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# Association Between Metabolic Syndrome and Liver Histology Among Children With Nonalcoholic Fatty Liver Disease

**Heather M. Patton, MD**[1], **Katherine Yates**[2], **Aynur Unalp-Arida, MD, PhD**[2], **Cynthia A. Behling, MD, PhD**[3], **Terry T.-K. Huang, PhD, MPH**[4], **Philip Rosenthal, MD**[5], **Arun J. Sanyal, MD**[6], **Jeffrey B. Schwimmer, MD**[1], **Joel E. Lavine, MD, PhD**[1], and **the NASH CRN**

[1]Departments of Medicine and Pediatrics, University of California, San Diego, San Diego, California, USA

[2]Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA

[3]Pacific Rim Pathology Group, San Diego, California, USA

[4]Obesity Research Strategic Core, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland, USA

[5]Department of Pediatrics, University of California, San Francisco, San Francisco, California, USA

[6]Department of Medicine, Virginia Commonwealth University, Richmond, Virginia, USA.

## Abstract

**OBJECTIVES—**Nonalcoholic steatohepatitis (NASH) is considered the hepatic manifestation of metabolic syndrome (MetS) among adults. Emerging data suggest that MetS may be associated with nonalcoholic fatty liver disease (NAFLD) in children as well. We sought to determine whether MetS or its component features are associated with specific histological features or severity of NAFLD.

**METHODS—**Children and adolescents aged 6 – 17 years enrolled in the Nonalcoholic Steatohepatitis Clinical Research Network (NASH CRN) with clinical data obtained within 6 months of liver biopsy were included. MetS was defined as the presence of three or more of the following features as determined by application of age-adjusted normative values: central obesity, dyslipidemia, impaired fasting glucose, and elevated blood pressure. Liver biopsies were evaluated by the Pathology Committee of the NASH CRN.

**RESULTS—**Two hundred fifty four children were included in the analysis, of whom 65 (26 %) met specified criteria for MetS. Among children with MetS, there is a higher proportion of females who were on average older in age and pubertal. The risk of MetS was greatest among those with



**Correspondence:** Joel E. Lavine, MD, PhD, Morgan-Stanley Children's Hospital of New York, 3959 Broadway, CHN7-702, New York, New York 10032, USA. jl3553@columbia.edu.

**Specific author contributions** : Heather Patton: conception of the study, study design, data collection, article preparation; Katherine Yates: study design, data analysis, article preparation; Aynur Unalp-Arida: study design, data analysis, article review; Cynthia Behling: data collection, article review; Terry Huang: study design, article review; Philip Rosenthal: data collection, article review; Arun Sanyal: data collection, article review; Jeffrey Schwimmer: study design, data collection, article review; Joel Lavine: conception of the study, study design, data collection, article review.

**SUPPLEMENTARY MATERIAL** is linked to the online version of the paper at http://www.nature.com/ajg

CONFLICT OF INTEREST

**Guarantor of the article** : Heather Patton, MD.

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severe steatosis (odds ratio (OR) = 2.58 for grade 3 vs. grade 1 steatosis, $P = 0.001$). The presence of hepatocellular ballooning was also significantly associated with MetS (OR = 2.10, $P = 0.03$). Those with advanced fibrosis (stage 3/4) had an OR for MetS of 3.21 ($P = 0.04$) vs. those without fibrosis (stage 0). Borderline zone 1 or definite NASH patterns compared with " not NASH " were strongly associated with MetS (OR = 4.44, $P = 0.005$ and OR = 4.07, $P = 0.002$, respectively). The mean NAFLD Activity Score (NAS) was greater among children with MetS vs. those without (4.8 ± 1.4 vs. 4.3 ± 1.4, $P = 0.01$). Central obesity was significantly associated with steatosis, fibrosis, hepatocellular ballooning, and NAFLD pattern. Insulin resistance was significantly associated with steatosis, fibrosis, hepatocellular ballooning, NAS, and NAFLD pattern.

**CONCLUSIONS—**MetS is common among children with NAFLD and is associated with severity of steatosis, hepatocellular ballooning, NAS, NAFLD pattern, and the presence of advanced fibrosis. Individual MetS features, particularly central obesity and insulin resistance, were also associated with severity of NAFLD. MetS features should be considered in children with NAFLD as individually and collectively they help identify children with more advanced disease.

## INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of pediatric chronic liver disease in North America. Data from the US National Health and Nutrition Examination Survey (NHANES) show elevated serum alanine aminotransferase (ALT) to be prevalent in 8 % of adolescents 12 – 19 years of age and histology-based population prevalence estimates are 8 % among children aged 2–19 years (1,2). The World Health Organization introduced the term " metabolic syndrome " in 1998 to label a constellation of cardiovascular risk factors that are associated with insulin resistance (3). The component features of metabolic syndrome (MetS) are central obesity, elevated blood pressure, atherogenic dyslipidemia, insulin resistance, proinflammatory state, and prothrombotic state (4). In adults, nonalcoholic steatohepatitis (NASH) is considered the hepatic manifestation of MetS (5). MetS is an independent predictor of steatosis, fibrosis, and NASH on liver biopsy among adults with NAFLD (6–8).

Evidence for a relationship between MetS and NAFLD in children is emerging. The Korean National Health and Nutrition Examination Survey found participants aged 10 – 19 years with three or more risk factors for MetS had an odds ratio of 6.2 (95 % CI 2.3 – 16.8) for an elevated serum ALT, which they used as an indicator of fatty liver (9). More recently, a case – control study of overweight children with biopsy-proven NAFLD and age-, sex-, and obesity-matched controls found that children with NAFLD were significantly more likely to have MetS than obese controls without evidence of fatty liver disease (50 vs. 15 % , $P < 0.001$)(10). A single center study from Italy reported MetS to be present in 65.8 % of children (3 – 18 years) with biopsy-proven NAFLD and found grade of fibrosis (fibrosis was absent in 33 % and none of the subjects had cirrhosis) to be the only histological feature significantly associated with MetS on univariate analysis (11). It is noted that the criteria used to define MetS in these studies was not uniform as a consensus on the definition of this syndrome in children remains in evolution.

The National Institute of Diabetes and Digestive and Kidney Diseases, with additional support from the National Institute of Childhood Health and Human Development, funded a Nonalcoholic Steatohepatitis Clinical Research Network (NASH CRN) beginning in 2002 to assess the etiology, natural history, and therapy of NAFLD (12). The prospective, multicenter ascertainment of clinical data concurrent with liver histology for the NASH CRN provides a unique opportunity to study the relationship between clinical variables and specific histological features of NAFLD (13). The aim of this study is to evaluate the

association between features of MetS and liver histology among children and adolescents with NAFLD enrolled in the NASH CRN. The study hypothesis is that features of MetS, correlates of risk for development of premature coronary artery disease and diabetes mellitus, may correspond to increased severity of particular histological features of NASH among children and adolescents with NAFLD.

