Chronic Liver Injury During Obstructive Sleep Apnea

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Patients with obstructive sleep apnea (OSA) are at risk for the development of fatty liver as a result of being overweight. Several data suggest that OSA *per se* could be a risk factor of liver injury; ischemic hepatitis during OSA has been reported, and OSA is an independent risk factor for insulin resistance. Therefore, we investigated liver damage and potential mechanisms in 163 consecutive nondrinking patients with nocturnal polysomnographic recording for clinical suspicion of OSA. Serum levels of liver enzymes were measured in all patients. Liver biopsy was offered to patients with elevated liver enzymes. Intrahepatic hypoxia was assessed by the expression of vascular endothelial growth factor (VEGF) on liver biopsy specimens. Severe OSA (apnea-hypopnea index [AHI] > 50/hr) was seen in 27% of patients; 52% had moderate OSA (AHI 10-50/hr), and 21% had no OSA. Overall, 20% had elevated liver enzymes. Independent parameters associated with elevated liver enzymes were body mass index (BMI) (OR: 1.13; CI: 1.03-1.2) and severe OSA (OR: 5.9; CI: 1.2-29). Liver biopsy was performed in 18 of 32 patients with elevated liver enzymes and showed steatohepatitis in 12 cases, associated with fibrosis in 7 cases. Patients with severe OSA were more insulin-resistant according to homeostasis model assessment, had higher percentage of steatosis as well as scores of necrosis and fibrosis, despite similar BMI. Hepatic immunostaining used as an indirect marker of hypoxia was not different between patients with or without severe OSA. In conclusion, severe OSA is a risk factor for elevated liver enzymes and steatohepatitis independent of body weight. Promotion of insulin resistance is probably involved. Further studies are needed to determine whether hypoxia contributes directly to liver injury. (HEPATOLOGY 2005;41:1290-1296.)

Obstructive sleep apnea (OSA) syndrome is a common condition with prevalence estimates of 2% to 4% in the general population.[1] The majority of patients are obese and therefore at risk for fatty liver.[2] However, several data suggest that OSA *per se* could be a risk factor of liver injury independent of overweight: several cases of ischemic hepatitis during severe OSA have been reported,[3,4] and epidemiological studies have shown that OSA is an independent risk factor for impairment of glucose homeostasis.[5-11] We therefore postulated that OSA-induced insulin resistance and direct liver hypoxia could possibly be involved in the pathogenesis of liver disease associated with OSA. There are few data concerning the prevalence and characterization of liver injury in patients with OSA independent of the body weight. This study aimed to assess prospectively the prevalence, type of liver injury, and potential mechanisms in patients with OSA, taking into account their overweight states.

Abbreviations: OSA, obstructive sleep apnea; VEGF, vascular endothelial growth factor; AHI, apnea-hypopnea index; BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyltransferase; α-GST, α-glutathion-S-transferase; HOMA, homeostasis model assessment; NASH, nonalcoholic steatohepatitis.

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Patients and Methods

Study Population. Between September 2000 and May 2001, 218 consecutive patients with clinical suspicion of OSA were referred to the Sleep Unit of Hospital Saint-Antoine. Exclusion criteria were as follows: decompensated cardiac or respiratory insufficiency, treatment for OSA, alcohol intake higher than 20 g/d, regular use of hepatotoxic drugs, known liver disease, and history of liver transplantation. All patients gave their informed consent, and the study was approved by the local ethics committee.

Demographic Data. The following data were recorded at the time of polysomnography: age, sex, body mass index (BMI), history of diabetes, hypertriglyceridemia, or high blood pressure. Overweight was defined as a BMI higher than 25 kg/m^2. Patients were interviewed via a standard questionnaire for their alcohol intake in the last 5 years and were considered drinkers if their mean daily consumption was higher than 20 g of pure alcohol.[12]

