.001	52.80 (12.10)	42.06 (11.28)	<0.001
Male (%)	1,257 (71.26)	57,276 (54.01)	<0.001	885 (70.29)	32,106 (55.72)	<0.001	652 (72.04)	18,696 (52.94)	<0.001	206 (83.74)	5,045 (58.22)	0.008
Current smoker (%)	163 (32.34)*	5715 (19.22)*	<0.001	129 (40.20)*	3,526 (21.55)*	<0.001	86 (32.70)*	1,901 (18.57)*	<0.001	37 (44.05)	518 (19.01)	<0.001
Current drinker (%)	23 (4.56)*	728 (2.45)*	0.016	12 (3.74)*	357 (2.18)*	0.042	9 (3.42)*	164 (1.60)*	0.094	5 (5.95)	53 (1.94)	0.054
Family history (%)	57 (3.23)	1799 (1.70)	<0.001	53 (4.21)	1,253 (2.17)	<0.001	50 (5.52)	886 (2.51)	<0.001	11 (4.47)	235 (2.71)	0.079
BMI (kg/m^2)	26.19 (3.53)	23.24 (3.39)	<0.001	26.11 (3.47)	23.14 (3.31)	<0.001	26.11 (3.45)	23.03 (3.23)	<0.001	26.57 (3.29)	23.18 (3.28)	<0.001
Initial FPG (mmol/L)	6.04 (0.67)	5.00 (0.55)	<0.001	5.86 (0.72)	4.83 (0.60)	<0.001	5.75 (0.74)	4.71 (0.63)	<0.001	5.70 (0.77)	4.73 (0.57)	<0.001
Cholesterol (mmol/L)	5.05 (0.96)	4.70 (0.90)	<0.001	5.05 (0.93)	4.70 (0.90)	<0.001	5.04 (0.94)	4.70 (0.90)	<0.001	5.06 (0.93)	4.69 (0.88)	<0.001
Triglyceride (mmol/L)	2.09 (1.56)	1.34 (1.03)	<0.001	2.08 (1.39)	1.33 (1.01)	<0.001	2.04 (1.44)	1.27 (0.97)	<0.001	2.35 (1.71)	1.31 (1.06)	<0.001
HDL (mmol/L)	1.32 (0.38)	1.41 (0.30)	<0.001	1.31 (0.30)	1.35 (0.31)	<0.001	1.22 (0.29)	1.32 (0.32)	<0.001	1.15 (0.23)	1.32 (0.32)	<0.001
LDL (mmol/L)	2.90 (0.70)	2.77 (0.67)	<0.001	2.91 (0.71)	2.79 (0.69)	<0.001	2.91 (0.71)	2.76 (0.69)	<0.001	2.78 (0.63)	2.63 (0.62)	0.003
ALT (U/L)	25 (18-25)	18 (13-27)	<0.001	26 (18-40)	18 (13-28)	<0.001	28 (18-45)	18 (13-27)	<0.001	29 (20-43)	19 (13-28)	<0.001
AST (U/L)	25 (20-32)	22 (18-26)	<0.001	26 (22-33)	22 (19-27)	<0.001	25 (20-32)	22 (19-26)	<0.001	27 (23-34)	22 (19-26)	0.001
BUN (mmol/L)	5.04 (1.28)	4.66 (1.18)	<0.001	5.02 (1.36)	4.63 (1.18)	<0.001	4.99 (1.23)	4.64 (1.18)	<0.001	4.91 (1.11)	4.69 (1.17)	0.003
CCR (μmol/L)	73.30 (15.90)	70.25 (16.13)	<0.001	72.40 (16.70)	70.06 (15.55)	<0.001	72.20 (15.1)	69.31 (15.37)	<0.001	71.90 (12.1)	69.61 (14.86)	0.01
Final FPG (mmol/L)	7.68 (1.73)	5.06 (0.51)	<0.001	7.87 (1.90)	5.08 (0.51)	<0.001	7.99 (2.04)	5.11 (0.50)	<0.001	8.23 (2.38)	5.08 (0.58)	<0.001

*There were missing data. Age, BMI, FPG, cholesterol, triglyceride, HDL, LDL, BUN, and CCR were described as mean and standard deviation; ALT and AST were described as medians (interquartile ranges (IQR)). Male, current smoker, current drinker, and family history were described as number and percentage. Abbreviation: BMI: body mass index; FPG: fasting plasma glucose; HDL: high-density lipoprotein; LDL: low-density lipoprotein; ALT: alanine aminotransferase; AST: aspartate aminotransferase; BUN: blood urea nitrogen; CCR: endogenous creatinine clearance rate.

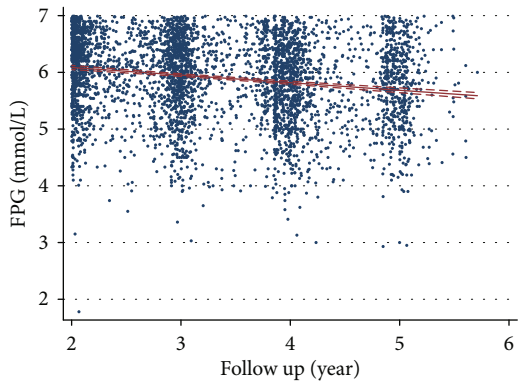

Figure 1: The colinear relationship between initial FPG and the follow-up year in diabetes patients.

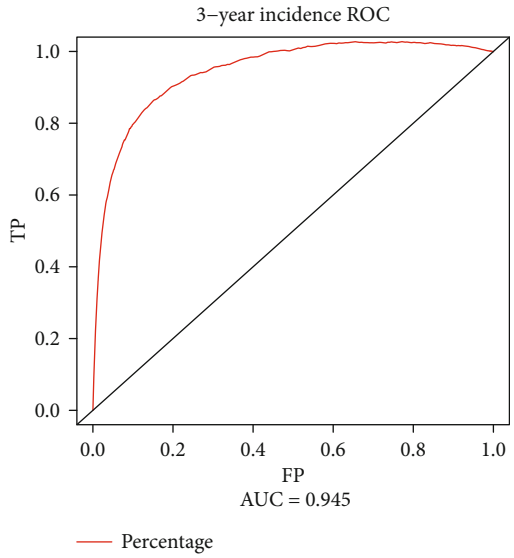

Figure 2: The time-dependent ROC evaluates the best cutoff and AUC of initial FPG (5.2 mmol/L) in predicting the further diagnosis of diabetes.

Table 3: The best cutoff and AUC of physical examination in predicting future diabetes.

Variables	Best cutoff	AUC
Age (years)	48.00	0.73
BMI (kg/m^2)	24.29	0.74
Initial FPG (mmol/L)	5.21	0.96
Cholesterol (mmol/L)	4.89	0.61
Triglyceride (mmol/L)	1.09	0.73
HDL (mmol/L)	0.51	0.42
LDL (mmol/L)	2.80	0.59
ALT (U/L)	17.30	0.67
AST (U/L)	23.80	0.66
BUN (mmol/L)	4.96	0.58
CCR (μmol/L)	61.90	0.55

lipids, and amino acids. It is also an important organ for insulin clearance and the production of inflammatory factors. Insufficiency of insulin secretion and/or function defect characteristic of diabetic patients are mainly manifested as glucose and lipid metabolism disorders [13]. Dysregulation of sugar metabolism may induce hyperglycemia, resulting in the accumulation of glycogen in the liver, thereby causing liver microvascular disease [14]. Lipid metabolism disorder leads to the increased amount of fat that cannot be catabolized and metabolized to accumulate in the liver, forming fatty liver, which impairs liver function. AST and ALT are important indicators that reflect the basic status of liver function, whose changes can sensitively indicate liver cell damage and its degree, as well as liver excretion function [15]. Therefore, continuous monitoring of liver enzyme changes reflects the degree of diabetic liver damage. Oka et al. [16] conducted an epidemiological study on the relationship between elevated liver enzymes and prediabetes. The subjects were 594 patients with normal baseline blood glucose levels, non-B viral hepatitis, or type C Japanese men who are patients or carriers of hepatitis virus. After 3.1 years of follow-up, 141 (23.7%) study subjects progressed to impaired glucose tolerance (IGT), 68 (11.4%) progressed to impaired fasting glucose (IFG), and 23 patients combined IGT and IFG. They also found that elevated ALT may be one of the early changes in the natural course of DM, which not only reflects the state of insulin resistance but also reflects the dysfunction of the gut-insulin axis.

In terms of cholesterol and triglyceride, changes in blood lipid levels in the body cause serious diseases in the body, mainly leading to coronary heart disease and atherosclerosis, and are also related to chronic diseases such as stroke and hypertension [17]. DM patients often have a higher rate of dyslipidemia. The survey results show that the prevalence of dyslipidemia in diabetic populations has reached more than 50% [18]. Although there is no consensus on the mechanism of the mutual influence between blood glucose and blood lipids, researchers in various countries have recognized that there is a certain correlation between blood lipid levels and blood glucose levels [19]. In addition to affecting the prevalence of DM, dyslipidemia is also significantly associated with several complications in diabetic patients. The level of triglycerides also has a significant impact on the development of many complications in diabetic patients. Hypertriglyceridemia can increase the risk of cardiovascular and cerebrovascular remnants in diabetic patients [20]. The transport form of triglyceride in the body is mainly lipoproteins, among which chylomicrons and very-low-density lipoproteins are the main carriers of triglyceride. When diabetic patients have hypertriglyceridemia, the above two lipoproteins can be decomposed into remnant lipoproteins, which accelerate the formation of arteriosclerotic plaques in the body; and as the plaques rupture, platelets accumulate in the body in large numbers, forming thrombi and occluding blood vessels, which ultimately leads to myocardial cell necrosis [21]. Abnormal blood triglycerides also have a certain impact on diabetic nephropathy. Studies have compared blood lipid levels in patients with three types of DM, without nephropathy, early nephropathy, and clinical nephropathy.

Table 4: The hazard ratio for calculating the incidence of diabetes.

	HR	95% CI	*P*
Age, >48 years vs. <48 years	1.70	1.63-1.77	<0.001
BMI, >24.49 kg/m^2 vs. <24.49 kg/m^2	1.50	1.43-1.57	<0.001
FPG, >5.21 mmol/L vs. <5.21 mmol/L	2.73	2.65-2.81	<0.001
Triglyceride, >1.09 mmol/L vs. <1.09 mmol/L	1.48	1.41-1.56	<0.001

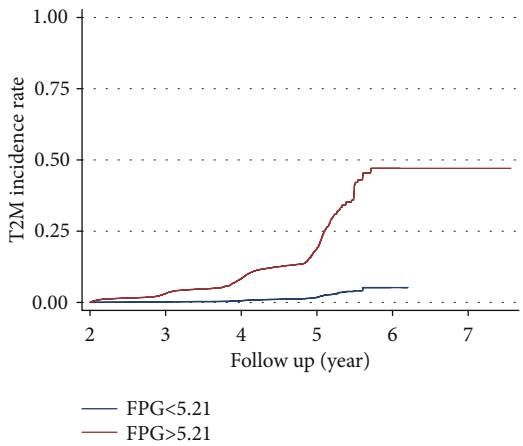

Figure 3: The incident rate of diabetes is based on initial FPG = 5.2 mmol/L.

The results indicate that the occurrence of diabetic nephropathy is related to elevated triglycerides [22].

In the time-dependent ROC, we confirmed that the participants' age, BMI, initial FPG, and triglyceride have higher predictive accuracy in the incidence of DM, in which AUCs were larger than 0.70. Among these, the initial FPG was the significant risk factor associating with the further incidence of DM. Not only in different predictive follow-up years, the FPG had a higher predictive AUC (>0.94), but also, we found that in the Cox regression the initial FPG had a significant impact on the incidence of DM. The incidence rate of DM was 0.21% in 3 years, 0.67% in 4 years, and 2% in 5 years if the initial FPG was less than 5.21 mmol/L, compared to 3.88%, 10.22%, and 24.35% if FPG was larger than 5.21 mmol/L. In 1997, the American Diabetes Association (ADA) and, in 1998, WHO set the critical value of impaired fasting blood glucose as 6.1 mmol/L [23]. Subsequently, in 2003, ADA lowered the threshold to 5.6 mmol/L [24]. In China's 2017 edition of the DM prevention and control guidelines, 6.1 mmol/L ≤ FPG < 7.0 mmol/L is defined as impaired fasting blood glucose [25]. Impaired fasting blood glucose and impaired glucose tolerance are collectively referred to as prediabetes, which are high-risk factors for the onset of DM and can also increase the risk of chronic kidney disease and Alzheimer's disease [26]. During this period, individuals can still be reversible to normal blood glucose. A large prospective cohort study in China shows that daily leisure sports activities (LTPA) are a protective factor for impaired fasting blood glucose and progression to DM, which could reverse the incidence of DM. Reaching the LTPA level recommended by the WHO can effectively reduce the risk of DM (population attributable risk: 19.2%, 95% CI: 5.6%~30.6%) [27].

There were some limitations in our study. Firstly, we only adopted FPG > 7.0 mmol/L as DM, but we did not distinguish the type of DM, including type 1, type 2, and gestational DM. Secondly, we only had the health examination indexes from the database; we did not contain other indexes which may influence the incidence of DM, HbA1c, for example, which might have a higher predictive effect in DM. Finally, we only have an initial experiment, instead of a dynamic test for one participant, which may have a good predictive value for predicting DM.

5. Conclusion

In conclusion, we suggested that there were differences in health examinations between participants who had the onset of the DM and those who are nondiabetic participants. Age, BMI, initial FPG, and triglyceride had a better predictive accuracy of DM. Patients who had a higher FPG have a high risk to develop DM; thus, blood glucose should be controlled no matter the circumstance.

Data Availability

The data and analytical methods of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Xiaomin Fu and Yingmin Jia contributed equally to the study.

Supplementary Materials

Supplementary 1. Supplement Figure 1: the time-dependent ROC evaluates the best cutoff and AUC of all physical experiment indexes.

Supplementary 2. Supplement Figure 2: the time-dependent ROC evaluates the best cutoff and AUC of initial FPG (5.3 mmol/L and 5.49 mmol/L) in predicting the further diagnosis of diabetes.

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Research Article

Prognostic Value of Atherosclerotic Extent in Diabetic Patients with Nonobstructive Coronary Artery Disease

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Background and Objective. Atherosclerotic extent was proved to be associated with adverse cardiac events. Risk scores derived by coronary computed tomography angiography (CCTA) could identify high-risk group among patients with nonobstructive coronary artery disease (CAD), but the ability is still uncertain in the presence of diabetes mellitus (DM). The purpose of this study was to investigate the prognostic value of the atherosclerotic extent shown by CCTA in diabetic patients with nonobstructive CAD. *Methods and Results*. 813 DM patients (mean age 58.9 ± 9.9 years, 48.1% male) referred for CCTA due to suspected CAD in 2015-2017 were consecutively included. During a median follow-up of 31.77 months, 50 major adverse cardiovascular events (MACEs) (6.15%) were experienced, including 2 cardiovascular deaths, 14 nonfatal myocardial infarctions, 27 unstable anginas requiring hospitalization, and 7 strokes. Three groups were defined based on coronary stenosis combined with Leiden score as normal, nonobstructive Leiden < 5, and nonobstructive Leiden ≥ 5. Cox models were used to assess the prognosis of plaque burden within these groups. An incremental incidence of MACE rates was observed. After adjustment for age, gender, and presence of high-risk plaque, the group of Leiden ≥ 5 showed a higher risk than Leiden < 5 (HR: 1.88, 95% CI: 1.03-3.42, $p = 0.039$). Similar results were observed when segment involvement score (SIS) was used for sensitivity analysis. *Conclusion*. Atherosclerotic extent was associated with the prognosis of DM patients with nonobstructive coronary artery disease, highlighting the importance of better risk stratification and management.

1. Introduction

It is well established that diabetes mellitus (DM) is associated with coronary artery disease (CAD) and a higher rate of mortality [1]. In turn, the rising prevalence of coronary artery disease, along with increased ischemic events, represents an important cardiac threat to DM patients. Early detection of CAD in this population has been an urgent requirement for the primary and secondary prevention of both fatal and nonfatal cardiac events [2–4].

Although there is no clear evidence suggesting the imaging evaluation of CAD in DM patients [5], the current practice guideline stands that coronary computed tomography angiography should be an access to cardiac risk assessment in the presence of DM with its high accuracy and acceptance [6]. Previous studies have shown that atherosclerotic extent derived by coronary computed tomography angiography (CCTA) has an extraordinary ability in risk stratification among nonobstructive CAD patients, to which little attention was paid due to the moderate stenosis [7]. However, few researches have been conducted on DM patients, despite the higher risk of major adverse cardiovascular events among them. Using comprehensive risk scores as a quantitative index, we aimed to investigate the stratification capability of atherosclerotic extent in DM patients with nonobstructive CAD.

