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Serum follistatin like 1 in children with obesity and metabolic-associated fatty liver disease

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Abstract

Background Follistatin-like protein 1 (FSTL1) has been identified as a secreted glycoprotein that plays an important role in obesity. However, its role in children with metabolic-associated fatty liver disease (MAFLD) has not been investigated. This study aimed at characterizing the relationship between serum FSTL1 concentration and MAFLD in children with obesity.

Methods A total of 121 subjects were recruited from the Second Affiliated Hospital of Xi'an Jiaotong University, including 45 obese children with MAFLD, 31 obese children without MAFLD, and 45 healthy controls. Anthropometric parameters, biochemical data were measured and circulating FSTL1 levels were detected by ELISA.

Results The levels of FSTL1 in obese children with MAFLD were higher than that in obese children without MAFLD: 1.31 (0.35–2.29) ng/mL vs. 0.55 (0.36–1.38) ng/mL. Correlation analysis illustrated that FSTL1 was associated with nonesterified free fatty acid and leptin ($r=0.278$, $P<0.05$ and $r=0.572$, $P<0.05$, respectively). Binary logistic regression suggested that increased FSTL1 was a risk factor for MAFLD in children (OR = 1.105, 95% CI: 1.066–1.269, $P<0.05$).

Conclusions Serum FSTL1 concentrations increase in obese children with MAFLD and may have the potential to be a risk factor for MAFLD in children with obesity.

Keywords Follistatin-like protein 1, metabolic-associated fatty liver disease, Obesity, Child

Introduction

The prevalence of obesity among children and adolescents is rising at an alarming rate globally, particularly in the aftermath of the COVID-19 pandemic [1]. Obesity is frequently associated with various metabolic disorders, including metabolic syndrome, insulin resistance, and fatty liver disease. In 2024, simplified criteria and redefinition of nonalcoholic fatty liver disease (NAFLD) known as metabolic-associated fatty liver disease (MAFLD) was proposed to identify fatty liver degeneration in children with overweight/obesity, prediabetes/diabetes, or metabolic dysfunction [2]. MAFLD, as a hepatic manifestation of obesity, is recognized as the most prevalent liver disease among children and adolescents worldwide. The prevalence of MAFLD increases alongside rising rates of obesity. A meta-analysis has indicated that the prevalence of NAFLD in children with normal weight is 9.6%, whereas in children with obesity, it exceeds three times that figure at 36.1% [3]. Despite these findings, there remains incomplete elucidation regarding the molecular mechanisms underlying the progression of MAFLD and limited therapeutic interventions are currently available.

Follistatin-like protein 1 (FSTL1), also known as follistatin-related protein (FRP) or transforming growth factor beta-stimulated clone (TSC-36), belongs to the TGF-β superfamily and was originally cloned from an

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osteoblast cell line [4]. As a matricellular glycoprotein, FSTL1 exhibits widespread expression across diverse tissues such as adipose tissue, liver, heart, lung, and skeletal muscle; participating in biological processes such as regulation of cell proliferation, differentiation, and migration. FSTL1 also plays a crucial role in the development of various diseases. Miyamae et al. initially defined FSTL1 as a pro-inflammatory factor in arthritis [5]. The possible involvement of FSTL1 in human metabolism diseases was first demonstrated in muscle ischemia and heart failure [6, 7]. The research revealed that FSTL1 was a myokine secreted by skeletal muscle and exerted a protective effect. Concerned with the role of FSTL1 in obesity, it was identified as a novel adipokine released from preadipocyte and a hallmark of preadipocyte to adipocyte conversion [7–9]. Fan et al. suggested that FSTL1 was a mediator of insulin resistance and inflammation in obesity [10]. In addition, methylation of FSTL1 in children's saliva was significantly associated with maternal body mass index (BMI) [11]. For liver disease, Hansen et al. demonstrated that FSTL1 was secreted from the liver at rest and during exercise [12]. The results examined the splanchnic release of FSTL1 by measuring arterial-to-hepatic veins in humans and identified that FSTL1 concentrations were significantly increased in the hepatic vein, which suggested the extremely critical role of the liver in the metabolism of FSTL1 [12]. Data obtained by animal models indicated that FSTL1 modulated liver fibrosis via attenuating hepatic stellate cell activation and reprogramming macrophage function [13–15]. For liver cancer, FSTL1 promoted tumor progression and activated fibroblasts [16, 17]. In addition, Li et al. corroborated that FSTL1 was a powerful ligand in accelerating hepatocyte replication in vivo [18]. These researches indicate that FSTL1 plays a critical role in obesity and hepatic disease.

