

# Serum Fibroblast Growth Factor 23 Level and Liver Fat Content in MAFLD: A Community-Based Cohort

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**Purpose:** Although fibroblast growth factor-23 (FGF23) is involved in the development of metabolic diseases, its association with metabolic-associated fatty liver disease (MAFLD) remains unknown. We explored the relationship between serum fibroblast growth factor-23 level, metabolic associated fatty liver disease, and liver fat content.

**Patients and Methods:** Participants were enrolled from communities in Shanghai. Serum fibroblast growth factor-23 level was determined using two-side sandwich enzyme-linked immunosorbent assays. MAFLD was diagnosed using the international expert consensus (2020) criteria. Liver fat content was assessed using ultrasound.

**Results:** We enrolled 1827 individuals aged 30–80 years (mean age, 59.4±7.3 years). MAFLD was diagnosed in 445/1393 (31.9%) non-diabetic participants and 245/434 (56.5%) diabetic participants. After adjusting for confounders, one standard deviation increase in serum FGF23 was associated with MAFLD in diabetic (odds ratio, 1.27; 95% confidence interval, 1.15–1.49;  $P<0.001$ ) and non-diabetic (odds ratio, 1.28; 95% confidence interval, 1.07–1.74;  $P=0.030$ ) groups. In a fully adjusted linear regression model, serum FGF23 emerged as a positive determinant of liver fat content in both diabetic and non-diabetic groups ( $P=0.039$ ;  $P=0.034$ ).

**Conclusion:** Participants with MAFLD had higher serum fibroblast growth factor-23 level than those without MAFLD, regardless of diabetes status. Serum fibroblast growth factor-23 was independently related to MAFLD and liver fat content.

**Keywords:** metabolism-associated fatty liver disease, fibroblast growth factor 23, liver fat content, diabetes

## Introduction

Fibroblast growth factor-23 (FGF23), a member of the endocrine FGF family, is mainly expressed in osteocytes and osteoblasts in bone and binds to FGF receptors with the cofactor  $\alpha$ Klotho to initiate downstream signaling events; thus, functioning as a circulating endocrine factor.<sup>1</sup> FGF23 is considered a link between the bone and kidney to regulate mineral balance.<sup>2,3</sup> Moreover, FGF23 plays a crucial role in the development of metabolic and cardiovascular diseases, including diabetes, obesity, and atherosclerosis.<sup>4–6</sup> Previous experimental studies show that FGF23 is related to oxidative stress, inflammatory response, insulin resistance, and ectopic fat deposition through the inflammatory pathway.<sup>7,8</sup> Clinical studies suggest that FGF23 is involved in the distribution and regulation of adipose tissue with an intensive relation to obesity and insulin resistance.<sup>9,10</sup>

Hepatic steatosis is one of the major etiologies of chronic liver disease with a global prevalence exceeding 25%.<sup>11</sup> Before 2019, the international guideline

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defined “nonalcoholic fatty liver disease (NAFLD)” by excluding other causes of liver steatosis such as alcoholism, hepatitis virus infection, and pregnancy.<sup>12,13</sup> With greater understanding of hepatic steatosis, growing evidence demonstrated that metabolic diseases contributed to the occurrence and development of fatty liver.<sup>14,15</sup> However, the influence and interactions of metabolic diseases with hepatic steatosis could not be clarified using traditional diagnostic criteria. Therefore, an international expert consensus on fatty liver proposed the definition of metabolism related fatty liver disease (MAFLD) in 2020. The diagnosis was based on histology, imaging, or blood biomarkers of fat accumulation in the liver in addition to one of three criteria, namely, overweight/obesity, presence of type 2 diabetes mellitus (T2DM), or evidence of metabolic dysregulation.<sup>16</sup>

Existing evidence indicates that serum FGF23 level is strongly associated with metabolic diseases and fat distribution.<sup>9,17</sup> Additionally, FGF23 is proven to be relevant to NAFLD in diabetic patients.<sup>18,19</sup> However, the relation of serum FGF23 with MAFLD has not been reported in community population yet. This study aimed to explore the link between serum FGF23 level, MAFLD, and liver fat content (LFC) assessed by standardized ultrasound hepatic/renal ratio and hepatic attenuation rate in a community-based population.

