

# Serum growth differentiation factor 11 is closely related to metabolic syndrome in a Chinese cohort

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## Keywords

Fat-free mass, Growth differentiation factor 11, Metabolic disorders

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## ABSTRACT

**Aims/Introduction:** Despite increasing interest in growth differentiation factor 11 (GDF11) based on its involvement in age-related disorders, clinical implications – especially for metabolic diseases – remain unclear. Therefore, we assessed the association between serum GDF11 levels and metabolic disturbance in the Chinese population.

**Materials and Methods:** A total of 381 individuals from the Shanghai Nичeng Cohort Study were included. In addition to anthropometry, laboratory and ultrasonography measurements, serum concentrations of GDF11 were measured by enzyme-linked immunosorbent assay.

**Results:** Circulating GDF11 concentrations were unchanged with age ( $r = -0.064$ ,  $P = 0.210$ ), but showed an inverse relationship to body mass index, waist circumference and fat-free mass index (all  $P < 0.05$ ). Correlation analysis showed decreased GDF11 concentrations accompanied by elevated diastolic blood pressure, fasting and 2-h plasma glucose, triglycerides, and low-density lipoprotein cholesterol after adjusting for sex, age and body mass index, whereas variations in aspartate aminotransferase and free thyroxine were consistent with GDF11 (all  $P < 0.05$ ). Furthermore, people, especially men, with abnormal glycometabolism, body mass index and/or fat accumulation in the liver had lower serum levels of GDF11 ( $P < 0.05$ ); an increase in metabolic syndrome morbidity along with the circulatory decline of GDF11 was found when stratified by GDF11-level quartiles ( $P$ -trend  $< 0.001$ ). Logistic regression showed that serum GDF11 levels were independently correlated with the presence of metabolic syndrome (odds ratio 0.665, 95% confidence interval 0.510–0.867,  $P = 0.003$ ).

**Conclusions:** We confirmed GDF11 as an endocrine factor playing a significant role in multiple metabolic processes and an indicator of metabolic syndrome in the Chinese population, particularly in males.

## INTRODUCTION

Growth differentiation factor 11 (GDF11) – widely accepted as a regulator playing pivotal roles in embryonic development, including skeletal formation and neurogenesis – is a member of the super family of transforming growth factor- $\beta$ <sup>1–4</sup>. Different from its homolog myostatin (MSTN or GDF8), which shares

90% sequence identity of the C-terminal signaling domain and is expressed specifically in skeletal muscle<sup>5</sup>, GDF11 shows a broader expression pattern, transcribed in practically all tissues<sup>6,7</sup>. Research reported in 2013 and 2014 proposed GDF11 as a circulating factor declining with age in mice that is able to rejuvenate age-related dysfunction in heart and skeletal muscle, as well as the central nervous system, bringing GDF11 to the forefront of aging research<sup>7–9</sup>. However, subsequent studies reported inconsistent or even opposite results, showing that

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GDF11 has no effect on cardiac structure or function, inhibits skeletal muscle regeneration and increases with age<sup>10,11</sup>. In addition, the correlation between age and serum GDF11 levels in humans is also controversial due to discrepant results<sup>10,12-20</sup>.

There can be little doubt that metabolic diseases, such as type 2 diabetes mellitus, obesity, and metabolic syndrome (MetS) represent key global healthcare challenges for the 21st century, considering the striking increase of prevalence, and the financial burden to families and societies. Recently, studies on GDF11 and metabolism have been published, showing that GDF11 can improve glucolipid metabolism in mice by exerting control over islet  $\beta$ -cell function and survival, as well as lipid content<sup>21,22</sup>. However, relatively few studies have investigated the association of serum GDF11 levels with body composition, type 2 diabetes mellitus and obesity in humans<sup>12,14-16,18</sup>, and none have been published in the field of MetS. Hence, the current study mainly aimed to investigate the relationship between serum GDF11 levels and metabolic disorders, especially metabolic syndrome, in the Chinese population.

## METHODS

### Study population

We enrolled 381 Chinese individuals without a validated history of diabetes (134 men, 247 women, age range 45.4–69.9 years) from the Shanghai Nicheng Cohort Study, which was designed to assess the prevalence and incidence of factors related to metabolic diseases among adults in Nicheng County, a suburb of Shanghai, China. Patients with cancer, severe disability, psychiatric disturbances, evidence for other chronic liver diseases, record of drug use including antihypertensive and lipid-lowering drugs, and individuals with missing laboratory measurements or missing samples/low sample volume were excluded. This study was approved by the ethics committee of Shanghai Jiao Tong University Affiliated Sixth People's Hospital and informed consent was provided by all participants.

### Anthropometric indices

Bodyweight and height were measured without shoes and with light clothing. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Waist circumference (WC) was measured midway between the lowest rib and the iliac crest with the participant in the standing position. Systolic blood pressure and diastolic blood pressure (DBP) were measured twice from the right arm after 5 min of sitting using a mercury sphygmomanometer at 3-min intervals, after which the mean value was calculated. Body fat and fat-free mass (FFM) was estimated with a Tanita body composition analyzer (TBF-418; Tanita Corp., Tokyo, Japan), and fat-free mass index (FFMI) was calculated as FFM in kg divided by the square of height in meters.