2. Materials and Methods

2.1. Patients. This study was approved by the local ethics committee, and informed consent was obtained from all

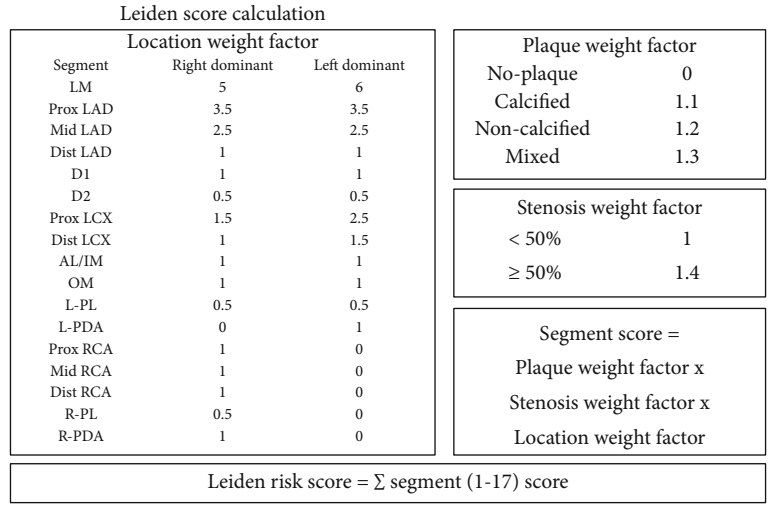

Figure 1: Schematic overview of the computed tomography angiography-derived risk score. Leiden score is calculated by the summation of segment score quantified as plaque weight factor × stenosis weight factor × location weight factor, i.e., a right dominant system with a noncalcified plaque with >50% stenosis in the left main segment (5 × 1.2 × 1.4) + a noncalcified plaque with <50% stenosis in the proximal left circumflex artery (1.5 × 1.2 × 1) + a calcified plaque with >50% stenosis in the right posterior descending artery (1 × 1.1 × 1.4), so the Leiden score is 11.74. Segment involvement score (SIS) was calculated by the summation of the segments exhibiting plaque; in the case above, SIS is 3. CTA = computed tomography angiography; AL = anterolateral segment; D1 = diagonal 1; D2 = diagonal 2; IM = intermediate segment; LAD = left anterior descending coronary artery; LCA = left coronary artery; LCX = left circumflex coronary artery; LM = left main segment; L-PDA = left posterior descending artery; L-PL = left posterolateral segment; OM = obtuse marginal segment; RCA = right coronary artery; R-PDA = right posterior descending artery; R-PL = right posterolateral segment.

participants. Between Jan 1, 2015, and Dec 31, 2017, 2135 DM patients who had undergone CCTA for suspected CAD in our institution were prospectively enrolled. Patients with known CAD, a history of percutaneous coronary intervention or coronary bypass surgery, a history of myocardial infarction or myocarditis, or revascularization driven by CCTA results within 3 months were excluded. Those with incomplete baseline data or uninterpretable CCTA results were ruled out of further analysis. In addition, only mild lesion was our concern, so the obstructive CAD was excluded according to CCTA definition mentioned below.

Basic demographic data were obtained by a review of medical records or patient interviews. DM was defined as fasting blood glucose ≥ 7.0 mmol/L or 2 h plasma glucose ≥ 11.1 mmol/L during oral glucose tolerance test or A1C ≥ 6.5 % (48 mmol/mol) or the use of oral hypoglycemic agents/insulin. The following cardiac risk factors were recorded: (1) hypertension (a systolic blood pressure ≥ 140 mmHg or a diastolic blood pressure ≥ 90 mmHg or administration of antihypertensive therapy), (2) hypercholesterolemia (known but untreated dyslipidaemia or current treatment with lipid-lowering medications), (3) positive family history of CAD (presence of CAD in first-degree relatives at <55 years in men and <65 years in women), and (4) smoking (current smoking or cessation of smoking within 3 months of CCTA).

2.2. Image Acquisition and Analysis. Multidetector CCTA scans were performed on a dual-source CT scanner (Somatom Definition Flash CT, Siemens Medical Solutions, Forchheim, Germany). All scans were analysed using a dedicated workstation (Syngo.via, Siemens) by two experienced cardiologists. When disagreements existed on diagnosis, the final decision would be made through consultation or the intervention of a third experienced researcher.

According to the modified American Heart Association classification, coronary lesions were assessed on the basis of the 17-segment model visually [8]. All segments were coded for the presence, composition, and severity of coronary plaque and were classified as normal, nonobstructive (1% to 49% luminal stenosis), or obstructive (>50% luminal stenosis). Calcified plaque was defined as having a density of >130 HU and further specified as "spotty" if its maximum diameter is <3 mm in any direction. Noncalcified plaque was defined as having an attenuation value lower than that of the contrasted vessel lumen. When both types existed, mixed plaque was defined. "Low CT attenuation plaques" were the presence of a central focal area within the plaque which has a low CT attenuation which is usually defined as at least 1 voxel with <30 HU. If the outer vessel diameter is >10% greater than the mean of the diameter of the normal adjoining segments, "positive remodelling" was recognized. "Napkin ring sign" was the presence of circumferential necrotic core. With at least two characteristics of "spotty calcification," "low CT attenuation plaques," "positive remodelling," and "napkin ring sign", high-risk plaque (HRP) was recorded [9, 10].

2.3. Comprehensive Risk Scores. Leiden score, a comprehensive risk score, was introduced as a quantitative index of atherosclerotic burden, containing information of plaque quantity, location, stenosis, and composition as shown in Figure 1. The segment involvement score (SIS) was obtained to quantify the atherosclerotic extent for sensitivity analysis, calculated as the total number of coronary artery segments

that exhibits plaque without consideration of stenosis (ranging from 0 to 16).

2.4. Follow-Up and Study Endpoint. Follow-up information was obtained by phone contact or the electronic medical record system. The primary endpoint was cardiovascular death, nonfatal myocardial infarction, stroke, or unstable angina requiring hospitalization that occurred >90 days after the CCTA examination from Jan 1, 2015, to Aug 31, 2020. Each event was identified by two physicians independently. In the case of divergence, consultation would be brought in.

2.5. Statistical Analysis. Analyses were performed with SPSS version 26.0 (SPSS, IL, USA) and R version 3.6.3. Baseline characteristics were presented as mean ± standard deviation or median (interquartile range (IQR)) for continuous variables and as proportions for categorical variables. Prevalence of no or nonobstructive CAD was calculated and stratified by the comprehensive risk score as normal group (no CAD), nonobstructive CAD with Leiden < 5, and nonobstructive CAD with Leiden ≥ 5. Sensitivity analysis was conducted with SIS, stratifying patients as the normal group (no CAD), nonobstructive CAD with SIS < 3, and nonobstructive CAD with SIS ≥ 3. Cumulative event rates were estimated using the Kaplan-Meier method and compared using the log-rank test. Cox proportional regression model was used to investigate multivariable-adjusted hazard ratios for increasing CAD severity mentioned above. A p value less than 0.05 was considered as statistically significant.

3. Results

3.1. Baseline Characteristics. A total of 2135 DM patients who underwent CCTA for suspected CAD were enrolled, among which 51 were lost during follow-up. 1271 patients were excluded because of known CAD, revascularization, incomplete data, or other criteria. A cohort of 813 diabetic patients (mean age 58.9 ± 9.9 years; 48.1% male; median follow-up 31.77 months) was included with full demographic characteristic and CCTA information. The prevalence of hypertension, hypercholesterolemia, current smoking, and a family history of CAD was 64.8%, 54.4%, 24.2%, and 23.6%, respectively (Table 1). For glucose control, 19.7% of the patients solely had a diet, 80.9% had oral hypoglycemic medication, and insulin was used in 14.3% of the patients. Overall, 190 (23.4%) of the 813 patients had no evidence of CAD on coronary CTA. In addition, high-risk plaques were found in 18 (2.2%) patients.

Table 1: Baseline characteristics.

Characteristic	Value (N = 813)
Age (years)	58.9 ± 9.9
Male	391 (48.1%)
Body mass index (kg/m^2)	26.2 ± 3.6
Cardiac risk factors	
Hypertension	527 (64.8%)
Hypercholesterolemia	442 (54.4%)
Current smoking	197 (24.2%)
Family history of CAD	192 (23.6%)
CCTA findings	
High-risk plaque	18 (2.2%)
CAD-RADS score	
0	190 (23.4%)
1	121 (14.9%)
2	502 (61.7%)
Segment involvement score	1 (1-1)
Segment stenosis score	1 (1-2)
Leiden risk score	2.8 (1.2-4.6)
Medication	
Antiplatelet	245 (30.1%)
Beta blocker	295 (36.3%)
ACEI/ARB	256 (31.4%)
Statin	245 (30.1%)
Calcium channel blocker	145 (17.8%)
Diabetic treatment	
Diet only	160 (19.7%)
Oral hypoglycemic agent	658 (80.9%)
Insulin	116 (14.3%)

Values are mean ± SD or n (%). CAD: coronary artery disease; CCTA: coronary computed tomography angiography; ACEI: angiotensin-converting enzyme inhibitor; ARB: angiotensin receptor blocker; CAD-RADS: Coronary Artery Disease-Reporting and Data System.

3.2. Cox Regression Analysis. In univariate analysis, age (HR: 1.04, 95% CI: 1.01-1.07) and the presence of HRP (HR: 11.66, 95% CI: 5.45-24.95) were associated with MACEs. Compared with the normal group, HR was 1.86 (95% CI: 0.70-5.00, p = 0.216) for the group of nonobstructive Leiden < 5 and 4.06 (95% CI: 1.56-10.56, p = 0.004) for nonobstructive Leiden ≥ 5, respectively.

In multivariate models, age (HR: 1.03, 95% CI: 1.00-1.07) and HRP (HR: 10.94, 95% CI: 5.00-23.92) remained significant in predicting outcome events (Table 2). Patients with nonobstructive Leiden ≥ 5 had an unadjusted hazard ratio of 4.06 (95% CI: 1.56 to 10.56, p = 0.004; log-rank test: p = 0.0015) (Table 2). After adjustment for age, gender, and presence of HRP, the hazard ratio remained significantly higher, which was 2.94 (95% CI: 1.11 to 7.79, p = 0.031) and 1.88 (95% CI: 1.03 to 3.42, p = 0.039), in comparison to the normal group and nonobstructive Leiden < 5, respectively.

3.3. Survival Analysis. Of the included 813 patients, 50 MACEs (6.15%) were experienced, including 2 cardiovascular deaths, 14 nonfatal myocardial infarctions, 27 unstable anginas requiring hospitalization, and 7 strokes (Figure 2). The annual MACE rate among patients in the normal group was 0.98 events per100 person-years, and the annual MACE rate among nonobstructive Leiden < 5 was 1.86 per 100 person-years, while the rate for nonobstructive Leiden ≥ 5 was 4.06 per 100 person-years (p < 0.01).

3.4. Sensitivity Analysis. For further sensitivity analysis, segment involvement score (SIS) was used to quantify the atherosclerotic extent instead. A comparable distribution of

Table 2: Univariate and multivariate analyses of clinical profile and CCTA findings for major cardiovascular events.

	Univariable HR (95% CI)	p value	Leiden × CAD Multivariable HR (95% CI)	p value
Age (years)	1.04 (1.01-1.07)	**0.009**	1.03 (1.00-1.07)	**0.027**
Male	0.75 (0.43-1.32)	0.325	0.84 (0.47-1.51)	0.556
BMI (kg/m^2)	1.03 (0.96-1.11)	0.388		
Cardiac risk factors				
Hypertension	1.23 (0.67-2.25)	0.505		
Hypercholesterolemia	1.42 (0.80-2.54)	0.231		
Current smoker	0.95 (0.50-1.82)	0.876		
Family history of CAD	0.69 (0.33-1.42)	0.310		
CCTA findings				
High-risk plaque	11.66 (5.45-24.95)	**<0.001**	10.94 (5.00-23.92)	**<0.001**
Leiden risk score	1.06 (1.00-1.13)	0.055		
Segment involvement score	1.17 (1.00-1.36)	0.048		
CAD severity (Leiden × CAD)				
Normal	Reference		Reference	
Nonobstructive Leiden < 5	1.86 (0.70-5.00)	0.216	1.56 (0.58-4.22)	0.379
Nonobstructive Leiden ≥ 5	4.06 (1.56-10.56)	**0.004**	2.94 (1.11-7.79)	**0.031**

Data in parentheses are 95% confidence intervals. In this analysis, gender and variables with significant ($p < 0.05$) impact on survival at a univariable level entered the multivariable model. HR: hazard rate; CAD: coronary artery disease; CCTA: coronary computed tomography angiography; BMI: body mass index.

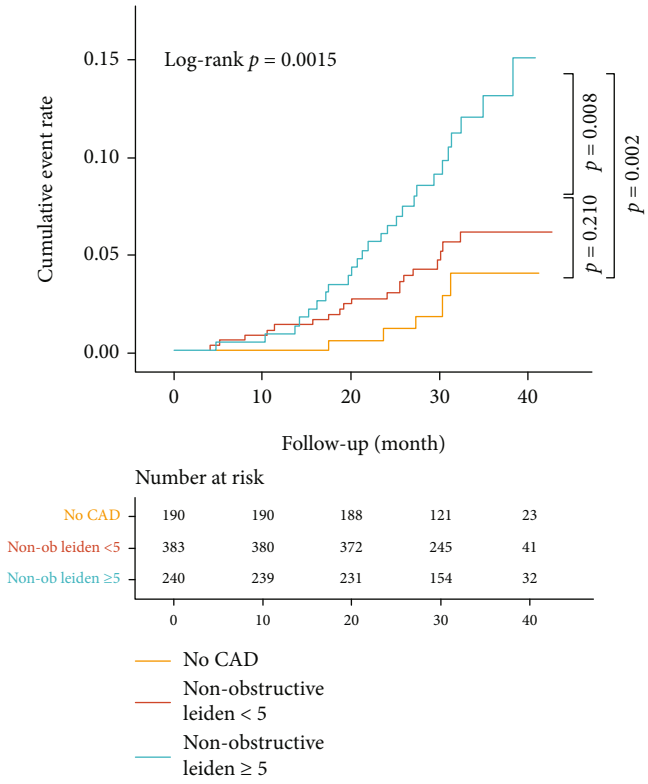

Figure 2: Cumulative risk of the composite endpoint on the basis of CAD severity with Leiden risk score (no CAD, nonobstructive CAD with Leiden < 5, and nonobstructive CAD with Leiden ≥ 5). CAD: coronary artery disease.

event rate has been noticed (Figure 3), of which the normal group, the nonobstructive SIS < 3 group, and the nonobstructive SIS ≥ 3 group were 2.63%, 5.54%, and 12.34%, respectively. In the adjusted Cox model, patients with nonobstructive SIS ≥ 3 conferred a significantly higher risk than those in both the normal group (HR: 3.49, 95% CI: 1.28-9.52, $p = 0.015$) and the nonobstructive SIS < 3 group (HR: 2.14, 95% CI: 1.17-3.91, $p = 0.013$).

4. Discussion

The main finding of this study was that in DM patients with nonobstructive CAD, higher atherosclerotic extent on CCTA provided incremental prognostic information and was associated with long-term cardiovascular outcome, even after adjustment for traditional risk factors including age, gender, and high-risk plaque profiles. Our results reinforced the notion that greater efforts are needed to promote risk stratification with nonobstructive CAD, especially in the presence of DM. Leiden risk score represented an effective and reliable tool for quantifying atherosclerotic extent, which had a substantial impact on clinical outcome in diabetic patients. The robustness of the conclusion was further evaluated with the sensitivity analysis using SIS, with similar main results observed.