To date, there is little clinical research supporting the role of FSTL1 in MAFLD, especially in children and adolescents. Consequently, this cross-sectional study was conducted with several objectives. First, detect serum concentrations of FSTL1 in children with obesity and MAFLD compared to those without MAFLD. Second, investigate the underlying association of serum FSTL1 concentrations with MAFLD in obese children. Third, evaluate the possibility of using circulating FSTL1 levels for the diagnosis of childhood MAFLD.

Materials and methods

Participants

A total of 76 children with obesity were enrolled from the Pediatric Department of the Second Affiliated Hospital of Xi'an Jiaotong University. The inclusion criteria for the obese children included children who were 6–14 years old and diagnosed as obesity. The exclusion criteria included having acute or chronic infection disease, having heart, lung, kidney, endocrine, or other systemic diseases, taking any medications, being diagnosed as having genetic obesity syndromes, having a history of alcohol consumption, viral hepatitis, autoimmune hepatitis or liver disease for causes other than simple obesity. Children who had comorbid diseases, mental retardation, or an autism spectrum disorder or whose parents did not give permission for inclusion in the study after detailed information were excluded. At the same time, 45 healthy children were recruited at child health care as healthy controls. The control group consisted of children whose weight was BMI-SDS ≥ 1 and ≤ 1. The inclusion criteria for the controls included being able to cooperate for study and willingness of parents to participate in the study after being given detailed information. All participants and their legal guardians agreed to participate and provided written informed consent. This study was conducted in conformity with the Declaration of Helsinki and approved by the Ethical Committee of the Second Affiliated Hospital of Xi'an Jiaotong University (20222456).

MAFLD diagnosis

The pediatric MAFLD criteria referred to the latest consensus: hepatic steatosis in addition to one of the three criteria: excess adiposity, presence of prediabetes or type 2 diabetes, or evidence of metabolic dysregulation [1]. Detections of hepatic steatosis were used ultrasound as recommended in guide. The upper abdominal ultrasonographic examination was performed for children with obesity using an ultrasound (SC5-1U ultrasound scanner, Reason 7 ultrasound system, Mindray) by the same trained sonographer with the same device and scanner.

Anthropometric collections and Laboratory measurements

All anthropometric data were measured twice by a professional pediatrician. The height, body weight and waist circumference (WC), hip circumference (HC) were measured while the children wore underclothes without shoes. BMI and waist-to-hip ratio (WHR) were calculated as body weight (kg) / square of height (m^2) and waist circumference (cm) / hip circumference (cm), respectively. According to the BMI reference for Chinese children and adolescents, children with a BMI > 95% for their age and sex were diagnosed as obesity. The BMI SD score (BMI-SDS) was converted by standardizing BMI data to age- and sex-specific centiles [19]. Tanner criteria were used to determine pubertal developmental stages, and children were divided into two groups: prepubertal (Tanner's stage I) and pubertal (Tanner's stage II–V). Serum samples were obtained at morning following 12 h of overnight fasting. Triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C),

low-density lipoprotein cholesterol (LDL-C), fasting blood glucose (FBG), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were tested by the Hitachi 747 autoanalyzer. Insulin levels were detected by radioimmunoassay (BeiFang Systems, Beijing, China). The homoeostasis model of insulin resistance (HOMA-IR) was used to estimate insulin resistance.