### Laboratory measurements

Blood samples were collected from participants after an overnight fast for measurements of fasting plasma glucose (FPG),

glycated hemoglobin A1c, glycated albumin, fasting plasma insulin, triglyceride (TG), total cholesterol, high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), alanine aminotransferase, aspartate aminotransferase (AST), creatinine, uric acid, blood urea nitrogen, thyroid-stimulating hormone (TSH), free triiodothyronine and free thyroxine. All individuals received an oral glucose tolerance test of 75 g glucose; 30-min plasma glucose, 30-min plasma insulin, 2-h plasma glucose (2h-PG) and 2-h plasma insulin (2h-Ins) were assessed. Standard laboratory measurements were carried out as described previously<sup>23</sup>. In addition, serum TSH, free triiodothyronine and free thyroxine concentrations were determined by a chemiluminescence immunoassay (Roche Cobas 6000; Roche Ltd., Basel, Switzerland), and glycated albumin values were measured using an enzyme-based assay (Lucica GA-L; Asahi Kasei Pharma, Tokyo, Japan) with the Glamour 2000 autoanalyzer (Molecular Devices, Sunnyvale, CA, USA). A sandwich enzyme-linked immunosorbent assay kit (Human GDF11 ELISA kit, E01G0124; BlueGene Biotech, Shanghai, China) was used to determine serum GDF11 concentrations, with intra- and interassay coefficients of variation of 5.5% and 7.8%, respectively. A declaration that no cross-reactivity exists with any other analog was provided by the manufacturer. Insulin resistance and  $\beta$ -cell function were assessed using the homeostasis model assessment for insulin resistance (HOMA-IR) and  $\beta$ -cell function (HOMA-% $\beta$ ), as described previously<sup>23</sup>. The estimated glomerular filtration rate was calculated on the basis of the Chronic Kidney Disease Epidemiology Collaboration creatinine equation<sup>24</sup>.

### Ultrasonography measurement

Ultrasound examination of the liver was carried out for all participants by experienced radiologists who were blinded to all clinical information using an ultrasound system (Z.One Ultra; Zonare Medical Systems, Inc., Mountain View, CA, USA). Fatty liver was identified according to the Asia-Pacific Guidelines<sup>25</sup>.

### Diagnostic criteria

Following the criteria of the 1999 World Health Organization<sup>26</sup>, the glucose regulation category was defined based on the oral glucose tolerance test as follows: normal glucose tolerance, impaired glucose regulation (IGR; a combination of impaired fasting glucose and glucose tolerance), as well as diabetes mellitus. On the basis of BMI, participants were classified into three groups according to the Chinese criteria<sup>27</sup>: normal ( $18.5 \leq \text{BMI} < 24 \text{ kg/m}^2$ ), overweight ( $24 \leq \text{BMI} < 28 \text{ kg/m}^2$ ) and obesity ( $\text{BMI} \geq 28 \text{ kg/m}^2$ ). Additionally, non-alcoholic fatty liver disease (NAFLD) was defined as ultrasound-diagnosed fatty liver in the absence of excessive drinking, hepatitis B surface antigen or hepatitis C virus antibody seropositivity and other causes of liver diseases<sup>25</sup>. Based on the Chinese Diabetes Society criteria for metabolic syndrome diagnosis<sup>28</sup>, patients were diagnosed with MetS when they had three or more of the following conditions: (i) central obesity (WC  $\geq 90$  cm for men

or  $\geq 85$  cm for women); (ii) hypertriglyceridemia (TG  $\geq 1.7$  mmol/L); (iii) low HDL-c (HDL-c  $< 1.04$  mmol/L); (iv) hypertension (systolic blood pressure/DBP  $\geq 130/85$  mmHg and/or medication); and (v) FBG  $\geq 6.1$  mmol/L or 2-h blood glucose after glucose overload (2h-BG)  $\geq 7.8$  mmol/L, or known history of type 2 diabetes mellitus.

### Statistical analysis

Statistical analysis was carried out using SAS software (version 9.4; SAS Institute, Cary, NC, USA). Data are presented as the mean  $\pm$  standard deviation or median (interquartile range 25–75%) according to their distribution after a Shapiro–Wilk test. Differences between/among groups were compared by the Wilcoxon rank test or Kruskal–Wallis test, as appropriate. Relationships between serum GDF11 levels and other variables were analyzed by Spearman's rank correlation or partial correlation analysis. Based on the quartiles of serum GDF11 levels, participants were divided into four groups (25th percentile Q1,  $\leq 1.548$  ng/mL; 25–50th Q2,  $\geq 1.552$  and  $\leq 2.025$  ng/mL; 50–75th Q3,  $\geq 2.032$  and  $\leq 2.781$  ng/mL; and 75th Q4,  $\geq 2.809$  ng/mL), and a Cochran–Armitage test for trend was applied to analyze the linear trend of the occurrence of MetS with GDF11 levels. Logistic regression analysis was carried out to identify the association of GDF11 with MetS with or without the adjustment of other confounding factors. Taking the difference of age and some metabolic parameters between men and women into consideration, subgroup analysis of GDF11 levels with age, as well as metabolic diseases, were carried out separately in men and women. A two-tailed  $P$ -value of  $< 0.05$  was considered statistically significant.

## RESULTS

### Characteristics of the study participants

Among the 381 Chinese individuals included in the present study, men were much older than women, with a median age of 66.9 years and 59.5 years, respectively ( $P < 0.001$ ), as shown in Table 1. Meanwhile, men had higher WHR, FFM, FFMI, creatinine, uric acid, blood urea nitrogen, free triiodothyronine, fT4 and serum GDF11 levels, but showed lower BMI, HC, body fat, body fat rate, 2h-PG, glycated hemoglobin A1c, fasting plasma insulin, 30-min plasma insulin, 2h-Ins, HOMA-IR, HOMA-% $\beta$ , TG, total cholesterol, LDL-c, HDL-c, estimated glomerular filtration rate and TSH levels compared with women (all  $P < 0.05$ ; Table 1).

### Association of circulating GDF11 levels with anthropometric and biochemical parameters in the study population

Although Spearman's rank correlation showed that serum GDF11 levels increased with increasing age ( $r = 0.151$ ,  $P = 0.003$ ; Table 2), no association was observed after adjusting for sex ( $r = -0.064$ ,  $P = 0.210$ ; Table 2). We carried out further subgroup analysis stratified by sex; the results showed that the GDF11 levels in neither men nor women were correlated with age ( $r = -0.027$ ,  $P = 0.754$  for men, and  $r = -0.090$ ,  $P = 0.157$

for women). As shown in Table 2, BMI, WC, FFM and FFMI were negatively related to GDF11 levels (all  $P < 0.05$ ), whereas body fat showed only a marginal correlation after adjustment for sex and age in model 2 ( $r = -0.094$ ,  $P = 0.077$ ). Also in the same model, serum GDF11 showed a negative association with DBP, FPG, 2h-PG, 2h-Ins, HOMA-IR, TG and LDL-c, whereas AST and fT4 were positively correlated (all  $P < 0.05$ ). Furthermore, even after adjusting for sex, age, and BMI, the association between DBP, FPG, 2h-PG, TG, LDL-c and AST, as well as fT4 and GDF11 levels, remained (all  $P < 0.05$ ; Table 2).

