Obstructive Sleep Apnea and Hypoxemia Are Associated with Advanced Liver Histology in Pediatric Nonalcoholic Fatty Liver Disease

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Objective To determine whether obstructive sleep apnea (OSA) and/or nocturnal hypoxemia are associated with the severity of liver injury in patients with pediatric nonalcoholic fatty liver disease (NAFLD).

Study design Obese children aged 10-18 years with liver biopsy–proven NAFLD were enrolled. Demographic, clinical, and laboratory data were collected, polysomnography was performed, and liver histology was scored. Subjects were divided into those with OSA/hypoxemia and those without OSA/hypoxemia for analysis.

Results Of 25 subjects with NAFLD, OSA/hypoxemia was present in 15 (60%) (mean age, 12.8 ± 1.9 years; 68% male; 88% Hispanic; mean body mass index *z*-score, 2.3 ± 0.3). Subjects with and without OSA/hypoxemia had similar levels of serum aminotransferases, serum lipids, and inflammatory and insulin resistance markers. Although there were no differences between groups in the histological severity of steatosis, inflammation, ballooning degeneration, NAFLD activity score, or histological grade, subjects with OSA/hypoxemia had significantly more severe hepatic fibrosis. Moreover, oxygen saturation nadir during polysomnography was related to hepatic fibrosis stage ($r = -0.49$; $P = .01$) and aspartate aminotransferase level ($r = 0.42$; $P < .05$). Increasing percentage of time with oxygen saturation ≤90% was related to NAFLD inflammation grade ($r = 0.44$; $P = .03$), degree of hepatic steatosis ($r = -0.50$; $P = .01$), NAFLD activity score ($r = 0.42$; $P = .04$), aspartate aminotransferase level ($r = 0.56$; $P = .004$), and alanine aminotransferase level ($r = 0.44$; $P = .03$).

Conclusion Moderate OSA/hypoxemia is common in pediatric patients with biopsy-proven NAFLD. OSA and the severity/duration of hypoxemia are associated with biochemical and histological measures of NAFLD severity. *(J Pediatr 2014;164:699-706)*.

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Nonalcoholic fatty liver disease (NAFLD) is a clinicopathological condition characterized by abnormal lipid deposition in hepatocytes in the absence of excess alcohol intake. The most common chronic liver disease affecting both children and adults, NAFLD is associated with the obesity epidemic.[1] NAFLD comprises a spectrum of diseases, ranging from isolated hepatic steatosis to nonalcoholic steatohepatitis (NASH), defined as steatosis, hepatocyte ballooning, and inflammation, which can be associated with fibrosis and may progress to cirrhosis.[1]

NAFLD affects up to 9.6% of all children and 38% of obese children.[2] Although isolated hepatic steatosis may have no significant consequences, NASH may progress to liver fibrosis and cirrhosis in approximately 20% of cases and is associated with hepatocellular carcinoma in adults.[1,3] Risk factors for pediatric NAFLD include Hispanic race, male sex, insulin resistance, and obesity.[4]

Obstructive sleep apnea (OSA), characterized by recurrent partial or complete upper airway obstruction during sleep, affects 1.2%-5.7% of the general pediatric population.[5-12] In addition, a relationship between obesity and OSA has been demonstrated in 2 large population-based studies using polysomnography.[5,10]

AHI	Apnea hypopnea index
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BMI	Body mass index
HPF	High-power field
NAFLD	Nonalcoholic fatty liver disease
NAS	Nonalcoholic fatty liver disease activity score
NASH	Nonalcoholic steatohepatitis
OSA	Obstructive sleep apnea
REM	Rapid eye movement
SaO_2	Oxygen saturation

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Symptoms of OSA include daytime sleepiness, poor school performance, and snoring, though many children are asymptomatic. Affected patients experience repeated episodes of nocturnal hypoxemia alternating with normoxia (so-called "chronic intermittent hypoxemia"), resembling the pathophysiological mechanisms involved in ischemia/reperfusion tissue injury.[13,14]

The relationship between NAFLD and OSA extends beyond their simple coexistence as obesity-related comorbidities. Obese mice fed a high-fat and high-cholesterol diet develop steatosis and, when exposed to chronic intermittent hypoxia, demonstrate significant increases in alanine aminotransferase (ALT) level, hepatic inflammation, and fibrosis.[15,16] Elevated serum aminotransferase (ALT and aspartate aminotransferase [AST]) levels are present in 20%-50% of adults with OSA,[11-13] and there is an increased prevalence of OSA in adults with NASH. In addition, morbidly obese adults with moderate to severe OSA and hypoxemia have more histologically severe hepatic inflammation compared with those with OSA without hypoxemia.[17] Precious little is known about the relationship of sleep-disordered breathing and pediatric NAFLD, however. A single study from a selected sleep medicine clinic population found OSA in 91% of obese children with elevated serum aminotransferase levels, although liver histology was not evaluated.[18,19]

Given that oxidative stress induced by hypoxia/reoxygenation is a potential factor in the progression from fatty liver to NASH, we conducted the present study to explore the relationship between pediatric NAFLD and sleep-disordered breathing. We hypothesized that OSA and/or nocturnal hypoxemia would be associated with the severity of biochemical or histological evidence of liver injury in patients with pediatric NAFLD.