Polysomnography. Measurements included sleep stage (electroencephalogram, electro-oculogram, and submental electromyogram), nasal airflow (nasal cannulae) and oral airflow (thermistor), rib cage and abdominal wall motion (respiratory inductance plethysmography), and arterial oxygen saturation (SaO_2). All polysomnographic data were analyzed visually. Sleep analysis was performed according to standard criteria.[13] Apnea was defined as cessation of airflow for more than 10 seconds. Hypopnea was defined as a reduction of airflow, regardless of its amplitude, with a more than 3% fall in SaO_2 or arousal. The number of apnea and hypopnea episodes per hour of sleep, or apnea hypopnea index (AHI), was calculated. OSA was defined as severe when the AHI was above 50/hr, moderate when it was 10 to 50/hr, and absent when it was less than 10/hr.[14,15]

Laboratory Investigations. A fasting venous blood sample was collected in the morning immediately after sleep. All measurements were performed on fresh serum. Plasma aminotransferases (alanine aminotransferase [ALT] and aspartate aminotransferase [AST]) and γ-glutamyltransferase (GGT) activities were routinely assayed on a Synchron CX4-CE analyzer (Beckman-Coulter Instruments, Palo Alto, CA) at 30°C. α-Glutathion-*S*-transferase (α-GST) was determined via immunoenzymatic assay (Biotrin International, Dublin, Ireland).[16] Patients with elevated liver enzymes were defined as having serum ALT, AST, GGT, and/or α-GST higher than the upper limit of normal (35, 30, 33, and 6 IU/L, respectively).

Patients with elevated liver enzymes were proposed for additional laboratory investigations at the time of liver biopsy. The following chronic liver diseases were excluded by using the appropriate tests: hepatitis B, hepatitis C, autoimmune hepatitis, genetic hemochromatosis, Wilson's disease, and primary biliary cirrhosis. After a 10-hour overnight fast, venous blood samples were drawn to determine levels of ALT, GGT, triglycerides, glucose, and insulin. Serum insulin level was determined via radioimmunoassay (Phadeseph insulin RIA; Pharmacia and Upjohn). Insulin resistance was calculated via homeostasis model assessment (HOMA) as follows: fasting serum insulin-fasting $\times$ serum glucose/22.5, where insulin is expressed in mU/L and glucose in μmol/L.

Pathology. Liver biopsy was proposed to patients with elevated liver enzymes and performed during the morning. Liver specimens were fixed in 10% buffered formalin, paraffin-embedded, and analyzed by a single pathologist who was unaware of clinical and biological data. For standard histology, sections were stained with hematoxylin-eosin-safran and sirius red. Liver biopsy was scored according to Brunt et al., with modifications.[17] Steatosis was graded as follows: mild (10%-30% of hepatocytes affected), moderate (30%-60% of hepatocytes affected), and severe (>60% of hepatocytes affected). Fibrosis was graded on a scale of 0 to 4 (0, absent; 1, perisinusoidal/pericellular fibrosis; 2, periportal fibrosis; 3, bridging fibrosis; 4, cirrhosis). Intralobular necrosis was graded on a scale of 0 to 3 (0, absent; 1, less than one necrosis injury per lobule; 2, one or more necrosis injury per lobule; 3, more than two necrosis injuries per lobule). Hepatocyte ballooning was graded on a scale of 0 to 2 (0, absent; 1, ballooned hepatocytes in less than 50% of lobules; 2, ballooned hepatocytes in more than 50% of lobules). Nonalcoholic steatohepatitis (NASH) was defined by the presence of steatosis associated with lobular necrosis (grade 1 or more) or hepatocyte ballooning (grade 1 or more), with or without fibrosis (grade 1 or more).

Immunohistochemistry. Vascular endothelial growth factor (VEGF) expression was studied via immunohistochemistry on formalin-fixed and paraffin-embedded liver biopsies. Four-millimeter tissue sections were deparaffinized in xylene and rehydrated in graded alcohol dilutions. Immunolabeling was performed using an Avidin-Biotin-peroxidase technique (Vectastain ABC Kit; Vector, Burlingame, CA) and an anti-VEGF rabbit polyclonal immunoglobulin G (A-20; Santa Cruz Biotechnology, Santa Cruz, CA) at a dilution of 1:100. Before immunostaining, endogenous peroxidase activity was inhibited with 0.1% hydrogen peroxide in methanol for 30 minutes. Color development was achieved with 3-amino-9-ethyl-carbazole, and sections were finally counterstained with hematoxylin. A VEGF-positive colorectal cancer was used as an external positive control. Negative control slides were obtained by omitting the primary antibody. The staining for VEGF was considered positive if there was staining of the lobular hepatocytes.