All enrolled participants were classified into three groups according to blood glucose or BMI successively; the Kruskal–Wallis test showed significant differences in GDF11 concentrations ( $P = 0.004$  and  $P < 0.001$ , respectively). Furthermore, circulating GDF11 levels were lower in IGR and participants with diabetes mellitus than in participants with normal glucose tolerance (Figure 1a). Furthermore, when compared with overweight and obese groups, people with normal weight had higher serum GDF11 levels (Figure 1d). Significantly higher circulating GDF11 levels were also observed in non-NAFLD participants than in NAFLD patients (Figure 1g). After subgroup analysis, difference of serum GDF11 levels among participants with distinct glycometabolism state only existed in men ( $P = 0.018$ ), with participants with diabetes mellitus having lower GDF11 concentrations than participants with normal glucose tolerance and IGR (Figure 1b). Consistently, only overweight men showed decreased circulating GDF11 levels compared with the lean group (Figure 1e). In addition, although GDF11 levels appeared higher in non-NAFLD than in NAFLD individuals of both sexes, statistical difference was not observed in women (Figure 1h and 1i).

### Relationship between GDF11 concentration and MetS

We divided the study population into four groups according to GDF11 level quartiles (Q1,  $n = 96$ ; Q2,  $n = 95$ ; Q3,  $n = 95$ ; Q4,  $n = 95$ ); the processed medians of variables associated with serum GDF11 levels in model 2 (Table 2) are shown in Figure 2 (unprocessed level of each parameter is shown in Table S1). The Kruskal–Wallis test showed a decreasing trend in BMI, WC, FPG, 2h-PG, 2h-Ins, HOMA-IR, TG and LDL-c, but an increasing trend in FFM, FFMI and fT4 accompanied by increasing serum GDF11 levels (all  $P < 0.05$ ). Further analysis carried out respectively in men and women showed that levels of FPG, 2h-PG, 2h-Ins, HOMA-IR, LDL-c, AST and fT4 in men, as well as fT4 in women, were different among the four groups divided by serum GDF11 level quartiles (all  $P < 0.05$ ; Table S2, S3).

Given that a majority of the traits in Figure 2 are components of MetS, we further investigated the relationship between GDF11 concentration and MetS. Using a Cochran–Armitage test for trend, we found that in the total study population and men, morbidity of MetS increased along with decreasing GDF11 levels (both  $P$ -trend  $< 0.001$ ). In addition, as shown in Figure 3, non-MetS individuals had higher GDF11 serum levels

**Table 1** | Clinical characteristics of the study participants

	All	Men	Women	<i>P</i>
<i>n</i>	381	134	247	
Age (years)	61.9 (57.1–66.8)	66.9 (65.4–67.8)	59.5 (56.3–62.9)	<0.001
BMI (kg/m <sup>2</sup> )	25.0 (22.7–28.3)	23.5 (21.8–25.2)	27.0 (23.2–29.3)	<0.001
WC (cm)	85 (79–92)	84 ± 8	87 (79–93)	0.084
HC (cm)	94 (90–100)	92 ± 6	96 (91–101)	<0.001
WHR	0.90 (0.86–0.94)	0.91 (0.88–0.95)	0.90 ± 0.06	0.012
Body fat (kg)	19.65 (14.60–27.30)	13.80 (10.80–17.50)	24.93 ± 7.44	<0.001
Body fat rate (%)	32.70 (23.70–39.90)	21.32 ± 4.86	38.65 (33.00–42.00)	<0.001
FFM (kg)	43.50 (39.10–49.80)	52.06 ± 4.88	40.50 (37.85–43.40)	<0.001
FFMI (kg/m <sup>2</sup> )	17.01 (16.16–18.01)	18.78 ± 1.28	16.54 (15.82–17.05)	<0.001
SBP (mmHg)	131 (122–143)	133 ± 16	132 (123–144)	0.651
DBP (mmHg)	81 (78–87)	82 ± 8	81 (78–87)	0.708
FPG (mmol/L)	5.83 (5.34–6.55)	5.79 (5.37–6.46)	5.84 (5.48–6.64)	0.282
30min-PG (mmol/L)	10.15 (8.83–11.59)	10.10 (8.81–11.33)	10.24 (8.85–11.69)	0.435
2h-PG (mmol/L)	7.77 (6.45–10.87)	7.27 (6.06–10.35)	8.03 (6.68–11.05)	0.020
HbA1c (%)	5.7 (5.4–6.0)	5.5 (5.3–5.9)	5.7 (5.5–6.1)	0.003
GA (%)	14.27 (13.25–15.52)	14.06 (13.12–15.32)	14.35 (13.32–15.61)	0.287
Flns (mU/L)	6.69 (4.77–10.34)	5.53 (3.85–7.48)	7.87 (5.41–12.03)	<0.001
30-min Ins (mU/L)	43.66 (28.19–65.73)	37.75 (22.44–59.54)	46.65 (30.78–70.67)	0.001
2h-Ins (mU/L)	38.78 (23.88–66.10)	28.51 (19.10–47.65)	45.96 (29.32–73.18)	<0.001
HOMA-IR	1.79 (1.15–2.92)	1.41 (0.92–2.00)	2.09 (1.37–3.32)	<0.001
HOMA-%β	56.65 (39.29–83.66)	46.26 (32.87–60.88)	69.28 (46.12–94.47)	<0.001
TG (mmol/L)	1.05 (0.82–1.47)	0.92 (0.73–1.35)	1.15 (0.90–1.54)	<0.001
TC (mmol/L)	4.67 (4.24–5.02)	4.52 (4.09–4.78)	4.74 (4.35–5.08)	<0.001
LDL-c (mmol/L)	2.70 (2.29–3.07)	2.59 ± 0.60	2.76 (2.34–3.12)	0.003
HDL-c (mmol/L)	1.27 (1.12–1.49)	1.22 (1.06–1.44)	1.29 (1.15–1.53)	0.023
ALT (U/L)	17 (14–24)	17 (13–24)	17 (14–24)	0.827
AST (U/L)	23 (20–27)	23 (20–28)	22 (19–26)	0.208
CR (μmol/L)	60 (53–70)	72(65–82)	55 (50–61)	<0.001
UA (mmol/L)	289 (245–338)	328 (282–364)	268 (324–319)	<0.001
BUN (mmol/L)	5.1 (4.3–6.0)	5.3 (4.6–6.4)	5.1 (4.3–5.9)	0.009
eGFR (mL/min/1.73 m <sup>2</sup> )	96.44 (90.73–101.85)	92.27 (87.34–96.46)	98.80 (93.65–102.96)	<0.001
TSH (mIU/L)	2.83 (2.03–4.06)	2.49 (1.84–3.68)	2.98 (2.12–4.20)	0.021
ft3 (pmol/L)	4.94 (4.59–5.24)	5.00 ± 0.58	4.87 (4.54–5.16)	0.001
ft4 (pmol/L)	15.29 (14.13–16.78)	15.83 ± 2.08	15.15 (13.97–16.43)	0.010
GDF11 (ng/mL)	2.03 (1.55–2.78)	2.69 (1.95–3.38)	1.83 (1.44–2.34)	<0.001

