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Circulating THBS1: A Risk Factor for Nonalcoholic Fatty Liver Disease in Obese Children

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Keywords
Thrombospondin 1 · Childhood obesity · Nonalcoholic fatty liver disease · Metabolic syndrome · Metformin

Abstract
Introduction: Thrombospondin 1 (THBS1) is a highly expressed adipokine in adults with obesity. In the present study, we aimed to investigate the clinical significance of THBS1in children with obesity and nonalcoholic fatty liver disease (NAFLD) and determine the effect of metformin on THBS1 expression in dietary-induced obese (DIO) mice. ***Methods:*** A cross-sectional study was conducted among 78 obese children and 35 nonobese children. Anthropometric parameters, clinical data, and circulating THBS1 levels were measured. The expression of THBS1 was detected in the serum and liver tissue from diet-induced obese mice (C57BL/6) with or without metformin treatment. ***Results:*** Higher THBS1 levels were observed in children with NAFLD and higher SDS-BMI. Individuals in the higher THBS1 quartile had a higher prevalence of hypo-high-density lipoprotein cholesterol (HDL-C). Logistic regression analysis showed a significant correlation between THBS1 and NAFLD, as well as between hip circumference and leptin levels. Receiver-operating characteristic (ROC) analysis revealed that THBS1 was a more sensitive predictor of NAFLD than leptin. Additionally, metformin ameliorated hepatic steatosis and decreased hepatic THBS1 expression in high-fat diet (HFD)-fed mice. ***Conclusions:*** Circulating THBS1 level may be a risk factor for NAFLD in obese children. Our findings provided a novel approach of metformin administration for the prevention and treatment of NAFLD. This study also confirmed that metformin decreased the expression of hepatic THBS in DIO mice.

Introduction

Childhood obesity has reached an epidemic level worldwide, posing a serious threat to public health [1, 2]. Childhood obesity is a major risk factor for metabolic-associated fatty liver disease (MAFLD), previously called nonalcoholic fatty liver disease (NAFLD) [3]. Herein, NAFLD will be referred to MAFLD. Currently, MAFLD is the most common chronic hepatopathy in children and adults worldwide [4] and is a multispectrum disease that

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ranges from simple fatty liver to nonalcoholic steatohepatitis (NASH) and fibrosis. The overall global prevalence of fatty liver disease in children with obesity is significantly higher than that in children with a normal weight [3, 4]. Furthermore, 45% of adolescents with obesity are estimated to have fatty liver disease in China [5].

Thrombospondin 1 (THBS1), also known as TSP-1, was initially identified in megakaryocytes and platelets [6]. However, recently, THBS1 was shown to be highly expressed in many tissues, including adipose and fibrotic tissue [7]. Thrombospondin 1 belongs to a family of glycoproteins that regulate transforming growth factor-β activity and display diverse biological activities, such as activation of angiogenesis, inflammation, cellular adhesion, migration, and growth. Varma et al. [8] have reported that THBS1 is a highly expressed adipokine in obese individuals and is correlated with adipose inflammation. Thrombospondin 1-null mice were protected from high-fat diet (HFD)-induced muscle fibrosis and insulin (INS) resistance [9]. Choline-deficient L-amino acid-defined HFD-fed THBS1-deficient mice exhibited a decrease in serum lipid levels and hepatic fibrosis compared with wild-type mice fed with choline-deficient L-amino acid-defined HFD [10].

Recently, increasing studies have shown that metformin is effective in treating obesity, NAFLD, metabolic syndrome (MetS), and even cancer [11, 12]. Metformin is a well-known oral antidiabetic drug that inhibits hepatic glucose output, enhancing lipolysis [13]. Interestingly, metformin is also known to increase THBS-1 synthesis in the serum of women with polycystic ovary syndrome (PCOS) and in certain cell types [14]. However, some studies have shown that metformin treatment results in a decrease in THBS1 expression in cultured endothelial precursor cells [15]. Limited data are available on the role of metformin in the expression of hepatic THBS1 in NAFLD.

To date, very few studies have investigated the relationship between obesity or NAFLD and serum THBS1 levels in obese children. To the best of our knowledge, THBS1 has not been studied in children with obesity and NAFLD compared to those without NAFLD. Therefore, the current study aimed to investigate the circulating THBS1 level in children with obesity and examine the association between serum THBS1 levels and NAFLD or metabolic syndrome's components in obese children. More importantly, we also investigated the possibility of using circulating THBS1 levels for the diagnosis of childhood NAFLD. In addition, we assessed the THBS1 expression in the serum and hepatic tissues of mice fed an HFD. We also utilized an obesity-associated fatty liver disease mouse model to assess the effects of metformin and to further characterize the THBS1 expression in response to metformin.

Methods

Human Subjects

A total of 78 obese Chinese children were consecutively enrolled at the Clinic of the Pediatric Department of the Second Affiliated Hospital of Xi'an Jiaotong University, and 35 children with a BMI below the 85th percentile were recruited as controls based on the BMI reference values for Chinese children [16]. The subjects were not taking any medications and were without acute, chronic infectious diseases, autoimmune diseases, endocrine disorders, or genetic obesity syndromes. All participants and their parents agreed to participate in the study and provided signed informed consent.

Diagnosis of NAFLD/NASH

An SC5-1U ultrasound scanner (Reason 7 ultrasound system, Mindray) was used for hepatic steatosis screening in obese children. Three ultrasonographic grades were used to diagnose fatty liver (none, mild, and severe), based on the degree of echogenicity of the liver and kidney parenchyma. Obese children were defined as having NASH as they had elevated alanine aminotransferase (ALAT) (>5 times the upper limit of normal) and were diagnosed with fatty liver using abdominal ultrasound. All ultrasound examinations for obese children were performed by the same person with the appropriate qualifications and experience with the same device and scanner.

Anthropometric Data Collection

The height, weight, waist circumference (WC), hip circumference (HC), systolic blood pressure (SBP), and diastolic blood pressure (DBP) were measured using an oscilloscopic sphygmomanometer and SDS-BMI and waist-to-hip ratio (WHR) were calculated for each child. According to the Tanner criteria, all participants were divided into the prepubertal and pubertal groups. Hypertension was defined as the SBP or DBP greater than the 95th percentile based on the BP reference standards for Chinese children [17].

Laboratory Assay Methods

Blood samples were drawn and collected from the antecubital vein at 8:00 a.m. in the next morning after 12 h fasting. Serum was separated by centrifugation and aliquoted into small vial. Circulating levels of fasting plasma glucose, total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein (LDL) cholesterol, ALAT, and aspartate aminotransferase (ASAT) were determined using an autoanalyzer (Hitachi 747; Hitachi, Tokyo, Japan) as described previously [18, 19]. Fasting INS levels were measured using radioimmunoassay instrument (BeiFang Systems, Beijing, China). Nonesterified fatty acids were using a commercial enzymatic kit (Applygen Technologies Inc., Beijing, China). The human leptin, adiponectin, oxidized LDL (ox-LDL), intercellular adhesion molecule 1 (ICAM-1), endothelial cell-specific molecule 1 (ESM1), Wnt family member 5A (Wnt5a), and THBS1 concentrations in serum were measured

using an enzyme-linked immunosorbent assay kit (Excell, Shanghai, China), all with interassay and intraassay coefficients of variation less than 10%.