## Materials and Methods

### Study Population

We enrolled participants from communities in Shanghai between October 2015 and July 2016. The collected data were derived from standardized questionnaires and included information on current and previous illnesses and medications, physical examinations, and biochemical measurements. Participants with a known history of cardiovascular or cerebrovascular diseases, malignant tumors or intracranial space occupying lesions, severe liver dysfunction, thyroid dysfunction, or hyperthyroidism/hypothyroidism treatment with steroids or thyroxine and an estimated glomerular filtration rate  $<60$  mL/min/1.73 m<sup>2</sup> were excluded. This study was approved by the Ethics Committee of the Sixth People’s Hospital Affiliated to Shanghai Jiao Tong University (approval number 2019-067). All participants provided written informed consent before participation. A total of 1827 eligible participants with complete data were recruited for the study.

Individuals with diabetes were diagnosed according to the 2021 American Diabetes Association criteria.<sup>21</sup>

### Anthropometric and Biochemical Measurements

Height, weight, waist circumference, and resting blood pressure were measured using the standardized methods.<sup>20</sup> Body mass index (BMI) was calculated as weight (kg)/height<sup>2</sup> (m<sup>2</sup>). The participants were examined on the morning following an overnight fast to collect their fasting blood samples. Thereafter, participants without diabetes underwent a 75-g oral glucose tolerance test, whereas those with diabetes took the 100-g steamed bread meal test. Biochemical variables, namely, fasting blood glucose (FPG), fasting insulin (FINS), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), C-reactive protein (CRP), alanine aminotransferase (ALT), alanine aminotransferase (AST), alkaline phosphatase (ALP), glutamyl transferase (GGT), creatinine (CR), 2-hour blood glucose (2hPG), and glycated hemoglobin (HbA<sub>1c</sub>) were detected as previously described.<sup>14</sup> Serum FGF23 level was determined using two-side sandwich enzyme-linked immunosorbent assays and fasting blood samples. The intra-assay and inter-assay coefficients of variations were 5.6% and 8.2%, respectively. The homeostasis model assessment of insulin resistance (HOMA-IR) was as follows:  $HOMA-IR = FINS (mU/L) \times FPG (mmol/L) / 22.5$ .<sup>22</sup>

### Diagnostic Criteria of MAFLD and Measurement of Liver Fat Content

MAFLD, in this study, was diagnosed in accordance with the international expert consensus statement on MAFLD in 2020.<sup>5</sup> Liver fat accumulation was determined using liver ultrasound and the presence of any one of the following three conditions: overweight/obesity (BMI  $\geq 23$  kg/m<sup>2</sup>), presence of T2DM, or evidence of metabolic dysregulation. Metabolic dysfunction was defined as the presence of at least two of the following metabolic risk factors: (1) a waist circumference  $\geq 90$  cm in men and  $\geq 80$  cm in women; (2) blood pressure  $\geq 130/85$  mmHg or ongoing antihypertensive therapy; (3) TG  $\geq 1.7$  mmol/L or ongoing lipid-lowering therapy; (4) HDL-C  $< 1$  mmol/L for men and  $< 1.3$  mmol/L for women or ongoing drug therapy; (5) prediabetes, ie, an FPG of 5.6–6.9 mmol/L, a 2hPG of 7.8–11.0 mmol/L, or an HbA<sub>1c</sub> of 5.7%–6.4%; (6) HOMA-IR  $\geq 2.5$ ; and (7) plasma CRP level  $> 2$  mg/L.