Our findings concur with a previous cohort study [11], which demonstrated that it is possible to identify high-risk diabetic patients based on assessment of CAD through CCTA. However, several disparities must be noted. A slightly higher MACE rate was presented, compared with an annual event rate ranging from 1.5% to 16.9% as a meta-analysis showed [1], in which diabetes examined by CCTA was investigated. One possibility is that we broadened enrollment to

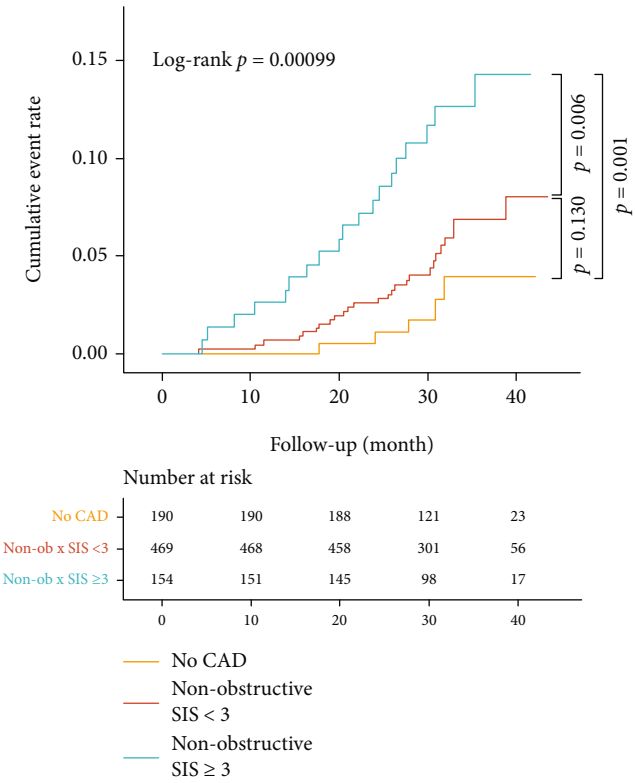

Figure 3: Cumulative risk of the composite endpoint on the basis of CAD severity with segment involvement score (no CAD, nonobstructive with SIS < 3, and nonobstructive with SIS ≥ 3). CAD: coronary artery disease; SIS: segment involvement score.

MACEs with strokes and an extended follow-up to a median of 31 months, which was a sufficient duration to capture more events. Moreover, up to 80% of the patients received hypoglycemic therapy in baseline, indicating a potentially long duration of diabetes and higher vascular risk. Another important observation from our study is that in risk-adjusted hazard analysis, the presence of HRP was found an independent predictor with a high HR of 3.15 (95% CI: 1.97-5.04). This corresponds with the result from the ICONIC study [12] that stressed the importance of HRP+ lesions in nonobstructive CAD, exhibiting a comparable risk of becoming a culprit lesion to obstructive HRP- lesions. In view of this, we bring it into analysis, which has rarely been studied before. However, after adjustment for HRP, extensive nonobstructive CAD was still found a significant indicator. This finding may inform future trials of the potential role of nonobstructive CAD in the setting of diabetes.

In the PROMISE (Prospective Multicenter Imaging Study for Evaluation of Chest Pain) trial, most cardiovascular deaths or myocardial infarctions (67%) occurred in patients with a normal stress test result at baseline, most of whom were found to have nonobstructive atherosclerotic disease by cardiac CT [13]. This suggests that we miss the opportunity to implement comprehensive preventive measures in most patients, especially in diabetic patients, by relying on stress test results. The SCOT-HEART (Scottish Computed Tomography of the Heart) trial revealed a reduction of 41% in the hazard of CAD-related death or nonfatal myocardial infarction for patients who were assigned to an anatomic versus functional strategy (2.4% vs. 3.9%) [14]. This was attributed to detection of nonobstructive coronary atherosclerosis and the initiation of directed preventive treatment. Our study was partly in line with the results above and further stressed the importance of medical management in diabetic patients with extensive nonobstructive coronary artery disease. The ability of noninvasively detecting nonobstructive atherosclerotic disease by CT, thus, should be rendered as a necessary opportunity to initiate earlier prevention or intensive treatment in the process of disease, a strategy proven effective in reducing MACEs [15].

Some previous studies have evaluated the extent and distribution of atherosclerosis with semiquantitative CCTA risk score in diabetes, mainly based on the SIS or the segment stenosis score (SSS) [16]. However, neither SIS nor SSS reflects the importance of relevant segment in coronary artery, because the proximal segment in the artery holds accountability for myocardial perfusion of larger territory. In this circumstance, the Leiden comprehensive risk score, being reported more strongly predictive than the SIS, integrates stenosis severity with the number and location of stenosis. A recent research from van den Hoogen et al. [17] evaluated the per-segment and per-patient weight scores to determine the contribution of the stenosis, composition, and location of CAD to the total score. As a result, all the per-patient weight scores were significantly higher in the setting of DM, while the per-segment location weight score was lower, which might be explained by the multisegment disease in DM patients. We also used SIS for sensitivity analysis to stratify the extent of atherosclerotic plaque, which demonstrated the similar result and further supported our hypothesis.

5. Study Limitation

First, as a retrospective single center study, referral decision for CCTA was made by physicians independently and certain patients were excluded finally due to various reasons, which may introduce selection bias. Second, diabetes is a dynamic risk factor; lack of the diabetes duration and treatment information on baseline may cause the misinterpretation of the subsequent data analysis. Third, although downstream treatment and management were recorded, relative treatments were not included in the final multivariate analysis, which may lead to potential confounders and over- or underestimation of the effect size of target variables.

6. Conclusion

In diabetic patients with nonobstructive CAD, atherosclerotic extent was associated with incremental risk of MACEs during a follow-up of about 3 years. Efforts should be made to determine risk stratification for the management of DM patients with nonobstructive CAD.

Data Availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest

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

Authors' Contributions

Yipu Ding and Zinuan Liu contributed to the work equally.

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Research Article

Unique Biomarker Characteristics in Gestational Diabetes Mellitus Identified by LC-MS-Based Metabolic Profiling

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Background. Gestational diabetes mellitus (GDM) is a type of glucose intolerance disorder that first occurs during women's pregnancy. The main diagnostic method for GDM is based on the midpregnancy oral glucose tolerance test. The rise of metabolomics has expanded the opportunity to better identify early diagnostic biomarkers and explore possible pathogenesis. *Methods*. We collected blood serum from 34 GDM patients and 34 normal controls for a LC-MS-based metabolomics study. *Results*. 184 metabolites were increased and 86 metabolites were decreased in the positive ion mode, and 65 metabolites were increased and 71 were decreased in the negative ion mode. Also, it was found that the unsaturated fatty acid metabolism was disordered in GDM. Ten metabolites with the most significant differences were selected for follow-up studies. Since the diagnostic specificity and sensitivity of a single differential metabolite are not definitive, we combined these metabolites to prepare a ROC curve. We found a set of metabolite combination with the highest sensitivity and specificity, which included eicosapentaenoic acid, docosahexaenoic acid, docosapentaenoic acid, arachidonic acid, citric acid, α-ketoglutaric acid, and genistein. The area under the curves (AUC) value of those metabolites was 0.984 between the GDM and control group. *Conclusions*. Our results provide a direction for the mechanism of GDM research and demonstrate the feasibility of developing a diagnostic test that can distinguish between GDM and normal controls clearly. Our findings were helpful to develop novel biomarkers for precision or personalized diagnosis for GDM. In addition, we provide a critical insight into the pathological and biological mechanisms for GDM.

1. Introduction

Gestational diabetes mellitus (GDM) is a glucose intolerance disorder that first emerges during women's pregnancy [1]. The prevalence of GDM ranges from 0.6% to 15% across different countries depending on the race and socioeconomic conditions of individuals [2]. It is considered that the increasing incidence of GDM worldwide is due to the growing prevalence of obesity in women of reproductive age and advanced maternal age [3]. GDM poses many health-related concerns with maternal and fetal complications, such as an increased risk for spontaneous preterm birth [4], neonatal hyperbilirubinemia, hypoglycemia [5], shoulder dystocia, stillbirth, acute hospitalization in the neonatal intensive care unit, and respiratory complications [6–9]. More notably, GDM may also lead to a significant increase in long-term incidence of type 2 diabetes and cardiovascular disease in pregnant women [10–18]. In view of these complications, it is important to detect women with GDM as early as possible. In addition, it is critical to utilize and implement current GDM risk-reduction strategies with the aim of minimizing the detrimental gestational complications for mother and

offspring. Currently, the accepted GDM diagnostic methods are both time consuming and laborious, which leads to low patient participation. Therefore, it is of utmost importance to find an alternative solution. The advent of advanced technology has made it possible to find more sensitive and specific molecules to distinguish between people with or without GDM. The emergence and development of metabolomics provide deeper insights into the discovery of new biomarkers for metabolic diseases, including GDM. More importantly, metabolomics can offer a noninvasive assessment using maternal biofluids and is a less expensive alternative to other approaches. Based on these advantages, metabolic profiling has the potential to meet the requirement for clinical application and provide critical insight into the pathological and biological mechanisms for GDM. Several studies have already identified early diagnostic markers and explored the pathophysiology of GDM using metabolomics. It has been reported that circulating fatty acids levels, including palmitic acid, stearic acid, and palmitoleic acid, were increased in GDM patients as compared with normal pregnancy groups [19–23]. Additional metabolites were also found to be significantly increased in women with GDM, such as prostanoic acid, sesaminol 2-O-triglucoside, tricin 7-neohesperidoside, dihydro-12-oxo-15-phytoenoic acid [24], acetylcarnitines [21, 22], bile acids [25], ketones [21, 26], creatinine, carbohydrate (primary glucose) [26], and other lipids and organic acids. Reduction of levels in phospholipids, (2E)-14-hydroxytetradec-2-enoic acid (or its isomer), (2E,13R)-13-hydroxytetradec-2-enoic acid, 2,15-dihydroxy-pentadecanoic acid (or its isomers), (7R,8S,9Z,12Z,15Z)-7,8-dihydroxy-9,12,15-octadecatrienoic acid, 11α,20,26-trihydroxyecdysone [24], glycerophospholipids [21, 22, 27], 1,5-anhydroglucitol [26], and gluconic acids [21, 27] was also reported. Moreover, decreases in amino acids [21, 22, 27, 28] and fatty acids have been shown [21, 22, 27]. However, some groups have reported that there were no significant changes in metabolite profiles between diagnosed GDM women and healthy controls [29–31]. These discrepancies may be due to differences in sample sizes, variations in study population composition, and statistical methods used.

Considering the promising diagnostic values of metabolomics in GDM, we subjected the serum from 34 normal controls and 34 GDM patients to metabolomics analysis in this study. The main objective of this study is to identify novel biomarkers for precision and personalized diagnosis for GDM and provide a critical insight into the pathological and biological mechanisms for GDM.

2. Materials and Methods

2.1. Study Population and Sample Collection. Clinical information was collected from 34 pregnant women with GDM and 34 healthy pregnant women with same the gestational weeks who gave birth in Women's Hospital School of Medicine Zhejiang University, Zhejiang, China, between 1 December 2018 and 31 March 2019. Inclusion criteria of pregnant women were as follows: (1) maternal ages at delivery ≥ 20 years; (2) gestational weeks at delivery ≥ 28 weeks; (3) detailed medical records; (4) singleton pregnancy; and (5) no presence of nonhereditary disease.

Exclusion criteria of pregnant women were as follows: (1) multiple pregnancies; (2) stillbirth; (3) in vitro fertilization-embryo transfer; and (4) had chronic diseases. Participants' blood samples were venously collected after 8-14 hours of fasting during their second trimester of pregnancy (24–28 gestational weeks). Sample transfer centrifugation (3500 rpm for 10 min at 4°C) and separation of serum were completed within 1 hour. Final samples were stored at -80°C until retrieval for metabolomics analysis.

2.2. The Diagnostic Criteria of GDM. The diagnosis of GDM cases were identified using the oral glucose tolerance test (OGTT) conducted between 24 and 28 gestational weeks. According to the International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria [32], pregnant women were considered to have GDM if one of the following plasma glucose values was met or exceeded: 0 h, 5.1 mmol/L; 1 h, 10.0 mmol/L; or 2 h, 8.5 mmol/L, after a 75 g glucose load.

3. Metabolomics Analysis

3.1. Metabolites Extraction. The serum samples had been thawed once prior to use for our study. The serum samples (100 μl) were resuspended with prechilled 80% methanol and 0.1% formic acid and vortex oscillation mix. Samples were placed in an ice bath for 5 min and then centrifuged at 15,000 rpm, 4°C for 10 min. A calculated amount of supernatant was diluted to a final concentration containing 60% methanol by LC-MS grade water and subsequently transferred to a fresh Eppendorf tube with 0.22 μm filter (Millipore, Bedford, MA, USA). Samples were then centrifugated at 15,000 g, 4°C for 10 min. Finally, the filtrate was injected into the LC-MS/MS system for analysis. Equal volume samples from each experimental sample were mixed as quality control (QC) samples. Blank sample is the blank matrix of the experimental sample, and the pretreatment process of the sample is the same as that of the experimental sample.

3.2. LC-MS Analysis (Liquid Chromatography-Mass Spectrometry). LC-MS/MS analyses were performed using a Vanquish UHPLC system (Thermo Fisher Scientific, Waltham, MA, USA) coupled with an Orbitrap Q Exactive series mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). Samples were injected into a Hypersil Gold column (C18) (Thermo Fisher Scientific, Waltham, MA, USA) using a 16 min linear gradient at a flow rate of 0.2 ml/min. The eluents for the positive polarity mode were eluent A (0.1% formic acid in water) and eluent B (methanol). The eluents for the negative polarity mode were eluent A (5 mM ammonium acetate, pH 9.0) and eluent B (methanol). The solvent gradient was set as follows: 2% B, 1.5 min; 100% B, 12.0 min; 100% B, 14.0 min; 2% B, 14.1 min; and 2% B, 16 min. The Q Exactive mass series spectrometer was operated in positive/negative polarity mode with spray voltage of 3.2 kV, capillary temperature of 320°C, sheath gas flow rate of 35 arb, and aux gas flow rate of 10 arb.

3.3. Identification of Metabolites. The raw data files generated by UHPLC-MS/MS were processed using the Compound Discoverer 3.0 (CD3.0, Thermo Fisher Scientific, Waltham, MA, USA) to perform peak alignment, peak picking, and quantitation for each metabolite. The main parameters were set as follows: retention time deviation of 0.1 min; quality deviation of 5 ppm; signal strength deviation of 30%; signal-to-noise ratio of 3; and minimum signal strength of 100,000. Peak intensities were normalized to the total spectral intensity. The normalized data was then used to predict the molecular formula based on additive ions, molecular ion peaks, and fragment ions. Lastly, peaks were matched with the mzCloud (https://www.mzcloud.org/) and ChemSpider (http://www.chemspider.com/) databases to obtain the accurate qualitative and relative quantitative results.

4. Data Analysis

After the serum metabolites assessment, the metabolites were annotated using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.genome.jp/kegg/), Human Metabolome Database (HMDB) database (http://www.hmdb.ca/), and Lipidmaps database (http://www.lipidmaps.org/). Principal components analysis (PCA), partial least squares discriminant analysis (PLS-DA), and fold change (FC) analysis were performed at metaX (a flexible and comprehensive software for processing metabolomics data). A univariate analysis (t-test) was applied to calculate the statistical significance (p-value). In this study, the Bonferroni method was used to reduce the false discovery rate (FDR). The metabolites with variable importance in the projection $(\mathrm{VIP}) > 1$, $p - \mathrm{value} < 0.05$, and $\mathrm{fold\,change} \geq 2$ or $\mathrm{FC} \leq 0.5$ were considered differential metabolites. Volcano plots were used to filter metabolites of interest, which were based on $\mathrm{Log}_2(\mathrm{FC})$ and $-\mathrm{Log}_{10}(p - \mathrm{value})$ of metabolites.

The data used for clustering heat maps was normalized using z-scores of the intensity areas of differential metabolites and was plotted by the Pheatmap package in *R* language (version 3.5.1). The correlation between differential metabolites was analyzed by *cor()* in *R* language (method: *Pearson*). Statistically significant values of correlation between differential metabolites were calculated by *cor.mtest* in *R* language. $p - \mathrm{value} < 0.05$ was considered statistically significant, and correlation plots were plotted by *corrplot* package in *R* language. The functions of these metabolites and metabolic pathways were studied using the KEGG database. The metabolic pathway enrichment of differential metabolites was performed when ratio was satisfied by $x/n > y/N$. Metabolic pathway enrichments were considered statistically significant when p-value of metabolic pathway is <0.05.

5. Statistical Analysis

The SPSS software version 20 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis and receiver operating characteristic (ROC) curve preparation. The modeling methods were selected on the basis of the logistic regression to increase the diagnostic accuracy. When the data was not normally distributed, normal transformations were attempted using of area normalization method. For the data processing portion, Log_2 conversion and standardization was completed; then, we performed UV scale and a two-tailed t-test to calculate the p-value. In this study, we used the Log_2 conversion to make the data meet the normality of the distribution. Chi-square test was used for categorical data and the Student's ***t***-test for measurement data between two groups. All collected data was expressed as the mean ± standard error of the mean (SEM), and the statistical significance level was set at $p < 0.05$.