Animals

Three-week-old male C57BL/6 mice were obtained from the Animal Center of the Xi'an Jiaotong University. The study was conducted according to the ARRIVE guidelines. Mice were fed with an HFD (n = 11) with 47.5% of total calories from fat or a normal chow diet (NCD, n = 8) as described previously [20]. Mice fed with HFD after 12 weeks were treated with metformin (n = 5) (150 mg/kg/day; intraperitoneal; Bristol-Myers Squibb Company) or 0.9% saline for 4 weeks (n = 6). Metformin was dissolved in 0.9% saline and administered daily via intraperitoneal injection at 9:00 a.m. All mice were housed in a specific pathogen-free facility with controlled room temperature and humidity (22°C; 60% relative humidity). All mice were maintained on a 12:12-h light-dark cycle and food and water were provided ad libitum. These experiments were approved by the Committee of Animal Research at Xi'an Jiaotong University and the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (No. 2021-962). Following the feeding or treatment regimen, the mice were sacrificed and blood and tissue samples after 8 h of fasting (at 8:00 a.m.) were collected. Liver tissues were isolated and weighted at 15 or 19 weeks of age. Three to six male mice were used in each group, and the experiments were performed twice. TC, TG, ALAT, and ASAT were determined as described in a previous study [19].

H&E Staining and Immunohistochemistry

Paraffin-embedded hepatic tissue specimens were cut into 5-μm-thick sections. The accumulation of lipids in the liver tissues was detected using H&E staining. Surplus sections were dewaxed and dehydrated with dimethylbenzene and ethanol at different concentrations for immunohistochemical analysis. The THBS1 staining was performed according to the protocol as described previously [20]. An antimouse THBS1 antibody (Elabscience Biotechnology) was used for immunohistochemistry. A 3DHISTECH Pannoramic Viewer (Hungary) was used to scan and capture H&E and immunohistochemical stained images. Image-Pro Plus 6.0 software (Media Cybernetics) was used to analyze and quantify the adipocyte size and percentage of the positive area in relation to the total area.

Total Protein Extraction and Western Blot Analysis

Total protein was isolated from approximately 50 mg samples of epididymal white adipose and liver tissue using TRIzol (Ambion) reagent, according to the manufacturer's protocols. The total extracted protein was then measured using the BCA Protein Assay Kit (Thermo Fisher) and western blotting was performed according to the manufacturer's protocol, as previously described [19]. Further, 50 μg of denatured protein was separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis using running buffer. The gel was subsequently blotted onto polyvinylidene difluoride membranes. Primary antibodies against THBS1 (Cat#37879) were purchased from Cell Signaling Technology. Antitubulin (Cat# 10094-1-AP) was purchased from Proteintech. All primary antibodies were incubated overnight in phosphate-buffered saline containing 0.1% Tween20 with 6% fat-free milk. After overnight incubation, the membrane was washed three times using TBS supplemented with 0.1% Tween20. The washed membrane was then incubated at room temperature for 2 h with horseradish peroxidase-conjugated secondary antibodies and washed as described above. The proteins were visualized on a polyvinylidene difluoride film using horseradish peroxidase-conjugated secondary antibodies and Immobilon Western Chemiluminescent HRP Substrate (Millipore). The visualization and analysis were further done using an imaging system (Bio-Rad).

Total RNA Extraction and Real-Time RT-PCR

Total RNA was purified using TRIzol reagent, as previously described. Complementary DNA was synthesized using a high-capacity reverse transcription kit (Takara) after removing genomic DNA using DNase I (Invitrogen), according to the manufacturer's protocol. Further, qRT-PCR for mRNA was conducted by using a SYBR Premix Ex Taq kit (Takara) as previously reported [19]. The mRNA expression levels were normalized to the reference gene tubulin and fold changes were calculated using $2^{-\Delta\Delta Ct}$ method. The primer sequences are shown in online supplementary Table S1 (see www.karger.com/doi/10.1159/000527780 for all online suppl. material).

Statistical Analysis

Statistical power analysis was conducted to determine the minimum sample size for this study. The normality of the data was detected using the Shapiro-Wilk test. Log transformations or normal score transformations were applied to the skewed data before parametric analysis. Data are presented as the mean ± SD or median and interquartile range. Comparisons between obese and nonobese children or between obese children with and without NAFLD were performed by the unpaired t test or χ^2 test or independent samples were analyzed using the Mann-Whitney U test as appropriate. Pearson's or Spearman correlation coefficient tests were performed to describe the linear association between metabolic variables and adipokines, and partial correlations to adjust age. Multiple logistic regressions were performed to determine risk factors of NAFLD. Statistical analyses were performed using the SPSS 22.0 software (SPSS Inc.) and GraphPad Prism version 8. The significance was set at $p < 0.05$.

Results

Clinical Characteristics

A total of 113 children with obesity (19 female and 59 male) and without obesity (12 female and 23 male) were enrolled in this study. A statistical power analysis showed that the minimum sample size for this study was 32 (16 children with obesity, 16 children without obesity), as shown in online supplementary Table S2. The number of samples in the present study was more than 32, which means that the number of participants in our study reached statistical significance. The clinical and biochemical characteristics of the patients are presented in Table 1. There were no differences in the sex distribution or age or pubertal stage between obese and nonobese chil-

Table 1. The clinical characteristics and metabolic parameters of the study subjects

	Variable	Nonobesity	Obesity	*p* value
Anthropometric parameters	Sex (M/F)	23/12	59/19	0.362
	Prepubertal/pubertal	23/12	52/26	1.000
	Age	9.84±1.99	10.18±2.33	0.465
	BMI, kg/m^2	16.22 (14.94, 18.07)	26.90 (23.21, 29.64)	<0.0001
	SDS-BMI	−0.28 (−0.85, 0.56)	3.94 (3.25, 4.85)	<0.0001
	WC, cm	68.91±8.17	89.68±11.69	<0.0001
	HC, cm	75.11±8.15	92.03±11.12	<0.0001
	WHR	0.92 (0.89, 0.94)	0.98 (0.94,1.00)	<0.0001
	SBP, mm Hg	101.09 + 6.62	116.15 + 16.58	<0.0001
	DBP, mm Hg	67.00 (63.00, 70.00)	70.50 (65.00,76.25)	0.020
Metabolic parameters	FPG, mmol/L	4.90±0.66	4.79±0.49	0.377
	INS, mIU/L	6.21 (5.18, 7.47)	20.73 (17.75, 23.07)	<0.0001
	HOMA-IR	1.39 (1.07, 1.61)	4.34 (3.64, 4.92)	<0.0001
	ALAT, IU/L	15.00 (12.00, 19.00)	27.00 (19.50, 46.50)	<0.0001
	ASAT, IU/L	23.00 (20.00, 25.00)	26.00 (22.00, 30.50)	0.012
	TC, mmol/L	3.72±0.68	4.19±0.60	0.001
	TG, mmol/L	0.65 (0.54, 1.02)	1.23 (0.86, 1.58)	0.005
	HDL-C, mmol/L	1.36±0.22	1.15±0.24	<0.0001
	LDL-C, mmol/L	2.42±0.55	2.56±0.43	0.156
	NEFA, mmol/L	0.63±0.31	0.70±0.32	0.283
	TC/HDL-C	2.76±0.44	3.76±0.94	<0.0001
Adipocytokines	Leptin, ng/mL	7.54±5.47	14.55±3.69	<0.0001
	Adiponectin, μg/mL	23.10±6.15	19.46±6.81	0.008
	LAR, ng/μg	0.26 (0.13, 0.50)	0.77 (0.58, 1.03)	<0.0001
	Ox-LDL, pg/mL	566.72±101.25	620.38±107.19	0.014
	THBS1, ng/mL	19.42 (16.18, 23.62)	35.57 (27.31, 47.77)	<0.0001
	ICAM-1, pg/mL	336.44±70.83	409.78±98.81	<0.0001
	ESM1, ng/mL	0.14 (0.12, 0.18)	0.21 (0.17, 0.27)	<0.0001
	Wnt5a, ng/mL	1.13 (0.80, 1.35)	1.62 (1.12, 2.57)	0.001