Statistical Analysis. Qualitative variables were expressed as a percentage and were compared using the Fisher exact test. Continuous variables were expressed as the mean $\pm$ SD and were compared using the Student *t* test or ANOVA in the case of more than two groups. The Mann-Whitney test or Spearman correlation was used for small groups. The following risk factors for elevated liver enzymes were taken into account in univariate and multivariate analysis: age, sex ratio, BMI, diabetes, hypertri-

Table 1. Characteristics of Patients According to Severity of OSA and of Controls

	Severe OSA (n = 44)	Moderate OSA (n = 84)	Controls (n = 35)	*P* Value*
Age† (yr)	52 ± 14‡	53 ± 13‡	44 ± 13	.003
Sex ratio (M/F)	4.5‡	2.8‡	1.3	.04
BMI† (kg/m^2)	30.3 ± 7§	27 ± 3.8	27 ± 6.4	.002
Diabetes mellitus (%)	11.4§	1.2	2.9	.02
Hypertriglyceridemia (%)	9	7	8.6	.9
High blood pressure (%)	38.6§	16.7	23	.02
AHI† (/hr)	80.7 ± 30§	27 ± 11.4‡	5.1 ± 2.9	<.0001
Mean SaO_2† (%)	92.4 ± 3.1§	94.4 ± 1.4‡	95.2 ± 1.4	<.0001
% of sleep time with SaO_2 <90† (%)	14.9 ± 19§	2.3 ± 5.2	0.6 ± 2.1	<.0001
AST† (UI/L)	14 ± 7	14 ± 12	11 ± 4	.14
ALT† (UI/L)	28 ± 16‡	24 ± 12	19 ± 8	.02
GGT† (UI/L)	21 ± 14‡	18 ± 16	13 ± 9	.05
α-GST† (UI/L)	4.6 ± 4.7	4.2 ± 8.7	2.1 ± 2.3	.2

*Fisher exact test or ANOVA.
†Mean ± SD.
‡Severe OSA versus controls ($P < .05$) or moderate OSA versus controls ($P < .05$).
§Severe OSA versus moderate OSA ($P < .05$) and severe OSA versus controls ($P < .05$).

glyceridemia, high blood pressure, and OSA. OSA was considered a multicategory variable (severe, moderate, none). Independent risk factors were assessed by using a logistic regression model taking into account the interaction between BMI and AHI. Variables with a *P* value less than 0.2 in the univariate analysis were included in the model. In patients with liver biopsy, multiple linear regression was used to examine associations between HOMA and AHI, percentage of steatosis and AHI, fibrosis score and HAI, and lobular necrosis score and HAI, controlling age and BMI as continuous variables. Differences were considered significant if the *P* value was less than .05.

Results

Characteristics of Patients According to the Severity of OSA. Between September 2000 and May 2001, nocturnal polysomnography was performed in 218 consecutive patients for clinical suspicion of OSA. Fifty-five patients were excluded: 10 because of incomplete data, 38 because of alcohol intake greater than 20 g/d, 5 because use of hepatotoxic drugs (*e.g.*, nonsteroidal anti-inflammatory drugs), 1 because of previous liver transplantation, and 1 because of known hepatitis C. Therefore, 163 patients were included in the study: OSA was severe in 44 patients (27%) (AHI >50/hr), moderate in 84 patients (52%) (AHI 10-50/hr), and absent in 35 patients (21%). The patients without OSA were used as internal controls. The characteristics of patients with severe or moderate OSA and controls are shown in Table 1. Patients with severe or moderate OSA were significantly older and more often male compared with controls. BMI was significantly higher in patients with severe OSA than in patients with moderate OSA or controls. Prevalences of diabetes or high blood pressure were significantly higher in patients with severe OSA than in patients with moderate or no OSA. There was no difference in the prevalence of hypertriglyceridemia in the three groups. ALT and GGT activities were significantly higher in patients with severe OSA than in controls. α-GST and AST activities were not statistically different between the three groups.

