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ORIGINAL ARTICLE

The Relationship Between Pediatric Nonalcoholic Fatty Liver Disease and Cardiovascular Risk Factors and Increased Risk of Atherosclerosis in Obese Children

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Abstract To investigate the relationship between nonalcoholic fatty liver disease and cardiovascular risk factors and increased risk of atherosclerosis in obese children. The study included 80 consecutive obese children who were stratified into group 1 [ultrasonographically diagnosed with NAFLD ($n = 50$)] and group 2 [not diagnosed with NAFLD ($n = 30$)]. The control group included 30 healthy children. The groups were compared in terms of clinical cardiovascular risk factors and carotid intimal medial thickness (CIMT) (as a marker of atherosclerosis) measured using B-mode ultrasound. Mean body mass index (BMI) and blood pressure (BP), as well as the frequency of dyslipidemia, metabolic syndrome (MetS), and insulin resistance (IR), were similar in groups 1 and 2. Mean BMI and triglyceride (TG) levels, and the frequency of IR and MetS, increased significantly as the grade of steatosis increased. Mean CIMT in group 1 was significantly greater than that in the control group ($P < 0.01$). There was a positive correlation between CIMT and age, BP, and BMI in groups 1 and 2. In addition, CIMT was correlated with TG, low high-density lipoprotein (HDL) cholesterol, MetS, and IR only in group 1. Linear regression analysis between CIMT and age, BP, BMI, TG level, HDL cholesterol level, IR, MetS, and grade of steatosis yielded a significant difference only for grade of steatosis. Cardiovascular risk factors are more impressive and CIMT was significantly higher in group 1 than in group 2 and the control group, indicating that they are associated with greater risk of atherosclerosis and future adverse cardiovascular events.

Keywords Atherosclerosis · Cardiovascular risk factors · Children · Nonalcoholic liver disease

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Introduction

Nonalcoholic fatty liver disease (NAFLD) is a clinicopathological entity characterized by macrovesicular fat accumulation in hepatocytes. It rarely occurs before age 8 years, and typical onset is age 12 years, although biopsy-proven cases have been reported in children age 2–17 years old [38]. Most children with NAFLD are obese as defined by a body mass index (BMI) <95th percentile for sex and age [38]. As such, an expert committee recommends targeted alanine aminotransferase (ALT) and/or ultrasound (US) screening of obese children [1, 43]. Several studies have reported that insulin resistance (IR) plays a role in pediatric NAFLD [37, 44, 49]. Furthermore, by the time of the diagnosis of type 2 diabetes mellitus (DM) in children, nearly one half have suspected fatty liver based on increased ALT [27].

Obesity and IR, as a part of metabolic syndrome (MetS), play an important role in every stage of atherosclerosis from initial endothelial dysfunction to final-stage plaque rupture. Traditionally, NAFLD is not considered a part of MetS, although it is accepted as a hepatic manifestation of MetS. NAFLD is strongly associated with obesity, IR, hypertension, and dyslipidemia [21]; therefore, it is also expected to be associated with atherosclerosis. Recent improvements in imaging technology have identified early vascular changes that can be assessed using US. Some studies have reported that NAFLD is associated with increased carotid intimal medial thickness (CIMT) [9, 18, 31], whereas others have reported that there is no such association [25].

The present study aimed to investigate the relationship between NAFLD and cardiovascular risk factors and increased risk of atherosclerosis in obese children. All of the study participants underwent carotid US for the measurement of CIMT as a marker of atherosclerosis. We hypothesized that the association with traditional cardiovascular risk factors, and thus the future risk of atherosclerosis, would be greater in obese children with NAFLD compared with obese children without NAFLD.

Materials and Methods

The study included 80 consecutive children diagnosed as obese based on BMI ≥95th percentile for sex and age [23]. The children were stratified into groups 1 and 2, which consisted of 50 children with NAFLD and 30 children without NAFLD, respectively. Exclusion criteria were systemic disease, including cystic fibrosis and inflammatory bowel disease, hepatitis, drug use, history of parenteral nutrition, cigarette use, alcohol use, and family history of hereditary hyperlipidemia, and/or premature atherosclerosis. Hepatotropic viruses, serum ceruloplasmin level, serum α1-antitrypsin level, autoantibodies against nuclear smooth muscle, and liver-kidney microsomal type-1 antigens were screened to eliminate infectious, metabolic, and autoimmune liver pathologies in both groups. The control group included 30 healthy children. None of the participants were excluded from the study.