Data are presented as mean±SD or median (25th percentile, 75th percentile). BMI, body mass index; SDS-BMI, BMI SD score; WC, waist circumference; HC, hip circumference; WHR, waist-to-hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; INS, fasting insulin; HOMA-IR, homeostasis model assessment of insulin resistance; TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; TC/HDL-C, TC-to-HDL-C ratio; LDL-C, low-density lipoprotein cholesterol; ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; NEFAs, nonesterified free fatty acids; LAR, leptin-to-adiponectin; Ox-LDL, oxidized low-density lipoprotein; THBS1, thrombospondin 1; ICAM-1, intercellular cell adhesion molecule-1; ESM1, endothelial cell-specific molecule-1; Wnt5a, Wnt family member 5A.

dren. As expected, BMI, SDS-BMI, WC, HC, and WHR were significantly higher in obese children. Children with obesity showed significant metabolic disorders with respect to systolic and diastolic blood pressure. Metabolic parameters including INS, HOMA-IR, ALAT, ASAT, TC, TG, HDL-C, and TC/HDL-C were significantly higher among children with obesity than among those without obesity. In terms of glucose and nonesterified fatty acid levels, no statistically significant difference was observed between the two groups. Leptin and adiponectin levels in obese children were higher and lower, respectively. A higher leptin to adiponectin ratio (LAR) was observed in children with obesity than in those without obesity. Compared to nonobese children, a significant increase in circulating ox-LDL, ICAM-1, ESM1, and Wnt5a levels was observed in obese children.

Circulating THBS1 Levels in Subgroups

Circulating THBS1 levels were significantly higher in children with obesity than in those without obesity. As observed in the total population, there were no significant differences in circulating THBS1 levels among children

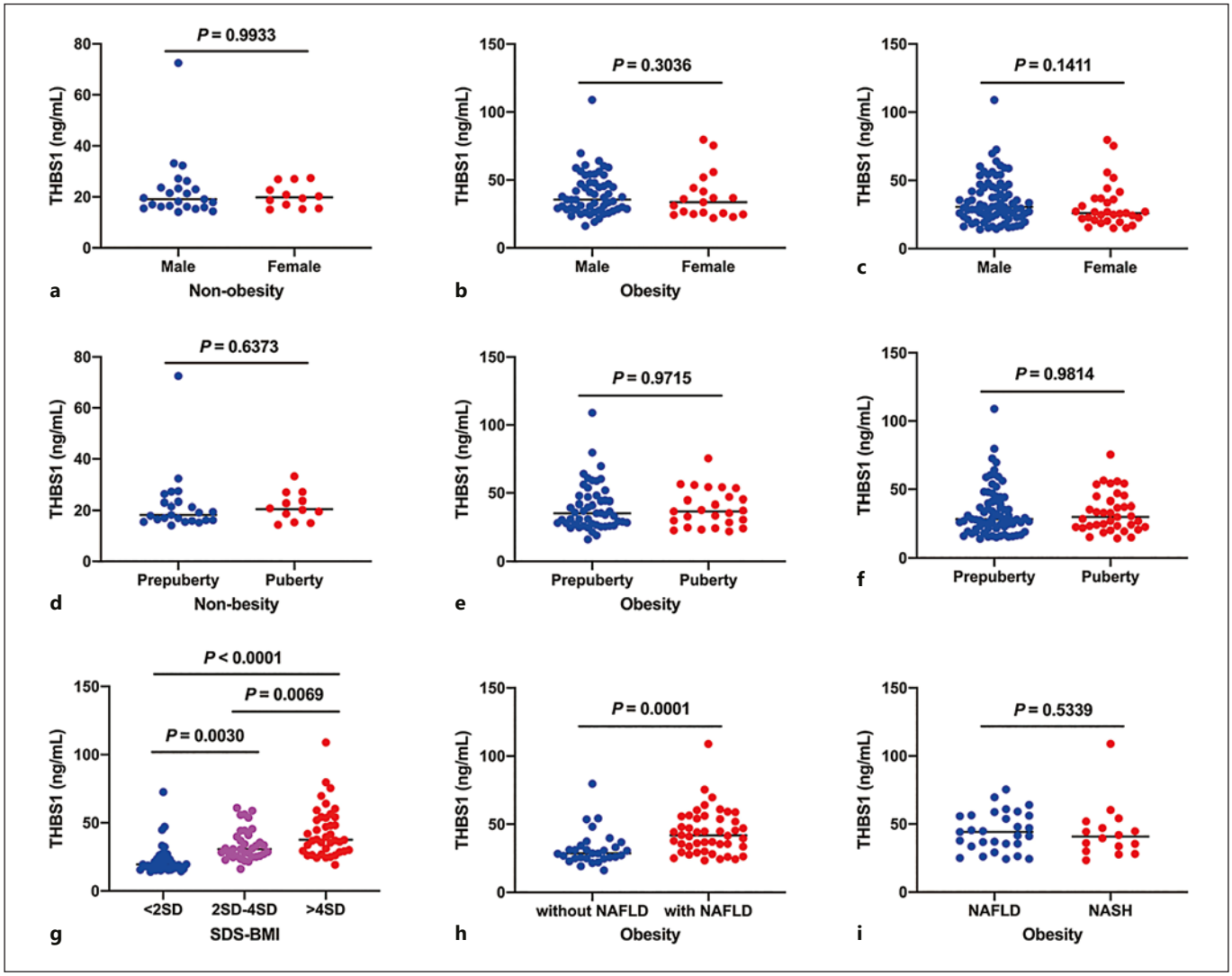


Fig. 1. Circulating THBS1 levels in subgroups. **a–c** Different gender subgroups in nonobesity groups, obesity groups, and all participants separately. **d–f** Different Tanner stage subgroups in nonobesity groups, obesity groups, and all participants separately. **g** Subgroups based on the SDS-BMI. **h** Among obesity group, with and without NAFLD diagnosed by using abdominal ultrasound. **i** Among NAFLD groups, with and without liver injury diagnosed by serum ALT levels. Mann-Whitney test was used for comparison between two groups.

based on sex or Tanner stages, or body weight. The children were divided into three groups based on their SDS-BMI. Higher circulating THBS1 levels were found in the children with higher SDS-BMI (Fig. 1a–g).

Children with obesity were divided into NAFLD and non-NAFLD groups based on the liver ultrasound results. Children with NAFLD were further divided into the NAFLD and NASH groups based on their serum ALAT levels. Higher circulating THBS1 levels were observed in children with NAFLD than those without NAFLD. However, there were no significant differences in circulating THBS1 levels between NAFLD and NASH subgroups (Fig. 1h, i).

Correlations of Circulating THBS1 Levels and Clinical Variables

Given that higher levels of circulating THBS1 are associated with a higher SDS-BMI, we determined the significance of circulating THBS1 as a biomarker of childhood obesity and MetS. To determine whether there was a significant correlation between the circulating THBS1 levels and clinical variables, we assessed the associations

Table 2. Correlations of serum log_{10}THBS1 with clinical variables

Variable	*r*	*p* value	*r**	*p* value*
Age	0.062	0.516		
SDS-BMI	0.549	0.000	0.507	0.000
WC	0.574	0.000	0.621	0.000
HC	0.505	0.000	0.510	0.000
WHR	0.365	0.000	0.405	0.002
SBP	0.462	0.000	0.379	0.004
DBP	0.187	0.100	0.073	0.592
ALAT	0.417	0.000	0.399	0.002
ASAT	0.182	0.062	0.116	0.391
TC	0.250	0.010	0.336	0.011
LnTG	0.264	0.006	0.241	0.070
HDL -C	−0.116	0.236	0.025	0.855
TC/HDL-C	0.272	0.005	0.235	0.078
Ox-LDL	0.310	0.001	0.207	0.122
Insulin	0.407	0.000	0.453	0.000
HOMA-IR	0.320	0.002	0.439	0.001
Leptin	0.432	0.000	0.369	0.005
Adiponectin	−0.172	0.073	−0.218	0.103
LAR	0.382	0.000	0.342	0.009
Wnt5a	0.158	0.099	0.165	0.086
ICAM-1	0.303	0.001	0.327	0.013
ESM1	0.175	0.067	0.087	0.521