## METHODS

### Study sample

The treatment of NAFLD in children (TONIC) and NAFLD Database studies have Institutional Review Board approval at each of the eight clinical centers participating in the NASH CRN (see Supplementary Appendix A online). Written consent was obtained from a parent or guardian and written assent obtained from all children 8 years and older before participation. Th e NAFLD Database is an observational study of patients 2 years and older and TONIC is a phase III, double-masked, randomized, placebo-controlled trial of metformin and vitamin E in children between the ages 7 and 17 years with NAFLD (13,14). Exclusion criteria for both studies include significant alcohol intake, other chronic liver diseases, history of parenteral nutrition, bariatric or hepatobiliary surgery, HIV infection, or short bowel syndrome. Participants in TONIC, but not NAFLD database, were required to have a baseline serum ALT value > 60 U / L. Additional exclusion criteria for the TONIC trial include diagnosis of diabetes mellitus, cirrhosis, use of drugs associated with NAFLD, anti-diabetic or anti-NAFLD drugs, metabolic acidosis, and renal dysfunction (these exclusion criteria do not apply to NAFLD database participants). Enrollment for the NAFLD database began in September 2004 and TONIC in August 2005. Participants from both studies, aged 6 – 17 years, were eligible for inclusion if they had baseline clinical data to evaluate features of the metabolic syndrome within 6 months of liver biopsy (only baseline, pre-treatment liver biopsies were used from children enrolled in the TONIC trial). Liver biopsy specimens underwent central uniform review by the Pathology Committee of the NASH CRN.

### Clinical data

Demographic data were obtained via structured interview and questionnaires. Height, weight, waist, and hip measurements were taken in duplicate with patients standing and wearing light clothing. Height and weight were measured without shoes to the nearest 0.1 cm and 0.1 kg, respectively. Body mass index (BMI) was calculated as the weight (kg) divided by the height (m) squared. BMI percentile was determined according to age and gender based on data from the Centers for Disease Control and Prevention (15). Waist measurement was taken at the midpoint between the highest point of the iliac crest and the lowest part of the costal margin. Hip measurement was taken at the fullest part of the hips. Resting blood pressure (mm Hg) was measured from an upper extremity using an appropriately sized cuff and an automated sphygmomanometer.

### Laboratory assays

Fasting whole blood samples were obtained via venipuncture following an (mg / dl), total cholesterol (mg / dl), HDL cholesterol (mg / dl), LDL cholesterol (mg / dl), overnight fast of 12 h and processed for plasma and serum within 2 h. Th e following laboratory assays were included at individual clinical centers: ALT (U / L), AST (U / L), triglycerides fasting insulin (μU/ml),fastingglucose (mg / dl), and HbA1c (%). Fasting insulin and glucose values were used to determine the homeostatic model assessment of insulin resistance (HOMA-IR) and the Quantitative Insulin Sensitivity CheckIndex(QUICKI)(16,17).

### Histology

Liver histology (H & E and Masson's trichrome stain) was centrally reviewed by the Pathology Committee of the NASH CRN blinded to clinical data. Biopsies were scored for the following features according to NASH CRN criteria published by Kleiner *et al.* (18): Steatosis (grade 0 (< 5% macrovesicular fat in hepatocytes), grade 1 (5–33%), grade 2 (34–66%), grade 3(>66%)), portal inflammation (0–2), lobular inflammation (0–3), hepatocellular ballooning (0–2),andfibrosis (stage 0, stage 1a (mild perisinusoidal), stage 1b (moderate perisinusoidal), stage 1c (portal / periportal fibrosis only), stage 2 (zone 3 and periportal), stage 3 (bridging fibrosis), stage 4 (cirrhosis)). A NAFLD activity score (NAS) was tabulated by summing the scores for steatosis, lobular inflammation, and ballooning degeneration (1–8 score possible). For regression analyses, all fibrosis stage 1 (1a, 1b, and 1c) biopsies were combined and treated as stage 1, and stage 3 and 4 biopsies were combined and treated as stage 3, advanced fibrosis and a categorical NAS score was defined as: 0 = 1 –3 (reference), 1 = 4–5, and 2 = 6–8.

In addition to determining the NAS score, an overall diagnostic categorization was determined for each case: “ Not NASH ” , “ Borderline ” or suspicious for NASH with a zone 3 or zone 1 pattern, or “Definite NASH” (19). “Definite NASH ” was unequivocally diagnostic of steatohepatitis, whereas the category of “ Not NASH ” encompasses cases of NAFLD in which the changes are so mild or non-specific that more specific classification cannot be made (20). The intermediate category of “ Borderline zone 3 ” was created for cases that had some, but not all, histological features of steato-hepatitis. “ Borderline zone 1 ” was used for cases that fit the zone 1 pattern of injury previously described in children and had a portal (Zone 1) predominant pattern of injury (21).

### Definitions and reference standards for metabolic syndrome

We used criteria for defining the presence of MetS as previously published (4). Gender- and age-specific criteria were used to define presence of the following MetS components: central obesity (waist circumference > 102 cm for males, > 88 cm for females) (22), high systolic blood pressure (SBP) and / or diastolic blood pressure (DBP) (> 95th percentile for age, sex, and height) (23), low HDL cholesterol (<5th percentile for age and race/ethnicity) (24), high triglycerides (TG) (>95th percentile for age and sex) (25–27) and impaired fasting glucose (IFG, fasting glucose ≥100mg/dl) (28). Pre-existing diagnosis or treatment for hypertension (HTN), high triglycerides or type 2 diabetes was also assessed from patient's baseline clinical history. As defined by the National Cholesterol Education Program Adult Treatment Panel III (ATP) for adults, children were classified as having the metabolic syndrome if they met 3 or more of the above criteria (4,22,29). Results for HOMAIR and QUICKI were analyzed as additional markers of insulin resistance.

The significance of the relationship between MetS and ethnicity groups and baseline characteristics or histology was determined from a Wilcoxon two-sample test for continuous variables, and either a $\chi^2$ test for non-ordered categories, a Cochran-Armitage trend test for ordered categories, Fisher's exact test, or binary or multinomial logistic regression for categorical variables (30,31). Adjusted odds ratios or cumulative odds ratios of each categorical histological feature with the presence of a component of MetS were estimated from either a logistic (for binary outcomes), multinomial or ordinal logistic regression model. *P* values were determined from a likelihood ratio test. If the assumption of a rank outcome was met, then an ordinal logistic regression model was used (steatosis, fibrosis, NAS); otherwise, the multinomial logistic model was used (NAFLD pattern) (32,33). Regression models presented were adjusted for sex, ethnicity (Hispanic / not), age group (6–11, 12–14, 15–17 years), puberty (yes/no). An ethnicity by risk factor interaction term was included in the model if the interaction was significant (*P* 0.01) and quantitative. Including

BMI *z*-score in the models did not significantly improve model fit, so this covariate was dropped. However, as BMI *z*-score had a highly significant correlation with fibrosis, additional analyses including BMI *z*-score as a covariate were computed for fibrosis on each component of MetS to test whether associations were independent of BMI *z*-score. All analyses were based on baseline data available through August 2009 and were performed using SAS statistical software (version 9.1; SAS Institute, Cary, NC) and STATA (release 10.0; Stata Corp, College Station, TX) software. *P* values presented are two-sided and have not been adjusted for multiplecomparisons.