ELISA

Serum FSTL1 concentrations were measured by the Human FSTL1 protein ELISA Kit (Westang, Shanghai, China), with the sensitivity less than 0.2 ng/mL. The inter-assay and intra-assay coefficients of variation (CV) values were less than 8% and 10%, respectively. Serum leptin concentrations were measured by the Human Leptin ELISA Kit (Excell, Shanghai, China), with the sensitivity less than 30 pg/mL. Serum adiponectin levels were measured by the Human Adiponectin ELISA Kit (Excell, Shanghai, China), with the sensitivity less than 100 pg/mL. The CV within and between plates of leptin and adiponectin ELISA kit were less than 10%.

Statistical analysis

The Shapiro Wilk test was used to test for normality of data. Continuous variables that were normally distributed were expressed as mean ± standard deviation (SD), whereas variables that did not follow the normal distribution were represented as median and a range between the first and third quartiles. Categorical variables were shown as count and percent. For more than two samples, the one-way ANOVA and the Kruskal–Wallis tests were used to determine differences. In addition, POST-HOC test (Games-Howell) was performed to compare data from subgroups. Correlations between FSTL1 and other variables were assessed by spearman correlations. Binary logistic regression was used to predict risk factors in children with obesity. All clinical statistical analysis and visualization were performed using SPSS 23.0 software and GraphPad Prism 8.0. *P* values (two-tailed) < 0.05 were regarded as statistically significant.

Results

The current study comprised 45 healthy children (26 boys and 19 girls), 31 obese children without MAFLD (17 boys and 14 girls), and 45 obese children with MAFLD (25 boys and 20 girls) within a range of ages between 6 and 14 years old. The clinical and laboratory parameters of all participants are shown in Table 1. According to the normality test, weight, weight SDS, BMI, BMI-SDS, waist circumference, waist/height, AST, HbA1c, and TSH were not distributed normally. No significant differences in age and gender among the three groups were observed. As expected, the obesity groups including obese children with MAFLD or without MAFLD had significantly higher BMI, BMI-SDS, WC, and WHR than those of the healthy controls ($P<0.05$). Metabolic parameters like triglycerides (TC) and total cholesterol (TG) were significantly higher among obese children than healthy controls, whereas low-density lipoprotein-cholesterol (LDL-C) was similar among the three groups. Alanine aminotransferase (ALT), a strong indicator of liver injury, was the highest in obese children with MAFLD, followed by obese children compared with healthy controls. There were significant differences between the MAFLD group

Table 1 Clinical and laboratory parameters of the study population

Variables	Healthy control (*n* = 45)	Obesity without MAFLD (*n* = 31)	Obesity with MAFLD (*n* = 45)	Test Statistics	*P*
Age (years)	10.95 ± 2.29	9.94 ± 2.13	9.59 ± 1.78	1.795 †	0.171
Gender, *n* (%)				10.887 *	0.144
Boy	26 (21.5)	17 (14.1)	25 (20.7)		
Girl	19 (15.7)	14 (11.6)	20 (16.5)		
Body mass index (kg/m^2)	16.21 ± 2.63	25.40 ± 3.54	26.70 ± 3.03	71.405 †	**< 0.05**
Body mass inde × SD score	0.01 ± 1.29	3. 95 ± 1.22	4.61 ± 1.33	9.213 †	**< 0.05**
Waist circumference (cm)	62.70 ± 5.46	85.41 ± 7.17	93.28 ± 9.77	65.804 †	**< 0.05**
Waist-to-hit ratio	0.92 (0.89–0.94)	0.95 (0.92-1.00)	0.98 (0.94-1.00)	20.573	**< 0.05**
Triglycerides (mmol/L)	0.85 ± 0.49	0.97 ± 0.29	1.51 ± 0.62	12.239 †	**< 0.05**
Total cholesterol (mmol/L)	3.72 ± 0.62	4.10 ± 0.52	4.24 ± 0.71	8.532 †	**< 0.05**
High-density lipoprotein-cholesterol (mmol/L)	1.34 ± 0.23	1.20 ± 0.22	1.16 ± 0.21	6.955 †	**< 0.05**
Low-density lipoprotein-cholesterol (mmol/L)	2.50 ± 0.50	2.50 ± 0.33	2.60 ± 0.52	0.443 †	0.643
Nonesterified free fatty acid (mmol/L)	0.68 ± 0.36	0.69 ± 0.41	0.73 ± 0.31	0.216 †	0.806
Alanine aminotransferase (IU/L)	15.89 ± 6.98	22.72 ± 6.45	53.80 ± 29.94	30.523 †	**< 0.05**
Aspartate aminotransferase (IU/L)	24.00 (21.75–26.25)	24.00 (21.00–27.00)	27.50 (23.00-35.75)	10.692 ‡	**< 0.05**
Leptin (ng/mL)	7.50 ± 3.83	13.20 ± 3.67	15.35 ± 2.75	35.938 †	**< 0.05**
Adiponectin (μg/mL)	23.57 ± 6.02	18.11 ± 6.12	19.67 ± 7.00	6.113 †	**< 0.05**
Leptin to Adiponectin (ng/μg)	0.35 ± 0.30	0.76 ± 0.41	0.89 ± 0.40	0.605 †	0.439