THBS1 was Log-transformed into normal distribution. TG was Ln-transformed into normal distribution. ALAT, ASAT, insulin, HOMA-IR, LAR, Wnt5a, and ESM1 were normal scores transformed into normal distribution. Correlation coefficients between SDS-BMI were estimated by Spearman correlations; all other data were estimated using Pearson's correlations. SDS-BMI, BMI SD score; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; HOMA-IR, homeostasis model assessment of insulin resistance; TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ox-LDL, oxidized low-density lipoprotein; ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; LAR, leptin-to-adiponectin; THBS1, thrombospondin 1; ICAM-1, intercellular cell adhesion molecule-1; ESM1, endothelial cell-specific molecule-1; Wnt5a, Wnt family member 5A. *Partial correlations after adjustment for age.

of THBS1 with other clinical variables using Pearson's correlation or Spearman correlation and partial correlation (Table 2). As expected, THBS1 was positively correlated with SDS-BMI, WC, HC, and WHR, independent of age. In terms of blood pressure, THBS1 was positively correlated with SBP but not DBP, which remained significant after adjustment for age. For glucose metabolism variables, we found that THBS1 was positively correlated with INS and HOMA-IR, independent of age. The correlations between THBS1 and ALAT, as well as between THBS1 and TC remained significant after the adjustment for age. Interestingly, we also observed that THBS1 was positively correlated with TG, TC/HDL-C, ox-LDL, leptin, LAR, and ICAM-1; however, only leptin, LAR, and ICAM-1 were significantly associated with THBS1 after adjusting for age. However, no significant correlation was found between THBS1 and ASAT, HDL-C, adiponectin, Wnt5a, or ESM1.

Prevalence of Metabolic Syndrome and Its Components by Quartile of Circulating THBS1 Levels

MetS was defined according to the Chinese pediatric MetS [21] as follows. Individuals with MetS were classified as having central obesity (WC >90th percentile for age and sex) and more than two of diagnostic components of MetS [22]. In this study, we found that individuals with a higher THBS1 quartile had a higher prevalence of hypo-HDL-C as shown in Table 3. There were no significantly differences in hypertriglyceridemia, hypertension, hyperglycemia, and MetS among the quartiles of serum THBS1 levels.

Binary Logistic Regression Analysis

Using NAFLD as dependent variable, and, age, sex, SDS-BMI, WC, HC, THBS1, ox-LDL, ICAM-1, Wnt5a, ESM1, leptin, and INS as independent variables, we performed binary logistic regression analysis (forward: LR) to screen for the potential associations between NAFLD and the variables. In this analysis, a significant correlation between the circulating THBS1 and NAFLD was identified, as well as between HC, leptin, and NAFLD (Table 4).

Receiver-Operating Characteristic Curves for the Identification of Subjects with NAFLD

The receiver-operating characteristic (ROC) method was used to predict the threshold value for the diagnosis of NAFLD by using THBS1 and leptin. The area under the ROC curve was 0.772 for the THBS1 level ($p < 0.001$). The sensitivity and specificity values for THBS1 were 76.1% and 75.9%, respectively (cutoff values = 33.34). The area under the curve was 0.719 ($p = 0.002$), and the cutoff value was 15.57 for leptin with a sensitivity of 54.3% and specificity of 82.8% (Fig. 2).

Expression of THBS1 Gene of Obese Mice

To test the expression of THBS1 in the serum and liver of DIO-related NAFLD mice, they were fed the NCD or HFD at 4 weeks old after adjusting feeding for 1 week. As previously reported, an increase in serum TG, ALAT, and ASAT levels in mice fed with HFD was observed as compared with mice fed with NCD, as well as in body

Table 3. Ratio of MetS and components of MetS in quartiles of serum THBS1 levels in obese children

	THBS1				*p* value
	Q1 (16.178–27.201 ng/mL), %	Q2 (27.653–35.564 ng/mL), %	Q3 (35.584–47.074 ng/mL), %	Q4 (47.999–108.904 ng/mL), %	
Hypo-HDL-C	2.6	5.1	9	14.1	0.024
Hypertriglyceridemia	3.8	5.1	7.6	13.9	0.082
Hypertension	7.7	9.0	5.1	10.3	0.657
Hyperglycemia	2.6	5.1	5.1	1.3	0.358
MetS	9	11.5	3.8	7.7	0.198

Pearson χ^2 test with Bonferroni correction. MetS, metabolic syndrome.

Table 4. Binary logistic regression (forward: LR) analysis using NAFLD as dependent variable

	B	SE	*p* value	Exp (B)
Constant	−29.052	12.571	0.021	0.000
Sex	−1.353	1.553	0.383	0.258
Age	−0.492	0.723	0.496	0.611
SDS-BMI	0.582	0.804	0.469	1.789
WC	−0.306	0.175	0.080	0.737
HC	0.541	0.275	0.049*	1.718
THBS1	0.243	0.112	0.031*	1.275
Ox-LDL	−0.007	0.006	0.258	0.993
ICAM-1	0.002	0.005	0.737	1.002
Wnt5a	−0.005	0.012	0.668	0.995
ESM1	2.433	3.099	0.432	11.395
Leptin	0.626	0.288	0.030*	1.871
Insulin	−0.067	0.149	0.653	0.935

BMI, body mass index; SDS-BMI, BMI SD score; WC, waist circumference; HC, hip circumference; ox-LDL, oxidized low-density lipoprotein; THBS1, thrombospondin 1; Wnt5a, Wnt family member 5A; ICAM-1, intercellular cell adhesion molecule-1; ESM1, endothelial cell-specific molecule-1. * $p < 0.05$.

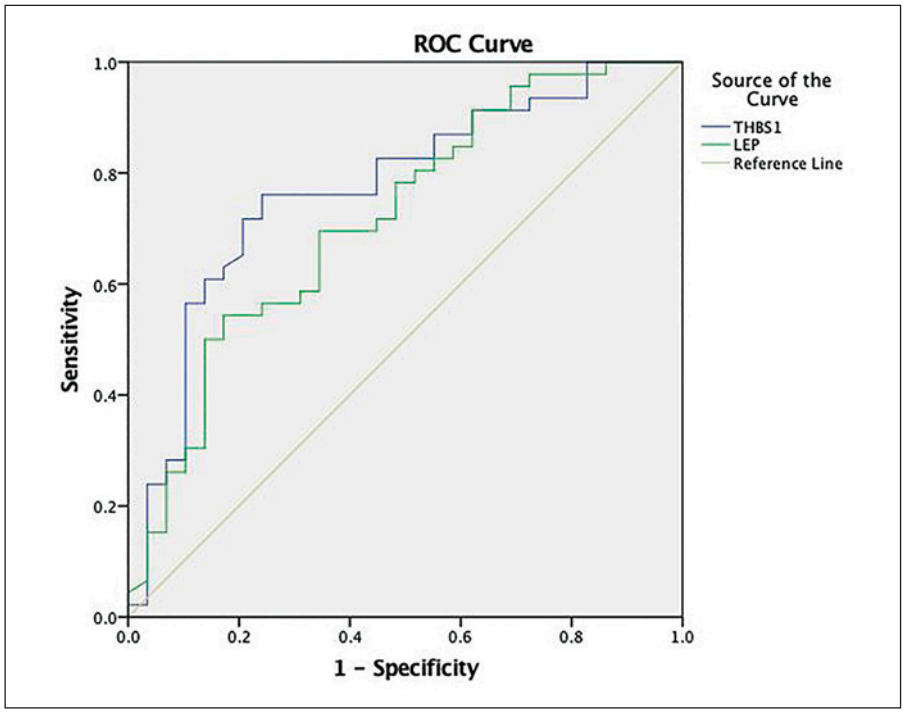
weight and organ weights (Fig. 3a–e). Mice fed an HFD had significantly increased serum THBS1 level compared with mice fed a regular chow diet (Fig. 3f).