6. Results

6.1. Comparison of Clinical Data. A total of 68 individuals, 34 normal pregnant women, and 34 pregnant women with GDM were included in this study. Detailed clinical data were recorded for all participants. The mean maternal age of two groups was 28.35 ± 3.03 and 31.78 ± 4.61 years, respectively. A comparison of height, weight, age for marriage, gravidity, systolic blood pressure, diastolic blood pressure, weight gain, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) was listed in Table 1. In addition, other laboratory data (Table S1) showed no significant differences between the GDM and control groups. Body mass index (BMI), fasting glucose and insulin, 1 h glucose, 2 h glucose and insulin, hemoglobin A1 (HbA1C), homeostasis model of assessment-insulin resistance (HOMA-IR), and triacylglycerides (TG) were significantly higher in the GDM group than in the control group ($p < 0.05$, Table 1). Gestational weeks and high-density lipoprotein- (HDL-) cholesterol were significantly lower in the GDM group compared with the control group ($p < 0.05$, Table 1). No differences between the two groups ($p > 0.05$, Table 1) were observed in the following parameters: age for marriage, height, weight, weight gain, gravidity, parity, systolic blood pressure, diastolic blood pressure, 1 h insulin, and total cholesterol (TCH).

6.2. Metabolic Results. To maximize the identification of different metabolites, we tested the samples in both the positive ion mode and negative ion mode. QC samples were included to determine the state of the instrument and to evaluate the stability of the system during the whole experiment. The correlations of QC samples were all close to 1, indicating that the method used has high stability and good data quality (Figures S2(a) and S2 (b)). The peak obtained from all experimental samples and QC samples were extracted and Pareto scaling was applied for PCA analysis. In the PCA analysis diagram, the distribution of QC samples, GDM samples, and control samples is clustered together. These results further indicate that the model we employed is reliable (Figures S2(c)–S2(f)).

Next, we analyzed the differential metabolites. PCA analysis was used as an unsupervised method, and PLS-DA was used as a supervised method to get an overview of the data and to detect trends within the experiment. A clear separation can be observed between GDM and control groups from the LC-MS data, indicating metabolic changes are inherent to GDM (Figures 1(a)–1(f)).

Table 1: General characteristics of study subjects.

Characteristic	No GDM	GDM	p-value
Age, years	28.35 ± 3.03	31.78 ± 4.61	0.001
Age for marriage, years	26.12 ± 2.56	27.2 ± 3.04	0.115
Height, cm	161 ± 5.26	159 ± 4.67	0.138
Weight, kg	57.85 ± 6.36	62.13 ± 7.77	0.016
BMI, kg/m^2	22.33 ± 2.6	24.45 ± 2.45	0.001
Weight gain, kg	6.26 ± 2.83	6.33 ± 3.91	0.930
BMI before pregnant, kg/m^2	19.91 ± 2.3	21.96 ± 2.4	0.001
Gravidity	1.53 ± 0.75	1.88 ± 1.00	0.149
Parity			
Nulliparous	22	16	
Multiparous	12	18	0.147
Gestational weeks	39.24 ± 1.07	38.44 ± 1.30	0.008
Systolic blood pressure (mm Hg)	113.6 ± 9.13	118.3 ± 10.6	0.055
Diastolic blood pressure (mm Hg)	66.6 ± 8.9	68.5 ± 10.4	0.420
Fasting glucose (mmol/l)	4.37 ± 0.34	5.07 ± 0.68	$p \leq 0.001$
1 h glucose, OGTT (mmol/l)	7.44 ± 1.21	10.70 ± 1.33	$p \leq 0.001$
2 h glucose, OGTT (mmol/l)	6.32 ± 0.75	9.43 ± 1.51	$p \leq 0.001$
HbA1C (%)	4.8 ± 0.21	5.3 ± 0.39	$p \leq 0.001$
Insulin (μU/ml)	7.21 ± 2.95	10.29 ± 4.35	$p \leq 0.001$
1 h insulin (μU/ml)	61.69 ± 24.55	68.73 ± 38.49	0.372
2 h insulin (μU/ml)	54.12 ± 25.81	86.26 ± 47.29	0.001
HOMA-IR	1.42 ± 0.64	2.33 ± 0.18	$p \leq 0.001$
Triacylglycerides (mmol/l)	1.95 ± 0.57	2.86 ± 1.44	0.001
Total cholesterol (mmol/l)	6.04 ± 0.79	5.65 ± 1.05	0.083
LDL-cholesterol (mmol/l)	3.08 ± 0.63	2.63 ± 0.70	0.007
HDL-cholesterol (mmol/l)	2.09 ± 0.42	1.78 ± 0.41	0.003
ALT, median(range), U/l	20.29 ± 9.43	18.26 ± 14.08	0.500
AST, median(range), U/l	19.71 ± 5.33	17.65 ± 7.28	0.188

GDM, gestational diabetes mellitus; HbA1c, hemoglobin A1; HOMA-IR, homeostasis model of assessment-insulin resistance; ALT, alanine aminotransferase; AST, aspartate aminotransferase. Data are mean ± SEM. t-test in continuous variables and chi-square test in categorical data were performed as appropriate. Results were considered significant when $p < 0.05$, compared with control group.

Based on the compounds identified by LC-MS, we generated a metabolite heat map and volcano map that revealed considerable differences between healthy controls and pregnant women with GDM (Figure 2). It can be seen that under the positive ion mode, a total of 2022 metabolites were detected with 184 increased metabolites and 86 decreased metabolites (Figures 2(a) and 2(c)). In the negative ion mode, a total of 1299 metabolites were detected with 65 increased metabolites and 71 decreased metabolites (Figures 2(b) and 2(d)). To better identify the potential biomarkers for GDM, we selected the top 40 differential changed metabolites between GDM patients and the normal pregnancy controls (20 for positive ion mode, and 20 for negative ion modes, respectively) (Table S3).

Next, correlation analysis of differential metabolites and KEGG pathway enrichment prediction were performed, and a KEGG enrichment bubble map was generated. The biosynthesis of unsaturated fatty acids, biosynthesis of phenylpropanoids, carbon fixation pathways in prokaryotes, biosynthesis of terpenoids and steroids, two-component system, ascorbate and aldarate metabolism, furfural degradation, isoflavonoid biosynthesis, biosynthesis of alkaloids derived from shikimate pathway, and biosynthesis of secondary metabolites were all found to be statistically different between the control group and the GDM group (Figures 3(a)–3(d); Table S4). In these pathways, docosahexaenoic acid, docosapentaenoic acid, arachidonic acid, citric acid, α-ketoglutaric acid, phosphoric acid, dehydroascorbic acid, 2-furoic acid, cephaeline, and methyl jasmonate were upregulated, and isoliquiritigenin, genistein, daidzein, and typhasterol were downregulated. In the positive ion mode, the only pathway with statistical differences was the biosynthesis of unsaturated fatty acids (Table S4). From these results, we selected the differential metabolites in this pathway

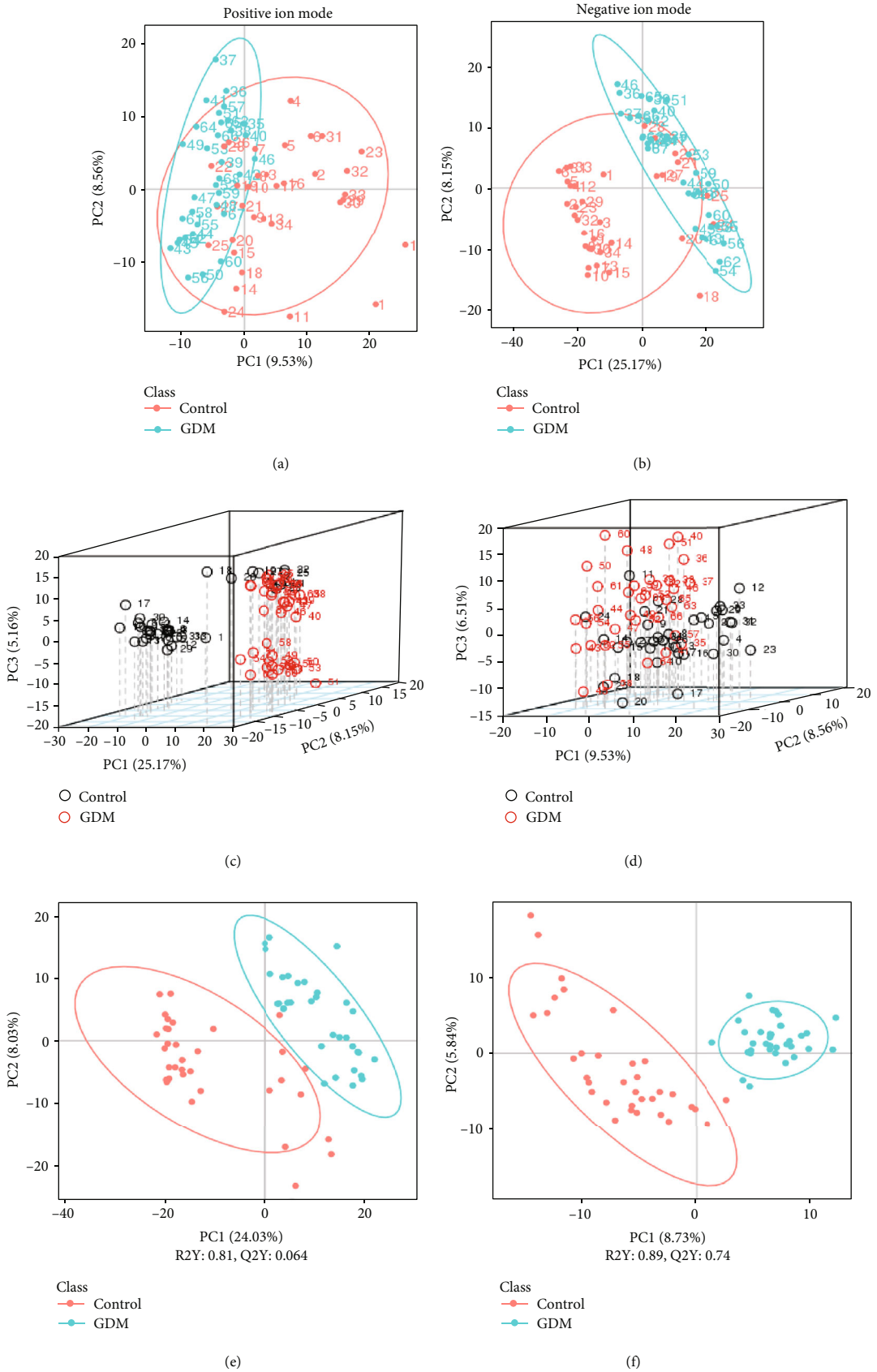

Figure 1: Metabolomic analysis of control and GDM. (a, b) PCA analysis between control and GDM. (c, d) 3D score plot of PCA analysis between control and GDM. (e, f) PLS-DA analysis between control and GDM, (a, c, e) Positive ion mode. (b, d, f) Negative ion mode.

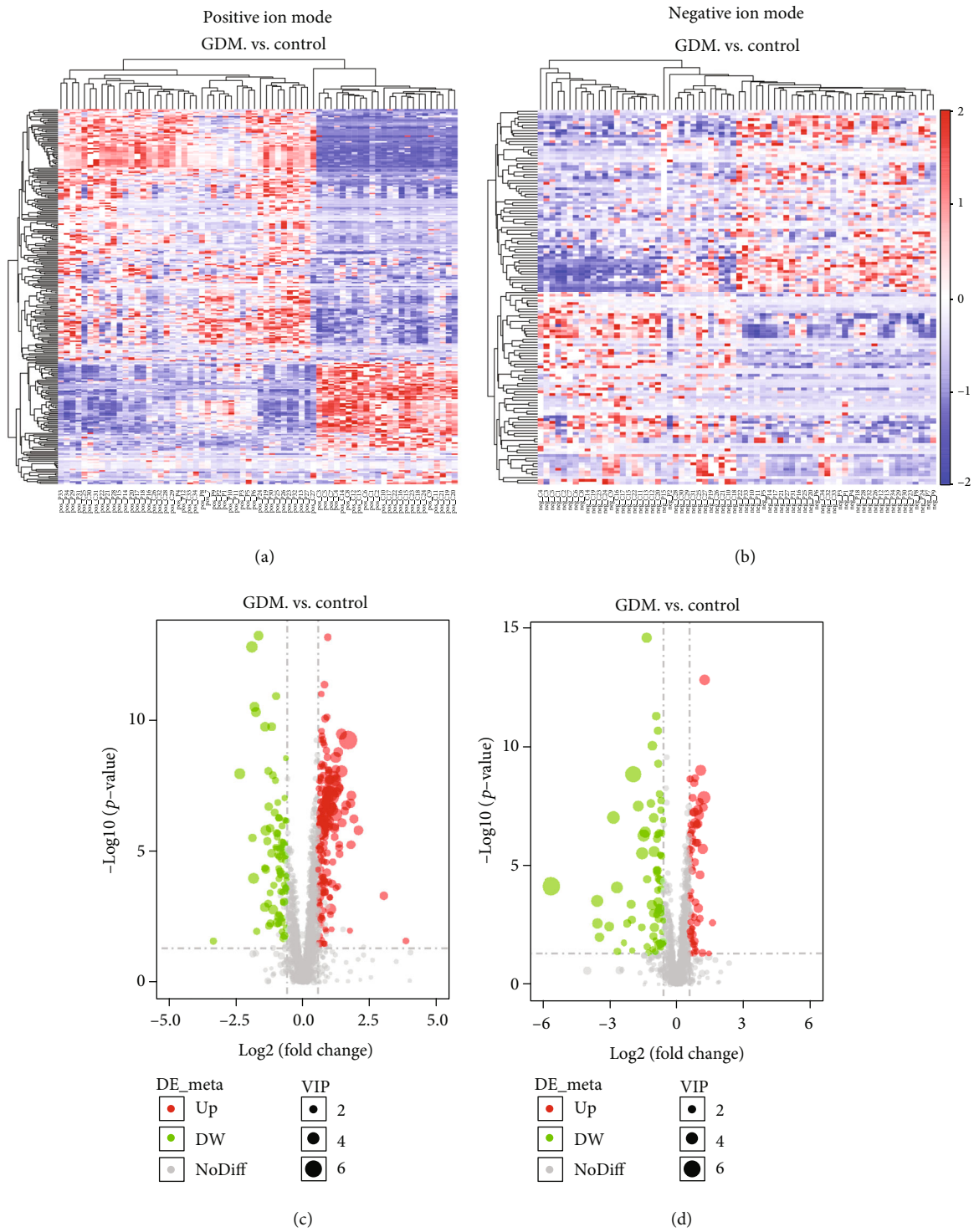

Figure 2: Analysis of differential metabolites between control and GDM through LC-MS. (a, b) Hierarchical clustering analysis was performed on each group of differential metabolites obtained, and the relative quantitative values of the differential metabolites were converted into z values ($z = (x - \mu)/\sigma$: x is a specific fraction, μ is average Number, and σ is the standard deviation) and clustering; different color regions represent different clustering group information, similar to the metabolic expression patterns in the same group, and may have similar functions or participate in the same biological process. (c, d) For each metabolite difference multiple, take the logarithm of 2 as the base, and take the p-value to the absolute value of the logarithm of 10 to make the volcano map. (a, c) Positive ion mode. (b, d) Negative ion mode.

for further analysis: eicosapentaenoic acid, docosahexaenoic acid, docosapentaenoic acid, and arachidonic acid. In the negative ion mode, differential metabolites were selected based on the following principles: (1) VIP values > 3; (2) KEGG metabolic pathway; and (3) 20 metabolites with the smallest p-value (Tables S4 and S5). The overlapping