Data are means ± standard deviation or median (interquartile range 25–75%). 2h-Ins, 2-h plasma insulin; 2h-PG, 2-h plasma glucose; 30min-Ins, 30-min plasma insulin; 30min-PG, 30-min plasma glucose; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BUN, blood urea nitrogen; CR, creatinine; DBP, diastolic blood pressure; eGFR, the estimated glomerular filtration rate; FFM, fat-free mass; FFMI, fat-free mass index; Flns, fasting plasma insulin; FPG, fasting plasma glucose; ft3, free triiodothyronine; ft4, free thyroxine; GA, glycated albumin; GDF11; growth differentiation factor 11; HbA1c, glycated hemoglobin A1c; HC, hip circumference; HDL-c, high-density lipoprotein cholesterol; HOMA-%β, homeostasis model assessment for β-cell function; HOMA-IR, homeostasis model assessment for insulin resistance; LDL-c, low-density lipoprotein cholesterol; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride; TSH, thyroid-stimulating hormone; UA, uric acid; WC, waist circumference; WHR, waist-to-hip ratio.

than people with MetS, and this was particularly so in men (both *P* < 0.001). Furthermore, as shown in Table 3, logistic regression analysis identified that serum GDF11 concentrations were significantly associated with the presence of MetS (odds ratio 0.627, 95% confidence interval 0.494–0.797, *P* < 0.001). Even after controlling for confounding factors, including sex, age and BMI, GDF11 levels remained an independent and

protective factor for MetS (odds ratio 0.665, 95% confidence interval 0.510–0.867, *P* = 0.003).

**Receiver operating characteristic curve analysis**

To investigate the predictive value of GDF11 for MetS, we analyzed the receiver operating characteristic curve of circulating GDF11. The area under the receiver operating characteristic

**Table 2** | Correlation analysis between serum growth differentiation factor 11 levels and other variables in the study population\*

	Unadjusted		Model 1		Model 2		Model 3	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Sex	-0.364	<0.001*						
Age	0.151	0.003*	-0.064	0.210				
BMI	-0.227	0.001*	0.111	0.031*	-0.109	0.034*		
WC	-0.138	0.007*	-0.114	0.026*	-0.112	0.029*	-0.044	0.394
HC	-0.156	0.002*	-0.074	0.153	-0.072	0.160	0.003	0.948
WHR	-0.033	0.523	-0.086	0.093	-0.083	0.107	-0.032	0.539
Body fat	-0.280	<0.001*	-0.093	0.080	-0.094	0.077	0.009	0.869
Body fat rate	-0.296	<0.001*	-0.055	0.299	-0.056	0.292	0.089	0.095
FFM	0.197	<0.001*	-0.111	0.036*	-0.114	0.031*	-0.072	0.176
FFMI	0.176	0.001*	-0.118	0.026*	-0.119	0.025*	-0.055	0.303
SBP	-0.083	0.105	-0.080	0.118	-0.075	0.143	-0.055	0.285
DBP	-0.121	0.019*	-0.122	0.017*	-0.126	0.014*	-0.104	0.043*
FPG	-0.139	0.007*	-0.128	0.013*	-0.130	0.012*	-0.105	0.043*
30min-PG	-0.032	0.533	-0.019	0.717	-0.022	0.665	0.000	0.999
2h-PG	-0.198	<0.001*	-0.167	0.001*	-0.164	0.001*	-0.135	0.009*
HbA1c	-0.141	0.006*	-0.092	0.074	-0.092	0.076	-0.070	0.178
GA	-0.105	0.042*	-0.091	0.077	-0.089	0.086	-0.086	0.095
Flns	-0.194	<0.001*	-0.087	0.091	-0.091	0.078	-0.036	0.482
30-min Ins	-0.044	0.387	0.018	0.721	0.019	0.714	0.041	0.425
2h-Ins	-0.220	<0.001*	-0.135	0.009*	-0.132	0.010*	-0.099	0.055
HOMA-IR	-0.202	<0.001*	-0.104	0.044*	-0.109	0.035*	-0.059	0.258
HOMA-%β	-0.136	0.008*	-0.026	0.616	-0.026	0.614	0.014	0.791
TG	-0.197	0.001*	-0.130	0.011*	-0.135	0.009*	-0.112	0.030*
TC	-0.135	0.008*	-0.070	0.177	-0.075	0.146	-0.077	0.137
LDL-c	-0.161	0.002*	-0.115	0.026*	-0.122	0.018*	-0.112	0.030*
HDL-c	0.013	0.799	0.060	0.242	0.061	0.238	0.030	0.565
ALT	-0.026	0.613	-0.032	0.530	-0.039	0.451	-0.003	0.960
AST	0.120	0.019*	0.104	0.044*	0.105	0.041*	0.116	0.025*
CR	0.237	<0.001*	-0.002	0.965	0.006	0.906	0.011	0.834
UA	0.158	0.002*	0.026	0.614	0.025	0.634	0.056	0.276
BUN	0.077	0.136	0.030	0.565	0.034	0.516	0.038	0.457
eGFR	-0.105	0.041*	0.037	0.479	0.004	0.944	-0.002	0.966
TSH	-0.051	0.325	-0.008	0.875	-0.002	0.976	-0.001	0.979
ft3	0.134	0.009*	0.079	0.122	0.074	0.153	0.083	0.106
ft4	0.195	<0.001*	0.159	0.002*	0.160	0.002*	0.170	0.001*