## RESULTS

Of the 123 / 195 (63 %) children in NAFLD Database with liver biopsies that underwent central review, 37 were excluded because they were not within 6 months of clinical data. All of the 173 (100%) children in TONIC with liver biopsies who underwent central review were within 6 months of clinical data. One child was excluded for age < 6 years and four were excluded for absence of steatosis on liver biopsy, leaving a total of 254 children with NAFLD eligible for study inclusion. Characteristics of children included in this study are summarized in Table 1. Overall, 65 (25.6 %) met criteria for diagnosis of MetS. Among children with MetS, there was a higher proportion of females who were older in age and pubertal (Tanner stage 2 – 4). Race / ethnicity did not differ between children with and without MetS. Measures of obesity were more severe among children with MetS (higher BMI, BMI *z*-score, and waist and hip circumferences). Children with MetS had a higher prevalence of previously diagnosed and / or treated diabetes, hyperlipidemia, and hypertension. As anticipated, children with MetS had higher mean values of blood pressure, triglycerides, and HOMA-IR with lower mean values of HDL cholesterol and QUICKI. Frequency and distribution of the individual components of MetS are shown in Figure 1. Among children with NAFLD, 33 (13.5 %) had no features of MetS and only two (0.8 %) had all five features present. Central obesity (67.1 %) and hypertension (44.6 %) were the most prevalent MetS components in this study population. About one quarter of children with NAFLD had dyslipidemia. Impaired fasting glucose was the least prevalent (12.2 %) of the MetS features.

Because of the high percentage of children of Hispanic ethnicity in the study population, the prevalence of MetS features was evaluated according to ethnicity to determine if any important differences were observed. Hispanic children ($n$ = 153) had no difference in measures of obesity (prevalence of central obesity 66.5 vs. 68.0 % , $P$ = 0.80) compared with non-Hispanic children ($n$ = 101). The prevalence of hypertriglyceridemia was lower among children of Hispanic ethnicity (22.9 vs. 33.3 % , $P$ = 0.07), whereas the prevalence of low HDL cholesterol was higher (30.1 vs. 19.2%, $P$ = 0.05). No difference was seen in the prevalence of HTN (47.0 % in Hispanic vs. 41.0 % in non-Hispanic children, $P$ = 0.35). Prevalence of IFG did not differ according to ethnicity, but severity of insulin resistance by fasting insulin (43.2 ± 52.7 μU/ml vs. 28.4±19.1μU/ml, $P$ = 0.003), HOMA-IR (9.8±13.0 vs. 6.2±4.2, $P$ = 0.003),and QUICKI (0.30±0.04 vs. 0.31±0.04, $P$ = 0.003) was greater among Hispanic children.

The frequency and severity of histological features of NAFLD was compared among children with and without MetS (Table 2). Severity of steatosis, hepatocellular ballooning, presence of advanced fibrosis, NAFLD pattern, and NAS were all significantly associated with a diagnosis of MetS after adjustment for sex, age, ethnicity, and Tanner stage. The risk of MetS was greatest among those with severe steatosis (OR = 2.85 for grade 3 vs. grade 1 steatosis, $P$ = 0.001). The COR was 2.11 ($P$ = 0.008) when comparing each level of steatosis to the ones before it. Higher levels of hepatocellular ballooning were significantly associatedwith MetS (OR = 2.01, $P$ = 0.03). There was no significant difference in the

association of MetS with the overall category of fibrosis, but those with advanced fibrosis (stage 3 / 4) had an OR for MetS of 3.21 ($P = 0.04$) vs. those without fibrosis (stage 0). Borderline zone 1 or definite NASH patterns compared with not NASH were strongly associated with MetS (OR = 4.44, $P = 0.005$ and OR = 4.07, $P = 0.002$, respectively). The mean NAS was greater among children with MetS vs. those without (4.8 +/− 1.4 vs. 4.3 +/− 1.4, $P = 0.01$).

Steatosis severity, fibrosis severity, NAFLD Activity Score (NAS), hepatocellular ballooning, and NAFLD pattern were further evaluated to see if the number of MetS features or the severity of individual MetS components had any relationship to these histological variables (Figure 2 and Table 3). In addition to an association with MetS, severity of steatosis was also significantly associated with the severity of certain component features of MetS. Steatosis grade was most strongly associated with severity of HTN (COR = 2.08, $P = 0.003$). The MetS score or total number of features (overall $P = 0.05$) and central obesity as reflected by waist hip ratio (COR = 1.48, $P = 0.05$) were also associated with steatosis severity. Although IFG was not significantly associated with steatosis severity, insulin resistance as measured by QUICKI was (COR 0.928, $P = 0.04$) (Figure 2a).

As noted earlier, overall severity of fibrosis was not significantly associated with MetS (Figure 2b). However, odds of more severe fibrosis were markedly increased if obesity was present as defined by BMI *z*-score (COR = 3.40, $P < 0.001$) as well as central obesity (COR = 1.78, $P = 0.03$), increased waist circumference (COR = 1.03, $P < 0.001$) and increased waist hip ratio (OR = 1.99, $P < 0.001$). The only other MetS feature associated with fibrosis stage was lower HDL cholesterol (COR = 0.97, $P = 0.04$), though insulin resistance, as measured by fasting insulin (COR = 1.03, $P = 0.002$), HOMAIR (COR = 1.15, $P = 0.001$), and QUICKI (COR = 0.90, $P = 0.002$) were associated with this histological feature. When the model included BMI *z*-score as an additional adjustment factor, an association with central obesity and increased severity of fibrosis remained as defined by the waist hip ratio (COR = 1.74, $P = 0.007$), as well as insulin resistance measured by fasting insulin, HOMA-IR and QUICKI (COR = 1.03, $P = 0.008$, COR = 1.14, $P = 0.006$ and COR = 0.92, $P = 0.03$, respectively). The association with lower HDL cholesterol no longer remained significant (COR = 0.97, $P = 0.08$).

Although the presence of MetS was predictive of a higher NAS (COR = 2.12, $P = 0.009$), associations were also presentwith higher triglyceride levels (COR = 1.003, $P = 0.04$), lower HDL cholesterol levels (COR = 0.97, $P = 0.02$), and a diagnosis of hypertension (COR = 1.65, $P = 0.04$). Insulin resistance as measured by QUICKI (COR = 0.86, $P < 0.001$) was also associated with NAS (Figure 2c).

Hepatocellular ballooning was associated with the presence of central obesity (OR = 2.15, $P = 0.01$), BMI *z*-score (OR = 2.17, $P = 0.04$), and higher triglycerides (OR = 1.0043, $P = 0.02$). Insulin resistance as measured by QUICKI (OR = 0.90, $P = 0.01$) was also associated, and for non-Hispanics, IFG was predictive of hepato-cellular ballooning (OR = 14.31, $P = 0.01$) (Figure 2d).