Prevalence and Risk Factors of Elevated Liver Enzymes. Overall, liver enzymes were elevated in 32 patients (20%) (14 [32%], 15 [18%], and 3 [8.6%] with severe OSA, with moderate OSA, and controls, respectively [$P = .02$]). The characteristics of patients with or without elevated liver enzymes are shown in Table 2. In univariate analysis, the parameters significantly associated with elevated liver enzyme were BMI ($P = .004$) and severe OSA ($P = .02$) (Table 2). In a multivariate analysis, the independent parameters associated with elevated liver enzymes were BMI (odds ratio [OR]: 1.13 [CI: 1.03-1.2] for each 1-point increment of BMI) and severe OSA (OR: 5.9 [CI: 1.2-29]) (Table 2). When considered as a continuous variable, AHI was still independently associated with elevated liver enzymes (OR: 1.1 [CI: 1.01-1.2]; $P = .03$).

Histopathological Findings. Liver biopsy was performed in 18 of 32 patients with elevated liver enzymes. Patients with or without liver biopsy were similar in terms of age (47 ± 10 vs. 52 ± 12 years), sex ratio (13 of 5 vs. 12 of 2), BMI (30 ± 6 vs. 29.5 ± 4.5), AHI (53 ± 40 vs. 44 ± 23 /hr), ALT (43 ± 14 vs. 40 ± 21 IU/L), and GGT (38 ± 31 vs. 32 ± 24 IU/L) activities at the time of polysomnography. Other causes of chronic liver injury (hepatitis B and C, autoimmune liver disease, genetic hemochromatosis, and Wilson's disease) were excluded

Table 2. Risk Factors for Elevated Liver Enzymes in Univariate and Multivariate Analysis

	Elevated Liver Enzymes* (n = 32)	Normal Liver Enzymes (n = 131)	*P* Value†	*P* Value‡	Odds Ratio (CI)
Age§ (yr)	50 ± 11	51 ± 14	.8	–	–
Percentage of male	84.4	69.5	.12	.2	2 (0.64-6.3)
BMI§ (kg/m^2)	30 ± 5.3	27.3 ± 5.6	.004	.007	1.13 (1.03-1.2)
Diabetes (%)	6.2	3.8	.6	?	?
Hypertriglyceridemia (%)	15.6	6	.13	.1	3 (0.8-11.7)
High blood pressure (%)	28	23	.6	–	–
Severe OSA (%)	44	23	.02	.02	5.9 (1.2-29)
Moderate OSA (%)	47	52		.15	3 (0.67-13.9)
No OSA (%)	9	25			1

*ALT, AST, GGT, and/or α-GST higher than upper limit of normal.
†Fisher exact test or Mann-Whitney test.
‡Logistic regression taking into account interaction between BMI and AHI ($P = .04$).
§Mean ± SD.

using appropriate tests. No patients had medications known to induce fatty liver. Nine patients had severe OSA, 6 had moderate OSA, and 3 had no OSA. Liver biopsy showed steatosis in 13 patients: mild in 7 patients, moderate in 3 patients, and severe in 3 patients. Steatosis was associated with lobular necrosis or hepatocyte ballooning in 12 patients, defining steatohepatitis. Among the 12 patients with steatohepatitis, perisinusoidal fibrosis was present in 3, periportal fibrosis in 3, and bridging fibrosis in 1. Three patients had lobular necrosis without steatosis. Two patients had normal histology.