Blood pressure (BP) was measured three separate times after the children had been sitting for ≥10 min, and the second and third measurements were averaged. Children with systolic BP and/or diastolic BP >95th percentile—adjusted for height, age, and sex—were considered to have high BP [29]. Fasting glucose, triglyceride (TG) levels, total cholesterol, low-density lipoprotein (LDL) cholesterol, and high-density lipoprotein (HDL) cholesterol were measured spectrophotometrically using a Beckman-Coulter LX-20 autoanalyzer (Brea, CA). The serum insulin concentration was measured by way of immunometric assay using an Immulite 2000 analyzer (Bio-DPC; Siemens Medical, Gywneed, UK).

Reference standards were used to classify the following five quantitative measurements: (1) normal range for serum ALT level in children (5–45 IU L^{-1}) [33]; (2) hyperlipidemia as defined by serum lipids >95th percentile for age and sex [28]; (3) increased levels of other biomarkers, including fasting plasma glucose ≥100 mg dL^{-1} [15]; (4) insulin resistance (IR) (homeostatic model assessment of insulin resistance [HOMA-IR]) calculated as insulin (mU L^{-1}) × glucose (mg dL^{-1})/405 [26]; and (5) a cut-off value for IR of 1.98 ± 0.57 in prepubertal children and 3.02 ± 0.76 in pubertal children [16, 19].

MetS was diagnosed based on a modification of the National Cholesterol Education Program's Adult Treatment Panel criteria [13]. Because body proportions normally change during pubertal development and can vary according to individuals, and waist circumference is difficult to interpret in children, BMI was used according to previously defined criteria [23] as a measure of obesity. As such, MetS was defined as the presence of equal to or more than the following five criteria: (1) obesity (BMI ≥95th percentile for sex and age); (2) hypertriglyceridemia (TGs >95th percentile for age, sex, and race); (3) low HDL cholesterol concentration (<5th percentile for age and sex); (4) increased BP (systolic or diastolic BP >95th percentile for age and sex); and (5) impaired fasting glucose or known type 2 DM.

Conventional hepatic US was performed by one radiologist using a GE logiq S6 (General Electric, USA) with convex transducers (frequency bandwidth 3.5 MHz). The radiologist was blinded to the clinical and laboratory data and risk factors. Before US examination, the participants rested quietly in a temperature-controlled dark room for 10–15 min. The ultrasonographic steatosis score was defined as follows: no steatosis (grade O) = normal liver echotexture; mild steatosis (grade 1) = slight and diffuse increase in fine parenchymal echoes with normal visualization of the diaphragm and portal vein borders; moderate steatosis (grade 2) = moderate and diffuse increase in fine echoes with slightly impaired visualization of the portal vein borders and diaphragm; and (4) severe steatosis (grade 3) = fine echoes with poor or no visualization of the portal vein borders, diaphragm, or posterior portion of the right lobe [20]. The same sonographer who was blinded to the participant's hepatic ultrasonographic data performed carotid US. Carotid scanning was performed with the patient in supine position with the neck extended using B-mode US (GE logiq, S6-USA) and a 14-MHz linear probe. The probe was placed in the longitudinal plane at the anterolateral position of the right side of the neck followed by the left side of the neck, and measurement of the common carotid artery intima media thickness (CIMT) was made at 1 cm below the bifurcation. The distance between the