In addition to the strong association between diagnosis of MetS and borderline zone 1 or definite NASH vs. not NASH patterns, certain component features of MetS were also associated with the NAFLD pattern as shown in Table 3. Central obesity was highly associated with borderline zone 1 or definite NASH patterns (OR = 2.71, $P = 0.02$ and OR = 4.08, $P = 0.001$ vs. not NASH, respectively). HTN was predictive of borderline zone 1 patternvs.notNASH (OR = 3.02, $P = 0.006$). Although IFG was not significantly associated with NAFLD pattern, severity of insulin resistance (Fasting insulin, HOMA-IR, and

QUICKI) were associated with the definite NASH pattern vs. not NASH (OR = 1.052, $P$ = 0.01, OR = 1.283, $P$ = 0.007, and OR = 0.786, $P$ <0.001, respectively).

## DISCUSSION

In adults, NASH is considered the hepatic manifestation of the metabolic syndrome (5). Although adult studies of NAFLD have found MetS to be an independent predictor of steatosis, fibrosis, and NASH on liver biopsy, ours is the first study to evaluate the association between components of MetS in children with features of NAFLD histology (6–8,34). The potential significance of understanding the association between MetS and NAFLD includes provision of prognostic markers, NAFLD relation to cardiovascular risk factors, insight into disease pathophysiology, and potential targets for therapeutic intervention. The potential power of MetS as a prognostic indicator of disease severity in NAFLD lies in the fact that determination of MetS components is relatively non-invasive. This may help to identify children at greatest risk for advanced fatty liver disease and facilitate appropriate evaluation of such individuals.

This study is the largest histology-based study of MetS in children with NAFLD and the only such study, which is prospective, or multi-center, or where liver biopsies were evaluated by a committee of pathologists with expertise in NAFLD histopathology. We applied age- and sex-adjusted normative values in defining the component features of MetS and required that these features had been evaluated within 6 months of liver biopsy. Using these criteria, the prevalence of MetS was 25.6 %, central obesity and hypertension (HTN) being the most common of the MetS features observed. In comparison, estimates of MetS in the general population among adolescents 8 to 19 years of age using data from NHANES range from 1.25 to 9.4 % (35–39). Only 13.5 % of children had zero features of MetS and the majority (77.2 %) had one to three features present. A diagnosis of MetS (three or more features present) was predictive of steatosis severity, NAS, hepatocellular ballooning and NAFLD pattern (borderline zone 1 or definite NASH). Fibrosis stage was not associated with a diagnosis of MetS; however, advanced fibrosis compared with no fibrosis was significant. This is in contrast with results of a single-center, Italian study with 120 Caucasian subjects (11). In this study, the reported prevalence of MetS was 65.8% and fibrosis was the only histological feature of NAFLD associated with the presence of MetS (details regarding distribution of the severity of fibrosis were not provided for this study for comparison). The significantly lower prevalence rate of MetS in our study population may be related to the criteria used to define MetS. We used age-adjusted normative values for blood pressure and lipids and used the most conservative cutoff values for HDL cholesterol and triglycerides (<5th percentile and > 95th percentile, respectively). We also used impaired fasting glucose to define insulin resistance as results for glucose tolerance were not available for the entire study sample. Furthermore, most of the children included in this analysis were from the TONIC study, which excluded those with impaired fasting glucose. Th e conservative criteria used to define MetS in our study may help to account for the associations that were identified with several histo-logical features of NAFLD in contrast to the study by Manco *et al.* (11) in which fibrosis was the only histological variable associated with MetS. As the criteria for MetS in this age group remains in flux, the prevalence of MetS among children with NAFLD and the strength of the relationship between MetS and features of NAFLD is likely to vary until consensus on the definition of MetS in children has been reached.

In addition to an overall diagnosis of MetS, we also evaluated the component features of MetS and their severity to see if these were associated with histological features of NAFLD. Of these, severity of insulin resistance was the component most consistently associated with histological features of NAFLD, showing significant associations with severity of steatosis,

fibrosis, NAS, hepatocellular ballooning, and NAFLD pattern. Importantly, fasting glucose values were not significantly associated with any of the histological features in this population of children with NAFLD. Thus while fasting glucose is the most common screening undertaken to assess for insulin resistance, it does not appear adequately sensitive, at least at levels currently recommended for diagnosis of IFG, to distinguish histological features of NAFLD in children. Central obesity was associated with steatosis grade, fibrosis stage, hepatocellular ballooning and NAFLD pattern and HTN with steatosis grade, NAS, and NAFLD pattern. The use of adult values for definition of central obesity may have resulted in misclassification error, particularly among the youngest children in the study. Thus, the true impact of central obesity may have been underestimated in our results.

The potential basis of NAFLD comorbidity with MetS may be common underlying abnormalities present in these conditions such as insulin resistance and oxidative stress. The most widely accepted paradigm of NASH pathogenesis is that of the “two-hit hypothesis” in which the primary abnormality, accumulation of triglyceride droplets within hepatocytes, occurs as a consequence of insulin resistance (40). Peripheral insulin resistance leads to hyperinsulinemia, which promotes *de novo* hepatic lipogenesis through upregulation of lipogenic transcription factors, and an increased flux of free fatty acids to the liver due to loss of insulin-mediated suppression of lipolysis (41,42). Hepatic export of triglycerides as very low-density lipoprotein may also be impaired (43–45). Following hepatocellular trigylceride accumulation, the steatotic liver is vulnerable to additional insults, the so-call “second hit.” Proposed sources of this secondary injury include oxidative stress, adipocytokines, and gut-derived bacterial endotoxins. The pathogenesis of the clustering of metabolic risk factors termed MetS is complex and may not have a single underlying cause, but appears to depend upon the presence of two major factors (excess body fat and metabolic susceptibility), which may be driven by several mediators. These include defects in insulin signaling and mitochondrial oxidative pathways (46). Ectopic fat deposition, including NAFLD, may also be an important factor in the pathogenesis of MetS among overweight and obese individuals (47).

A recent case – control study compared 150 overweight children with biopsy-proven NAFLD to 150 age-, sex-, and obesity-matched children without evidence of NAFLD and found that, after adjustment for age, sex, race, ethnicity, and hyperinsulinemia, children with MetS had an odds ratio of 5.0 (95% CI 2.6 – 9.7) for NAFLD compared with children without MetS (10). This is the most compelling data to support a significant relationship between NAFLD and MetS, not explicable merely by the coexistence of overweight or obesity in these two conditions, and lend support to the hypothesis that fat accumulation in the liver has an important role in the pathogenesis of other obesity-related comorbidities.

While an understanding of the clinical significance of MetS in childhood is still evolving, there are data that show pediatric MetS to be predictive of pre-clinical and clinical cardiovascular disease. Childhood (age 3 – 18 years) LDL cholesterol, SBP, BMI, and smoking have been shown to be predictive of intima-medial thickness, a marker of preclinical atherosclerosis, in young adults (ages 24 – 39 years) (48). Long-term follow-up of participants in the National Heart, Lung, and Blood Institute Lipid Research Clinics (LRC) Princeton Prevalence Study who were included in the Princeton Follow-up Study assessed the association of MetS in childhood with adult cardiovascular disease 25 years later (49). In a multivariable analysis, pediatric MetS (OR 14.7, $P < 0.0001$) and age (OR 1.2, $P = 0.03$) were significant predictors of cardiovascular disease in adulthood. These data support the concept of pediatric MetS as an important marker for future comorbidity and highlight the importance of further studies to investigate the implications of MetS in this age group, including its relationship with NAFLD.