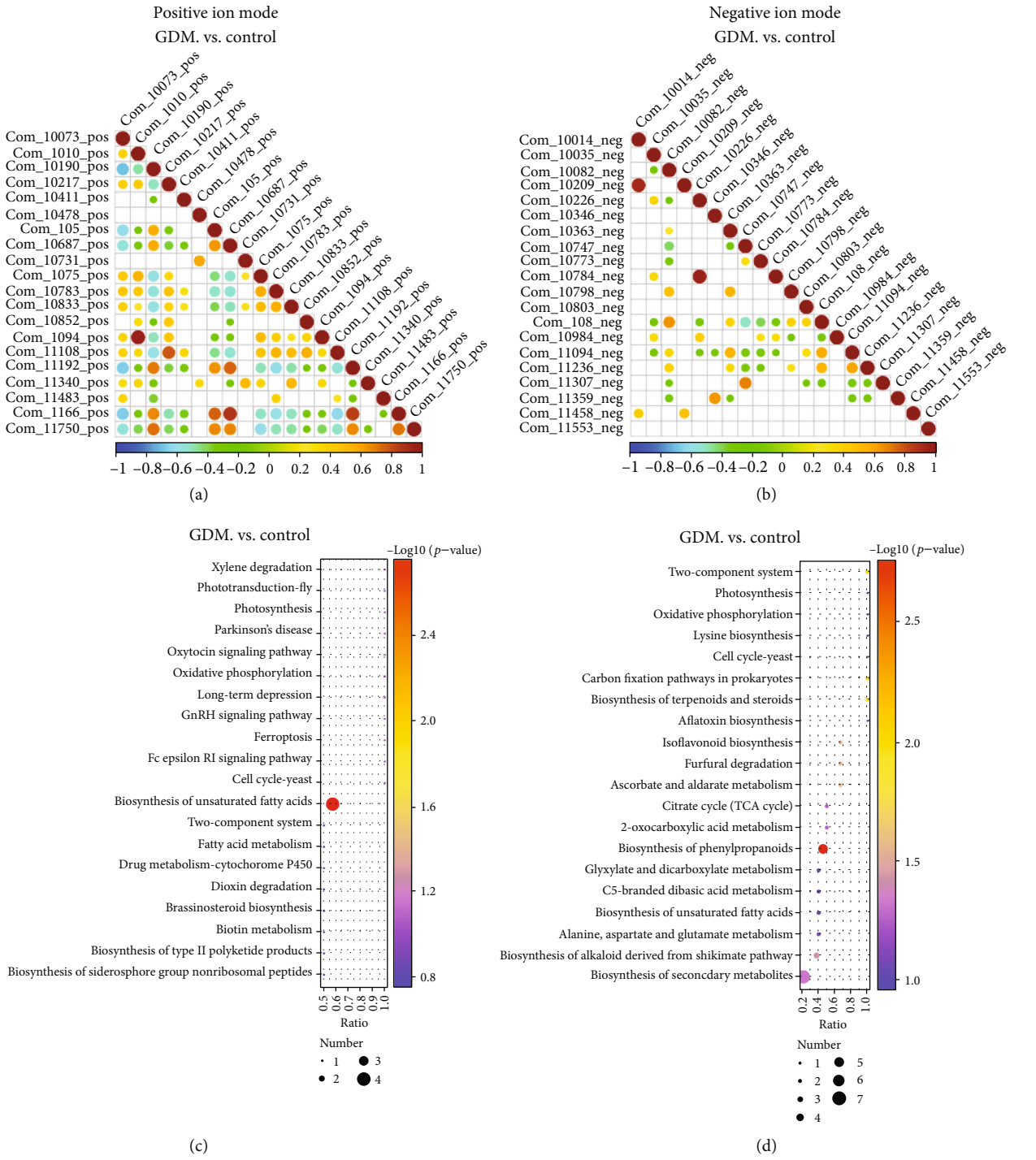

FIGURE 3: Differential metabolite KEGG pathway enrichment map. (a, b) When the linear relationship of the two metabolites is enhanced, the correlation coefficient tends to 1 or -1; when positive correlation, it tends to 1, and when it is negatively correlated, it tends to -1. The correlation is a maximum of 1, a complete positive correlation (red), a correlation of -1, and a complete negative correlation (blue). (c, d) KEGG analysis was used to identify the pathways which were significantly enriched by differential metabolites compared to all identified metabolite backgrounds. (a, c) Positive ion mode. (b, d) Negative ion mode.

metabolites that fell within criteria of these three conditions were selected for subsequent analysis. These metabolites include citric acid, α-ketoglutaric acid, genistein, daidzein, phosphoric acid, and 2-furoic acid, which can be screened in both positive ion mode and negative ion modes. The FC, VIP, and AUC of these ten metabolites are listed in Table 2.

6.3. Validation and Diagnostic Performance of Selected Metabolite. The levels of the selected metabolites in a group of participants comprising of 34 normal women and 34 women with GDM were measured using LC-MS and analyzed by the Student's *t*-test. Figures 4 and 5 depict the boxplots of their concentration levels in the GDM and control.

Table 2: Ten major differential metabolites for future analysis.

ID	Name_des	Formula	Molecular weight	RT (min)	FC	*p*-value	VIP	Up/down
ESI+								
Com_962_pos	Eicosapentaenoic acid	C20 H30 O2	302.22	14.10	0.58	4.95E-03	2.28	Down
Com_384_pos	Docosahexaenoic acid	C22 H32 O2	328.24	15.07	1.77	1.62E-06	1.60	Up
Com_2412_pos	Docosapentaenoic acid	C22 H34 O2	330.26	15.24	2.36	6.97E-09	2.23	Up
Com_1075_pos	Arachidonic acid	C20 H32 O2	304.24	15.13	1.50	3.85E-06	1.06	Up
Com_7642_pos	α-Ketoglutaric acid	C5 H6 O5	146.02	1.40	1.18	1.22E-03	0.44	Up
Com_332_pos	Phosphoric acid	H3 O4 P	97.98	1.68	1.65	9.31E-07	1.80	Up
ESI-								
Com_108_neg	Citric acid	C6 H8 O7	192.03	1.49	2.11	1.02E-09	3.43	Up
Com_5332_neg	α-Ketoglutaric acid	C5 H6 O5	146.02	1.72	1.78	2.17E-09	2.00	Up
Com_7586_neg	Genistein	C15 H10 O5	270.05	9.61	0.63	6.15E-03	1.17	Down
Com_8251_neg	Daidzein	C15 H10 O4	254.06	9.10	0.52	4.12E-02	1.50	Down
Com_740_neg	Phosphoric acid	H3 O4 P	97.98	1.55	2.33	1.42E-08	4.31	Up
Com_783_neg	2-Furoic acid	C5 H4 O3	112.02	1.50	2.25	1.97E-06	3.15	Up

FC, fold change (GDM case/control). VIP, variable importance in the projection. ESI+, positive ion mode; ESI-, negative ion mode.

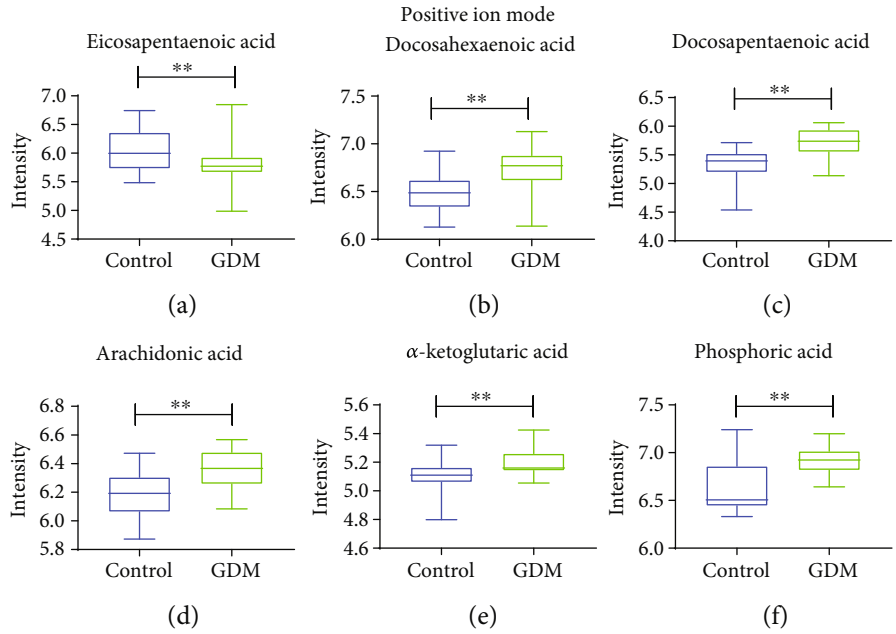

Figure 4: Boxplots of metabolites between control and GDM under positive ion mode. Student's *t*-test for measurement data between two groups was performed for significant difference. All data are expressed as the mean ± SEM, and the statistical significance level was set at $^{*}p < 0.05$. $^{**}p < 0.01$.

Table 3 shows the group AUC values of targeted metabolites obtained through multiple comparison analysis. The AUC values indicate the diagnostic potentials of the metabolites as unique biomarkers for identification of GDM and control.

The area under the curve for the individual differential metabolites we selected was less than 0.882 (Table 2). However, we hope to find a combination of metabolites which has higher sensitivity and specific to distinguish between GDM and controls. We decided to combine the metabolites into various sets and subjected them to AUC analysis to evaluate their diagnostic performances as combined biomarkers for GDM. Based on the principle of selecting the least metabolites and the highest area under the curve, we have selected the following combination: eicosapentaenoic acid, docosahexaenoic acid, docosapentaenoic acid, arachidonic acid, citric acid, α-ketoglutaric acid, and genistein. The AUC value of the combined metabolites was 0.984 between the GDM and control groups (Table 3; Figure S6).

7. Discussion

Alteration in metabolites, like bile acid metabolism, amino acid metabolism, and fatty acid metabolism, has all been involved with the development of metabolic diseases and is characterized as a hallmark of metabolic diseases, such as GDM [31–33]. The emergence and development of

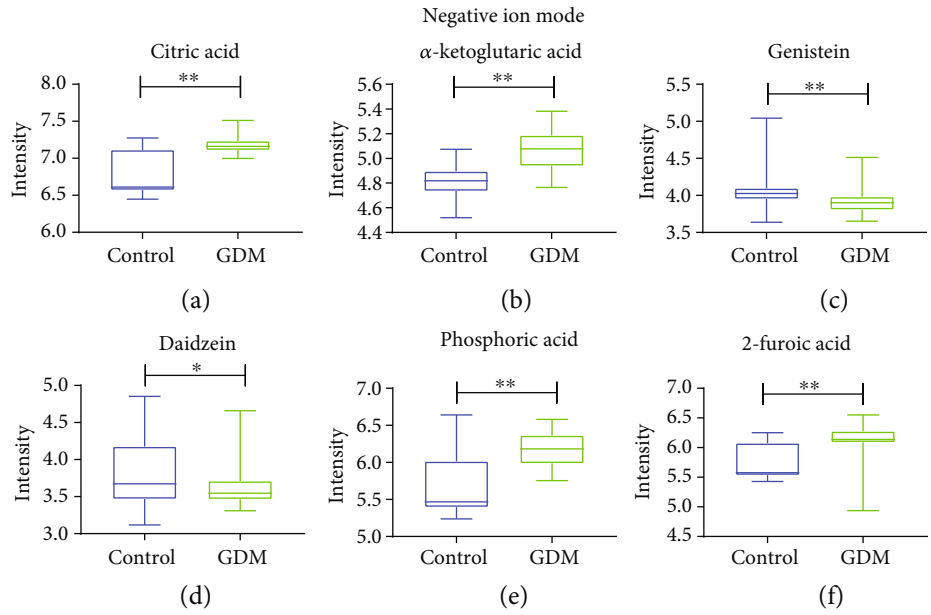

Figure 5: Boxplots of metabolites between control and GDM under negative ion mode. Student's t-test for measurement data between two groups was performed for significant difference. All data are expressed as the mean ± SEM, and the statistical significance level was set at $^{*}p < 0.05$. $^{**}p < 0.01$.

metabolomics provide deeper insights in the discovery of new biomarkers of these diseases [34]. More importantly, metabolomics can offer a noninvasive assessment by using maternal biofluids and is a less expensive alternative to other approaches [35]. GDM can be diagnosed using the OGTT method, which is a cheap "golden diagnostic standard". Unfortunately, it may be not the ideal biomarker to predict the potential mechanism related to GDM [36]. In this study, we identified several differential metabolites, such as eicosapentaenoic acid, docosahexaenoic acid, docosapentaenoic acid, and arachidonic acid, that were closely correlated to the disease process of GDM. Thus, the metabolomic biomarkers have the potential to serve as an innovative approach for the predictive, preventive, and personalized medicine in the future.

Our analysis of clinical data shows that triacylglycerides were significantly increased in the GDM group, while HDL-cholesterol and low-density lipoprotein- (LDL-) cholesterol decreased significantly in the GDM group (Table 2). This finding suggests there is a change in lipid metabolism during women's pregnancy. Our study identified four molecules in the pathway of unsaturated fatty acid metabolism, which includes eicosapentaenoic acid, docosahexaenoic acid, docosapentaenoic acid, and arachidonic acid.

Pregnancy is a complicated physiological process, and pregnant women need sufficient nutrition to support the growth and development of themselves and their fetus. It has been found that arachidonic acid (AA) and docosahexaenoic acid (DHA) play important roles in fetal growth and development [37, 38]. However, these enzyme expressions to synthesize long-chain polyunsaturated fatty acids (LC-PUFA) are quite low in the fetus. This shows that AA and DHA, which is necessary for fetal growth and development, are supplied by the placental transport [39]. Therefore, alterations in maternal polyunsaturated fatty acid (PUFA) metabolism during gestation would significantly impact the growth and development of the fetus. In addition, research has been reported that in placental transfer of AA in vitro in perfused placentas of women with insulin-dependent diabetes mellitus was impaired [40].

Williams et al. found that linoleic acid, oleic acid, myristic acid, D-galactose, D-sorbitol, O-phosphocolamine, L-alanine, L-valine, 5-hydroxy-l-tryptophan, L-serine, sarcosine, L-pyroglutamic acid, L-mimosine, L-lactic acid, glycolic acid, fumaric acid, and urea differentiated GDM cases from controls using GC-MS technology in early pregnancy and identified combinations of metabolites in early pregnancy that are associated with subsequent risk of GDM [41].

In this study, we also found some additional branched-chain amino acids that can be used to distinguish GDM from the control group using untargeted metabolomics. It was found that DL-β-leucine, L-threonine, L-(+)-alanine, DL-serine, valine, L-tyrosine, α-linolenic acid, oleic acid, tryptophan, and glutamine have no significant change between controls and GDM; L-isoleucine, L-theanine, L-aspartic acid, L-phenylalanine, cystine, L-glutamic acid, and DL-lysine have significant changes. Among the 17 metabolites discovered by Williams et al., most of them were not detected in our study. Based on these results, it is reasonable to infer that metabolism is different in the early and middle trimesters of pregnancy.

Although we find α-linolenic acid (ALA) has no significant change between controls and GDM in our study, our results show that DHA was increased in GDM. DHA can be converted from ALA [42, 43]. Circulating levels of DHA can reflect the ability of synthesize by the liver and the amount of dietary intake. Previously, White et al. performed to compare obese women with GDM with obese non-GDM

Table 3: Area under the curves among GDM and control.