Subsequently, we performed histological analysis of hepatic steatosis and THBS1 expression using H&E staining and immunohistochemistry. Mice fed with HFD exhibited hepatocellular lipid accumulation in the surrounding the perisinusoidal areas as compared with the mice fed with NCD. The NAFLD activity score [23] for H&E-stained images of livers from mice is shown in online supplementary Table S3. Positive THBS1 staining was observed in the liver of HFD-fed mice (Fig. 4a). Quantification of THBS1 in the positive areas revealed that the HFD cohort had a significantly upregulated hepatic THBS1 expression (Fig. 4b). Additionally, the protein and mRNA levels of THBS1 in the liver of HFD-fed mice fed were significantly upregulated compared to those in the NCD group (Fig. 4c, d). Metformin ameliorates hepatic steatosis and decreases hepatic THBS1 expression in DIO mice.

The treatment of obese mice with metformin has been shown to improve hypertrophic adipocytes and hepatic steatosis [12, 20]. We examined the effects of metformin on THBS1 expression in HFD-fed mice. Five obese mice were treated with metformin for 4 weeks. Metformin treatment inhibited the lipid accumulation in the liver and ameliorated hepatic steatosis (Fig. 4a). In addition, metformin decreased the increase in serum TG, ALAT, and ASAT levels in HFD-fed but did not affect the serum THBS1 levels (Fig. 3d–f). Interestingly, we also found that metformin significantly downregulated the expression of hepatic THBS1 mRNA and protein levels of DIO mice (Fig. 4b–d).

Discussion

An increasing body of data has revealed the role of THBS1 as a novel biomarker in obesity, prediabetes, and MetS in adults [8, 24]. However, the role of THBS1 in obesity and MetS remains controversial. Some studies reported that THBS1 can improve hepatic steatosis in diet-induced INS -resistant mice [25]. Others showed a positively association between serum THBS1 levels and BMI in women, as well as between serum THBS1 levels and fasting plasma glucose [24]. Recently, Gwag et al. [26] reported that THBS1 promotes obesity-associated NAFLD. However, studies on the role of THBS1 in children with obesity and MetS are lacking.



Color version available online

Fig. 2. The area under the ROC curve for NAFLD in children with obesity.

Higher Circulating THBS1 Levels in Children with Obesity and NAFLD

Matsuo et al. [24] did not observe any difference in the THBS1 levels in adult male subjects with obese and non-obese phenotypes, but they reported higher circulating THBS1 levels in female subjects with obesity than in lean individuals. Tahergorabi et al. [27] reported a significantly lower serum THBS1 level in obese PCOS patients than in the control group. In the present study, we observed higher THBS1 levels in obese children, especially in those with NAFLD. Additionally, higher THBS1 levels were observed in children with greater SDS-BMI. Consistent with our founding, Buras et al. [28] reported that THBS1 levels were markedly increased in obese mice fed an HFD. In addition, it has been reported that THBS1 shows significant genetic and sex interactions [29]. However, in our cohort, we did not observe significantly differences in circulating THBS1 levels between males and females, or between prepuberty and puberty in individuals.

Interestingly, our study revealed a higher THBS1 level in obese children with NAFLD than those without NAFLD, which is consistent with Bai's observations [25]. Bai et al. [25] also showed a significant reduction in serum THBS1 levels as well as an improvement in liver steatosis after lifestyle intervention. They also reported that treatment with recombinant human THBS1 attenuated hepatic steatosis in DIO mice. Recently, Gwag et al. [26] showed that macrophage-specific THBS1 deletion in adipocytes protected mice against obesity-associated liver injury. We defined children with elevated ALAT and diagnosed fatty liver by using abdominal ultrasound as the NASH group [30]. No significant differences were found between children with and without liver injury. The inconsistency in the clinical characteristics of subjects, species, and the methodological differences in biomedical experiments may result in discrepancies in the results. In addition, we confirmed that several other adipokines, including ox-LDL, ICAM-1, ESM1, and Wnt5a, which have been reported to be increased in subjects with obesity or NAFLD, were also elevated in children with obesity. Meanwhile, we were able to replicate the findings with regard to the increasing serum leptin and decreasing serum adiponectin levels in children with obesity. However, data on the association between serum THBS1 and other adipokines are scarce.

In this study, the correlations between THBS1 and ALAT, as well as between THBS1 and TC, remained significant after adjusting for age. THBS1 was also positively correlated with leptin, LAR, and ICAM-1 levels after adjusting for age. No significant association between THBS1 and adiponectin levels was observed. Leptin is thought to participate in the development of NAFLD. Previous data indicated the need for leptin or leptin receptor-mediated signaling to ensure adequate liver func-