In conclusion, MetS appears to be common among children with NAFLD, affecting approximately one quarter of children enrolled in the NASH CRN. Central obesity and HTN were the most prevalent MetS features present in children with NAFLD. The presence of MetS was associated with steatosis, NAS, advanced fibrosis, hepato-cellular ballooning, and a diagnosis of borderline zone 1 or definite NASH, thus may be useful as a clinical indicator of children who are likely to have more severe histological findings. Of the MetS features, central obesity and insulin resistance (indicated by fasting insulin, HOMA-IR, or QUICKI) were most consistently associated with NAFLD histology. These results indicate that an evaluation for MetS features should be considered in children being evaluated for NAFLD as individually and collectively they may help to identify children with more advanced disease. Future studies will be required to establish the relationship of MetS with long-term prognosis and response to treatment in children with NAFLD.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## REFERENCES

1. Fraser A, Longnecker MP, Lawlor DA. Prevalence of elevated alanine aminotransferase among US adolescents and associated factors: NHANES 1999–2004. Gastroenterology. 2007; 133:1814–20. [PubMed: 18054554]
2. Schwimmer JB, Deutsch R, Kahen T, et al. Prevalence of fatty liver in children and adolescents. Pediatrics. 2006; 118:1388–93. [PubMed: 17015527]
3. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med. 1998; 15:539–53. [PubMed: 9686693]
4. Grundy SM, Brewer HB Jr, Cleeman JI, et al. Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. Circulation. 2004; 109:433–8. [PubMed: 14744958]
5. Marchesini G, Marzocchi R, Agostini F, et al. Nonalcoholic fatty liver disease and the metabolic syndrome. Curr Opin Lipidol. 2005; 16:421–7. [PubMed: 15990591]
6. Kang H, Greenson JK, Omo JT, et al. Metabolic syndrome is associated with greater histologic severity, higher carbohydrate, and lower fat diet in patients with NAFLD. Am J Gastroenterol. 2006; 101:2247–53. [PubMed: 17032189]
7. Ryan MC, Wilson AM, Slavin J, et al. Associations between liver histology and severity of the metabolic syndrome in subjects with nonalcoholic fatty liver disease. Diabetes Care. 2005; 28:1222–4. [PubMed: 15855597]
8. Marchesini G, Bugianesi E, Forlani G, et al. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. Hepatology. 2003; 37:917–23. [PubMed: 12668987]
9. Park HS, Han JH, Choi KM, et al. Relation between elevated serum alanine aminotransferase and metabolic syndrome in Korean adolescents. Am J Clin Nutr. 2005; 82:1046–51. [PubMed: 16280437]

10. Schwimmer JB, Pardee PE, Lavine JE, et al. Cardiovascular risk factors and the metabolic syndrome in pediatric nonalcoholic fatty liver disease. Circulation. 2008; 118:277–83. [PubMed: 18591439]
11. Manco M, Marcellini M, Devito R, et al. Metabolic syndrome and liver histology in paediatric non-alcoholic steatohepatitis. Int J Obes (Lond). 2008; 32:381–7. [PubMed: 18087267]
12. Lavine JE, Schwimmer JB. Pediatric initiatives within the Nonalcoholic Steatohepatitis-Clinical Research Network (NASH CNR). J Pediatr Gastroenterol Nutr. 2003; 37:220–1. [PubMed: 12971361]
13. Patton HM, Lavine JE, Van Natta ML, et al. Clinical correlates of histopathology in pediatric nonalcoholic steatohepatitis. Gastroenterology. 2008; 135:1961–71e2. [PubMed: 19013463]
14. Lavine JE, Schwimmer JB, Molleston JP, et al. Treatment of nonalcoholic fatty liver disease in children: TONIC trial design. Contemporary Clinical Trials. 2010; 31:62–70. [PubMed: 19761871]
15. Kuczmarski RJ, Ogden CL, Guo SS, et al. CDC Growth Charts for the United States: methods and development. Vital Health Stat. 2000; 2002; 11:1–190.
16. Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985; 28:412–9. [PubMed: 3899825]
17. Katz A, Nambi SS, Mather K, et al. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. J Clin Endocrinol Metab. 2000; 85:2402–10. [PubMed: 10902785]
18. Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology. 2005; 41:1313–21. [PubMed: 15915461]
19. Kleiner DE, Behling CB, Brunt EM, et al. Comparison of adult and pediatric NAFLD-confirmation of a second pattern of progressive fatty liver disease in children. Hepatology. 2006; 44:259A–60A.
20. Brunt EM. Pathology of nonalcoholic steatohepatitis. Hepatol Res. 2005; 33:68–71. [PubMed: 16214395]
21. Schwimmer JB, Behling C, Newbury R, et al. Histopathology of pediatric nonalcoholic fatty liver disease. Hepatology. 2005; 42:641–9. [PubMed: 16116629]
22. National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation. 2002; 106:3143–4421. [PubMed: 12485966]
23. National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents. The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. Pediatrics. 2004; 114:555–76. [PubMed: 15286277]
24. Hickman TB, Briefel RR, Carroll MD, et al. Distributions and trends of serum lipid levels among United States children and adolescents ages 4–19 years: data from the Third National Health and Nutrition Examination Survey. Prev Med. 1998; 27:879–90. [PubMed: 9922071]
25. Schulpis K, Karikas GA. Serum cholesterol and triglyceride distribution in 7767 school-aged Greek children. Pediatrics. 1998; 101:861–4. [PubMed: 9565415]
26. Jolliffe CJ, Janssen I. Distribution of lipoproteins by age and gender in adolescents. Circulation. 2006; 114:1056–62. [PubMed: 16940191]
27. Yip PM, Chan MK, Nelken J, et al. Pediatric reference intervals for lipids and apolipoproteins on the VITROS 5, 1 FS Chemistry System. Clin Biochem. 2006; 39:978–83. [PubMed: 16962571]
28. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2008; 31(Suppl 1):S55–60. [PubMed: 18165338]
29. Alberti KG, Zimmet P, Shaw J. The metabolic syndrome--a new worldwide definition. Lancet. 2005; 366:1059–62. [PubMed: 16182882]
30. Agresti, A. Categorical Data Analysis. John Wiley & Sons, Inc.; New York: 1990.
31. McCullagh P. Regression models for ordinal data (with discussion). Journal of the Royal Statistical Society, B. 1980; 42:109–42.