All participants underwent abdominal ultrasonographic examination using a Voluson 730 Expert B-mode ultrasound machine (5.0-MHz transducer, GE Healthcare, Waukesha, WI, USA) by a trained sonographer, who was blind to the study design and clinical details of the participants. Fatty liver was diagnosed through ultrasonography with the presence of at least two of the following four findings:<sup>23</sup> (1) diffusely increased echogenicity of the liver relative to the kidney or spleen, (2) ultrasound beam attenuation with decreased vessel signal, (3) poor visualization of intrahepatic structures, and (4) slightly enlarged liver with blunt margin. LFC, as an index of fat accumulation in the liver, was determined through an ultrasound fatty liver quantification. The regions of interest in the images captured by the ultrasound device were analyzed using image software certified by the National Institutes of Health (ImageJ 1.41o, National Institutes of Health, Bethesda, MD, USA). All the instrument settings, including “gain,” “depth,” and “time-gain compensation,” were fixed for each measurement. A 3D abdominal organ-mimicking phantom (Model 057; Computerized Imaging Reference Systems, Norfolk, VA) was used for standardization of the ultrasound hepatic/renal echo-intensity ratio and hepatic echo-intensity attenuation rate. LFC was then calculated based on the following equation:  $LFC = 62.592 \times \text{hepatic/renal echo-intensity ratio} + 168.076 \times \text{hepatic echo-intensity attenuation rate} - 27.863$ .<sup>24</sup>

## Statistical Analyses

Data were analyzed using SPSS, version 20.0 (SPSS Inc, Chicago, IL, USA). All variables were tested for normality. Normally distributed variables are presented as mean  $\pm$  standard deviation (SD), and non-normally distributed variables are presented as median and interquartile range. Student's *t*-test was used to compare two groups with normal distribution whereas Wilcoxon rank sum test was used for comparing two groups with skewed distribution. Multivariable logistic regression was used to analyze the relationship between serum FGF23 level and MAFLD. The results of logistic regression analysis were expressed as odds ratio (OR) and 95% confidence interval (CI). Factors affecting LFC were investigated using multivariate linear regression. All *P* values were two-tailed tests, and *P*<0.05 was considered statistically significant.

## Results

### Clinical Characteristics of Study Subjects

A total of 1827 individuals including 740 men and 1087 women, aged between 30 and 80 years, with a mean age of  $59.4 \pm 7.3$  years, were enrolled. The serum FGF23 level was

$37.2 \pm 10.8$  in all participants. Table 1 shows the characteristics of the participants according to their diabetes and MAFLD status. MAFLD was diagnosed in 445/1393 (31.9%) non-diabetic participants and 245/434 (56.5%) diabetic groups. Participants with MAFLD presented with significantly higher serum FGF23 level than those without MAFLD in both diabetic and non-diabetic groups (both *P*<0.05). LFC of the total population was  $14.59 \pm 9.5\%$ . In both the non-diabetic group and diabetic group, participants with MAFLD had a higher LFC than those without MAFLD (both *P*<0.05). The values of BMI, waist circumference, systolic and diastolic blood pressures, FPG, 2hPG, FINS, HbA<sub>1c</sub>, HOMA-IR, ALT, AST, ALP, GGT, TG, CRP, and LDL-C in participants with MAFLD were significantly higher than in those without MAFLD. Contrastingly, participants with MAFLD had lower HDL-C levels than did those without MAFLD in both diabetic and non-diabetic groups (all *P*<0.05).

### Associations Between Serum FGF23 and MAFLD

The sample was divided into three groups according to the tertiles of serum FGF23 level: T1,  $\leq 30.8$  ng/mL; T2, 30.9–40.8 ng/mL; and T3,  $>40.8$  ng/mL. Overall, with increasing serum FGF23 level, the proportions of MAFLD in T1, T2, and T3 were 30.77%, 39.84%, and 42.86%, respectively, showing a significant upward trend (*P* for trend <0.01). Moreover, the LFC in T1, T2, and T3 increased to 12.8%, 14.7%, and 16.1%, respectively (*P* for trend <0.05). After stratifying the sample as per diabetes status, the proportions of MAFLD showed a significant upward trend in both diabetic and non-diabetic groups (both *P* for trend <0.05). In the diabetic group, the proportions of MAFLD in T1, T2, and T3 were 25.11%, 33.60%, and 37.23%, and the LFC were 11.7%, 13.5%, and 14.5%, respectively. In the non-diabetic group, the proportions of MAFLD were 48.3%, 59.0%, and 62.4%, and LFC were 16.3%, 18.8%, and 20.9% (Figure 1).