Table 1 Patient clinical and laboratory data

Patient and laboratory data	Group 1	Group 2	Group 3
Mean ± SD age (y)	11.7 ± 3.8	10.7 ± 3.0	11.2 ± 3.6
Male sex (%)*	36 (72.0)	13 (43.3)	14 (46.7)
BMI (kg/m^2)**	27.3 ± 6.3	26.4 ± 4.7	17.5 ± 2.7
Mean ± SD systolic BP (mmHg)	108.9 ± 15.5	111.6 ± 14	104.1 ± 7.5
Mean ± SD diastolic BP (mmHg)**	70.3 ± 10.5	71.3 ± 11.4	62.6 ± 6.7
Mean ± SD (range) ALT (IU/L)***	39.3 ± 22.6 (13–107)	21.4 ± 5.1 (13–33)	17.1 ± 6.1 (9–30)
Mean ± SD total cholesterol (mg/dl)	152.3 ± 34.4	158.9 ± 26.1	–
Mean ± SD LDL cholesterol (mg/dl)	93.2 ± 24.9	96.6 ± 22.1	–
Mean ± SD HDL cholesterol (mg/dl)	36.1 ± 13.4	35.5 ± 8.7	–
Mean ± SD triglycerides (mg/dl)	117.6 ± 70.1	123.9 ± 79.8	–
HOMA-IR	3.42 ± 2.32	2.90 ± 1.81	–
No. insulin resistance (%)	20 (42.6)	10 (38.5)	
No. MetS (%)	17 (36.2)	9 (33.3)	–
Mean ± SD RCIMT (mm)****	0.46 ± 0.21	0.35 ± 0.09	0.30 ± 0.13
Mean ± SD LCIMT (mm)****	0.44 ± 0.09	0.35 ± 0.08	0.27 ± 0.04

Statistical tests: one-way ANOVA, Student *t*, Chi-square, and Tukey

RCIMT right carotid artery intimal medial thickness; *LCIMT* left carotid artery intimal medial thickness

* $P < 0.05$ group 1 versus 2 and group 1 versus 3, ** $P < 0.005$ group 1 versus 3 and group 2 versus 3, *** $P < 0.001$ group 1 versus 2, **** $P < 0.01$, group 1 versus 2, group 1 versus group 3, and group 2 versus group 3

echogenicity of the lumen–intima interface and the adventitia–media interface was accepted as intima–media thickness. CIMT was defined as the mean of three distinct measurements from each side. The study was performed in accordance with the Declaration of Helsinki [48]; the study protocol was approved by the Ethics Committee of Dr. Sami Ulus Training and Research Hospital; and informed consent was provided by the parents of each participant.

Statistical Analysis

Analyses were performed using SPSS v.15.0 for Windows (SPSS, Chicago, IL). Numeric variables are expressed as mean ± SD, and nominal variables are shown as number and percentage. Parametric and nonparametric variables were compared using Student *t* test, one-way analysis of variance, Mann–Whitney *U* test, Kruskal–Wallis test, and X^2 test. Tukey analysis was used for multiple group comparisons. Spearman's test was used to investigate the relationship between variables. Linear regression analysis was used to identify risk factors associated with atherosclerosis. Statistical significance was set at $P < 0.05$. Bonferroni correction greater than the *P* value was required for multiple group comparisons.

Results

The study included 80 obese children stratified into groups 1 and 2, which consisted of 50 (62.5 %) children with NAFLD and 30 (38.5 %) without NAFLD, respectively. Mean age in both groups was similar. There were 36 boys (72.0 %) in group 1, which was significantly more than in group 2 ($n = 13$ [43.3 %]) and the control group ($n = 14$ [46.7 %]) ($P < 0.05$). The frequency of family history including DM and hypertension was similar in groups 1 and 2. None of the controls had a family history of diabetes or hypertension ($P < 0.05$). Mean body weight and BMI in groups 1 and 2 were similar, but they significantly higher than in the control group ($P < 0.005$). Mean systolic BP in groups 1 and 2, as well as the control group, were similar (108.9 ± 15.5, 111.6 ± 14.0, and 104.17 ± 7.5 mmHg, respectively). Mean diastolic BP measurements in groups 1 and 2 were similar but significantly higher than in the control group ($P < 0.005$) (Table 1). Hypertension was noted in 7 children (14 %) in group 1, 5 children (16.7 %) in group 2, and no children in the control group ($P = 0.052$). The frequency of hypertension was similar in groups 1 and 2 ($P = 0.756$).