32. Brant R. Assessing proportionality in the proportional odds model for ordinal logistic regression. Biometrics. 1990; 46:1171–8. [PubMed: 2085632]
33. Hosmer, D.; Lemeshow, S. Applied Logistic Regression. Second edn.. John Wiley & Sons, Inc.; New York: 2000.
34. Patton HM, Unalp A, Yates K, et al. Metabolic syndrome among children with variable features of nonalcoholic fatty liver disease. Hepatology. 2007; 46:730A. [PubMed: 17668885]
35. Pan Y, Pratt CA. Metabolic syndrome and its association with diet and physical activity in US adolescents. J Am Diet Assoc. 2008; 108:276–86. discussion 286. [PubMed: 18237576]
36. Ford ES, Li C, Zhao G, et al. Prevalence of the metabolic syndrome among US adolescents using the definition from the International Diabetes Federation. Diabetes Care. 2008; 31:587–9. [PubMed: 18071007]
37. Messiah SE, Arheart KL, Luke B, et al. Relationship between body mass index and metabolic syndrome risk factors among US 8- to 14-year-olds, 1999–2002. J Pediatr. 2008; 153:215–21. [PubMed: 18534237]
38. Cook S, Auinger P, Li C, et al. Metabolic syndrome rates in United States adolescents, from the National Health and Nutrition Examination Survey, 1999–2002. J Pediatr. 2008; 152:165–70. [PubMed: 18206683]
39. Kranz S, Mahood LJ, Wagstaff DA. Diagnostic criteria patterns of US children with Metabolic Syndrome: NHANES 1999–2002. Nutr J. 2007; 6:38. [PubMed: 17986354]
40. Day CP, James OF. Steatohepatitis: a tale of two “hits”? Gastroenterology. 1998; 114:842–5. [PubMed: 9547102]
41. Lewis GF, Carpentier A, Adeli K, et al. Disordered fat storage and mobilization in the pathogenesis of insulin resistance and type 2 diabetes. Endocr Rev. 2002; 23:201–29. [PubMed: 11943743]
42. Azzout-Marniche D, Becard D, Guichard C, et al. Insulin effects on sterol regulatory-element-binding protein-1c (SREBP-1c) transcriptional activity in rat hepatocytes. Biochem J. 2000; 350(Part 2):389–93. [PubMed: 10947952]
43. Namikawa C, Shu-Ping Z, Vyselaar JR, et al. Polymorphisms of microsomal triglyceride transfer protein gene and manganese superoxide dismutase gene in non-alcoholic steatohepatitis. J Hepatol. 2004; 40:781–6. [PubMed: 15094225]
44. Letteron P, Sutton A, Mansouri A, et al. Inhibition of microsomal triglyceride transfer protein: another mechanism for drug-induced steatosis in mice. Hepatology. 2003; 38:133–40. [PubMed: 12829995]
45. Charlton M, Sreekumar R, Rasmussen D, et al. Apolipoprotein synthesis in nonalcoholic steatohepatitis. Hepatology. 2002; 35:898–904. [PubMed: 11915037]
46. Grundy SM. Metabolic syndrome: a multiplex cardiovascular risk factor. J Clin Endocrinol Metab. 2007; 92:399–404. [PubMed: 17284640]
47. Unger RH. Minireview: weapons of lean body mass destruction: the role of ectopic lipids in the metabolic syndrome. Endocrinology. 2003; 144:5159–65. [PubMed: 12960011]
48. Raitakari OT, Juonala M, Kahonen M, et al. Cardiovascular risk factors in childhood and carotid artery intima-media thickness in adulthood: the Cardiovascular Risk in Young Finns Study. Jama. 2003; 290:2277–83. [PubMed: 14600186]
49. Morrison JA, Friedman LA, Gray-McGuire C. Metabolic syndrome in childhood predicts adult cardiovascular disease 25 years later: the Princeton Lipid Research Clinics Follow-up Study. Pediatrics. 2007; 120:340–5. [PubMed: 17671060]

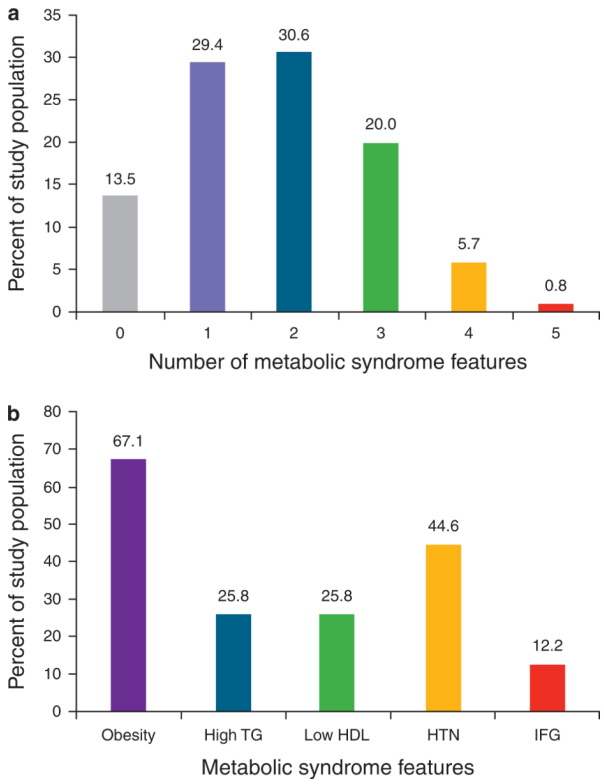


**Figure 1.**

Percentage of children with NAFLD and metabolic syndrome (MetS) features. (**a**) Percent with 0, 1, 2, 3, 4, or 5 features of MetS. (**b**) Percent with each MetS feature. Obesity: central obesity, High TG: high triglycerides, Low HDL: low level of high-density lipoprotein cholesterol, HTN: hypertension, IFG: impaired fasting glucose.