Insulin Resistance and Liver Injury According to the Severity of OSA in Patients With Elevated Liver Enzymes. Patients with liver biopsy had serum level determination of ALT, GGT, triglycerides, glucose, and insulin. Because of similar clinical, biochemical, and histological characteristics (mean age 52 ± 12 vs. 49 ± 5 yr; BMI 30 ± 5 vs. 31 ± 7; ALT 35 ± 7 vs. 35 ± 20 IU/L; HOMA 1.2 ± 0.6 vs. 1.6 ± 0.8; steatosis 16 ± 24 vs. 5 ± 5%; necrosis score 0.8 ± 0.4 vs. 0.4 ± 0.6; and fibrosis score 0 vs. 0, respectively), liver-biopsed patients with moderate or no OSA were pooled and compared with patients with severe OSA. Clinical and biochemical characteristics of patients according to the severity of OSA are shown in Table 3. Patients with or without severe OSA were similar in terms of age, sex ratio, BMI, and triglyceride levels. Patients with severe OSA had significantly higher levels of insulin and were more insulin-resistant according to HOMA than patients with moderate or no OSA (Table 3). When HOMA was considered as a dependent variable, multiple linear regression showed that AHI was correlated with HOMA independent of BMI and age ($r = 0.48 \pm 0.023$; $P = .02$). Regarding histological findings, percentage of steatosis, score of lobular necrosis, and score of fibrosis were significantly higher in patients with severe OSA than in patients with moderate or no OSA (35% ± 7% vs. 13% ± 7%, $P = .03$; 1.3 ± 0.2 vs. 0.7 ± 0.2, $P = .01$; and 1.1 ± 0.4 vs. 0.2 ± 0.1, $P = .03$, respectively) (Fig. 1). The percentage of steatosis, score of lobular necrosis, and score of fibrosis were positively correlated with AHI ($r = 0.65$, $P = .01$; $r = 0.65$, $P = .01$; and $r = 0.6$, $P = .02$, respectively) and the percentage of sleep time with SaO_2 less than 90% ($r = 0.5$, $P = .05$; $r = 0.54$, $P = .03$; and $r = 0.53$, $P = .03$, respectively). In a multivariate analysis, the percentage of steatosis, lobular necrosis, and fibrosis scores were correlated with AHI independent of age and BMI ($P = .01$, $r = 0.58 \pm 012$; $P = .05$, $r = 0.41 \pm 0.005$; and $P = .02$, $r = 0.54 \pm 0.006$, respectively). When HOMA was added in the multivariate analysis, there was no more significant association between AHI and any of the histopathological findings. Finally, the number of patients with steatohepatitis was 8 of 9 in those with severe OSA versus 4 of 9 in those with moderate or no OSA ($P = .04$).

VEGF Immunostaining According to OSA and Liver Histology. Among the 18 liver-biopsied patients, only 3 had positive VEGF immunostaining. All had le-

Table 3. Characteristics of Patients at the Time of Liver Biopsy According to Severity of OSA

	Severe OSA (n = 9)	Moderate or No OSA (n = 9)	*P* Value*
Age† (yr)	44 ± 6	51 ± 10	.07
Sex ratio (M/F)	8/1	5/4	.1
BMI† (kg/m^2)	30 ± 5	30 ± 6	.9
AHI (/hr)	84 ± 24	18 ± 13	<.0001
Percentage of sleep time with SaO_2 <90%$^\$$ (%)	17 ± 20	0.7 ± 0.8	.03
ALT† (IU/L)	56 ± 29	35 ± 11	.02
GGT† (IU/L)	42 ± 27	44 ± 39	.9
Triglycerides† (mmol/L)	1.7 ± 0.8	1.3 ± 0.7	.25
Glucose† (mmol/L)	5.9 ± 3.2	5.6 ± 0.8	.25
Insulin† (mU/L)	15.2 ± 12.3	5.5 ± 3.5	.01
HOMA†	5.4 ± 7	1.3 ± 0.6	.05

*Fisher exact test or Mann-Whitney test.
†Mean ± SD.