Mean ALT in group 1 was significantly higher than in group 2 ($P < 0.001$). Mean total cholesterol, LDL cholesterol, TGs, and HDL cholesterol in groups 1 and 2 were similar. Accordingly, dyslipidemia was observed in 23 (51.1 %) and 15 patients (51.7 %), respectively, in groups 1 and 2 (Table 1). Group 1 was divided into three subgroups according to grade of steatosis: subgroup 1a had grade I steatosis ($n = 18$ patients [36 %]); subgroup 1b had grade II steatosis ($n = 21$ [42 %]); and subgroup 1c had grade III steatosis ($n = 11$ [22 %]). The subgroups did not differ in mean age, sex, BP, cholesterol, or ALT,

Table 2 Clinical and laboratory characteristics of the children with NAFLD stratified according to degree of steatosis

Characteristics	Group 1a	Group 1b	Group 1c
Mean ± SD age (y)	11.3 ± 4.6	11.4 ± 3.6	13.0 ± 2.6
Male sex (%)	13 (72.2)	16 (76.2)	7 (63.6)
BMI (kg/m^2)*	24.8 ± 5.3	27.3 ± 5.2	31.6 ± 8.1
Mean ± SD (range) ALT (IU/L)	34.1 ± 23.2 (13.0–90.0)	40.2 ± 21.6 (20.0–107.0)	46.5 ± 23.7 (14.0–92.0)
Mean ± SD total cholesterol (mg/dl)	158.4 ± 33.7	155.2 ± 36.4	138.5 ± 31.2
Mean ± SD LDL cholesterol (mg/dl)	96.4 ± 26.3	98.3 ± 25.1	80.1 ± 19.6
Mean ± SD HDL cholesterol (mg/dl)	37.0 ± 6.8	34.6 ± 8.0	37.5 ± 7.1
Mean ± SD TG (mg/dl)**	90.4 ± 48.9	121.7 ± 69.7	150.0 ± 86.2
HOMA–IR***	2.26 ± 1.77	3.59 ± 2.31	4.93 ± 2.33
MetS (%)**	2 /17(11.8)	7/19 (36.8)	8/11 (72.7)
Median (minimum–maximum) RCIMT (mm)****	0.40 (0.23–0.26)	0.40 (0.33–0.60)	0.53 (0.40–1.80)
Median (minimum–maximum) LCIMT****	0.41 (0.26–0.50)	0.43 (0.30–0.63)	0.56 (0.36–0.60)

Statistical tests: one-way ANOVA, Kruskal–Wallis, Student *t*, Mann–Whitney *U*, Chi-square, Tukey

RCIMT right carotid artery intimal medial thickness; *LCIMT* left carotid artery intimal medial thickness

* $P < 0.05$, group 1a versus 1c, ** $P < 0.005$ group 1a versus 1c, *** $P < 0.05$ group 1a versus 1b and $P < 0.005$ group 1a versus 1c, **** $P < 0.05$, group 1a versus 1c and group 1b versus 1c

whereas they did differ significantly in terms of MetS, TGs, BMI, and HOMA-IR (Table 2).

Mean HOMA-IR in group 1 was higher than in group 2 (3.42 ± 2.32 vs 2.90 ± 1.81, respectively), but the difference was not significant. IR was noted in 20 children (42.6 %) in group 1 and 10 children (38.5 %) in group 2 (Table 1). Mean HOMA-IR increased as the degree of steatosis increased. Mean HOMA-IR was 2.27 ± 1.70, 3.50 ± 2.30, and 4.90 ± 2.30 in subgroups 1a, 1b, and 1c, respectively. There was a significant difference in mean HOMA-IR between subgroups 1a and 1b and between subgroups 1a and 1c ($P < 0.05$ and $P < 0.005$, respectively) (Table 2). In total, 17 children (36.2 %) in group 1 and 9 children (33.3 %) in group 2 had MetS ($P = 1.000$) (Table 1). MetS was noted in 2 of 17 children (11.7 %) in subgroup 1a, in 7 of 19 children (36.8 %) in subgroup 1b, and in 8 of 11 children (72.7 %) in subgroup 1c. The occurrence of MetS increased significantly as the grade of steatosis increased ($P < 0.01$) (Table 2).