Group	AUC	95% CIs
Eicosapentaenoic acid; docosahexaenoic acid; docosapentaenoic acid; arachidonic acid	0.909	0.840-0.979
Eicosapentaenoic acid; docosahexaenoic acid; docosapentaenoic acid; arachidonic acid; citric acid	0.959	0.921-0.998
Eicosapentaenoic acid; docosahexaenoic acid; docosapentaenoic acid; arachidonic acid; α-ketoglutaric acid	0.981	0.957-1.000
Eicosapentaenoic acid; docosahexaenoic acid; docosapentaenoic acid; arachidonic acid; genistein	0.913	0.846-0.981
Eicosapentaenoic acid; docosahexaenoic acid; docosapentaenoic acid; arachidonic acid; daidzein	0.920	0.858-0.983
Eicosapentaenoic acid; docosahexaenoic acid; docosapentaenoic acid; arachidonic acid; phosphoric acid	0.952	0.906-0.997
Eicosapentaenoic acid; docosahexaenoic acid; docosapentaenoic acid; arachidonic acid; 2-furoic acid	0.957	0.916-0.997
Eicosapentaenoic acid; docosahexaenoic acid; docosapentaenoic acid; arachidonic acid; citric acid; α-ketoglutaric acid	0.984	0.962-1.000
Eicosapentaenoic acid; docosahexaenoic acid; docosapentaenoic acid; arachidonic acid; citric acid; genistein	0.960	0.922-0.998
Eicosapentaenoic acid; docosahexaenoic acid; docosapentaenoic acid; arachidonic acid; citric acid; daidzein	0.958	0.918-0.997
Eicosapentaenoic acid; docosahexaenoic acid; docosapentaenoic acid; arachidonic acid; citric acid; phosphoric acid	0.958	0.917-0.998
Eicosapentaenoic acid; docosahexaenoic acid; docosapentaenoic acid; arachidonic acid; citric acid, 2-furoic acid	0.960	0.922-0.998
Eicosapentaenoic acid; docosahexaenoic acid; docosapentaenoic acid; arachidonic acid; α-ketoglutaric acid; genistein	0.982	0.959-1.000
Eicosapentaenoic acid; docosahexaenoic acid; docosapentaenoic acid; arachidonic acid; α-ketoglutaric acid; daidzein	0.981	0.957-1.000
Eicosapentaenoic acid; docosahexaenoic acid; docosapentaenoic acid; arachidonic acid; α-ketoglutaric acid; phosphoric acid	0.982	0.959-1.000
Eicosapentaenoic acid; docosahexaenoic acid; docosapentaenoic acid; arachidonic acid; α-ketoglutaric acid; 2-furoic acid	0.981	0.957-1.000
Eicosapentaenoic acid; docosahexaenoic acid; docosapentaenoic acid; arachidonic acid; genistein; daidzein	0.922	0.860-0.984
Eicosapentaenoic acid; docosahexaenoic acid; docosapentaenoic acid; arachidonic acid; genistein; phosphoric acid	0.951	0.905-0.997
Eicosapentaenoic acid; docosahexaenoic acid; docosapentaenoic acid; arachidonic acid; genistein; 2-furoic acid	0.958	0.919-0.998
Eicosapentaenoic acid; docosahexaenoic acid; docosapentaenoic acid; arachidonic acid; daidzein; phosphoric acid	0.956	0.913-0.999
Eicosapentaenoic acid; docosahexaenoic acid; docosapentaenoic acid; arachidonic acid; daidzein; 2-furoic acid	0.956	0.915-0.997
Eicosapentaenoic acid; docosahexaenoic acid; docosapentaenoic acid; arachidonic acid; phosphoric acid; 2-furoic acid	0.958	0.915-0.997
Eicosapentaenoic acid; docosahexaenoic acid; docosapentaenoic acid; arachidonic acid; citric acid; α-ketoglutaric acid; genistein	0.984	0.962-1.000
Eicosapentaenoic acid; docosahexaenoic acid; docosapentaenoic acid; arachidonic acid; citric acid; α-ketoglutaric acid; daidzein	0.984	0.962-1.000
Eicosapentaenoic acid; docosahexaenoic acid; docosapentaenoic acid; arachidonic acid; citric acid; α-ketoglutaric acid; phosphoric acid	0.984	0.962-1.000
Eicosapentaenoic acid; docosahexaenoic acid; docosapentaenoic acid; arachidonic acid; citric acid; α-ketoglutaric acid; 2-furoic acid	0.984	0.962-1.000
Eicosapentaenoic acid; docosahexaenoic acid; docosapentaenoic acid; arachidonic acid; citric acid; genistein; daidzein	0.957	0.916-0.997

Table 3: Continued.

Group	AUC	95% CIs
Eicosapentaenoic acid; docosahexaenoic acid; docosapentaenoic acid; arachidonic acid; citric acid; genistein; phosphoric acid	0.958	0.919-0.998
Eicosapentaenoic acid; docosahexaenoic acid; docosapentaenoic acid; arachidonic acid; citric acid; genistein; 2-furoic acid	0.960	0.922-0.998
Eicosapentaenoic acid; docosahexaenoic acid; docosapentaenoic acid; arachidonic acid; citric acid; α-ketoglutaric acid; genistein; daidzein	0.983	0.960-1.000
Eicosapentaenoic acid; docosahexaenoic acid; docosapentaenoic acid; arachidonic acid; α-ketoglutaric acid; genistein; daidzein; phosphoric acid	0.981	0.957-1.000
Eicosapentaenoic acid; docosahexaenoic acid; docosapentaenoic acid; arachidonic acid; α-ketoglutaric acid; genistein; daidzein; 2-furoic acid	0.981	0.957-1.000
Eicosapentaenoic acid; docosahexaenoic acid; docosapentaenoic acid; arachidonic acid; citric acid; α-ketoglutaric acid; genistein; daidzein; phosphoric acid	0.984	0.962-1.000
Eicosapentaenoic acid; docosahexaenoic acid; docosapentaenoic acid; arachidonic acid; citric acid; α-ketoglutaric acid; genistein; daidzein; 2-furoic acid	0.984	0.962-1.000
Eicosapentaenoic acid; docosahexaenoic acid; docosapentaenoic acid; arachidonic acid; citric acid; α-ketoglutaric acid; genistein; daidzein; phosphoric acid; 2-furoic acid	0.984	0.962-1.000

ROC curves were prepared for different metabolite combinations. AUC, area under the curves.

women at time point 1 (mean gestational weeks 17 weeks 0 days) and time point 2 (mean gestational weeks 27 weeks 5 days) using a targeted NMR metabolome [44]. The results showed that total fatty acids were higher at time point 1 and were marginally increased at time point 2. Additionally, monounsaturated fatty acid and saturated fatty acid concentrations were greater at both time points. At time point 2, a decreased proportion of DHA and increased proportion of saturated fatty acids both reached significance in GDM women. On the contrary, our results show that DHA was increased in GDM. The difference in DHA findings may be attributed to the choice of different groups of people. Furthermore, White et al.'s research found that tricarboxylic acid (TCA) cycle intermediate citrate concentrations were also higher but lactate had no notable difference between the two groups at either time [44]. This is basically consistent with our findings that citric acid was increased in GDM, while ethyl lactate and n-butyl lactate do not change significantly.

Findings from metabolomics of GDM have generally been inconsistent in the past. Alterations in branched-chain amino acids, free fatty acids, fatty acid oxidation products, and gluconeogenic precursors have been reported by several studies [41, 45–47]. Yet, Graça et al. and Sachse et al. found no significant changes in metabolite profiles between women with GDM and controls [30, 31]. Surprisingly, our results are not consistent with the findings of others. The reasons for the different results may be as follows: utilization of metabolome profiling platforms, differences in specimen collections, GDM diagnostic criteria, intrinsic biological characteristics of individual participants, methods in data processing, and statistical analysis. The implementation of strict study guidelines and consistent recommendations across studies are needed to improve replication of findings [48].

Based on our results, we can see that under the positive ion mode, a total of 2022 metabolites were detected and a total of 1299 metabolites were detected in the negative ion mode. Through more rigorous selection, we selected the most obvious metabolites to prepare an ROC curve. Moreover, the results of eicosapentaenoic acids, docosahexaenoic acid, docosapentaenoic acid, and arachidonic acid are inconsistent [49–51]. Wheeler's experimental results are relatively similar to ours; they found that eicosapentaenoic acid, docosahexaenoic acid, docosapentaenoic acid, and arachidonic acid were all upregulated [23]. The only difference in our study is that eicosapentaenoic acid was reduced. Also, his research found another furan fatty acid metabolite 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid (CMPF) can impair β cell function by inhibiting insulin biosynthesis and secretion through organic anion transporters-3 (OAT3). This can cause abnormal glucose metabolism and increased oxidative stress [23]. This may be further explained by glucolipotoxicity, which means hyperglycemia and hyperlipidemia arise and may exert additional damaging effects on the β cell. Many studies have associated glucolipotoxicity with β cell dysfunction in type 2 diabetes, suggesting that metabolites are likely causally related to diabetes development [52]. This proposition may offer some insight on the results of Wheeler's and ours. Fatty acids have been proven to induce β cell apoptosis under high glucose conditions [53]. Pancreatic β cells exposed to fatty acids for a long period can lead to increased oxidative stress products like ROS. High levels of ROS can increase cell membrane permeability through oxidation of lipid, leading to calcium influx, and phospholipase activation, which may further induce β cell apoptosis [54]. Busch et al. have also found that the expression of the enzyme, stearoyl coenzyme A desaturase, correlates with the resistance of β cells to the proapoptotic by the effect of palmitate. This may indicate that the capability of cell to desaturate fatty acids serves as some form of protection against glucolipotoxicity [55]. These results suggest that unsaturated fatty acids are at least partially involved in the development of GDM. Under normal physiological conditions, eicosapentaenoic acid, docosahexaenoic acid, and

arachidonic acid can be synthesized from essential fatty acid precursors that might otherwise be inadequate during periods of rapid intrauterine growth, which are considered essential for intrauterine development and specific functions such as retinal and cerebral development [38, 56–58].

Ultimately, we want to develop an effective discriminant model based on a ten-metabolite panel that can predict GDM early. We screened only one pathway in positive ion mode where eicosapentaenoic acid, docosahexaenoic acid, docosapentaenoic acid, and arachidonic acid were included in this pathway. We combined those four molecules with other metabolites into various sets and subjected them to AUC analysis in order to evaluate their diagnostic performances as combined biomarkers for controls and GDM. Based on the principle of selecting the least metabolites and the highest area under the curve, we have selected the following combinations: eicosapentaenoic acid, docosahexaenoic acid, docosapentaenoic acid, arachidonic acid, citric acid, α-ketoglutaric acid, and genistein. The AUC value of those metabolites was 0.984 between the GDM and control groups. This result shows that these metabolite combinations can clearly distinguish between GDM and normal controls. However, this would require a larger sample size for subsequent verification. In continuation from our initial findings, we will work on verifying these results with hopes of using them clinically in the near future.

Overall, our study had several limitations. First, the number of patients included might not be of a substantial amount and could have affected the robustness of our statistical analysis. Therefore, the conclusions of this study need to be verified using a larger group of participants. Another limitation of our study is that some external factors outside of the controlled screening parameters may have affected the metabolome outcomes. These factors include some lifestyle elements, such as dietary habits, previous macrosomia, and previous GDM. Similarly, glycemic control for all women included in the present study was not able to be characterized because these data was not available. Lastly, the LC-MS analysis we performed might not be a feasible screening technique for large populations because of its high cost. In this study, we found several differential factors, such as gestational weeks and BMI, and these factors may be related to the differences in metabolites. As a metabolic disease, GDM may be related to the dysfunctional lipid metabolism, obesity, and glucose metabolism. In this study, we mainly focus on the differential metabolites between the controls and GDM groups, and these metabolites may be the candidates or biomarkers for GDM diagnosis. We will further our studies to explore the potential mechanism and correlation between these differential metabolites and other factors in the future.

In this study, we found that unsaturated fatty acid metabolism was impaired in GDM by identifying key metabolites differences between the controls and GDM groups. In addition, we discovered a set of metabolite combination that can clearly distinguish between GDM and normal controls. These results demonstrate the possibility of developing a diagnostic test that can distinguish between GDM and normal controls clearly.

Data Availability

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

Ethical Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Women's Hospital, School of Medicine, Zhejiang University Committee (Approval number: IRB-20200004-R), and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Consent

Informed consent was obtained from all individual participants included in the study.

Conflicts of Interest

None of the authors has any conflict in relation to the study.

Authors' Contributions

Methodology was done by Y.L.Z., Y.N.Z., and X.J.M. Investigation was done by X.J.M., B.Z., Y.L., L.F., B.B.Y., Y.N.S, M.N.M, and Y.L.H. Writing, original draft, was done by X.J.M. and Y.L.Z. Writing, review and editing, was done by Y.L.Z. Supervision was done by Y.L.Z. Funding acquisition was done by Y.L.Z. and Y.N.Z. Xingjun Meng, Bo Zhu, and Yan Liu contributed equally to this work.

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Supplementary Materials

Table S1: other general characteristics of study subjects. We included some clinical data other than Table 1 in the article in this part, including FIB, APTT, PT, TT, HGB, LY#, LY%, MCH, MCHC, MCV, NE#, NE%, P-LCR, PDW, PLT, RBC, WBC, FT3, FT4, TT3, TT4, TSH, A-G, ALB, and albumin; D-BIL, GGT, ID-BIL, T-BIL, TBA, TP, and URIC may be better for us to understand the basic situation of patients. Figure S2: evaluation of system stability throughout the experiment. QC samples are used to determine the status of the instrument and balance chromatography-mass spectrometry system before the sample and to evaluate the stability of the system throughout the experiment. The

correlation of QC samples was all close to 1, indicating that the method used has high stability and good data quality. In the PCA analysis diagram, the distribution of QC samples, GDM samples, and control samples is clustered together. These results further indicate that the model we employed is also reliable. Table S3: twenty metabolite molecules with the most significant differences. In this part of the supplementary materials, we selected 20 metabolites with the lowest *p*-value under different models. *p*-value, area under ROC curve, VIP value, and trends are list in the table to make more clearer to the readers. Table S3: the most obvious metabolite on the KEGG pathway. In this part of the supplementary materials, we list the most obvious metabolite on the KEGG pathway, including *p*-value, area under ROC curve, VIP value, and trends. Table S5: the largest metabolites of VIP. In this part of the supplementary materials, we list the largest metabolites of VIP, including *p*-value, area under ROC curve, VIP value, and trends. Figure S6: area under the curves among GDM and controls. This part of the result is the same as that of Table 3. Table 3 shows area under the curves among GDM and controls in a form mode, while Figure S6 shows the picture format. *(Supplementary Materials)*

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Research Article

Association of the Ratio of Triglycerides to High-Density Lipoprotein Cholesterol Levels with the Risk of Type 2 Diabetes: A Retrospective Cohort Study in Beijing

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Background. Previous studies have shown that the ratio of triglyceride to high-density lipoprotein cholesterol level (TG/HDL-C) is a risk factor for type 2 diabetes mellitus (T2DM). The aim of this study was to investigate the nonlinear relationship between TG/HDL-C and the incidence of T2DM in a Chinese population. *Methods*. We used logistic regression models to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the incidence of T2DM among 7,791 participants from the Risk Evaluation of cAncers in Chinese diabeTic Individuals: a lONgitudinal (REACTION) cohort study at baseline. *Results*. After adjusting for age, sex, body mass index, smoking status, alcohol intake, low-density lipoprotein cholesterol level, strenuous activity, education level, family histories of T2DM and tumors, and the presence of hypertension, tumor, stroke, and coronary heart disease, we showed that TG/HDL-C was positively associated with the incidence of T2DM at the 4-year follow-up (OR = 1.49, 95%CI = 1.26 – 1.78). TG/HDL-C and incidence of T2DM showed a nonlinear relationship; the inflection point of TG/HDL-C was 1.50. The ORs (95% CI) on the left and right sides of the inflection point were 2.50 (1.70–3.67) and 0.96 (0.67–1.37), respectively. After adjusting for age, sex, and body mass index (BMI) in the linear relationship, the OR of the incidence of T2DM was 1.60 (95%CI = 1.37 – 1.87). When the TG/HDL-C was less than 1.50 or greater than 1.76, the ORs (95% CI) were 2.41 (1.82–3.18) or 0.81 (0.53–1.25), respectively. Subgroup analysis showed no relationships of T2DM incidence with sex, BMI, family history of T2DM, or TG/HDL-C. *Conclusion*. TG/HDL-C is positively associated with diabetes risk. In our study, with each increasing quintile, the risk of T2DM after 4 years was 1.60 or 1.49 depending on the variables adjusted. In addition, our cohort study showed a nonlinear relationship between TG/HDL-C and T2DM incidence, with an inflection point of 1.76 or 1.50, depending on the variables adjusted. When the TG/HDL was less than 1.50, the ORs (95% CI) were 2.41 (1.82–3.18) and 2.50 (1.70–3.67). When the TG/HDL-C was greater than 1.76 or 1.50, there was no significant difference in the change in OR.

1. Introduction

Approximately 463 million adults (20–79 years) were living with diabetes in 2019, and this figure will increase to 700 million by 2045 [1]. Of these cases, 90% are type 2 diabetes mellitus (T2DM) [2], which can lead to complications if untreated. Acute complications include diabetic ketoacidosis, hyperosmolar hyperglycemic state, and even death [3].

Serious long-term complications include cardiovascular disease, stroke, chronic kidney disease, foot ulcers, damage to the nerves, damage to the eyes, and cognitive impairment [4]. Given the global burden of diabetes, it is important to understand the impact of modifiable risk factors on prevention.

The main cause of T2DM is insulin resistance (IR), which drives a series of metabolic processes leading to a proatherogenic lipid profile and T2DM [5, 6]. This is characterized by the decreased ability of insulin to stimulate muscle and adipose tissues to use glucose and inhibit liver glucose production and output [7]. The glucose clamp technique, which was first reported by DeFronzo et al. [8], is a classic method for assessing IR. However, it is a complex, time-consuming, and invasive method that is not feasible for routine clinical applications. Therefore, numerous indicators of IR have been evaluated, among which the ratio of triglyceride to high-density lipoprotein cholesterol levels (TG/HDL-C) was shown to be associated with IR [9–16]. However, those studies were mainly cross-sectional and did not reveal a nonlinear relationship between TG/HDL-C and T2DM incidence.