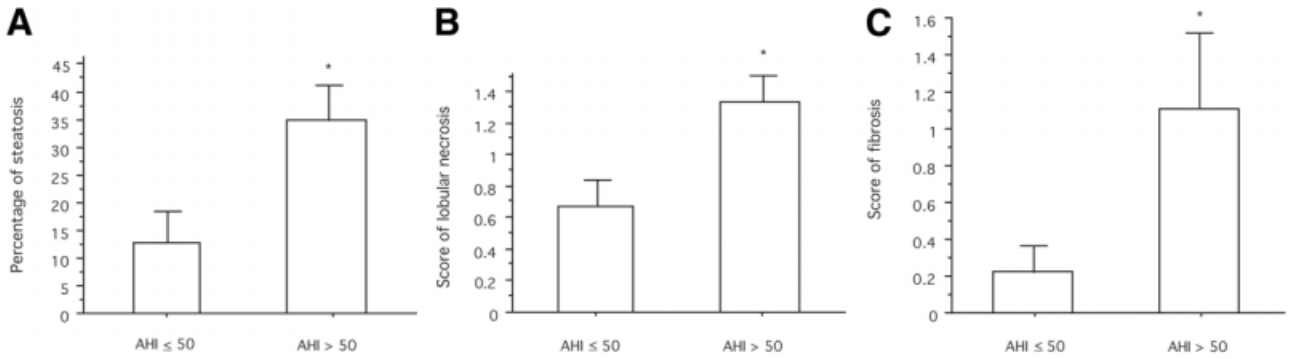

Fig. 1. Histological findings according to the severity of OSA in patients with elevated liver enzymes. Patients were divided into two groups: severe OSA (AHI >50/hr; n = 9) and moderate or no OSA (AHI ≤50/hr; n = 9). Body mass index was similar in both groups (30 ± 5 vs. 30 ± 6). (A) Percentage of steatosis in patients with severe OSA (AHI >50/hr) and in patients with moderate or no OSA (AHI ≤50/hr). Values are expressed as the mean ± SE. *P = .04. (B) Score of lobular necrosis in patients with severe OSA (AHI >50/hr) and in patients with moderate or no OSA (AHI ≤50/hr). Values are expressed as the mean ± SE. *P = .02. (C) Score of fibrosis in patients with severe OSA (AHI >50/hr) and in patients with moderate or no OSA (AHI ≤50/hr). Values are expressed as the mean ± SE. *P = .03. AHI, apnea-hypopnea index.

sions of steatohepatitis, 2 had perisinusoidal fibrosis. The mean AHI was similar between patients with or without positive immunostaining (51 ± 46 vs. 54 ± 39, respectively). The number of patients with positive immunostaining was 1 of 9 in those with severe OSA versus 2 of 9 in those with moderate or no OSA (nonsignificant).

Discussion

This study shows that severe OSA is a risk factor for abnormal liver enzymes and steatohepatitis independent of overweight states. Promotion of insulin resistance is likely to be involved in the pathogenesis of liver injury.

This study assesses liver injury in patients with OSA. Patients included in this study were consecutively hospitalized for clinical suspicion of OSA. Thirty-five patients had no polysomnographic criteria for OSA and were considered a control group. No patient had nasal continuous positive airway pressure therapy. Patients with a daily alcohol intake greater than 20 g and regular use of potential hepatotoxic drugs were excluded from the study. In the first part of the study, the main evaluation criteria were serum abnormal liver enzymes (*i.e.*, elevated aminotransferases, GGT, and/or α-GST). α-GST has a panlobular distribution and is considered a marker of hepatocellular injury.[16] In the second part of the study, liver biopsy was performed in the subgroup of patients with abnormal liver tests. Considering the limited number of patients with liver biopsy, the results must be considered with caution.