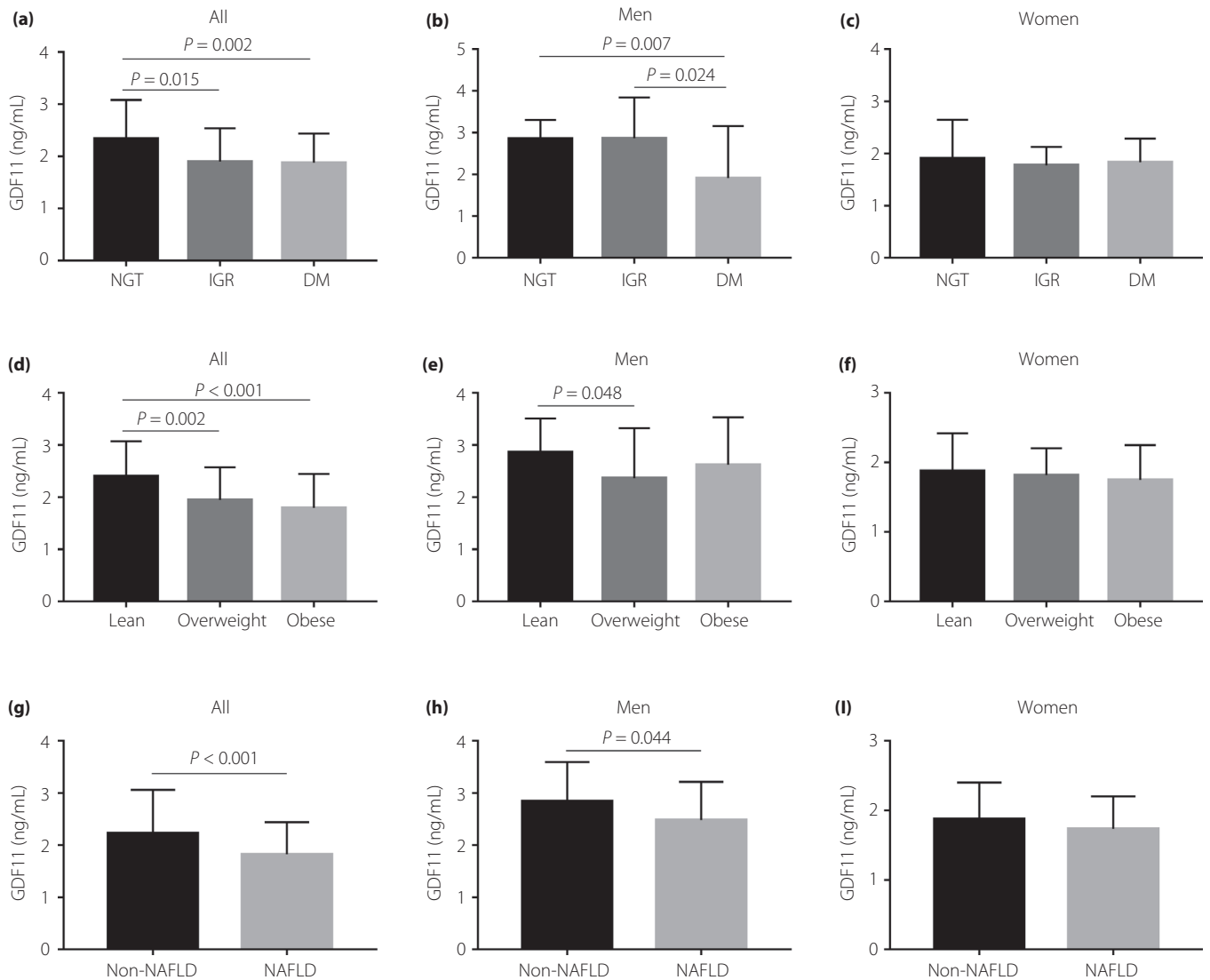
Values are Spearman's correlation coefficients and associated *P*-values. Traits were adjusted for sex in model 1, adjusted for both sex and age in model 2 and adjusted for sex, age and body mass index (BMI) in model 3. Male = 1 and female = 2 were used in the analysis. \**P*-values <0.05. 2h-Ins, 2-h plasma insulin; 2h-PG, 2-h plasma glucose; 30min-Ins, 30-min plasma insulin; 30min-PG, 30-min plasma glucose; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CR, creatinine; DBP, diastolic blood pressure; eGFR, the estimated glomerular filtration rate; FFM, fat-free mass; FFMI, fat-free mass index; Flns, fasting plasma insulin; FPG, fasting plasma glucose; ft3, free triiodothyronine; ft4, free thyroxine; GA, glycated albumin; GDF11; growth differentiation factor 11; HbA1c, glycated hemoglobin A1c; HC, hip circumference; HDL-c, high-density lipoprotein cholesterol; HOMA-%β, homeostasis model assessment for β-cell function; HOMA-IR, homeostasis model assessment for insulin resistance; LDL-c, low-density lipoprotein cholesterol; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride; TSH, thyroid-stimulating hormone; UA, uric acid; WC, waist circumference; WHR, waist-to-hip ratio.

curve (AUC) was 0.63 (*P* < 0.001), with a sensitivity of 75.59% and specificity of 48.43% (Figure 4a). Although in men, the area under the receiver operating characteristic curve was 0.73 (*P* < 0.001), and the sensitivity and specificity were 64.71% and 78%, respectively (Figure 4b). The best cut-off value for serum GDF11 to predict MetS in all participants or the male individuals was 2.27 ng/mL. However, circulating GDF11 appeared of

no value to identify metabolic syndrome in the women of the present study.