Therefore, the purpose of this retrospective cohort study was to evaluate the associations of clinical parameters related to lipid profiles with the incidence of T2DM in Chinese participants from the Risk Evaluation of cAncers in Chinese diabeTic Individuals: a lONgitudinal (REACTION) cohort study. To the best of our knowledge, few studies have assessed this nonlinear relationship among Chinese individuals with different glycemic statuses.

2. Methods

2.1. Study Participants. We used data from the REACTION cohort study, which was designed to investigate the associations of T2DM and prediabetes with the risk of cancer in a Chinese population [17]. All permanent residents aged 40 years or older of the Jingding, Laoshan, and Gucheng communities of Beijing (China) were invited to complete baseline questionnaires and medical examinations between March 2011 and December 2011.

A total of 10,216 individuals participated in the study. The diagnosis of T2DM was considered according to the American Diabetes Association criteria of 2003 [18]. People who have been diagnosed with T2DM or treated with hypoglycemic drugs were considered to be diabetic patients. The exclusion criteria were as follows: participants with missing information, participants with a history of liver cancer or related diseases, and pregnant women. The remaining 7,791 people participated in the study, of whom 394 people were diagnosed with T2DM in 2015 as shown in Figure 1.

2.2. Clinical Evaluation and Laboratory Measurements. TG/HDL-C was divided into five quintiles: <20%, 20–39%, 40–59%, 60–79%, and ≥80%. The participants underwent standardized questionnaires, body measurements, and blood collection. Trained clinicians conducted the standardized questionnaires assessing histories of tumors, stroke, coronary heart disease, hypertension, and dyslipidemia; marital status; strenuous activity; walking; education level; levels of alanine aminotransferase, aspartate aminotransferase, creatinine, fasting plasma glucose, and hemoglobin A1c; and family histories of T2DM and tumors. All data were collected according to standardized methods by the same highly trained clinicians. Physical examination included measurements of height, weight, waist circumference, hip circumference, blood pressure, and heart rate. Height was measured with bare feet to the nearest 0.01 m. Weight was measured in light clothes to the nearest 0.1 kg. Waist and hip circumferences were measured to the nearest 0.01 m by the same staff. Body mass index (BMI) was calculated as weight (kg)/height (m^2). After resting for at least 5 min, blood pressure was measured in the seated position three times at 1 min intervals using an OMRON electronic blood pressure monitor; the average value was used for the analysis. Smoking frequency was divided into three categories: never or previous smoker, occasionally (smoking less than once a week or less than 7 cigarettes weekly), and frequently (smoking one or more cigarettes daily for at least a half year). Similarly, alcohol intake frequency was divided into three categories: never or previous drinker, occasionally (less than once a week), and frequently (more than once a week for at least a half year). Stroke, including all subtypes, was determined based on self-report, including a history of language or physical dysfunction and a history of ischemic or hemorrhagic stroke by imagological diagnosis over 24 hours. Coronary heart disease events were defined as any self-reported history of myocardial infarction, angina pectoris, or coronary revascularization.

2.3. Statistical Analysis. Normal distribution data were expressed as the mean ± standard deviation. Skewed distribution data were expressed as median (*P*25, *P*75). Categorical variables data were expressed as frequency or percentage. The Kolmogorov-Smirnov test was utilized for normal distribution and homogeneity test for a variance. The Kruskal-Wallis test was used in skewed distribution data to compare the differences among multiple groups of measurement data. The chi-square test was used for categorical variables. Proportional hazards models were used to calculate odds ratios (ORs) and 95% confidence intervals (95% CIs) for T2DM according to serum TG/HDL-C. Both nonadjusted and multivariate-adjusted models were used. Binary logistic models were adjusted for age, sex, BMI, smoking status, alcohol intake, low-density lipoprotein cholesterol (LDL-C), strenuous activity, education level, family histories of T2DM and tumors, and the presence of hypertension, tumors, stroke, and coronary heart disease. Trend tests were conducted using linear regression by entering the medians for each TG/HDL-C quintile in the models as continuous variables. A generalized additive model was used to evaluate the nonlinear relationship between TG/HDL-C and the incidence of T2DM. Based on the smooth curve, we further developed a two-piecewise linear regression model to determine the threshold effect, adjusting for potential confounding factors. The threshold level of TG/HDL-C was determined using a recurrence method, including selecting the turning points along predetermined intervals and selecting the turning point that produces the maximum likelihood model. The log-likelihood ratio test was used to compare the two-piecewise

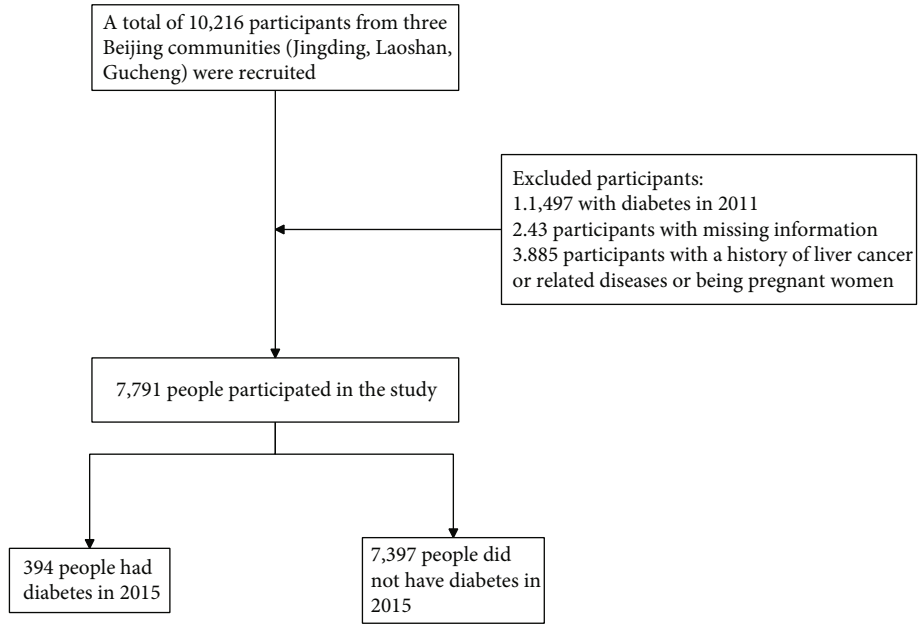

Figure 1: Flow chart of the study participant selection process.

linear regression model with the one-line linear model. A stratified logistic regression model was used to perform subgroup analyses based on sex, BMI, and family history of T2DM. The likelihood ratio test was used to test the interactions among subgroups. For all statistical analyses, we used R version 3.4.3 (The R Foundation, Vienna, Austria). A two-way P value < 0.05 was considered significant.

3. Results

3.1. Baseline Characteristics of the Study Participants according to Serum TG/HDL-C. Of the 7,791 participants, 394 were diagnosed with T2DM at 4 years of follow-up. The changing tendency of blood lipids and TG/HDL-C through 4 years between T2DM patients and non-T2DM controls is shown in Supplementary Table 1. Table 1 lists the baseline characteristics of all participants. The mean age was 56.03 ± 7.82 years, and one-third (2,613, 33.54%) of the participants were male. The mean TG/HDL-C was 1.10 ± 0.62. Participants with higher TG/HDL-C values were more likely to be male and smokers and to have hypertension, stroke, hyperlipidemia, lower walking frequency, and a family history of T2DM. In addition, serum TG/HDL-C was directly proportional to systolic and diastolic blood pressure, BMI, waist circumference, hip circumference, presence of fatty liver, and the levels of alanine aminotransferase, creatinine, total cholesterol, TGs, LDL-C, fasting plasma glucose, and hemoglobin A1c, but inversely proportional to the HDL-C level.

3.2. Association between Serum TG/HDL-C and T2DM Incidence. Table 2 shows the ORs and 95% CIs for developing T2DM according to TG/HDL-C quintile. In the nonadjusted model, the risk of T2DM increased as the TG/HDL-C increased by 20% (P for trend <0.01). Participants whose TG/HDL-C was between the highest and lowest quintile had a nearly fourfold increased risk of developing T2DM (OR = 3.71, 95%CI = 2.51 – 5.51). After adjusting for age, sex, BMI, histories of hypertension, tumors, stroke, and coronary heart disease, smoking status, alcohol intake, LDL-C, strenuous activity, education level, and family histories of T2DM and tumors, the ORs (95% CI) were 1.34 (0.82–2.20) ($P = 0.24$), 1.55 (0.96–2.51) ($P = 0.07$), 2.23 (1.41–3.53) ($P < 0.01$), and 2.42 (1.53–3.84) ($P < 0.01$) for TG/HDL-C quintiles 2–5, respectively (P for trend <0.01).

3.3. Threshold Effect Analysis of TG/HDL-C on the Incidence of T2DM. To evaluate whether a dose-response relationship exists between TG/HDL-C and the incidence of T2DM, we used a smooth function analysis. After adjusting for age, sex, and BMI, a nonlinear relationship between TG/HDL-C and T2DM was observed (Figure 2(a)). The risk of developing T2DM was positively correlated with TG/HDL-C until the ratio reached 1.76 (OR = 2.41, 95%CI = 1.82 – 3.18, $P < 0.01$). However, at TG/HDL − C > 1.76, the OR for T2DM was 0.81 (95%CI = 0.53 – 1.25), indicating that the risk of T2DM did not increase significantly with increasing TG/HDL-C ($P = 0.35$) (Table 3).

After adjusting for age, sex, body mass index, histories of hypertension, tumors, stroke, and coronary heart disease, smoking status, alcohol intake, LDL-C level, strenuous activity, education level, and family histories of T2DM and tumors, a nonlinear relationship between TG/HDL-C and T2DM incidence was observed (Figure 2(b)). The risk of T2DM was positively correlated with serum TG/HDL-C until the ratio reached 1.50 (OR = 2.50, 95%CI = 1.70 – 3.67, $P < 0.01$). However, when TG/HDL-C exceeded 1.50, the OR for developing T2DM was 0.96 (95%CI = 0.67 – 1.37), indicating that the risk of T2DM did not increase significantly as TG/HDL-C increased ($P = 0.82$) (Table 4).

Table 1: Baseline characteristics of the participants in the REACTION study according to serum TG/HDL-C.

Variable	All participants	TG/HDL-C Q1 (1558)	Q2 (1557)	Q3 (1560)	Q4 (1557)	Q5 (1559)	*P* value
Age (years)	56.03 ± 7.82	55.15 ± 7.88	56.22 ± 8.10	56.36 ± 7.64	56.21 ± 7.80	56.24 ± 7.60	<0.01
Male	2613 (33.54%)	419 (26.89%)	493 (31.66%)	504 (32.31%)	591 (37.96%)	606 (38.87%)	<0.01
TG/HDL-C	1.10 ± 0.62	0.46 ± 0.07	0.68 ± 0.07	0.94 ± 0.08	1.31 ± 0.14	2.12 ± 0.44	<0.01
SBP (mmHg)	131.22 ± 16.24	127.98 ± 16.67	129.42 ± 15.86	131.95 ± 16.20	132.76 ± 16.02	134.02 ± 15.68	<0.01
DBP (mmHg)	75.84 ± 9.52	73.47 ± 9.13	74.74 ± 9.5	76.21 ± 9.43	76.99 ± 9.56	77.78 ± 9.35	<0.01
Heart rate	78.65 ± 11.29	77.93 ± 11.25	78.06 ± 11.26	78.72 ± 11.41	79.22 ± 11.38	79.32 ± 11.07	<0.01
Height (kg)	161.12 ± 7.76	160.52 ± 7.36	160.61 ± 7.67	160.81 ± 7.64	161.8 ± 7.98	161.87 ± 8.04	<0.01
Weight (cm)	66.96 ± 10.84	62.15 ± 9.64	65.23 ± 10.17	67.26 ± 10.29	69.60 ± 10.95	70.55 ± 10.96	0.31
BMI (kg/m^2)	25.74 ± 3.41	24.09 ± 3.16	25.26 ± 3.32	25.97 ± 3.30	26.53 ± 3.30	26.86 ± 3.25	<0.01
WC (cm)	83.77 ± 17.14	78.81 ± 8.15	82.12 ± 8.47	84.44 ± 24.72	86.01 ± 8.30	87.46 ± 24.59	<0.01
HC (cm)	94.71 ± 16.07	92.06 ± 6.44	93.68 ± 6.54	95.46 ± 23.93	95.69 ± 7.04	96.66 ± 23.91	<0.01
ALT (U/L)	18.30 (14.20-24.70)	15.90 (12.80, 20.70)	17.10 (13.30, 22.60)	18.50 (14.60, 24.40)	19.50 (15.20, 27.10)	21.00 (16.30, 28.70)	<0.01
AST (U/L)	19.60 (16.70-23.20)	19.50 (16.90-22.40)	19.30 (16.50-22.80)	19.80 (16.90-23.40)	19.70 (16.80-23.70)	19.80 (16.80-23.60)	0.051
Cr (mmol/L)	67.18 ± 13.97	64.77 ± 12.82	66.00 ± 13.55	67.27 ± 14.15	68.74 ± 14.58	69.13 ± 14.24	<0.01
TC (mmol/L)	5.28 ± 1.53	5.10 ± 1.44	5.23 ± 1.84	5.29 ± 0.95	5.32 ± 1.54	5.43 ± 1.71	<0.01
TG (mmol/L)	1.45 ± 0.63	0.80 ± 0.15	1.06 ± 0.19	1.32 ± 0.22	1.66 ± 0.28	2.39 ± 0.54	<0.01
HDL-C (mmol/L)	1.43 ± 0.32	1.76 ± 0.29	1.56 ± 0.26	1.41 ± 0.22	1.28 ± 0.20	1.13 ± 0.18	<0.01
LDL-C (mmol/L)	3.26 ± 0.80	2.96 ± 0.71	3.19 ± 0.78	3.34 ± 0.77	3.39 ± 0.77	3.44 ± 0.86	<0.01
FPG	5.43 ± 0.53	5.31 ± 0.49	5.41 ± 0.53	5.42 ± 0.53	5.46 ± 0.54	5.53 ± 0.56	<0.01
HbA1C (%)	5.87 ± 0.62	5.73 ± 0.52	5.83 ± 0.55	5.86 ± 0.57	5.90 ± 0.63	6.02 ± 0.77	<0.01
Hypertension	2167 (27.82%)	307 (19.72%)	401 (25.75%)	432 (27.69%)	494 (31.73%)	533 (34.19%)	<0.01
Tumor	156 (2.00%)	22 (1.41%)	34 (2.18%)	34 (2.18%)	26 (1.67%)	40 (2.57%)	0.15
Stroke	189 (2.43%)	23 (1.48%)	34 (2.18%)	43 (2.76%)	39 (2.50%)	50 (3.21%)	0.03
CHD	474 (6.08%)	82 (5.27%)	91 (5.84%)	94 (6.03%)	97 (6.23%)	110 (7.06%)	0.33
Hyperlipidemia	1067 (13.70%)	107 (6.87%)	152 (9.76%)	204 (13.08%)	234 (15.03%)	370 (23.73%)	<0.01
Marital status							0.67
Married	7316 (93.92%)	1445 (92.81%)	1465 (94.09%)	1469 (94.17%)	1455 (93.45%)	1482 (95.06%)	
Unmarried	29 (0.37%)	5 (0.32%)	6 (0.39%)	6 (0.38%)	6 (0.39%)	6 (0.38%)	
Widowed	262 (3.36%)	65 (4.17%)	48 (3.08%)	53 (3.40%)	54 (3.47%)	42 (2.69%)	
Divorced	179 (2.30%)	40 (2.57%)	38 (2.44%)	31 (1.99%)	41 (2.63%)	29 (1.86%)	
Else	4 (0.05%)	2 (0.13%)	0 (0.00%)	1 (0.06%)	1 (0.06%)	0 (0.00%)	
Education level							0.048
Illiteracy	77 (0.99%)	17 (1.09%)	17 (1.09%)	12 (0.77%)	16 (1.03%)	15 (0.96%)	
Primary school	370 (4.75%)	68 (4.37%)	94 (6.05%)	79 (5.07%)	72 (4.63%)	57 (3.66%)	
Junior high school	2547 (32.73%)	456 (29.31%)	496 (31.90%)	535 (34.32%)	525 (33.74%)	535 (34.36%)	
High school or technical secondary school	3460 (44.46%)	729 (46.85%)	687 (44.18%)	682 (43.75%)	689 (44.28%)	673 (43.22%)	
College or above	1329 (17.08%)	286 (18.38%)	261 (16.78%)	251 (16.10%)	254 (16.32%)	277 (17.79%)	
Smoking (%)							<0.01
Never	6370 (81.88%)	1358 (87.22%)	1291 (83.18%)	1293 (83.04%)	1226 (78.79%)	1202 (77.15%)	
Occasionally	169 (2.17%)	27 (1.73%)	32 (2.06%)	33 (2.12%)	40 (2.57%)	37 (2.37%)	
Frequently	1241 (15.95%)	172 (11.05%)	229 (14.76%)	231 (14.84%)	290 (18.64%)	319 (20.47%)	
Alcohol intake							0.78
Never	5515 (70.85%)	1120 (71.89%)	1095 (70.46%)	1129 (72.37%)	1083 (69.65%)	1088 (69.88%)	
Occasionally	1470 (18.88%)	285 (18.29%)	302 (19.43%)	277 (17.76%)	304 (19.55%)	302 (19.40%)	
Frequently	799 (10.26%)	153 (9.82%)	157 (10.10%)	154 (9.87%)	168 (10.80%)	167 (10.73%)	

Table 1: Continued.