Significant *P* values are indicated in bold. * Pearson's chi-square statistic. † One-way ANOVA F Statistic; ‡ Kruskal-Wallis statistic

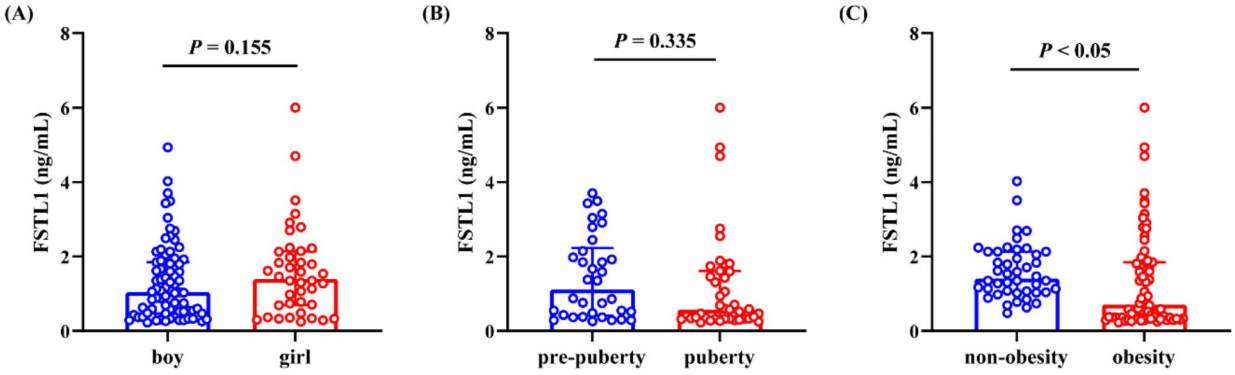

Fig. 1 FSTL1 levels between the different groups. (**a**) boy vs. girl; (**b**) pre-puberty vs. puberty; (**c**) non-obesity vs. obesity

Table 2 Comparison of serum FSTL1 levels among different subgroups using POST-HOC test

Group	Group	Mean diff	SE	*P*	95% CI Lower	Upper
control	obesity without MAFLD	0.862	0.272	**<0.05**	0.209	1.515
	obesity with MAFLD	0.283	0.318	0.649	-0.476	1.042
obesity without MAFLD	control	-0.862	0.272	**<0.05**	-1.515	-0.209
	obesity with MAFLD	-0.679	0.259	**<0.05**	-1.201	-0.043
obesity with MAFLD	control	-0.283	0.318	0.649	-1.042	0.476
	obesity without MAFLD	0.679	0.259	**<0.05**	0.043	1.201

SE, Standard Error; CI, Confidence Interval

and the non-MAFLD group in terms of Aspartate aminotransferase (AST). In addition, nonesterified fatty acid (NEFA), leptin, and leptin to adiponectin ratio were significantly higher whereas adiponectin concentrations were significantly lower in obese children than those in healthy controls.