In our study population, 27% and 52% of patients had polysomnographic criteria for severe or moderate OSA, respectively, while 21% had no criteria for OSA. As expected, risk factors for OSA were age, male sex, and overweight condition.[1,2] The prevalence of abnormal liver enzymes in our OSA population (20%) was close to that observed in obese patients, reaching 32% in patients with severe OSA.[18] It could be hypothetized that these findings are almost exclusively related to the overweight states, which is a major risk factor of OSA. However, in a multivariate analysis, severe OSA was an independent risk factor, suggesting a role of OSA in liver injury independent of BMI. This is consistent with the findings of Chin et al., who showed in a longitudinal study that continuous positive airway pressure treatment of OSA in obese patients was associated with a significant decrease in aminotransferase levels.[19]

Liver damage was probably underestimated in our study, because diagnosis was based on elevated liver enzymes, whereas cases of NASH without abnormal liver tests have been reported.[20] However, it has been shown that elevated ALT was an independent predictor of NASH in obese patients, suggesting that patients with normal liver enzymes may have less severe liver injury.[21] Moreover, in clinical practice, detection of liver injury is based on abnormal liver tests, and liver biopsy is usually proposed only for patients with elevated liver enzymes. As expected, liver biopsy revealed steatosis in the majority of patients. Because usual causes of liver injury were excluded, we considered these patients as having criteria for nonalcoholic fatty liver disease.[22,23] Steatosis was associated with hepatocyte ballooning or lobular necrosis, allowing the diagnosis of NASH in 12 cases, associated with fibrosis in 7 cases.[22,23] Among the 18 liver-biopsed patients, 9 belonged to the initial group with severe OSA, 6 to the group with moderate OSA, and 3 to the control group. Severe OSA was associated with more severe liver injury in terms of the degree of steatosis, lobular necrosis, and fibrosis. Furthermore, the prevalence of NASH was

higher in patients with severe OSA than in patients with moderate or no OSA. In a multivariate analysis, steatosis, lobular necrosis, and fibrosis were related to AHI independent of age and BMI, both of which are known risk factors for NASH.[23] However, there was no more significant relationship when HOMA was added to the model.

Insulin resistance is thought to be the "first hit" in nonalcoholic fatty liver disease, leading to excess free fatty acid accumulation in the liver.[21] In our study, we found a significant link between insulin resistance and OSA, independent of BMI, confirming previous published data.[5-11] The presence of recurrent hypoxemia and abnormal nocturnal sympathetic output has been proposed as the mediating mechanism in the causal link between insulin resistance and OSA.[24] Improvement in insulin responsiveness after continuous positive airway pressure treatment was reported.[8] Therefore, it could be postulated that OSA contributes to increased insulin resistance and steatosis.

The pathogenesis of NASH (*i.e.*, the nature of the "second hit") remains unclear.[25,26] The striking relationship between AHI and lobular necrosis or fibrosis in our biopsed patients suggests that OSA may play a role in the pathogenesis of NASH. A possible mechanism may be CYP2E1 activation, as recently shown by Chalasani et al., who found a significant correlation between hepatic CYP2E1 activity, nocturnal hypoxia, and serum insulin levels in nondiabetic patients with biopsy-proven NASH.[27]

Liver injury in OSA could also be the result of direct hypoxia, as suggested in two previous case reports.[3,4] Indeed, some of our patients with liver biopsy had lobular necrosis without steatosis. Accordingly, we performed immunohistochemical staining of VEGF on liver biopsy specimens. Because VEGF is a hypoxia-induced gene regulated by hypoxia-inducible factor 1, it may be considered an indirect marker of hypoxia.[28] Only 3 patients had positive immunostaining, none with lobular necrosis alone. Moreover, we found no relationship between the severity of OSA and VEGF immunostaining. Because the VEGF protein remains present in the liver 4 to 6 hours following hypoxia, VEGF immunostaining is likely to detect previous night liver hypoxia in our patients who underwent liver biopsy during the morning. However, we cannot rule out mild hepatic hypoxia because of the lack of sensitivity of the VEGF assay.

In conclusion, OSA is a risk factor for abnormal liver enzymes independent of BMI and should be investigated in patients without other cause of liver disease. In addition, our results suggest that OSA could play a role in the pathogenesis of NASH by increasing insulin resistance. However, we cannot rule out the direct contribution of hypoxia in liver injury. Further studies are needed to assess the prevalence of OSA in patients with NASH and to evaluate whether treatment of OSA may improve liver injury.

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