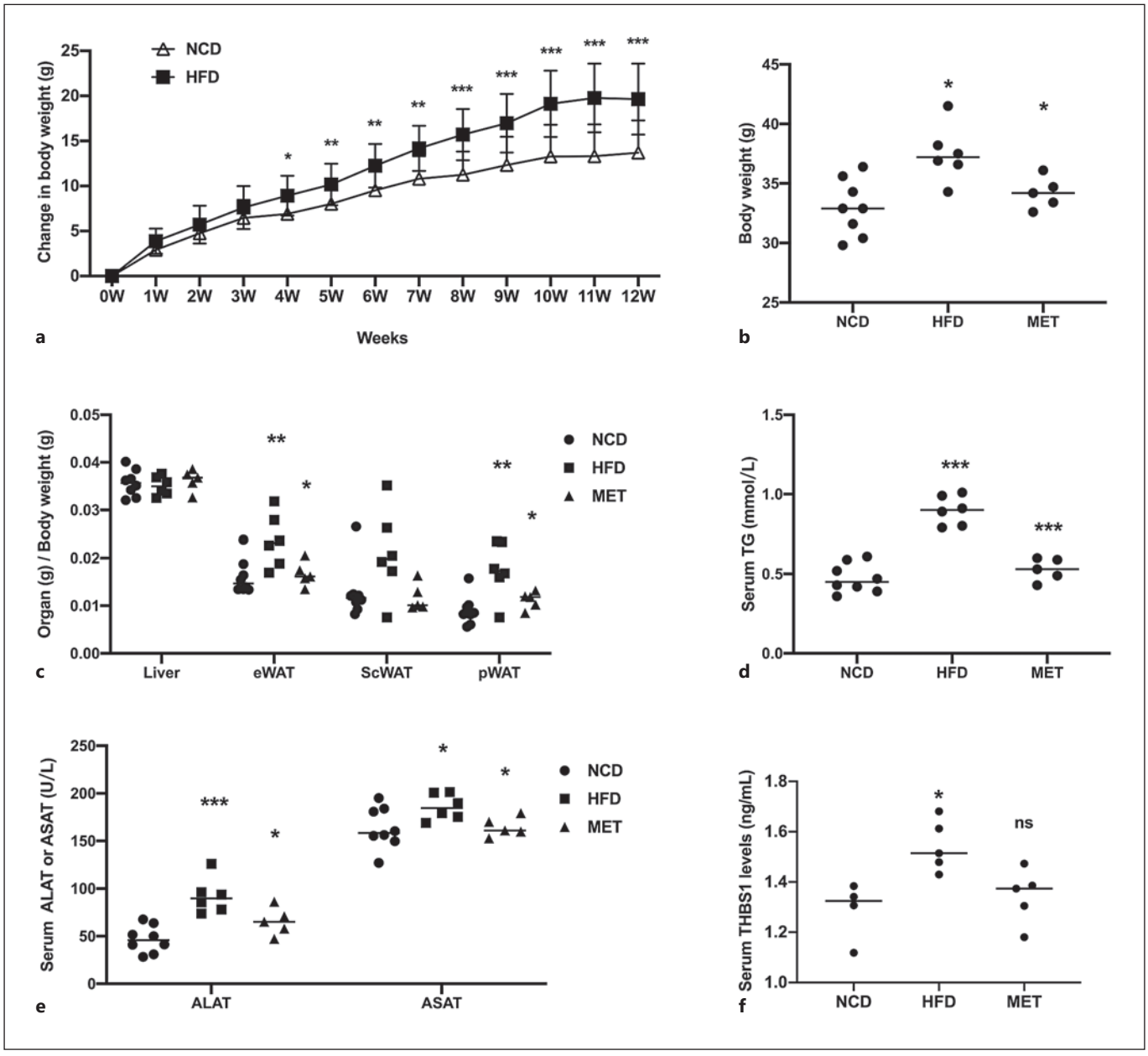


Fig. 3. Effects of metformin on body weight, serum TG, ALT, and AST and THBS1 levels of DIO mice. **a** Changes in body weight of mice fed with high-fat diet. **b**, **c** Changes in body weight and percentage of organ weight of DIO mice with metformin treatment. **d**, **e** Serum TG, ALT, and AST levels in NCD, HFD, or MET groups. **f** Serum THBS1 levels in above three groups. TG, triglyceride; ALT, alanine aminotransferase; ASAT, aspartate aminotransferase; NCD, normal chow diet; HFD, high-fat diet; MET, HFD + metformin treatment. Data are presented as the mean ± SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

tion. Specific deletion of leptin receptor in the liver leads to increased hepatocellular TG content and lipid accumulation [31]. Chavez et al. [32] reported that leptin upregulated THBS1 expression in vascular smooth muscle cells via the JAK2 and MAPK pathways. In an obese diabetic mouse model, THBS1 inhibited leptin-induced matrix metalloproteinase-2 activation in cardiac fibroblasts populating the collagen pads [33]. Sahu et al. [34] revealed that leptin augments recruitment of IRF-1 and CREB to the THBS1 gene promoter in vascular smooth muscle

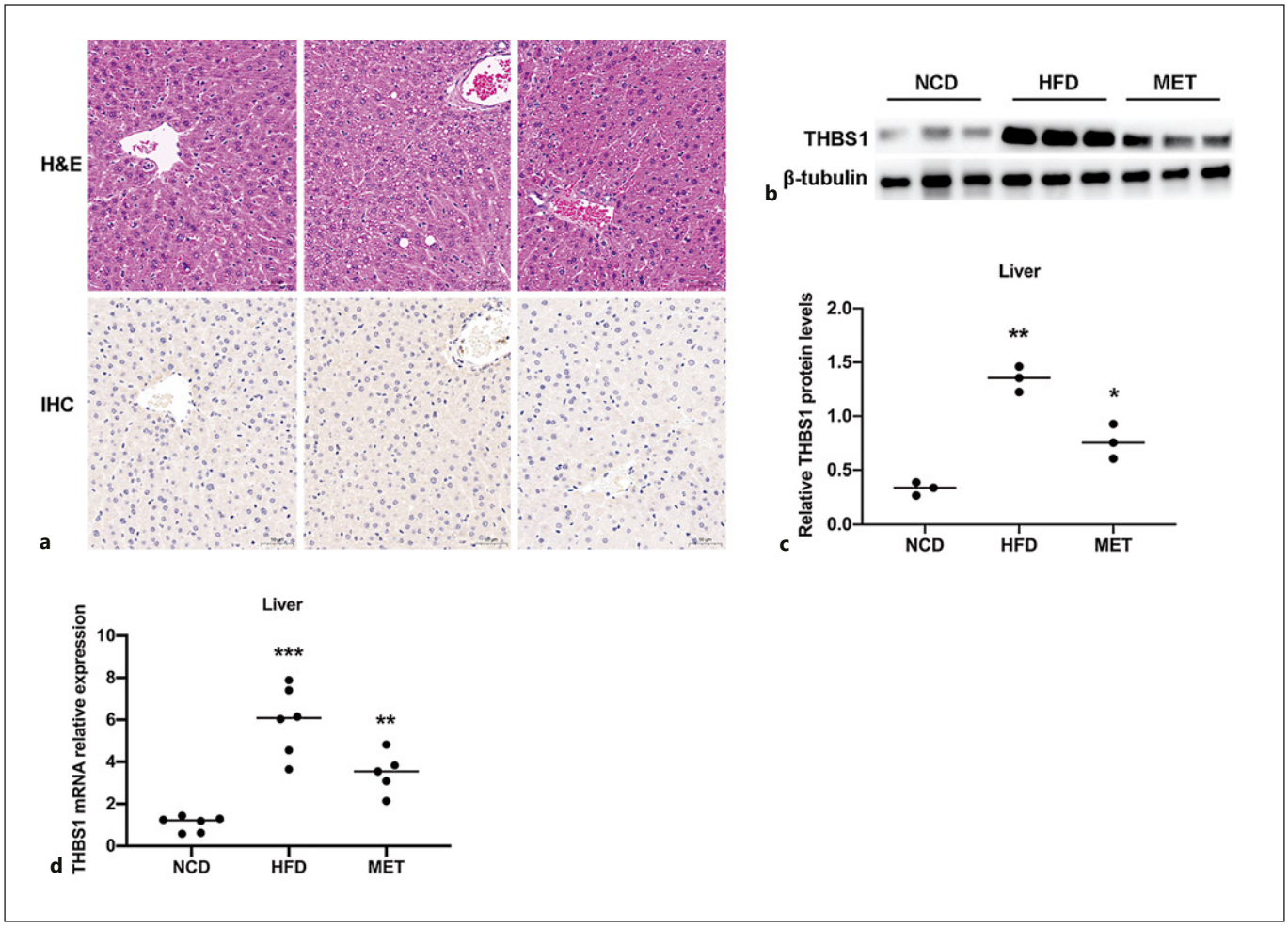


Fig. 4. Metformin treatment ameliorates high-fat diet-induced hepatic steatosis and decreases the expression of hepatic THBS1 in DIO mice. **a** Microphotographs of liver tissues from mice fed with NCD, HFD, and HFD + metformin treatment, following H&E and IHC staining for THBS1. **b**, **c** THBS1 protein levels (western blot) in liver tissue from mice fed with NCD, HFD, and HFD + metformin treatment. **d** RNA relative expression of THBS1 in liver tissue from the above three mice groups. Data are presented as the mean ± SD. H&E, hematoxylin and eosin; IHC, immunohistochemistry. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

cells. Recently, Ganguly et al. [35] showed that THBS1 is a potential target for inducing vasculopathy. Nevertheless, studies on the role of THBS1 in leptin deficiency-associated NAFLD development are unavailable; therefore, further studies are warranted.

THBS1 Is a More Sensitive Predictor of NAFLD than Leptin

To further elucidate the relationship between serum THBS1 and obesity, we compared the correlation of hypertension, hyperglycemia, hypertriglyceridemia, hypo-high-density lipoproteinemia, and MetS among different THBS1 levels. In children with obesity, with increasing quartiles of THBS1, the prevalence of hypo-HDL-C increased significantly, while the prevalence of hypertension, hyperglycemia, hypertriglyceridemia, and MetS remained unchanged. The above results showed that the increase in circulating THBS1 levels plays an important role in the development of obesity and dyslipidemia. As mentioned above, we found that serum TG levels were elevated in children with obesity compared to that in children without obesity, and also observed a positive association between TG and circulating THBS1. Roth et al. [36] reported that the THBS1 gene was overexpressed in injured arteries from hypercholesterolemic atherosclerosis. Hida et al. [37] showed the differential expression of

THBS1 in the visceral adipose tissue of obese OLETF rats, an animal model characterized by abdominal obesity and dyslipidemia. Recently, it has also been reported that an overexpression of THBS1 in cholesterol-enriched diet-treated animals was observed [38]. The present study showed an important role of THBS1 in dyslipidemia of children with obesity. Taken together, these results demonstrated that THBS1 is closely related to childhood obesity, dyslipidemia, and NAFLD.