## DISCUSSION

The primary results obtained in the present study are as follows: (i) age had no effect on serum GDF11 levels; (ii) GDF11 concentrations were negatively associated with FFM and FFMI

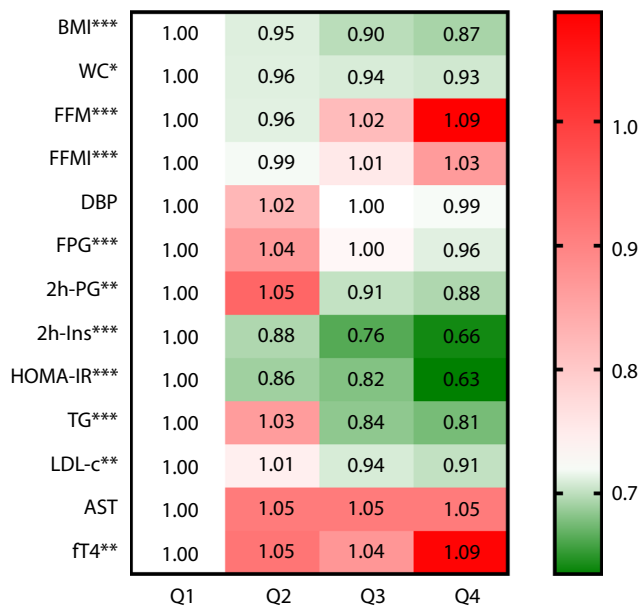


**Figure 1** | Distribution of serum growth differentiation factor 11 (GDF11) concentrations according to glucose levels, body mass index and fat accumulation in the liver. (a–c) Circulating GDF11 levels in individuals with normal glucose tolerance (NGT;  $n = 162$  for the total population,  $n = 65$  for men and  $n = 97$  for women), impaired glucose regulation (IGR;  $n = 112$  for the total population,  $n = 36$  for men and  $n = 76$  for women) and diabetes (DM;  $n = 107$  for the total population,  $n = 33$  for men and  $n = 74$  for women). (d–f) Distribution of serum GDF11 concentrations in lean ( $n = 149$  for the total population,  $n = 75$  for men and  $n = 74$  for women), overweight ( $n = 127$  for the total population,  $n = 46$  for men and  $n = 81$  for women) and obese ( $n = 105$  for the total population,  $n = 13$  for men and  $n = 92$  for women) individuals. (g–i) Comparison of serum GDF11 levels between individuals without non-alcoholic fatty liver disease (non-NAFLD;  $n = 202$  for the total population,  $n = 90$  for men and  $n = 112$  for women) and those with non-alcoholic fatty liver disease (NAFLD;  $n = 174$  for the total population,  $n = 40$  for men and  $n = 134$  for women). Data are shown as the median with 25th and 75th percentiles.

– indirect surrogates of skeletal muscle mass; (iii) GDF11 might play a role in metabolic disorders by influencing glucose and lipid homeostasis, as well as thyroid function, thereby serving as a potential indicator for MetS, especially in men.

The relationship between age and circulating GDF11 concentrations has long been debated. GDF11/8 in human sera has been reported to increase or have an increasing tendency<sup>10,19,20</sup>,

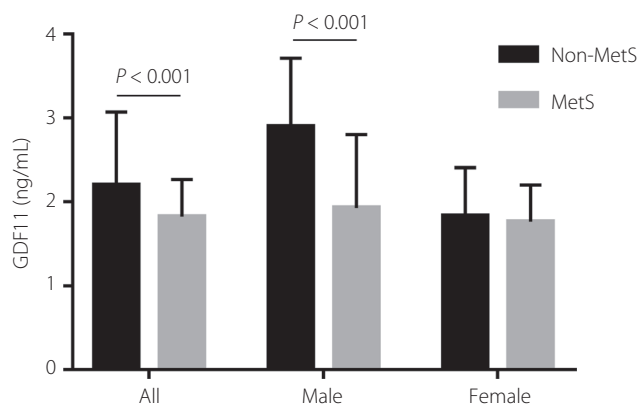
decrease<sup>12–14,17,18</sup>, or remain unchanged<sup>15,16</sup> with aging, with methodological diversity partly contributing to the inconsistencies. We used a commercial enzyme-linked immunosorbent assay kit that claims no cross-reactivity with any other analog when detecting GDF11; no association was observed between serum GDF11 and age in not only the entire group of individuals, but also in the subgroup analysis of different sexes. One



**Figure 2** | Clinical and biological traits in four groups evenly classified by serum growth differentiation factor 11 levels. Variables shown here were those significantly correlated to growth differentiation factor 11 concentrations after adjusting for sex and age. All parameters were divided by the corresponding median of quartile 1 (Q1), and each cell in the figure is labeled with the processed median of the relevant group. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . 2h-Ins, 2-h plasma insulin; 2h-PG, 2-h plasma glucose; AST, aspartate aminotransferase; BMI, body mass index; DBP, diastolic blood pressure; FFM, fat-free mass; FFMI, fat-free mass index; FPG, fasting plasma glucose; HOMA-IR, homeostasis model assessment for insulin resistance; LDL-c, low-density lipoprotein cholesterol; TG, triglyceride; WC, waist circumference.

limitation of the present study involves the advanced age of the participants – especially the men; a broader age range would be helpful to reduce bias.