FSTL1 concentrations between the different groups are presented in Fig. 1. There were no significant differences in FSTL1 concentrations between sex and puberty stages. Compared with healthy children, serum FSTL1 concentrations were significantly lower in the obesity group. To address the main point of this study, we divided all the subjects into three subgroups, healthy controls, obesity without MAFLD and obesity with MAFLD. Levene's test indicated that the homogeneity of variances for FSTL1 levels were not equal. Kruskal Wallis test showed that there was a statistically significant difference in levels of FSTL1 across three groups ($P<0.05$). In addition, the POST-HOC analysis (Games-Howell) data were reported in the Table 2. Serum FSTL1 concentrations were significantly higher in obese children with MAFLD [1.31 (0.35–2.29) ng/mL] than those in obese children without MAFLD [0.55 (0.36–1.38) ng/mL]. The levels of FSTL1 between healthy controls and obesity with MAFLD were significantly different ($P<0.05$). No significant difference in FSTL1 levels between healthy controls and obesity without MAFLD.

Serum FSTL1 concentrations were positively correlated with ALT ($r=0.248$, $P=0.022$), NEFA ($r=0.278$, $P=0.014$), leptin ($r=0.572$, $P<0.05$), and leptin to adiponectin ratio ($r=0.501$, $P<0.05$), as shown in Fig. 2. The serum levels of FSTL1 were negatively related to FBG and there was no significant correlation between FSTL1 and HOMA-IR. After adjustment for age, sex, and BMI-SDS, the serum FSTL1 concentrations were still remained significant correlations with NEFA ($r=0.363$, $P<0.05$) and leptin ($r=0.381$, $P<0.05$), as shown in Table 3. Binary logistic regression was used to predict the value of FSTL1 in MAFLD with obesity. The Hosmer-Lemeshow test was used to evaluated the goodness of fit. The test statistics was 7.484 ($P=0.485$), which indicated that the goodness of fit of the model was good. As shown in Fig. 3, increased FSTL1 was a risk factor for MAFLD in children with obesity (OR = 1.105, 95% CI: 1.066–1.269, $P<0.05$).

Discussion

An increasing body of data has revealed the role of FSTL1 as a novel biomarker in obesity and diabetes in adults and children. However, studies on the role of FSTL1 in children with MAFLD are lacking. The current data revealed higher FSTL1 concentrations in obese children with