Mean right CIMT in groups 1 and 2, as well as the control group, was 0.46 ± 0.21, 0.35 ± 0.09, and 0.30 ± 0.13 mm, respectively. The corresponding mean left CIMT was 0.44 ± 0.09, 0.35 ± 0.08, 0.27 ± 0.04 mm, respectively. Mean right and left CIMT in group 1 was significantly higher than that in group 2 as well as the control group ($P < 0.01$). Mean CIMT in group 2 was also significantly higher than that in the control group ($P < 0.01$) (Table 1). There was a positive correlation between right and left CIMT and age in group 1 and between left CIMT and age in group 2. Sex was not associated with CIMT. Systolic and or diastolic BP values were weakly correlated with mean right and left CIMT in group 1. In contrast, there was a weak positive correlation between systolic BP and left CIMT in group 2 (Table 3). Subgroup 1c had a significantly higher CIMT than subgroups 1a and 1b ($P < 0.005$ and $P < 0.01$, respectively) (Table 2).

There was a significant but weak correlation between BMI and right and left CIMT in group 1 ($P < 0.01$, $r = 0.495$ and $r = 473$, respectively). In contrast, the correlation between BMI and CIMT in group 2 was only observed in the right carotid artery ($P < 0.05$, $r = 0.382$). There was a weak correlation between TGs and right and left CIMT in group 1 ($P < 0.05$, $r = 0.358$ and $r = 0.304$ respectively). No correlation was observed between CIMT and blood cholesterol levels in groups 1 and 2 (Table 3). Right and left CIMT in children with IR in group 1 was significantly higher than in the children without IR. In contrast, mean CIMT in the children with and without IR in group 2 were similar. Right and left mean CIMT in the children with MetS in group 1 was significantly higher than in those without MetS ($P < 0.01$). Mean CIMT in the children with and without MetS in group 2 was similar.

Multivariate linear regression analysis was performed to analyze the effect of age, sex, BMI, BP, TGs, HDL cholesterol, IR, MetS, and steatosis on CIMT. Steatosis was the only factor that had an effect on increased CIMT (right CIMT: $P < 0.05$, odds ratio [OR] = 2.12; left CIMT: $P < 0.01$, OR = 4.44) (Table 4).

Discussion

NAFLD is a growing problem, especially in obese children. NAFLD comprises a disease spectrum ranging from simple steatosis to steatohepatitis, is characterized by a variable

Table 3 The relationship between clinical and laboratory characteristics and CIMT in the obese children with and without steatosis (Spearman's correlation test)

	Group 1				Group 2			
	RCIMT		LCIMT		RCIMT		LCIMT	
	P	*r*	*P*	*r*	*P*	*r*	*P*	*r*
Age (y)	0.002	0.432	0.013	0.349	0.061	0.352	0.017	0.441
Sex	0.298	–0.150	0.491	–0.100	0.226	0.228	0.879	–0.29
Systolic BP	<0.001	0.493	0.002	0.436	0.111	0.297	0.044	0.370
Diastolic BP	0.001	0.467	0.016	0.340	0.151	0.269	0.093	0.312
BMI	<0.001	0.495	0.001	0.473	0.301	0.195	0.037	0.382
ALT	0.586	0.079	0.384	0.126	0.158	0.274	0.480	0.139
TG	0.015	0.358	0.040	0.304	0.151	0.274	0.473	0.151
Total cholesterol	1.000	0.00	0.599	–0.800	0.832	–0.041	0.712	–0.072
HDL cholesterol	0.067	–0.273	0.016	–0.354	0.172	–0.266	0.333	–0.190
MetS	<0.001	0.554	<0.001	0.556	0.745	0.066	0.329	0.195
Insulin resistance	<0.001	0.604	<0.001	0.491	0.466	0.149	0.147	0.293

RCIMT right carotid artery intimal medial thickness; *LCIMT* left carotid artery intimal medial thickness