To further explore the association between serum FGF23 level and MAFLD, we performed a logistic regression analysis, in which the presence of MAFLD was designated as the dependent variable. In all subjects, model 1—a crude model—showed that for each one SD increment (corresponding to the actual value of 10.8 ng/mL) in serum FGF23, the OR for MAFLD was 1.32 (95% CI, 1.20–1.45; *P*<0.001). Model 2, which was adjusted for age, sex, smoking status, ALT, AST, ALP, and GGT, showed that for each one SD increment in serum FGF23,

**Table 1** Characteristics of the Study Subjects

Variables	Non-Diabetic Group (n = 1393)		Diabetic Group (n = 434)	
	Non-MAFLD (n = 948)	MAFLD (n = 445)	Non-MAFLD (n = 189)	MAFLD (n = 245)
Age (years)	60.0.0 (54.7–64.4)	59.1 (54.1–64.0)	62.0 (56.9–66.8)	62.0 (56.9–66.1)
BMI (kg/m <sup>2</sup> )	23.1 ± 2.8	26.2 ± 3.0**	23.9 ± 2.7	26.3 ± 3.0**
WC (cm)	80 (75–85)	90 (84–95)**	84 (78–90)	90 (85–94)**
LFC (%)	7.13 ± 2.31	26.15 ± 7.14**	6.99 ± 1.82	27.56 ± 7.17**
SBP (mmHg)	128 ± 16	136 ± 18**	137 ± 18	139 ± 17*
DBP (mmHg)	76 ± 10	81 ± 11**	78 ± 10	81 ± 10*
FPG (mmol/L)	5.6 (5.3–6.0)	5.7 (5.4–6.1)**	7.1 (6.2–8.1)	7.4 (6.7–8.5)*
2hPG (mmol/L)	6.7 (5.6–7.9)	7.5 (6.1–8.8)**	12.1 (9.7–14.5)	12.8 (11.1–15.8)*
FINS (uU/mL)	7.5 (5.7–10.1)	12.2 (8.9–16.2)**	8.7 (6.0–11.4)	13.6 (9.8–17.9)**
HbA <sub>1c</sub> (%)	5.6 (5.4–5.8)	5.7 (5.4–5.9)*	6.3 (5.9–6.7)	6.6 (6.1–7.3)**
HOMA-IR	1.9 (1.4–2.6)	3.2 (2.3–4.1)**	2.5 (1.8–3.9)	4.5 (3.2–6.0)**
ALT (U/L)	16 (13–21)	22 (17–30)**	18 (14–24)	25 (18–34)**
AST (U/L)	20 (17–24)	21 (18–25)**	20 (17–24)	22 (18–28)**
ALP (U/L)	72 (61–86)	74 (64–87)*	74 (61–87)	79 (67–93)*
GGT (U/L)	21 (16–29)	29 (21–40)**	24 (18–35)	24 (34–50)**
TC (mmol/L)	5.3 (4.7–6.0)	5.4 (4.8–6.1)	5.5 (4.7–6.2)	5.4 (4.7–6.3)
TG (mmol/L)	1.2 (0.9–1.7)	1.8 (1.4–2.6)**	1.4 (1.2–1.7)	1.9 (1.4–2.7)**
HDL-C (mmol/L)	1.5 (1.2–1.7)	1.3 (1.1–1.4)**	1.4 (1.2–1.7)	1.2 (1.1–1.4)**
LDL-C (mmol/L)	3.2 (2.7–3.7)	3.3 (2.9–3.9)**	3.3 (2.6–4.0)	3.4 (2.8–4.0)*
CRP (mg/L)	0.71 (0.34–1.31)	1.21 (0.70–2.20)**	0.97 (0.39–1.80)	1.42 (0.72–2.68)**
Cr (umol/L)	62 (54–73)	64 (55–76)	66 (55–77)	60 (51–70)
eGFR (mL/min/1.73m <sup>2</sup> )	97.1 (91.0–103.5)	97.4 (89.8–102.5)	95.6 (90.1–102.2)	98.3 (91.9–104.1)
FGF23 (pg/mL)	35.0 (28.3–42.5)	37.5 (30.8–46.5)**	34.2 (27.5–43.3)	37.5 (30.0–45.0)*

**Notes:** Continuous variables are expressed as means ± standard deviation or medians with interquartile range. Categorical variables are expressed as numbers with percentages. Non-MAFLD versus MAFLD, \**P* < 0.05, \*\**P* < 0.01.