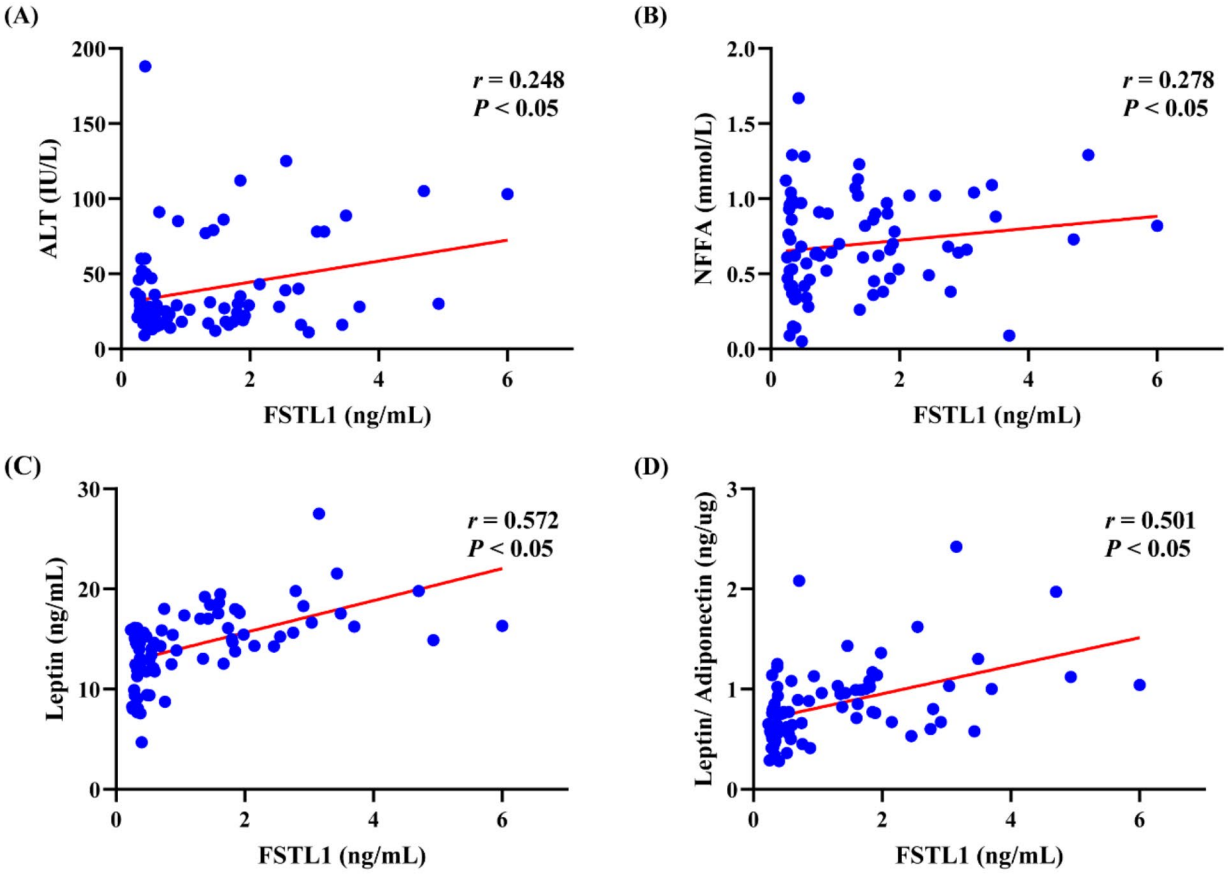

Fig. 2 Correlation analysis between serum FSTL1 levels and (**a**) ALT; (**b**) NEFA; (**c**) leptin; (**d**) leptin to adiponectin ratio

Table 3 The correlations analysis of variables associated with FSTL1 in the children with obesity

Variable	*r*	*P*	*r*[a]	*P*[a]
Waist-to-hit ratio	0.021	0.058	0.012	0.940
Triglycerides (mmol/L)	0.078	0.522	0.232	0.139
Total cholesterol (mmol/L)	0.130	0.286	0.024	0.879
High-density lipoprotein-cholesterol (mmol/L)	0.125	0.301	0.098	0.539
Low-density lipoprotein-cholesterol (mmol/L)	0.155	0.203	0.028	0.859
Alanine aminotransferase (IU/L)	0.248	**< 0.05**	0.249	0.112
Aspartate aminotransferase (IU/L)	−0.021	0.862	0.022	0.890
Nonesterified free fatty acid (mmol/L)	0.278	**< 0.05**	0.363	**< 0.05**
Leptin to adiponectin (ng/μg)	0.501	**< 0.05**	0.150	0.344
Leptin (ng/mL)	0.572	**< 0.05**	0.381	**< 0.05**
Adiponectin (μg/mL)	−0.157	0.186	−0.030	0.851

[a] Partial correlation after adjustment for age, sex, and BMI-SDS

MAFLD than those without MAFLD, which was consistent with results in adults [15]. BMI, BMI-SDS, and WHR were significantly higher in obese children with MAFLD than in obese children without MAFLD, indicating that children with higher body weight and abdominal obesity were more likely to develop MAFLD. As expected, higher ALT and AST concentrations were observed in children with MAFLD. This indicated that children in the obese group had a higher degree of hepatocyte damage than those in the control group. In conclusion, it is reasonable to assume that FSTL1 plays an important role in children MAFLD.