As aforementioned, GDF11 shares 90% amino acid sequence identity with myostatin, a protein already well documented to decrease muscle mass and interfere with muscle repair. Given that both GDF11 and myostatin activate the same signaling pathways (SMAD2/3, extracellular signal-regulated kinase, c-Jun N-terminal kinase and p38 mitogen-activated protein kinase)<sup>29</sup>, and GDF15, another member of the transforming growth factor- $\beta$  family, was reported to be a novel biomarker for identifying high-risk patients with muscle wasting<sup>30</sup>, GDF11 was speculated to play a role in skeletal muscle. However, mutually exclusive findings were reported in mice. Soon after the idea that circulating GDF11 protein acts as a youthful systemic factor for skeletal muscle<sup>9</sup> was presented, a Novartis team cast doubt on the rejuvenating ability of GDF11, declaring its potent inhibitory effect on skeletal muscle regeneration<sup>10</sup>. Further studies showed that the overexpression of certain proteins by plasmids or adeno-associated virus-mediated systems in the liver identified GDF11 as a deleterious biomarker in muscle wasting diseases<sup>31,32</sup>. The current study showed that serum GDF11



**Figure 3** | Distribution of serum growth differentiation factor 11 (GDF11) concentrations with and without metabolic syndrome (MetS). Comparison of circulating GDF11 levels between non-MetS ( $n = 254$  for the total population,  $n = 100$  for men and  $n = 154$  for women) and MetS ( $n = 127$  for the total population,  $n = 34$  for men and  $n = 93$  for women) participants. Data are shown as the median with 25th and 75th percentiles.

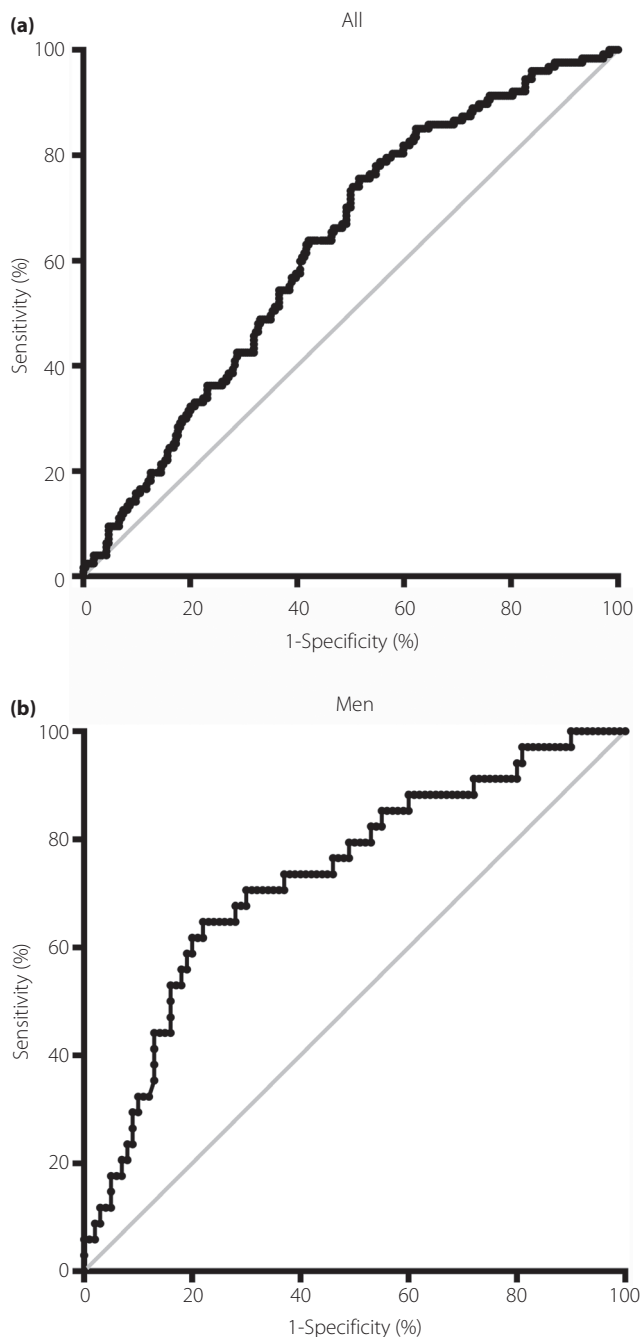
levels were negatively related to FFM and FFMI in a Chinese cohort, whereas Fife *et al.*<sup>16</sup> found a positive association between GDF11 and FFMI in a smaller group of older women ( $n = 56$ ) of European descent, but no significant correlation with muscle function. Furthermore, in a cross-sectional analysis of 319 patients (126 men and 193 women) of European descent, the results showed no effect of GDF11 in the regulation of skeletal muscle mass<sup>18</sup>. These discrepancies might derive from different races and sample sizes, as well as other potential confounding factors.

As early as 2004, Harmon *et al.*<sup>33</sup> proposed that GDF11 is essential to regulate the production and maturation of islet progenitor cells during pancreas development. Furthermore, administration of recombinant GDF11 conferred obvious

**Table 3** | Logistic regression analysis of association between growth differentiation factor 11 and metabolic syndrome

Model	Variable	OR (95% CI)	P
Model 1	GDF11	0.627 (0.494–0.797)	<0.001
			<0.001
Model 2	GDF11	0.657 (0.513–0.842)	0.001
	Sex	1.314 (0.752–2.295)	0.338
	Age	0.996 (0.954–1.040)	0.867
Model 3	GDF11	0.665 (0.510–0.867)	0.003
	Sex	0.536 (0.276–1.041)	0.066
	Age	0.993 (0.945–1.045)	0.796
	BMI	1.411 (1.294–1.539)	<0.001

BMI, body mass index; CI, confidence interval; GDF11, growth differentiation factor 11; OR, odds ratio.