**Abbreviations:** BMI, body mass index; WC, waist circumference; LFC, liver fat content; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; 2hPG, 2-h plasma glucose; FINS, fasting insulin; HbA<sub>1c</sub>, glycated hemoglobin A<sub>1c</sub>; HOMA-IR, homeostasis model assessment-insulin resistance index; Alb, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT,  $\gamma$ -glutamyl transpeptidase; ALP, alkaline phosphatase; Cr, creatinine; TC, total cholesterol; TG, triglyceride; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; CRP, C-reactive protein; FGF23, fibroblast growth factor23; eGFR, estimated glomerular filtration rate.

the OR for MAFLD was 1.23 (95% CI, 1.16–1.43; *P*<0.001). In model 3, which was further adjusted for TC, LDL-C, and CR, a reduction was noted in the association of per SD of serum FGF23 with the risk of MAFLD; however, the association remained significant in all participants (OR, 1.22; 95% CI, 1.12–1.17; *P*<0.001) (Table 2).

Similarly, on stratifying the sample as per diabetes status and adjusting for all confounding factors (Model 3), each one SD increment in serum FGF23 level was significantly related to the risks of MAFLD in both non-diabetic (OR, 1.27; 95% CI, 1.15–1.49; *P*<0.001) and diabetic (OR, 1.28; 95% CI, 1.07–1.74; *P*=0.030) groups.

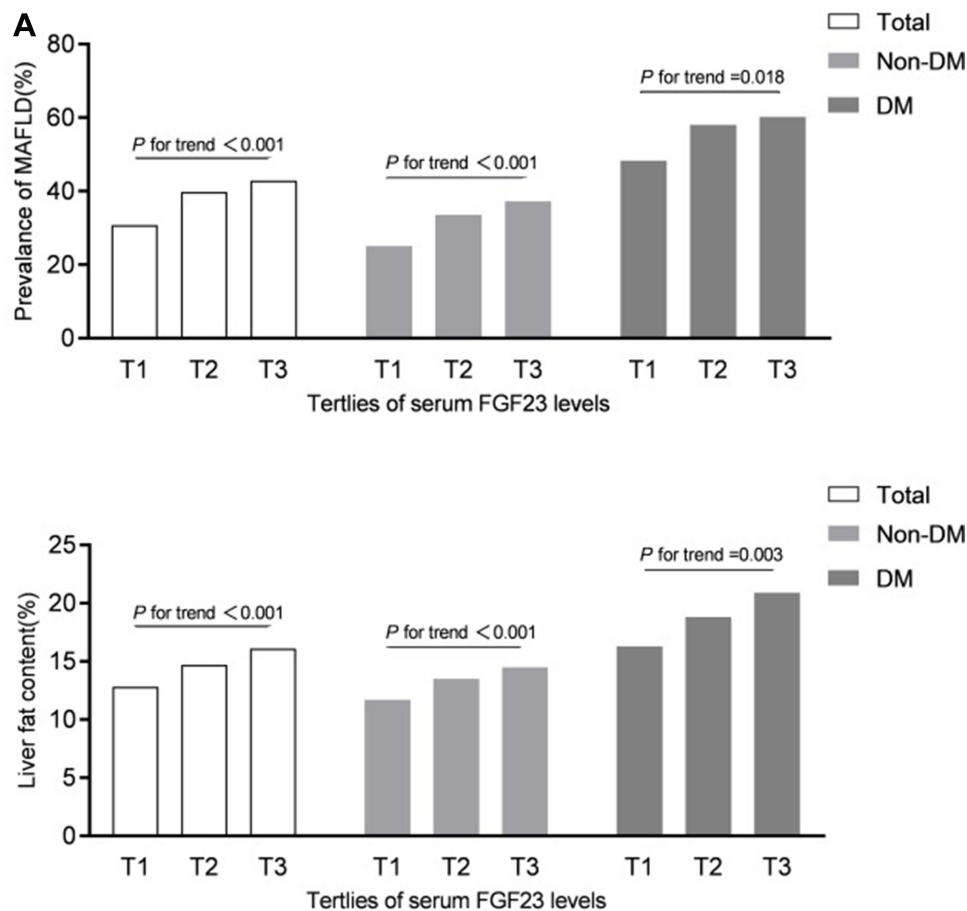
## Analysis of Multiple Factors Affecting LFC