Previous study found that FSTL1 and glucose metabolism were closely related, but there were few studies on FSTL1 in lipid metabolism [20]. This work demonstrated a positive correlation between serum FSTL1 concentrations and NEFA, which was consistent with the experimental results conducted by Xu et al. [21]. Integrating the current findings with previous literature, two potential hypothesized mechanisms have been proposed to elucidate the association between altered serum FSTL1 levels and MAFLD in children with obesity. Firstly, FSTL1 may participate in the process of MAFLD by regulating NEFA metabolism. In the initial stage of obesity without MAFLD, there is a significant reduction in FSTL1 levels, leading to predominant accumulation of NEFA in adipose tissue [22]. As adipose tissue reaches its capacity for NEFA storage saturation, FSTL1 compensates by inhibiting further expansion of adipose tissue. NEFA that fails to be sequestered into lipid droplets is deposited in

Variable	OR (95%CI)	*P* value
FSLT1	1.105 (1.066-1.269)	< 0.05
ALT	1.247 (1.029-1.632)	< 0.05
AST	0.658 (0.948-1.366)	0.776
TG	1.006 (0.995-1.011)	0.113
TC	1.021 (1.005-1.022)	< 0.05
BMI-SDS	1.313 (0.503-2.107)	0.461
leptin	1.682 (0.806-2.733)	0.166
adiponectin	0.921 (0.751-1.093)	0.432

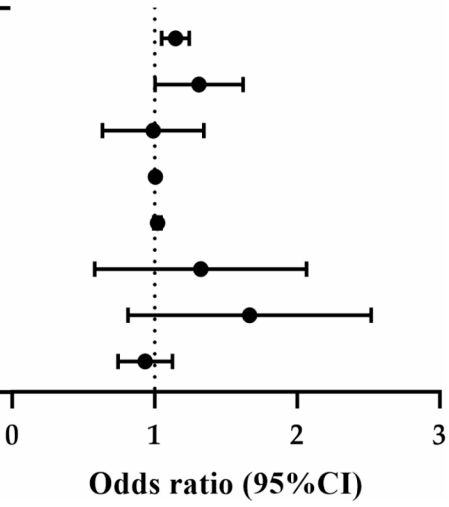

Fig. 3 Binary logistic regression using MAFLD as dependent variable

tissues such as the liver and muscle. Previous research showed that FSTL1 was a myokine that is secreted by skeletal muscle and exerted a protection effect through Akt-eNOS signaling [23]. Dittlfeld et al. [24] found a tissue-different expression of FSTL1 in T2DM patients, indicating different functions of FSTL1 in different locations. Therefore, we hypothesized that FSTL1 may restrict adipose tissue expansion by promoting NEFA influx into the liver, thereby initiating ectopic hepatocyte fat deposition which constitutes an early event in MAFLD pathogenesis. This could potentially explain why children who have obesity without MAFLD exhibit lower levels of FSTL1 compared to those with concurrent MAFLD. Furthermore, elevated NEFA levels can exacerbate hepatic steatosis, while lipotoxicity induces immune activation and chronic low-grade inflammation, thereby facilitating the progression of MAFLD to steatohepatitis [25]. FSTL1 is closely associated with inflammation and fibrosis. Suppression of *Fstl1* expression can attenuate the activation of hepatic stellate cells by downregulating the TGF-β1 signaling pathway [26]. In murine models, *Fstl1* binds to the TLR4 receptor, leading to fibroblast activation and hepatocellular carcinoma regeneration [17]. These findings suggest that FSTL1 exhibits further elevation during inflammatory progression. In addition, the current study found that serum FSTL1 concentrations were positively correlated with leptin and leptin to adiponectin ratio. In humans, leptin concentrations are higher in MAFLD patients than in controls and gradually increase with disease severity. Leptin can inhibit key enzymes of fatty acid synthesis and participate in the regulation of hepatic lipogenesis [27]. In obese children, circulating leptin is positively correlated with BMI and the severity of hepatic steatosis [28]. Increased leptin plays a role in hepatic fibrogenesis, and decreased adiponectin may contribute to hepatic insulin resistance [29]. Consistent with Xu et al. [21], the current data also showed an inverse correlation between FSTL1 and adiponectin, although not statistically significant. Therefore, it is reasonable to assume that FSTL1 concentrations pathologically increase when the disease progresses to fibrosis and overprotection of the FSTL1 can lead to increased liver fibrosis, eventually forming a vicious cycle. Once the liver healing process exceeds the body's compensatory capacity, it will cause a second blow to the liver, making MAFLD irreversibly progress [30]. In conclusion, FSTL1 plays a vital role in the pathogenesis of MALFD in obese children through lipid metabolism disorder, inflammatory response, and progression of fibrosis.