Variable	All participants	TG/HDL-C Q1 (1558)	Q2 (1557)	Q3 (1560)	Q4 (1557)	Q5 (1559)	*P* value
Strenuous activity							0.24
Yes	222 (2.86%)	51 (3.28%)	32 (2.07%)	47 (3.02%)	50 (3.22%)	42 (2.70%)	
No	7543 (97.14%)	1502 (96.72%)	1517 (97.93%)	1510 (96.98%)	1502 (96.78%)	1512 (97.30%)	
Walking							<0.01
Yes	6742 (86.80%)	1369 (88.15%)	1376 (88.83%)	1336 (85.81%)	1349 (86.92%)	1312 (84.32%)	
No	1025 (13.20%)	184 (11.85%)	173 (11.17%)	221 (14.19%)	203 (13.08%)	244 (15.68%)	
Family history of T2DM	1916 (24.61%)	363 (23.30%)	381 (24.49%)	373 (23.96%)	374 (24.04%)	425 (27.28%)	0.09
Family history of tumors	1655 (21.26%)	340 (21.82%)	319 (20.50%)	325 (20.87%)	340 (21.85%)	331 (21.25%)	0.86

TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; SBP: systolic blood pressure; DBP: diastolic blood pressure; BMI: body mass index; WC: waist circumference; HC: hip circumference; ALT: alanine aminotransferase; AST: aspartate aminotransferase; Cr: creatinine; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; FPG: fasting plasma glucose; HbA1c: hemoglobin A1c; CHD: coronary heart disease.

Table 2: Association between serum TG/HDL-C and the incidence of T2DM in the REACTION study.

	Crude	Model I	Model II
TG/HDL-C	1.78 (1.53–2.06) <0.01	1.60 (1.37–1.87) <0.01	1.49 (1.26–1.78) <0.01
		TG/HDL-C quintile	
Q1	1	1	1
Q2	1.53 (0.98–2.39) 0.06	1.36 (0.87–2.13) 0.17	1.34 (0.82–2.20) 0.24
Q3	1.98 (1.29–3.03) <0.01	1.66 (1.08–2.55) 0.02	1.55 (0.96–2.51) 0.07
Q4	2.94 (1.96–4.39) <0.01	2.32 (1.53–3.50) <0.01	2.23 (1.41–3.53) <0.01
Q5	3.71 (2.51–5.51) <0.01	2.88 (1.92–4.31) <0.01	2.42 (1.53–3.84) <0.01
P for trend	<0.01	<0.01	<0.01

Data are ORs (95% CI). Model I was adjusted for age, sex, and body mass index. Model II was adjusted for the variables in model I plus histories of hypertension, tumors, stroke, and coronary heart disease, smoking status, alcohol intake, low-density lipoprotein cholesterol level, strenuous activity, education level, and family histories of T2DM and tumors.

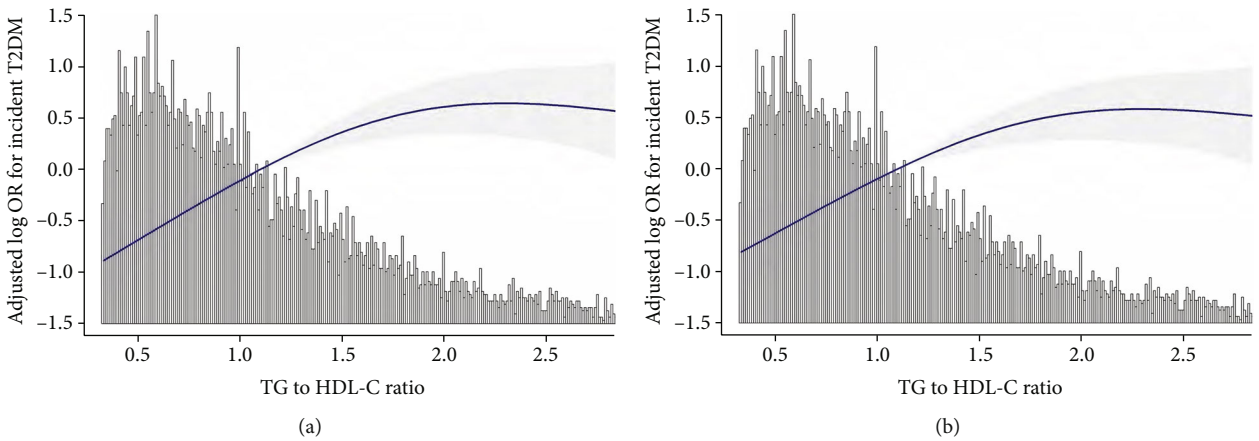

Figure 2: (a) Threshold effect analysis of TG/HDL-C on the incidence of T2DM in the REACTION study. Notes: adjusted for age, sex, and body mass index. (b) Threshold effect analysis of TG/HDL-C on the incidence of T2DM in the REACTION study. Notes: adjusted for age, sex, body mass index, histories of hypertension, tumor, stroke, and coronary heart disease, smoking status, alcohol intake, low-density lipoprotein cholesterol level, strenuous activity, education level, and family histories of T2DM and tumors.

3.4. Subgroup Analyses. To explore whether the correlation between TG/HDL-C and T2DM incidence exists among different subgroups, we conducted stratified analyses and interactive analyses (Table 5). The data showed that age played an important role in the association between TG/HDL-C and incidence of T2DM (*P* for interaction <0.01). The associations in the top four quintiles of TG/HDL-C were stronger for participants aged <60 years (quintile 2, 1.67 (0.91–0.67);

Table 3: Threshold effect analysis of TG/HDL-C on the incidence of type 2 diabetes mellitus in the REACTION study.

Outcomes	OR (95% CI)	*P* value
One-line linear regression model	1.60 (1.37–1.87)	<0.01
Two-piecewise linear regression model		
<1.76	2.41 (1.82–3.18)	<0.01
>1.76	0.81 (0.53–1.25)	0.35
Log-likelihood ratio test		<0.01

Adjusted for age, sex, and body mass index.

Table 4: Threshold effect analysis of TG/HDL-C on the incidence of type 2 diabetes mellitus in the REACTION study.

Outcome	OR (95% CI)	*P* value
One-line linear regression model	1.49 (1.26–1.78)	<0.01
Two-piecewise linear regression model		
<1.50	2.50 (1.70–3.67)	<0.01
>1.50	0.96 (0.67–1.37)	0.82
Log-likelihood ratio test		<0.01

Adjusted for age, sex, body mass index, histories of hypertension, tumor, stroke, and coronary heart disease, smoking status, alcohol intake, low-density lipoprotein cholesterol level, strenuous activity, education level, and family histories of T2DM and tumors.

quintile 3, 2.59 (1.47–4.56); quintile 4, 3.28 (1.88–5.70); and quintile 5, 4.50 (2.61–7.75) vs. quartile 1, 1.00; *P* for trend <0.01). No significant associations were observed among the other subgroups.

4. Discussion

In this cohort study, TG/HDL-C was shown to be associated with an elevated risk of T2DM, independent of age, sex, body mass index, histories of hypertension, tumor, stroke, and coronary heart disease, smoking status, alcohol intake, LDL-C, strenuous activity, education level, and family histories of T2DM and tumors. We showed a nonlinear relationship between serum TG/HDL-C and risk of T2DM; in that, the risk of T2DM after 4 years increased significantly with an increase in TG/HDL-C when the ratio was less than 1.76 or 1.50, depending on the variables adjusted.

The diagnosis of IR is based on simultaneous measurements of glucose and insulin. The classic method for IR measurement is the metabolic euglycemic clamp, but this method is laborious and expensive and thus is used mainly for research purposes. IR affects the metabolism of TGs, HDL-C, and LDL-C [6]. High TG and low HDL-C levels are associated with IR and T2DM [19], but TG and HDL-C levels alone are weaker risk factors compared with the TG/HDL-C [9, 20]. McLaughlin et al. [21] were the first to demonstrate the clinical utility of TG/HDL-C for identifying healthy Caucasians with IR, including 258 healthy nondiabetic individuals. The results showed that TG/HDL-C was closely related to specific indicators of insulin-mediated glucose disposal and the fasting plasma insulin concentration. This ratio has been widely used to assess the associations between IR and various clinical syndromes. Most previous studies on the relationship between TG/HDL-C and the incidence of T2DM reported a positive association [10, 16, 22], and similar results can be found for type 1 diabetes mellitus [23]. Our findings on TG/HDL-C are consistent with those studies. As shown in Table 2, the incidence of T2DM increased with increasing TG/HDL-C. In the lowest TG/HDL-C quintile, the OR (95% CI) for developing T2DM after 4 years of follow-up was 2.88 (1.92–4.31) or 2.42 (1.53–3.84) depending on the variables adjusted. Previous studies have also explored the relationship between TG/HDL-C and the incidence of T2DM. Cheng et al. [24] also showed a nonlinear relationship between TG/HDL-C and the overall risk of T2DM ($P < 0.01$). The risk of T2DM continued to increase with increasing TG/HDL-C, with a gradual increase as TG/HDL-C exceeded 2.5 in males. Our study showed that when TG/HDL was <1.76 or <1.50, depending on the variables adjusted, the OR (95% CI) was 2.41 (1.82–3.18) or 2.50 (1.70–3.67), respectively. When TG/HDL was >1.76 or >1.50, there was no statistical difference in the change in OR.

Lipid and glucose metabolism is affected by many factors. We analyzed whether there were differences in the relationship according to different subgroups. The associations between TG/HDL-C and risk of T2DM were significant only with age < 60 years, which was similar with the result of Zhang et al. [11]. As compared with young people, the anabolism of older people is significantly lower [25]. Pramfalk et al. [26] reported that despite no significant sex difference in the plasma total cholesterol (TC) level, men had significantly higher fasting plasma TG levels, while women had lower plasma LDL-C and higher HDL-C. However, the *P* value for interaction was 0.53 in our subgroup analysis, and there was no effect of sex on TG/HDL-C or T2DM incidence, suggesting that the association between TG/HDL-C and T2DM incidence after 4 years is similar between the sexes. The effect of sex on blood lipid metabolism is still controversial, and we will further explore the influence of gender on the relationship between TG/HDL-C and the incidence of diabetes in the next follow-up study. Iwani et al. [27] showed that TG/HDL-C is significantly associated with IR. TG/HDL-C is an inexpensive predictor of IR and may be a useful tool to identify high-risk individuals for early intervention, thereby preventing or delaying the development of IR-associated diseases such as T2DM. The interaction of this study showed that in people with BMI less than 25 kg/m^2 and BMI greater than 25 kg/m^2, the incidence of T2DM increased as the TG/HDL-Co increased. This indicated that at different BMIs, the ability of TG/HDL-C to predict the incidence of diabetes is the same. As they share genetic and environmental factors with T2DM patients, the first-degree relatives of T2DM patients show early signs of metabolic abnormalities [28]. In our model, there was no interaction between family history of T2DM and TG/HDL-C; that is, the relationship between T2DM incidence and TG/HDL-C was independent of a family history of T2DM. We have only been followed up for 4 years, and the incidence of T2DM is only 5%. So it may lead to the influence of the family history of diabetes on the incidence of T2DM. We will continue to analyze the impact of family history of T2DM on the incidence of diabetes after the next follow-up.

Table 5: Subgroup analyses of the association between TG/HDL-C and incidence of type 2 diabetes mellitus in the REACTION study.

Confounding factor	Serum TG/HDL-C quintile					*P* for trend	*P* for interaction
	Q1	Q2	Q3	Q4	Q5		
Age							0.01
<60 years	1	1.67 (0.91, 3.07) 0.10	2.59 (1.47, 4.56) <0.01	3.28 (1.88, 5.70) <0.01	4.50 (2.61, 7.75) <0.01	<0.01	
>60 years	1	0.96 (0.48, 1.92) 0.90	0.74 (0.36, 1.54) 0.43	1.30 (0.67, 2.54) 0.44	1.08 (0.55, 2.12) 0.82	0.50	
Sex							0.53
Male	1	1.51 (0.69–3.31) 0.30	1.48 (0.68–3.22) 0.33	1.65 (0.78–3.50) 0.19	2.19 (1.05–4.57) 0.04	0.03	
Female	1	1.21 (0.69–2.11) 0.50	1.75 (1.04–2.96) 0.03	2.61 (1.58–4.31) <0.01	2.97 (1.81–4.88) <0.01	<0.01	
BMI							0.83
<25	1	1.43 (0.73–2.79) 0.30	1.85 (0.95–3.60) 0.07	2.39 (1.22–4.67) 0.01	3.71 (1.97–6.99) <0.01	<0.01	
≥25	1	1.26 (0.68–2.35) 0.46	1.55 (0.86–2.79) 0.14	2.19 (1.25–3.83) 0.01	2.40 (1.38–4.18) <0.01	<0.01	
Family history of T2DM							0.20
Yes	1	1.09 (0.50–2.34) 0.8309	1.76 (0.87–3.6) 0.1155	2.79 (1.42–5.47) 0.0029	2.18 (1.09–4.34) 0.0267	0.01	
No	1	1.55 (0.88–2.73) 0.1281	1.69 (0.97–2.93) 0.0639	2.18 (1.28–3.72) 0.0041	3.31 (1.98–5.55) <0.0001	<0.01	

Adjusted for age, sex, body mass index, histories of hypertension, tumors, stroke, and coronary heart disease, smoking status, alcohol intake, low-density lipoprotein cholesterol level, strenuous activity, education level, and family histories of T2DM and tumors.

5. Limitations

There are potential limitations of this study to note. First, T2DM is associated with region and ethnicity. As this cohort study was conducted in Beijing, our findings may not be applicable to other regions, ethnicities, or special groups such as children and pregnant women. Second, the presence of T2DM was reported only at the 4-year follow-up, while the specific date of diagnosis was not recorded; thus, we could only perform logistic regression, which is weaker than Cox regression. The date of T2DM diagnosis during the next follow-up needs to be determined to obtain more information for analysis.

6. Conclusions

TG/HDL-C was positively associated with diabetes risk. In our study, for every increase in TG/HDL-C quintile, the risk of T2DM after 4 years was 1.60 or 1.49 depending on the variables adjusted. In addition, a nonlinear relationship between TG/HDL-C and T2DM incidence was found in our cohort study. The inflection point of TG/HDL-C was 1.76 or 1.50, depending on the variables adjusted. When the TG/HDL-C was less than 1.76 or 1.50, the ORs (95% CI) were 2.41 (1.82–3.18) and 2.50 (1.70–3.67), respectively. When the TG/HDL-C was greater than 1.76 or 1.50, there was no statistical difference in the change in OR.

Data Availability

The data and analytical methods of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

All authors declare that there is no conflict of interest associated with this research.

Authors' Contributions

Hongzhou Liu and Shuangtong Yan were responsible for data curation. Zhaohui Lyu and Junping Wen were responsible for funding acquisition and supervision. Hongzhou Liu, Shuangtong Yan, Gang Chen, Bing Li, and Ling Zhao contributed in writing the original draft. Yajing Wang, Xiaodong Hu, Xiaomeng Jia, Jingtao Dou, and Yiming Mu contributed in writing, review, and editing. Hongzhou Liu and Shuangtong Yan contributed equally to the study.

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Supplementary Materials

During the 4 years, the level of blood lipid and TG/HDL-C changed significantly ($P < 0.05$). (Supplementary Table 1) We are all grouped according to the data of the first time. *(Supplementary Materials)*

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