Partial correlation analysis showed that serum FGF23 level was positively correlated with LFC after adjustment for sex and age (*r*=0.162; *P*<0.001). Furthermore, multiple linear regression analysis was performed with LFC as the

dependent variable and BMI, WC, ALT, AST, ALP, GGT, FPG, 2hPG, HOMA-IR, TG, HDL-C, LDL-C, CRP, and serum FGF23 as the independent variables. In addition to traditional risk factors such as BMI, WC, HDL-C, and HOMA-IR, serum FGF23 level influenced the LFC (standardized  $\beta$ =0.038; *P*=0.039) in the total population after further adjusting for presence of diabetes. Even on stratifying the sample as per diabetes status, serum FGF23 level remained an independent risk factor of LFC in non-diabetic (standardized  $\beta$ =0.036; *P*=0.039) and diabetic (standardized  $\beta$ =0.033; *P*=0.034) groups (Table 3).

## Discussion

Our study findings show that participants with MAFLD had higher serum FGF23 level than those without MAFLD, regardless of their diabetes status. Serum FGF23 level was positively related to MAFLD and LFC, independent of other metabolic factors.



**Figure 1** Prevalence of MAFLD (%) and liver fat content (%) according to tertiles of serum FGF23 level in total, non-diabetic and diabetic population.

A new positive definition for the MAFLD was proposed by an international expert consensus in 2020. The expanded criteria of inclusion rather than exclusion are expected to provide a more comprehensive overview of the widespread hepatic steatosis disease and lay more emphasis on the contribution of metabolic diseases in the presence and progression of hepatic steatosis. The international expert consensus hopes to identify participants at early stages of metabolic dysfunction and higher risk of disease progression through this definition.<sup>16</sup> Since the definition for MAFLD is a conceptual one, a retrospective study with population-based data from the National Health and Nutrition Examination Surveys in the United States from 1988 to 1994 showed that the definition of MAFLD was more practical than that of NAFLD for identifying participants with fatty liver disease with high risk of disease progression.<sup>25</sup> Additionally, Huang et al analyzed the liver biopsy results of 166 patients with fatty liver and found that the novel diagnostic criteria for MAFLD could better identify those with a high degree

of disease severity for early intervention than the previous NAFLD criteria.<sup>26</sup> Moreover, a study which enrolled 765 Japanese patients revealed that using the definition of MAFLD rather than that of NAFLD led to better identification of liver stiffness evaluated by non-invasive methods including Fibro-Scan and FIB-4 index.<sup>27</sup>

Increasing evidence indicates the involvement of bone in the regulation of homeostasis including adiposity metabolism and energy balance. Through the interaction of bone-derived factors and adipocytokines, bone and adipose tissue establish a steady feedback system of homeostasis.<sup>28</sup> FGF23 was considered to play a role in the regulation of fat accumulation and distribution as a factor mainly produced and secreted by osteoblasts and osteoclasts.<sup>29</sup> Clinical studies demonstrate that serum FGF23 level are associated not only with overall obesity but also with central obesity. Moreover, it is believed that serum FGF23 level was related to the increase of fat mass and occurrence and development of metabolic syndrome and its components.<sup>9</sup> Our previous study revealed that