The study of FSTL1 in obese people is still controversial. The concentrations of FSTL1 were significantly reduced in obese adults, which was consistent with the current study that obese children had lower FSTL1 concentration [31]. Oelsner et al. [11]observed decreased FSTL1 concentrations and increased FSTL1 methylation in children's saliva. Contradicted results reported by Fan et al. [10] that an elevation of serum FSTL1 concentrations was detected in obese adults. However, their study was limited to adult males. Although the median of FSTL1 was significantly lower in obese children in the current study, there were individuals in the obese group who had higher concentrations. It is well known that obesity is often accompanied by chronic low-grade inflammation. In healthy Japanese populations, FSTL1 was defined as an anti-inflammatory protective factor that was increased during inflammation and acted as a counterregulatory factor [32]. Serum FSTL1 concentrations were significantly higher in patients with newly

diagnosed metabolic syndrome than in controls [33]. Yamazaki et al. and Widera et al. elucidated that plasma FSTL1 concentrations were higher in people with coronary artery disease [34, 35]. Accumulated evidence has shown that the concentrations of FSTL1 may act as a protective factor in the pathological process of obesity. These contradictory results can be explained by the role of FSTL1 in different stages of obesity. In the early stage of obesity, FSTL1 concentrations are decreased. This is consistent with FSTL1 concentrations being significantly reduced during preadipocyte differentiation and adipogenesis [36]. When obesity further progresses to inflammation and other complications, FSTL1 acts as a reactive factor and its concentrations increase on the contrary. Therefore, it is important to illustrate the role of FSTL1 in obesity complications. In addition, no differences in serum FSTL1 concentrations between males and females or between pubertal and prepubertal subjects were observed in the current study.

The current study has some limitations. First, this study is a case-control study and cannot reflect the causal and temporal relationship between FSTL1 and childhood MAFLD. Second, liver ultrasound is used for the diagnosis of MAFLD, which has a slightly lower diagnostic value than liver biopsy but is noninvasive. Third, sequencing of the biopsy tissue would be of great interest to further clarify the mechanism of FSTL1 in pediatric MAFLD if a liver biopsy is available.

In conclusion, the current study clarified that serum FSTL1 concentrations were elevated in obese children with MAFLD. The serum FSTL1 concentrations were significantly higher in obese children with MAFLD than those in obese children without MAFLD, which were associated with ALT, NEFA, and leptin. Additionally, elevated FSTL1 may be one of the risk factors for MAFLD in obese children. Further studies are needed to explore the underlying molecular mechanism of FSTL1 in children's obesity and MAFLD.

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Author contributions
Lujie Liu collected the clinical sample, carried out experiments, analyzed data and wrote the manuscript. Meng Li carried out experiments.Yujie Qin collected the clinical sample , Luyang Liu analyzed data and Yanfeng Xiao conceived and carried out the experiments.All authors reviewed the manuscript.

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Data availability
All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

Declarations

Ethics approval and consent to participate
This study was conducted in conformity with the Declaration of Helsinki and approved by the Ethical Committee of the Second Affiliated Hospital of Xi'an Jiaotong University (20222456). All participants and their legal guardians agreed to participate and provided written informed consent.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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