Table 4 Factors affecting CIMT per linear regression analysis

Factors	Right CIMT		Left CIMT	
	Beta	*P*	Beta	*P*
Age (y)	0.272	0.077	0.193	0.134
BMI	–0.098	0.530	0.209	0.114
Blood pressure	–0.06	0.971	0.244	0.808
Insulin resistance	0.268	0.081	0.147	0.253
Triglyceride	0.069	0.569	0.063	0.537
HDL cholesterol	–0.025	0.841	–0.117	0.273
MetS	0.107	0.575	–0.93	0.572
Presence of steatosis	0.241	0.038	0.425	<0.001

degree of inflammation and fibrosis, and has the potential to progress to cirrhosis and hepatocellular carcinoma. The prevalence of NAFLD in children is 1.3–9.6 % based on US estimation and autopsy findings [40, 45, 50]; however, the prevalence is greater in obese children and ranges from 38.7 to 77 % [7, 14, 17]. In the present study, 62.5 % of obese children had NAFLD, which is in agreement with previous reports. NAFLD was reported to occur more frequently in boys [45], and the prevalence was also reported to be 3-fold greater in boys [50]. In the present study, the rate of NAFLD was significantly greater in the boys. It remains unknown why the prevalence of NAFLD in male patients is greater. One theory is that male individuals are more likely to distribute excess body fat in the intra-abdominal compartment, which some researchers think is related to the presence of steatosis [39]; however, this hypothesis remains to be proven in children.

Obesity is associated with a higher frequency of cardiovascular risk factors, including abnormal values for HDL cholesterol, insulin, ALT, TG levels, CRP, CIMT, and BP in adolescents [3, 5, 10, 24]. Epidemiological data show that fatty liver is predictive of—independent of other factors—MetS, type 2 diabetes, and cardiovascular disease [22]. All components of MetS were reported to correlate with liver fat content [22]. Although the prevalence of steatosis increases as the prevalence of obesity increases [46], the relationship between the components of MetS and steatosis remains strong even after adjusting for BMI. In the present study, mean diastolic BP in obese children with and without steatosis was significantly higher than that in the controls ($P = 0.001$). Mean systolic BP and diastolic BP in the obese children with steatosis was higher than in the obese children without steatosis, but the difference was not significant. This finding is in accord with the results of

most studies that have reported obese children with NAFLD having higher systolic BP and/or diastolic BP than their counterparts without NAFLD [6, 25, 31, 32, 41].

It is known that NAFLD is associated with IR [21]. IR and NAFLD appear to be correlated from childhood [36]. Both hepatic IR and impaired insulin clearance are likely to contribute to fasting hyperinsulinemia. Whereas IR promotes fatty acid accumulation in the liver, the latter causes hepatic IR, which is characterized by a lack of suppression of endogenous liver glucose production. As such, NAFLD may act as a stimulus for further increasing whole-body IR and dyslipidemia, leading to accelerated atherosclerosis [12]. Serum insulin correlates closely with liver fat content independent of age, sex, and BMI [3]. Some studies in children have reported a close relationship between NAFLD and IR [31, 32, 47], whereas others reported no such relationship [25, 32]. In the present study, the percentage of children with IR was similar between children with NAFLD and those without NAFLD. In contrast, mean HOMA-IR in the children with NAFLD was higher than in those without NAFLD, but not significantly. Nevertheless, NAFLD appears to be associated with IR.

Most studies have reported that the number of patients with hypertriglyceridemia and low HDL cholesterol were significantly higher in children with NAFLD compared with obese children without it [6, 25, 32]. Schwimmer et al. [41] reported that total cholesterol and LDL cholesterol levels were higher in obese people with NAFLD. In contrast, Pacifico et al. [31] reported similar frequency of dyslipidemia. In the present study, mean total cholesterol, LDL cholesterol, TGs, and HDL cholesterol in the obese children with and without steatosis were similar as was the rate of dyslipidemia. Nevertheless, NAFLD may be related to dyslipidemia as are other cardiovascular risk factors; however, additional research is necessary to better understand the relationship between NAFLD and dyslipidemia beyond its association with obesity.

Only a few studies have evaluated the relationship between severity of steatosis and cardiovascular risk factors. Demircioglu et al. [9] reported that children with grade II and III steatosis had significantly higher BMI than obese children with grade I steatosis and those without steatosis. In contrast, serum TGs, total cholesterol, HDL and LDL cholesterol, and fasting glucose were similar regardless of the degree of steatosis. However, the researchers did not evaluate IR. Nobili et al. [30] reported that the severity of liver injury was an independent predictor of the proatherogenic lipid profile after adjusting for BMI, HOMA-IR, impaired glucose tolerance, and MetS. In the present study, children with NAFLD who were stratified according to grade of steatosis did not differ with respect to mean age, sex, total cholesterol, LDL and HDL cholesterol, BP, or ALT. In contrast, the occurrence of MetS and BMI and HOMA-IR and TG levels in the children with grade III steatosis was significantly greater than in those with grade I steatosis (Table 2). These findings suggest that severity of steatosis may be associated with increased cardiovascular risk.