Methods

Pediatric patients receiving care at the Children's Hospital Colorado Pediatric Liver Center between June 2009 and January 2013 were enrolled if they had suspected NAFLD and were scheduled to undergo a clinically indicated liver biopsy (**Figure 1**; available at www.jpeds.com). In our center, NAFLD is suspected in overweight or obese children (body mass index [BMI] >85% for age and sex) with chronically elevated serum aminotransferase levels and negative screening results for Wilson disease, autoimmune hepatitis, viral hepatitis, and alpha-1 antitrypsin deficiency.

The study subjects were males and females aged 8-18 years and at Tanner stage 2-4. This Tanner stage range was chosen to minimize variations in insulin sensitivity that could possibly confound the interpretation of potential associations between OSA/hypoxia and NAFLD. Exclusion criteria included a history of Wilson disease, alpha-1 antitrypsin deficiency, viral hepatitis, autoimmune hepatitis, another known chronic liver disease, or cholelithiasis, as well as the use of anticonvulsants, sedatives, oral or intravenous steroids, drugs that promote or reduce insulin resistance (including insulin sensitizers, thiazolidenediones, and metformin), or medications known to induce hepatic steatosis (eg, steroids, amiodarone, total parenteral nutrition) in the previous 2 weeks. Additional exclusion criteria included current use of continuous positive airway pressure, insulin-dependent diabetes, regular tobacco or alcohol use, a neuromuscular disorder, or a genetic or craniofacial abnormality. This study was approved by the Colorado Multiple Institutional Review Board. Informed consent was obtained from parents/guardians, and written assent was obtained from all subjects.

Demographic and medical history data, including results of clinical testing for autoimmune hepatitis, alpha-1 antitrypsin deficiency, Wilson disease, and viral hepatitis, and abdominal ultrasound or computed tomography imaging data were obtained. Subjects were asked about clinical symptoms of sleep apnea (ie, snoring, witnessed apnea, nonrestorative sleep, and daytime sleepiness). Height, weight, and waist and hip circumferences were measured, and BMI was calculated. BMI *z*-scores were determined based on age and sex using Centers for Disease Control and Prevention data.[20] Waist measurements were obtained at the midpoint between the highest point of the iliac crest and the lowest part of the costal margin. Hip measurements were obtained at the fullest part of the hips.

Liver biopsy specimens were obtained for clinical indications by a standard percutaneous technique. Liver histology (hematoxylin and eosin and Masson trichrome stain) was reviewed and scored by a single pediatric pathologist blinded to patient information. Biopsy specimens with histologically confirmed NAFLD (defined as ≥5% of hepatocytes containing macrovesicular fat) were assigned a grade of necroinflammation (0-3) and a stage of fibrosis (0-4) based on the standard histological criteria of Brunt et al.[21] Biopsy specimens were also scored for the following criteria established by the NASH Clinical Research Network[22]: steatosis (grade 0, <5 % of hepatocytes containing macrovesicular fat; grade 1, 5%-33%; grade 2, 34%-66%; grade 3, >66%); lobular inflammation (grade 0, no foci of inflammation; grade 1, <2 foci per high-power field [HPF]; grade 2, 2-4 foci per HPF; grade 3, >4 foci per HPF); ballooning degeneration (grade 0, none; grade 1, few balloon cells; grade 2, many/prominent balloon cells). An NAFLD activity score (NAS) was calculated by summing the scores for steatosis, lobular inflammation, and ballooning degeneration.[22] Fibrosis was scored as stage 0 (none), stage 1a (mild perisinusoidal), stage 1b (moderate perisinusoidal), stage 1c (portal/periportal fibrosis only), stage 2 (zone 3 and periportal), stage 3 (bridging fibrosis), or stage 4 (cirrhosis).[20] Subjects were also classified as type 1 NASH (classic adult pattern), type 2 NASH (portal-based), or an overlap of the 2 NASH histological subtypes.[23]

Subjects with histologically confirmed NAFLD underwent a standard multichannel sleep study (polysomnography), which was scored by a research-trained technician and interpreted by a single sleep medicine physician, both of whom were masked to liver biopsy results. The following data were