**Table 2** Logistic Regression Analysis for the Association of Serum FGF23 Level and MAFLD

	FGF23			P for Trend	Per 1 SD Increment	P value
	T1	T2	T3			
Total (n=1827)						
Model 1	1.00	1.49 (1.18–1.88)	1.69 (1.33–2.14)	<0.001	1.32 (1.20–1.45)	<0.001
Model 2	1.00	1.41 (1.10–1.81)	1.60 (1.24–2.07)	0.010	1.29 (1.16–1.43)	<0.001
Model 3	1.00	1.34 (1.02–1.76)	1.45 (1.12–1.93)	0.015	1.22 (1.12–1.47)	<0.001
Non-diabetic (n=1393)						
Model 1	1.00	1.51 (1.14–2.01)	1.77 (1.33–2.36)	<0.001	1.39 (1.16–1.43)	<0.001
Model 2	1.00	1.41 (1.05–1.90)	1.73 (1.27–2.35)	0.002	1.28 (1.15–1.43)	<0.001
Model 3	1.00	1.38 (1.02–1.86)	1.72 (1.27–2.38)	0.012	1.27 (1.15–1.49)	<0.001
Diabetic (n=434)						
Model 1	1.00	1.68 (1.06–2.67)	1.72 (1.12–2.69)	0.003	1.36 (1.11–1.66)	0.003
Model 2	1.00	1.42 (0.86–2.34)	1.68 (1.02–2.74)	0.021	1.30 (1.06–1.61)	0.016
Model 3	1.00	1.32 (0.98–2.85)	1.65 (1.04–2.85)	0.031	1.28 (1.07–1.74)	0.030

**Notes:** Data are odds ratio (OR) and 95% confidence interval (CI). P values were calculated from the logistic regression models. Model 1: crude model. Model 2: adjusted for age, gender, current smoking, ALT, AST, ALP, and GGT. Model 3: Model 2+TC, LDL-C, and Cr on model 2.

**Abbreviations:** OR, odds ratio; FGF23, fibroblast growth factor 23; CI, confidence interval; SD, standard deviation; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT,  $\gamma$ -glutamyl transpeptidase; ALP, alkaline phosphatase; TC total cholesterol; HDL-C, high-density lipoprotein cholesterol, Cr, Creatinine;

**Table 3** Multivariate Regression Analyses Showing Factors Independently Associated with LFC

Variable	In Total (n=1827)*		In Non-Diabetic (n=1393)		In Diabetic (n=434)	
	Standardized $\beta$	P	Standardized $\beta$	P	Standardized $\beta$	P
FGF23	0.038	0.039	0.036	0.039	0.033	0.034
BMI	0.164	<0.001	0.162	<0.001	0.137	<0.001
WC	0.207	<0.001	0.202	<0.001	0.225	<0.001
ALT	0.233	<0.001	0.164	<0.001	0.201	<0.001
TG	0.151	<0.001	0.140	<0.001	0.153	<0.001
HDL-C	-0.080	<0.001	-0.053	0.025	-0.153	<0.001
CRP	0.043	0.019	0.060	0.035	0.051	0.028
HOMA-IR	0.100	<0.001	0.160	<0.001	0.109	0.006

**Notes:** Multivariate model included age, gender, BMI, WC, ALT, AST, GGT, ALP, FPG, 2hPG, HOMA-IR, TG, HDL-c, LDL-c, CRP, and FGF23. \*Further adjusted for DM + Multivariate model in total.

**Abbreviations:** BMI, body mass index; WC, waist circumference; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; HOMA-IR, homeostasis model assessment-insulin resistance index; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; CRP, C-reactive protein; FGF23, fibroblast growth factor 23.

serum FGF23 level was independently associated with visceral fat accumulation.<sup>19</sup>