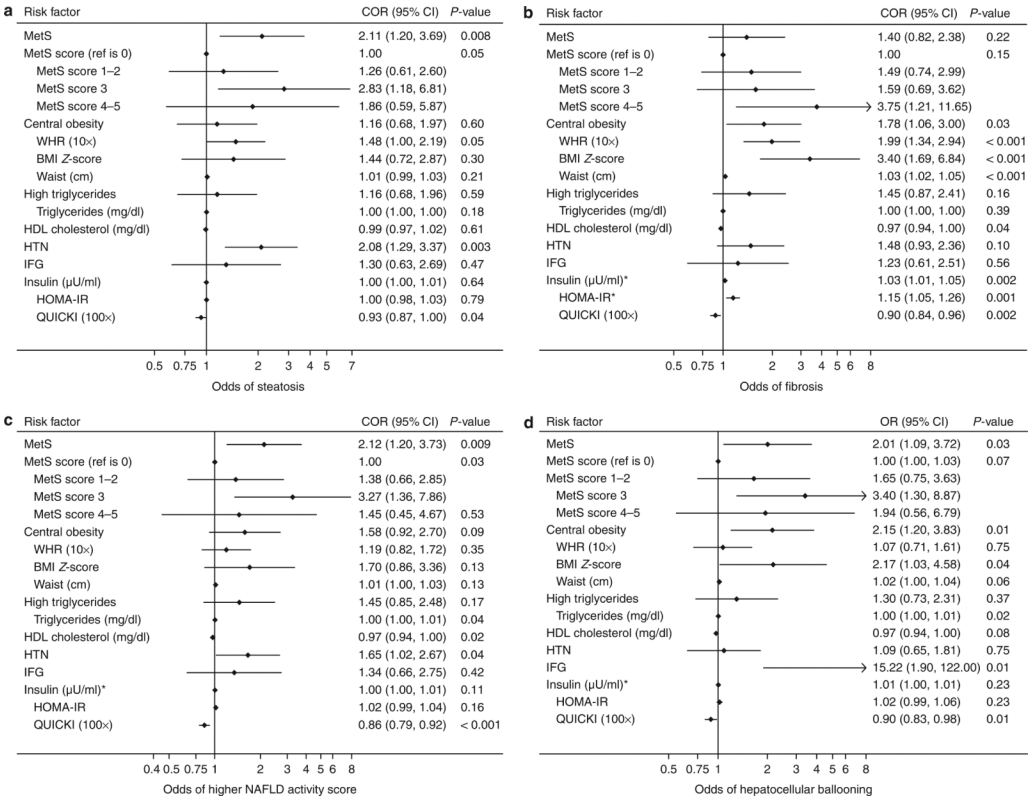


**Figure 2.**

Relationship between metabolic syndrome (MetS) risk factors and selected histological features in children with nonalcoholic fatty liver disease (NAFLD). (**a**) Ordinal logistic regression analyses of steatosis. (**b**) Ordinal logistic regression analyses of fibrosis. (**c**) Ordinal logistic regression analyses of NAFLD activity score. (**d**) Logistic regression analyses of hepatocellular ballooning.

## Table 1

Baseline characteristics of children with nonalcoholic fatty liver disease with and without metabolic syndrome (MetS)

| Baseline characteristic | All children[a] (*n* = 254) | Children with MetS[a] (*n* = 65) | Children with no MetS[a] (*n* = 189) | *P* value[*] |
|---|---|---|---|---|
| *Demographic* | | | | |
| Males vs. females: *n* (%) | 195 (76.8%) | 43 (66.2%) | 152 (80.4%) | **0.02** |
| Age (years) | 13.2 ± 2.6 | 13.8 ± 2.6 | 13.0 ± 2.5 | **0.05** |
| Tanner stage 1 (pre-pubertal) vs. other: *n* (%) | 82 (32.3%) | 10 (15.4%) | 72 (38.1%) | **0.0007** |
| White vs. other: *n* (%) | 184 (72.4%) | 46 (70.8%) | 138 (73.0%) | 0.73 |
| Hispanic vs. not Hispanic: *n* (%) | 153 (60.2%) | 40 (61.5%) | 113 (59.8%) | 0.80 |
| *Anthropomorphic* | | | | |
| Waist circumference (cm) | 106.9 ± 15.3 | 113.4 ± 13.0 | 104.6 ± 15.4 | < **0.0001** |
| Hip (cm) | 108.4 ± 14.6 | 113.2 ± 13.2 | 106.7 ± 14.7 | **0.001** |
| Waist-to-hip ratio | 0.99 ± 0.07 | 1.00 ± 0.06 | 0.98 ± 0.06 | **0.02** |
| BMI (kg/m$^2$) | 33.3 ± 6.3 | 35.6 ± 5.8 | 32.5 ± 6.3 | < **0.0001** |
| BMI z-score < 2 (97.7%) vs. ≥2: *n* (%) | 43 (16.9%) | 6 (9.2%) | 37 (19.6%) | **0.01** |
| BMI *z*-score | 2.3 ± 0.3 | 2.4 ± 0.3 | 2.3 ± 0.4 | **0.0005** |
| *Clinical* | | | | |
| Diabetes vs. none: *n* (%) | 6 (2.4%) | 5 (7.7%) | 1 (0.5%) | **0.005** |
| Hyperlipidemia vs. none: *n* (%) | 20 (7.9%) | 10 (15.4%) | 10 (5.3%) | **0.009** |
| Hypertension vs. none: *n* (%) | 10 (3.9%) | 7 (10.8%) | 3 (1.6%) | **0.003** |
| Systolic BP (mm Hg) | 124.2 ± 15.2 | 135.4 ± 13.2 | 120.3 ± 13.8 | < **0.0001** |
| Diastolic BP (mm Hg) | 68.9 ± 10.5 | 73.8 ± 10.9 | 67.2 ± 9.8 | < **0.0001** |
| Cholesterol, total (mg/dl) | 172.3 ± 39.2 | 176.8 ± 44.1 | 170.8 ± 37.7 | 0.61 |
| Cholesterol, HDL (mg/dl) | 37.9 ± 9.0 | 33.7 ± 8.5 | 39.4 ± 8.7 | < **0.0001** |
| Cholesterol, LDL (mg/dl) | 106.4 ± 29.4 | 103.9 ± 25.1 | 107.2 ± 30.7 | 0.34 |
| Triglycerides (mg/dl) | 146.8 ± 95.0 | 205.3 ± 129.2 | 126.5 ± 63.8 | < **0.0001** |
| Fasting insulin (μU/ml) | 37.0 ± 42.6 | 55.3 ± 70.1 | 30.7 ± 24.6 | < **0.0001** |
| Fasting glucose (mg/dl) | 88.3 ± 9.6 | 90.7 ± 12.8 | 87.4 ± 8.1 | **0.03** |
| Glucose, 2-hour post (mg/dl) | 121.2 ± 25.8 | 119.2 ± 23.0 | 121.9 ± 26.83 | 0.52 |
| HbA1c (%) | 5.33 ± 0.75 | 5.36 ± 0.48 | 5.32 ± 0.83 | 0.17 |
| HOMA-IR | 8.3 ± 10.4 | 12.8 ± 17.4 | 6.7 ± 5.7 | < **0.0001** |
| QUICKI | 0.30 ± 0.04 | 0.29 ± 0.04 | 0.31 ± 0.03 | < **0.0001** |

BMI, body mass index; HDL, high-density lipoprotein; HbA1c, glycosylated hemoglobin; HOMA-IR, homeostatic model assessment of insulin resistance; LDL, low-density lipoprotein; QUICK1, quantitative insulin sensitivity check index.

[a] Data are means ± s.d. or number (*n*) and percent (%) in each category.

[*] *P* values (2-sided) determined from either a $\chi^2$ test for non-ordered categories, Cochran-Armitage trend test for ordered categories or Fisher's exact test for categorical variables, and a Wilcoxon two-sample test for continuous variables. *P* values in bold type are statistically significant (< 0.05).