**Figure 4** | Receiver operating characteristic curve analysis. (a) Receiver operating characteristic curve analysis of the prediction of metabolic syndrome in all individuals. (b) Receiver operating characteristic curve analysis of the prediction of metabolic syndrome in men.

advantages to adult pancreatic islets, improving  $\beta$ -cell function and glucose metabolism in non-genetic and genetic mouse models of type 2 diabetes mellitus<sup>21</sup>. Both discoveries showed the important role of GDF11 in glucose metabolism as regulated by the pancreas. In terms of lipid homeostasis, an *in vitro* experiment showed that GDF11 can inhibit adipogenic

differentiation in both bone marrow-derived human mesenchymal stem cells and 3T3-L1 pre-adipocytes by activating the Smad2/3-dependent transforming growth factor- $\beta$  pathway<sup>22</sup>. Furthermore, a recent study confirmed a comprehensive function of GDF11 in preventing a series of high-fat diet-induced disorders, including obesity, hyperglycemia, insulin resistance and fatty liver, as well as ameliorating metabolic homeostasis in obese mice and mice with STZ-induced diabetes<sup>34</sup>. The above evidence from mice and cultured cells validates the function of GDF11 in regulating metabolic homeostasis and energy balance. In humans, however, just a few studies have focused on circulating GDF11 concentrations and metabolic disorders, and those that do exist report discrepant conclusions. The first human study investigating GDF11 levels and diabetes implied that the higher concentrations of GDF11 observed in diabetes were attributed exclusively to macroangiopathy in a relatively small sample size (38 type 2 diabetes mellitus and 38 age- and sex-matched non-diabetic individuals)<sup>12</sup>. In a cohort of individuals suffering from severe aortic stenosis, patients with higher levels of GDF11 tended to have diabetes, yet BMI seemed unaffected<sup>15</sup>. Apart from fat-free mass, Fife *et al.*<sup>16</sup> further extended their analysis to the association of GDF11 with anthropometric parameters and other indices of body composition, showing inverse correlations between GDF11 levels and BMI, WC and fat mass together with percentage of fat mass in women. Nevertheless, Anon-Hidalgo *et al.*<sup>17,18</sup> failed to find the aforesaid negative relevance after adjusting for age, instead proposing that serum GDF11 levels were unaltered in type 2 diabetes mellitus and obesity, but increased with higher TSH levels.

In the current study, BMI and WC were significantly negatively associated with serum GDF11 levels in the model adjusting for sex and age. Furthermore, even with BMI static, DBP and glucolipid metabolism traits, including FPG, 2h-PG, TG and LDL-c, still increased along with declining GDF11 in circulation. Unsurprisingly, individuals, particularly men, with impaired glucose regulation or diabetes mellitus had lower levels of GDF11 compared with normal controls, as is the case for overweight or obese individuals, in accordance with results obtained from another Chinese male population<sup>14</sup>. Recently, Dai *et al.*<sup>35</sup> showed an upregulation of hepatic GDF11 transcript in patients with fibrotic livers, and uncovered a protective role of GDF11 during liver fibrosis in a mouse model. In contrast, we found that GDF11 concentrations decreased in the circulation of NAFLD patients. The present study – to the best of our knowledge – is the first to analyze the change in GDF11 serum levels in patients with lipid deposition in the liver. Furthermore, it is worth noting that the present results confirmed the participation of GDF11 in thyroid metabolism with *t*T4, as reported by Anon-Hidalgo<sup>17</sup>, but not TSH, showing a positive correlation thereto, suggesting its role in the regulation of energy expenditure. Further studies investigating the relationship of thyroid antibodies, ultrasonography, resting energy expenditure and so on with serum GDF11 levels will help to clarify this finding. It is well recognized that metabolic syndrome is a constellation of metabolic abnormalities composed of



visceral obesity, dyslipidemia, hyperglycemia and hypertension, all of which are risk factors for type 2 diabetes mellitus and cardiovascular disease<sup>36</sup>. As one of the major public health challenges worldwide, finding biomarkers to predict the development of MetS is of great importance. Our investigations showed an increased tendency of the incidence of MetS along with decreased serum GDF11 levels, and demonstrated that the decline in circulating GDF11 concentrations was an independent risk factor for MetS, suggesting GDF11 as an indicator of MetS in a Chinese cohort. However, it is worth noting that the strong relationship between GDF11 and MetS, as well as its components, only existed in men when carrying out sex-specific analysis. In addition, the receiver operating characteristic analysis only showed a predictive value of GDF11 for MetS in all or male participants. The difference between the sexes might be explained by the age range of the women in the present study (47–70 years), as women experienced remarkable fluctuation of gonadal hormone levels, which might have an impact on metabolism.

The present study had four limitations. First, the ages of the participants were overly homogeneous; hence, certain results might not stand on analysis in the general population. Second, we lacked data of serum gonadal hormone levels, resulting in the existence of a potential confounding factor. Third, the cross-sectional design restricted our ability to elucidate the causal relationship between serum GDF11 levels and metabolic disorders. Finally, the study was carried out exclusively with Chinese individuals; therefore, the conclusions might not be applicable to individuals of other ethnicities.

In conclusion, the present results showed that GDF11 was closely associated with metabolic diseases, such as diabetes, obesity and NAFLD, in a Chinese cohort, especially in men. As such, the assessment of its levels might serve as an effective indicator of MetS in the Chinese population writ large. A prospective study design is crucial to provide further information on the causal relationship between GDF11 and metabolic syndrome, and future study is expected to further explore its potential as a novel therapeutic target.

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## DISCLOSURE

The authors declare no conflict of interest.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Table S1** | Clinical characteristics of the study participants in four groups according to GDF11 level quartiles.

**Table S2** | Clinical characteristics of the male study participants in four groups according to GDF11 level quartiles.

**Table S3** | Clinical characteristics of the female study participants in four groups according to GDF11 level quartiles.