Ectopic fat distribution is a stronger determinant of metabolic health than increased fat mass itself.<sup>30</sup> Increased liver fat content is closely related to insulin resistance, obesity, diabetes, and other metabolic dysfunctions.<sup>31,32</sup> It is generally considered that magnetic resonance spectroscopy (MRS) is the best noninvasive way to detect liver fat content. In 2012, Xia et al proposed an improved method in assessing liver fat by ultrasound hepatic/renal echo-intensity ratio and ultrasound hepatic

echo-intensity attenuation rate.<sup>24</sup> It showed no significant difference between ultrasound and MRS in the LFC of moderate to severe fatty liver. Difference of estimated liver fat contents between different ultrasound machines and operators was reasonably well. Thus, computer-aided US is a valid method to estimate liver fat content and can be applied extensively after standardization of ultrasound quantitative parameters. Moreover, Li et al found that LFC assessed by ultrasound was positively correlated with sub-clinical atherosclerosis and bone density in middle-aged and elderly people recruited from communities.<sup>33,34</sup>

Previous studies have established the correlation between FGF23 and NAFLD in diabetic inpatients. He et al have exhibited a positive correlation of serum FGF23 level with NAFLD and fatty liver index in 331 diabetic inpatients,<sup>17</sup> and Hu et al have found that serum FGF23 level was positively correlated with the presence and severity of NAFLD using Fibro-Scan to evaluate hepatic steatosis in 296 diabetic inpatients.<sup>18</sup> Since the concept of MAFLD was proposed recently, the correlation between serum FGF23 level and MAFLD remains unclear and only inpatients with diabetes have been included in previous studies that examine this association. We selected a large sample of participants (n=1827) from the community and we found that per SD increase in serum FGF23 was associated with a higher risk of MAFLD (19%) after adjusting for age, sex, liver enzymes, and metabolism indices. Additionally, this association persisted in the sample even after stratifying it into non-diabetic (n=1393) and diabetic (n=434) groups.

Fatty liver is one of the manifestations of liver fat deposition. Ultrasound can quantify LFC to evaluate hepatic steatosis.<sup>24,35</sup> We revealed that serum FGF23 was a positive risk factor for LFC after adjusting for age, sex, and metabolic indicators. Similar results were found in participants with or without diabetes. Previous studies show that serum FGF23 is related to the distribution of visceral adiposity.<sup>8,19</sup> FGF 1C, a potential receptor of FGF23, is highly expressed in macrophages, known as Kupffer cells in the liver, and increases fat deposition in the liver via increased activity of diacylglycerol transferase when polarized to the M1 phenotype.<sup>36</sup> Xu et al have revealed that FGF receptor inhibitor could increase the level of FGF23 and activate the phosphorylation of *JAK2*, whereas anti-FGF23 could inhibit its phosphorylation. The activation of *JAK/STAT* signaling pathway is involved in insulin resistance and triglyceride accumulation in the liver.<sup>7</sup> The emerging evidence shows that the activation of *JAK1/STAT3* signaling pathway leads to liver inflammation and liver fat deposition, which can significantly increase the synthesis of FGF23 in the liver, suggesting a bidirectional effect between liver fat deposition and FGF23.<sup>37</sup>

To the best of our knowledge, this is the first study of the associations of serum FGF23 level and MAFLD. However, this study had a few limitations. First, the study population only included participants from the Shanghai community, limiting the generalizability of the findings. Second, the cross-sectional design of the study could not determine the

causal relationship between increased serum FGF23 level and MAFLD. Therefore, further large-scale, prospectively designed studies are needed. Third, compared with the constantly updated detection methods (such as fibro scan), abdominal ultrasound may miss mild fatty liver. In addition, serum iron and ferritin were not collected in our study, and hemochromatosis could not be ruled out. Moreover, Vit D was strongly related to FGF23; it was a pity that we did not collect the date of Vit D.

In conclusion, serum FGF23 level was positively correlated with MAFLD and LFC in both non-diabetic and diabetic populations, suggesting that serum FGF23 is a supplementary biomarker for screening MAFLD.

## Data Sharing Statement

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

## Ethical Approval and Informed Consent

The study was approved by the Ethics Committee of Shanghai Jiao Tong University Affiliated Sixth People's Hospital, and all procedures were performed in accordance with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. The study methods and potential risks were fully explained to all participants, and each participant provided a written informed consent prior to enrollment.

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## Disclosure

The authors declare that they have no conflicts of interest.

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