**Table 2**

Relationship between presence of metabolic syndrome (MetS) and histological features among children with non-alcoholic fatty liver disease

| | MetS[a] (*n* = 65) | No MetS[a] (*n* = 189) | Unadjusted[†] | | Adjusted[†] | |
|---|---|---|---|---|---|---|
| | | | OR | *P* value | OR | *P* value |
| *Steatosis grade* | | | | 0.21 | | **0.003** |
| Mild | 13 (20%) | 58 (31%) | 1.00 | | 1.00 | |
| Moderate | 21 (32%) | 60 (32%) | 1.56 | | 1.98 | 0.10 |
| Severe | 31 (48%) | 71 (38%) | 1.95 | | 2.85 | 0.001 |
| Steatosis grade (test for trend)[‡] | | | 1.60 | 0.08 | 2.11 | **0.008** |
| *Lobular inflammation score* | | | | 0.06 | | 0.16 |
| < 2 Under ×20 magnification | 27 (42%) | 105 (56%) | 1.00 | | 1.00 | |
| ≥2 Under ×20 magnification | 38 (58%) | 34 (44%) | 1.76 | | 1.54 | |
| *Chronic portal inflammation score* | | | | 0.95 | | 0.36 |
| None/mild | 59 (91%) | 172 (91%) | 1.00 | | 1.00 | |
| More than mild | 6 (9%) | 17 (9%) | 1.03 | | 1.64 | |
| *Hepatocellular ballooning* | | | | **0.01** | | **0.03** |
| None | 22 (34%) | 98 (52%) | 1.00 | | 1.00 | |
| Few/many | 43 (66%) | 91 (48%) | 2.10 | | 2.01 | |
| *Fibrosis stage, categorized* | | | | 0.58 | | 0.34 |
| None | 16 (25%) | 58 (31%) | 1.00 | | 1.00 | |
| Mild/moderate | 37 (58%) | 106 (56%) | 1.27 | | 1.40 | 0.35 |
| Bridging/cirrhosis | 11 (17%) | 25 (13%) | 1.60 | | 2.07 | 0.15 |
| *Bridging/cirrhosis* | | | | 0.31 | | **0.04** |
| No fibrosis | 16 (59%) | 58 (70%) | 1.00 | | 1.00 | |
| Yes | 11 (41%) | 25 (30%) | 1.60 | | 3.21 | |
| *Non-alcoholic fatty liver disease pattern* | | | | **0.02** | | **0.003** |
| Not NASH | 8 (12%) | 52 (28%) | 1.00 | | 1.00 | |
| Borderline zone 3 | 9 (14%) | 38 (20%) | 1.54 | | 1.69 | 0.39 |
| Borderline zone 1 | 17 (26%) | 42 (22%) | 2.63 | | 4.44 | 0.005 |
| Definite NASH | 31 (48%) | 57 (30%) | 3.54 | | 4.07 | 0.002 |
| NAFLD activity score (NAS), mean ± s.d. | 4.8 ± 1.4 | 4.3 ± 1.4 | | **0.01** | | **0.01** |

NAFLD, non-alcoholic fatty liver disease; NAS, NAFLD Activity Score; NASH, nonalcoholic steatohepatitis.

[a] Data are number and percent (%) in each category except for the NAFLD Activity Score (NAS).

[†] Odds ratios and *P* values (two-sided) determined from either a logistic (binary) or a multinomial logistic regression; multiple linear regression analysis used for continuous NAS score. Estimates derived from multivariable regression were adjusted for sex, ethnicity, age (6–11, 12–14, 15–17), and puberty (yes/no).

[‡] Ordinal logistic regression used to determine COR and *P* value to test for trend, if model assumptions were met. *P* values in bold type are statistically significant ($P < 0.05$).

**Table 3**

Relationship between metabolic syndrome (MetS) risk factors and NAFLD pattern in children with NAFLD

| | NAFLD pattern | | | | | | |
|---|---|---|---|---|---|---|---|
| | Borderline zone 3 vs. not NASH (*n* = 47) | | Borderline zone 1 vs. not NASH (*n* = 59) | | Definite NASH vs. not NASH (*n* = 88) | | Overall *P* value* |
| | OR | *P* value | OR | *P* value | OR | *P* value | |
| MetS (yes vs. no) | 1.69 | 0.34 | 4.44 | 0.005 | 4.07 | 0.002 | **0.003** |
| *MetS score* | | | | | | | **0.02** |
| 0 (Reference) | 1.00 | | 1.00 | | 1.00 | | |
| 1–2 | 1.39 | 0.57 | 0.99 | 0.98 | 2.58 | 0.09 | |
| 3 | 3.34 | 0.15 | 6.67 | 0.02 | 13.86 | 0.001 | |
| 4–5 | 0.89 | 0.91 | 2.08 | 0.43 | 3.86 | 0.12 | |
| *Central obesity (yes vs. no)* | 1.53 | 0.35 | 2.71 | 0.02 | 4.08 | 0.001 | **0.004** |
| BMI z-score | 1.35 | 0.59 | 2.77 | 0.08 | 3.07 | 0.03 | 0.09 |
| Waist circumference (cm) | 1.006 | 0.71 | 1.017 | 0.26 | 1.032 | 0.01 | 0.06 |
| Waist to hip ratio (10X) | 1.40 | 0.30 | 1.48 | 0.21 | 1.56 | 0.10 | 0.49 |
| *High triglycerides (yes vs. no)* | 0.81 | 0.65 | 0.94 | 0.88 | 1.21 | 0.62 | 0.80 |
| Triglycerides (mg/dL) | 1.004 | 0.12 | 1.003 | 0.34 | 1.006 | 0.02 | 0.06 |
| *Low HDL cholesterol (yes vs. no)* | 1.69 | 0.25 | 1.01 | 0.99 | 1.51 | 0.32 | 0.51 |
| Cholesterol, HDL (mg/dl) | 1.012 | 0.60 | 1.042 | 0.07 | 0.967 | 0.14 | **0.005** |
| *HTN (yes vs. no)* | 1.41 | 0.40 | 3.02 | 0.006 | 1.51 | 0.25 | **0.05** |
| Systolic BP (mm Hg) | 1.024 | 0.08 | 1.030 | 0.03 | 1.020 | 0.09 | 0.13 |
| Diastolic BP (mm Hg) | 1.000 | 0.96 | 1.013 | 0.52 | 1.021 | 0.21 | 0.57 |
| *IFG (yes vs. no)* | 0.79 | 0.76 | 1.77 | 0.39 | 2.61 | 0.08 | 0.14 |
| Fasting insulin (μU/ml)[a] | 1.005 | 0.13 | 1.013 | 0.58 | 1.052 | 0.01 | **0.007** |
| Fasting glucose (mg/dl) | 0.985 | 0.47 | 1.007 | 0.74 | 1.021 | 0.11 | 0.12 |
| HOMA-IR[a] | 1.028 | 0.80 | 1.091 | 0.43 | 1.283 | 0.007 | **0.004** |
| QUICKI (100X) | 0.951 | 0.41 | 0.985 | 0.76 | 0.786 | < 0.001 | < **0.001** |

BMI, body mass index; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; HTN, hypertension; IFG, impaired fasting glucose; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis.

* Odds ratios determined from a multinomial logistic regression of NAFLD pattern (reference category = not NASH (*n* = 60)) adjusted for sex, ethnicity, age (6–11, 12–14, 15–17), and puberty (yes/no). *P* values (2-sided) determined from a likelihood ratio test statistic. Overall *P* values in bold type are deemed statistically significant.

[a]Model included interaction between risk factor and Hispanic demographic.