To determine the efficacy of predicting the role of THBS1 in the development of NAFLD among children with obesity, we analyzed the optimal cutoff points, AUC, sensitivity, specificity, and *p* value of the ROC curves of leptin and THBS1. The AUC values for THBS1 were higher than those for leptin. These results suggest that THBS1 is a more sensitive predictor of NAFLD than leptin in children with obesity. This study also revealed that circulating THBS1 higher than 33.34 ng/mL could lead to the development of NAFLD. However, the cutoff points for circulating THBS1 levels in predicting NAFLD have not yet been studied in children before.

Administration of Metformin Decreased the Expression of Hepatic THBS1 Not Serum Levels in DIO Mice

Under physiological conditions, it has been reported that the expression of THBS1 in the adult liver is very low or nearly undetectable [39]. Previous studies have showed that a significant upregulation in the gene expression of THBS1 was associated with intracellular lipid accumulation in an in vitro model of NAFLD [40]. Macrophage-specific THBS1 deletion protects mice from obesity-associated NAFLD [26]. Consistent with these findings, Li et al. [41] revealed that THBS1 deficiency alleviated macrophage accumulation in adipose tissue in HFD-fed mice. However, other studies have shown that the administration of THBS1 can improve aberrant lipid metabolism and attenuate hepatic steatosis in diet-induced NAFLD mice [25]. To clarify the expression of the hepatic THBS1 gene in obese and NAFLD mice, and to reveal the role of the THBS1 gene in the development of NAFLD in mice, we established a DIO mouse model and evaluated the pathological stage by using the NAFLD activity score. In the current study, we found that serum THBS1 and hepatic THBS1 levels were upregulated in HFD-induced obese mice.

As already known, administration of metformin could attenuate the development of obesity and NAFLD in children and adolescents [42]. According to a previous report, metformin treatment induces weight loss and improved steatosis of the liver induced by an HFD [13, 20]. Interestingly, in our study, metformin did not change the serum THBS1 levels but decreased the expression of hepatic THBS in DIO mice. Inconsistent with our results, Tan et al. [14] showed that metformin treatment significantly increased serum THBS1 levels in overweight PCOS women. Varma et al. [8] reported that pioglitazone (but not metformin) treatment resulted in a decrease in adipose THBS1 gene expression in obese subjects. These inconsistent results could be explained by the different disease model and tissue species. The detailed molecular mechanism underlying the involvement of THBS1 in obesity and NAFLD is needed to be studied further.

In summary, the present study had a limitation that should be noted. Our clinical study was a single-center clinical observation with a relatively small size for subanalysis. We did not confirm the expression of THBS1 in the liver because of the difficulty in obtaining tissue materials from children. Our study is the first to reveal the differential circulating THBS1 levels between children with and without obesity, as well as between non-NAFLD and NAFLD subgroups in obese children. Hence, further studies with larger cohorts are necessary to confirm or disprove circulating THBS1 levels in obese children. The current study also reported the sensitivity, specificity, and cutoff points as predictors and risk factors for NAFLD in children with obesity. These findings are novel and have been reported for the first time in Chinese children. More importantly, we present novel data showing that metformin treatment significantly attenuated the effects on THBS1 expression in a DIO mouse model. Understanding the pathological role of THBS1 in childhood obesity and obesity-related NAFLD and the mechanisms by which metformin decreases the expression of THBS1, which contributes to attenuated NAFLD, could potentially identify novel therapeutic targets for obesity-related NAFLD.

Conclusion

This is the first study to report elevated circulating THBS1 levels in children with obesity, clarify the association of circulating THBS1 levels with obesity, dyslipidemia, and NAFLD, reveal the cut-off points for circulating THBS1 levels in predicting NAFLD among children with obesity, and supplement the role of metformin in the expression of hepatic THBS1 gene level of DIO mice.

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Statement of Ethics

The current study was conducted in accordance with the Declaration of Helsinki and approved by the Human Research Ethics Committee of the Second Affiliated Hospital of Xi'an Jiaotong University (No. 2020-068). The current study was conducted in compliance with the ARRIVE guidelines. All participants and their parents agreed to participate in the study and provided signed informed consent. Animal experiments were approved by the Committee of Animal Research at Xi'an Jiaotong University and the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (No. 2021-962).

Author Contributions

Yanfeng Xiao provided funding for the study and directed it. Min Li designed the study. Lujie Liu performed animal experiments. Yurong Kang was involved in the clinical data collection, sample cleaning, and follow-up. Shanlong Huang was involved animal experiments. Min Li and Lujie Liu analyzed the data. Min Li drafted the manuscript. Yanfeng Xiao reviewed the manuscript and edited it. All the authors have read and approved the final manuscript.

Conflict of Interest Statement

The authors declare no conflicts of interests.

Data Availability Statement

All data generated or analyzed during this study are included in this article and its online supplementary material. Further enquiries can be directed to the corresponding author.

References

1 Kumar S, Kelly AS. Review of childhood obesity: from epidemiology, etiology, and comorbidities to clinical assessment and treatment. Mayo Clin Proc. 2017 Feb;92(2):251–65.
2 Drozdz D, Alvarez-Pitti J, Wójcik M, Borghi C, Gabbianelli R, Mazur A, et al. Obesity and cardiometabolic risk factors: from childhood to adulthood. Nutrients. 2021 Nov 22;13(11):4176.
3 Eslam M, Alkhouri N, Vajro P, Baumann U, Weiss R, Socha P, et al. Defining paediatric metabolic (dysfunction)-associated fatty liver disease: an international expert consensus statement. Lancet Gastroenterol Hepatol. 2021 Oct;6(10):864–73.
4 Younossi Z, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, et al. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. Nat Rev Gastroenterol Hepatol. 2018 Jan;15(1):11–20.
5 Zhou X, Hou DQ, Duan JL, Sun Y, Cheng H, Zhao XY, et al. Prevalence of nonalcoholic fatty liver disease and metabolic abnormalities in 387 obese children and adolescents in Beijing, China. Zhonghua Liu Xing Bing Xue Za Zhi. 2013 May;34(5):446–50.
6 Bornstein P. Thrombospondins as matricellular modulators of cell function. J Clin Invest. 2001 Apr;107(8):929–34.
7 Kong P, Gonzalez-Quesada C, Li N, Cavalera M, Lee DW, Frangogiannis NG. Thrombospondin-1 regulates adiposity and metabolic dysfunction in diet-induced obesity enhancing adipose inflammation and stimulating adipocyte proliferation. Am J Physiol Endocrinol Metab. 2013 Aug 1;305(3):E439–50.
8 Varma V, Yao-Borengasser A, Bodles AM, Rasouli N, Phanavanh B, Nolen GT, et al. Thrombospondin-1 is an adipokine associated with obesity, adipose inflammation, and insulin resistance. Diabetes. 2008 Feb;57(2):432–9.
9 Inoue M, Jiang Y, Barnes RH 2nd, Tokunaga M, Martinez-Santibañez G, Geletka L, et al. Thrombospondin 1 mediates high-fat diet-induced muscle fibrosis and insulin resistance in male mice. Endocrinology. 2013 Dec;154(12):4548–59.
10 Min-DeBartolo J, Schlerman F, Akare S, Wang J, McMahon J, Zhan Y, et al. Thrombospondin-I is a critical modulator in non-alcoholic steatohepatitis (NASH). PLoS One. 2019;14(12):e0226854.
11 Hopkins BD, Goncalves MD, Cantley LC. Obesity and cancer mechanisms: cancer metabolism. J Clin Oncol. 2016 Dec 10;34(35):4277–83.
12 Lv Z, Guo Y. Metformin and its benefits for various diseases. Front Endocrinol. 2020;11:191.
13 Szymczak-Pajor I, Wenclewska S, Śliwińska A. Metabolic action of metformin. Pharmaceuticals. 2022 Jun 30;15(7):810.
14 Tan BK, Adya R, Chen J, Farhatullah S, Heutling D, Mitchell D, et al. Metformin decreases angiogenesis via NF-κB and Erk1/2/Erk5 pathways by increasing the antiangiogenic thrombospondin-1. Cardiovasc Res. 2009 Aug 1;83(3):566–74.
15 Han X, Tao Y, Deng Y, Yu J, Sun Y, Jiang G. Metformin accelerates wound healing in type 2 diabetic db/db mice. Mol Med Rep. 2017 Dec;16(6):8691–8.
16 Ji CY; Working Group on Obesity in China. Report on childhood obesity in China (1): body mass index reference for screening overweight and obesity in Chinese school-age children. Biomed Environ Sci. 2005 Dec;18(6):390–400.
17 Consortium T. Recommended standards for assessing blood pressure in human research where blood pressure or hypertension is a major focus. Clin Exp Hypertens. 2018;40(6):509–13.
18 Tan X, Wang X, Chu H, Liu H, Yi X, Xiao Y. SFRP5 correlates with obesity and metabolic syndrome and increases after weight loss in children. Clin Endocrinol. 2014 Sep;81(3):363–9.
19 Li M, Wang M, Liu Y, Huang S, Yi X, Yin C, et al. TNF-A upregulates IKKε expression via the Lin28B/let-7a pathway to induce catecholamine resistance in adipocytes. Obesity. 2019 May;27(5):767–76.
20 Li M, Sharma A, Yin C, Tan X, Xiao Y. Metformin ameliorates hepatic steatosis and improves the induction of autophagy in HFD-induced obese mice. Mol Med Rep. 2017 Jul;16(1):680–6.
21 Liang L, Fu JF, DU JB. Significance of exploring the definition of metabolic syndrome in Chinese children and adolescents. Zhonghua Er Ke Za Zhi. 2012 Jun;50(6):401–4.
22 Yin C, Zhang H, Zhang M, Xiao Y. Adropin and apelin-12 efficiently predict metabolic syndrome in obese children. Pediatr Diabetes. 2020 Nov;21(7):1132–9.