Pacifico et al. [31] reported that obese patients with NAFLD have significantly increased CIMT independent of anthropometric and metabolic features. Kelishadi et al. [18] reported that CIMT was strongly associated with IR and NAFLD and suggested that the liver and vessels might share common mediators. Demircioglu et al. [9] observed an association between US-detected NAFLD and CIMT measured at the common carotid artery, carotid bulb, and internal carotid artery. Manco et al. [25], in contrast, reported that there was not a significant difference in CIMT between children with and without NAFLD, although median CIMT in obese children was greater than in non-obese children. Moreover, they did not observe an association between CIMT and histologic severity of biopsy-proven NAFLD. In a population-based study [41] the presence of fatty liver, together with BMI, waist circumference, and systolic BP, was independently associated with increased CIMT. Moreover, a recent study with a large sample [32] reported that obese children with NAFLD had significantly higher CIMT than obese children without NAFLD independent of other cardiovascular risk factors, including MetS.

In the present study, CIMT in obese children with and without NAFLD was significantly greater than in controls. In addition, CIMT in the obese children with NAFLD was significantly greater than in those without NAFLD. CIMT was positively correlated with age, BP, BMI, TGs, IR, and MetS; the associations between these factors were stronger in the children with NAFLD. In particular, MetS, IR, and TG level were correlated with CIMT only in the children with NAFLD. Moreover, HOMA-IR increased significantly as the grade of steatosis increased. After adjusting for potential confounders—e.g., age, BMI, BP, IR, MetS, TGs, and HDL cholesterol—only NAFLD was observed to be strongly correlated with CIMT. In this study, CIMT increased as the grade of steatosis increased, a finding similar to that reported by Demircioglu et al. [9]. In addition, the difference was statistically significant. Together, the present study's findings indicate that children with NAFLD may have an increased risk cardiovascular disease in the future.

The most important limitation of the present study is its cross-sectional design, which permitted only an examination of associations, not causation. In addition, some of the observed differences in IR between the study groups may be attributed to sex, which was not well-matched, and to differences in Tanner stage, which was not assessed. The diagnosis of NAFLD was based on US data, and the severity of liver disease was not confirmed histologically.

Indeed, a recent study [42] showed that US is useful for quantifying steatosis and strongly correlates with grade of steatosis on liver biopsy. In addition, US is an ethically sound procedure, easily reproducible, and is not associated with any complications, which are all major drawbacks of liver biopsy [4, 11, 34]. Moreover, liver biopsy is associated with sampling error [8, 35], as well as intraobserver and interobserver discrepancy [2]. Liver fat content may be more reliably measured by magnetic resonance spectroscopy [47]. However, this does not reliably distinguish between simple steatosis and steatohepatitis as in the case of sonography. Admittedly, it is more expensive method, not easily reproducible, and may be difficult to perform in children. Finally, the present study's sample size was not calculated *a priori*, but the power of the study was calculated *a posteriori* using the observed results. The study had a power of 98 % to observe significant differences between mean CIMT in obese children with NAFLD and those without NAFLD at the 5 % level.

The present study's findings indicate that the obese children with and without NAFLD have a greater risk of future cardiovascular disease. The risk was greater in obese children with NAFLD, but not significantly. Similarly, CIMT in obese children with NAFLD was significantly greater than in those without NAFLD and the controls, theoretically placing them at higher risk of atherosclerosis. To the best of our knowledge, the present study is the first to report that CIMT values and the occurrence of MetS increased as degree of steatosis increased. Nevertheless, NAFLD is not a single factor but rather acts in concert with other metabolic abnormalities. The relationship between NAFLD and other metabolic abnormalities associated with increased risk of atherosclerosis is only a statistical reflection and may not be relevant to clinical outcome. Long-term longitudinal studies are required to more definitively determine if pediatric NAFLD is a risk factor for future cardiovascular disease.

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