23 Brunt EM, Kleiner DE, Wilson LA, Belt P, Neuschwander-Tetri BA; NASH Clinical Research Network (CRN). Nonalcoholic fatty liver disease (NAFLD) activity score and the histopathologic diagnosis in NAFLD: distinct clinicopathologic meanings. Hepatology. 2011 Mar;53(3):810–20.
24 Matsuo Y, Tanaka M, Yamakage H, Sasaki Y, Muranaka K, Hata H, et al. Thrombospondin 1 as a novel biological marker of obesity and metabolic syndrome. Metabolism. 2015 Nov; 64(11):1490–9.
25 Bai J, Xia M, Xue Y, Ma F, Cui A, Sun Y, et al. Thrombospondin 1 improves hepatic steatosis in diet-induced insulin-resistant mice and is associated with hepatic fat content in humans. EBioMedicine. 2020 Jul;57: 102849.
26 Gwag T, Reddy Mooli RG, Li D, Lee S, Lee EY, Wang S. Macrophage-derived thrombospondin 1 promotes obesity-associated non-alcoholic fatty liver disease. JHEP Rep. 2021 Feb; 3(1):100193.
27 Tahergorabi Z, Salmani F, Jonaidabad SH, Behdani B, Yazdi P, Zardast M, et al. Association of serum levels of vascular endothelial growth factor and thrombospondin-1 to body mass index in polycystic ovary syndrome: a case-control study. Obstet Gynecol Sci. 2019 Nov; 62(6):420–8.
28 Buras ED, Converso-Baran K, Davis CS, Akama T, Hikage F, Michele DE, et al. Fibro-adipogenic remodeling of the diaphragm in obesity-associated respiratory dysfunction. Diabetes. 2019 Jan;68(1):45–56.
29 McCarthy JJ, Meyer J, Moliterno DJ, Newby LK, Rogers WJ, Topol EJ. Evidence for substantial effect modification by gender in a large-scale genetic association study of the metabolic syndrome among coronary heart disease patients. Hum Genet. 2003 Dec; 114(1):87–98.
30 Lin CH, Kohli R. Emerging new diagnostic modalities and therapies of nonalcoholic fatty liver disease. Curr Gastroenterol Rep. 2020 Aug 19;22(10):52.
31 Jiménez-Cortegana C, García-Galey A, Tami M, Del Pino P, Carmona I, López S, et al. Role of leptin in non-alcoholic fatty liver disease. Biomedicines. 2021 Jun 30;9(7):762.
32 Chavez RJ, Haney RM, Cuadra RH, Ganguly R, Adapala RK, Thodeti CK, et al. Upregulation of thrombospondin-1 expression by leptin in vascular smooth muscle cells via JAK2- and MAPK-dependent pathways. Am J Physiol Cell Physiol. 2012 Jul 15;303(2): C179–91.
33 Gonzalez-Quesada C, Cavalera M, Biernacka A, Kong P, Lee DW, Saxena A, et al. Thrombospondin-1 induction in the diabetic myocardium stabilizes the cardiac matrix in addition to promoting vascular rarefaction through angiopoietin-2 upregulation. Circ Res. 2013 Dec 6;113(12):1331–44.
34 Sahu S, Ganguly R, Raman P. Leptin augments recruitment of IRF-1 and CREB to thrombospondin-1 gene promoter in vascular smooth muscle cells in vitro. Am J Physiology-Cell Physiol. 2016 Aug 1;311(2):C212–24.
35 Ganguly R, Khanal S, Mathias A, Gupta S, Lallo J, Sahu S, et al. TSP-1 (Thrombospondin-1) deficiency protects ApoE(-/-) mice against leptin-induced atherosclerosis. Arterioscler Thromb Vasc Biol. 2021 Feb;41(2):e112–27.
36 Roth JJ, Gahtan V, Brown JL, Gerhard C, Swami VK, Rothman VL, et al. Thrombospondin-1 is elevated with both intimal hyperplasia and hypercholesterolemia. J Surg Res. 1998 Jan;74(1):11–6.
37 Hida K, Wada J, Zhang H, Hiragushi K, Tsuchiyama Y, Shikata K, et al. Identification of genes specifically expressed in the accumulated visceral adipose tissue of OLETF rats. J Lipid Res. 2000 Oct;41(10):1615–22.
38 Kerforne T, Favreau F, Khalifeh T, Maiga S, Allain G, Thierry A, et al. Hypercholesterolemia-induced increase in plasma oxidized LDL abrogated pro angiogenic response in kidney grafts. J Transl Med. 2019 Jan 14;17(1): 26.
39 Kong P, Cavalera M, Frangogiannis NG. The role of thrombospondin (TSP)-1 in obesity and diabetes. Adipocyte. 2014 Jan 1;3(1):81–4.
40 Chavez-Tapia NC, Rosso N, Tiribelli C. Effect of intracellular lipid accumulation in a new model of non-alcoholic fatty liver disease. BMC Gastroenterol. 2012 Mar 1;12:20.
41 Li Y, Tong X, Rumala C, Clemons K, Wang S. Thrombospondin1 deficiency reduces obesity-associated inflammation and improves insulin sensitivity in a diet-induced obese mouse model. PLoS One. 2011;6(10):e26656.
42 Soliman A, De Sanctis V, Alaaraj N, Hamed N. The clinical application of metformin in children and adolescents: a short update. Acta Biomed. 2020 Sep 7;91(3):e2020086.