Obstructive Sleep Apnea, Insulin Resistance, and Steatohepatitis in Severe Obesity

Vsevolod Y. Polotsky¹, Susheel P. Patil¹, Vladimir Savransky¹, Alison Laffan¹, Shannon Fonti¹, Leigh A. Frame¹, Kimberly E. Steele², Michael A. Schweitzer², Jeanne M. Clark^{3,4}, Michael S. Torbenson⁵, and Alan R. Schwartz¹

¹Division of Pulmonary and Critical Care Medicine, Department of Medicine; ²Department of Surgery; ³Division of General Internal Medicine, Department of Medicine; ⁴Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health; and ⁵Department of Pathology, Johns Hopkins University, Baltimore, Maryland

Rationale: Obstructive sleep apnea is associated with insulin resistance and liver injury. It is unknown whether apnea contributes to insulin resistance and steatohepatitis in severe obesity.

Objectives: To examine whether sleep apnea and nocturnal hypoxemia predict the severity of insulin resistance, systemic inflammation, and steatohepatitis in severely obese individuals presenting for bariatric surgery.

Methods: We performed sleep studies and measured fasting blood glucose, serum insulin, C-reactive protein, and liver enzymes in 90 consecutive severely obese individuals, 75 women and 15 men, without concomitant diabetes mellitus or preexistent diagnosis of sleep apnea or liver disease. Liver biopsies (n = 20) were obtained during bariatric surgery.

Measurements and Main Results: Obstructive sleep apnea with a respiratory disturbance index greater than 5 events/hour was diagnosed in 81.1% of patients. The median respiratory disturbance index was 15 ± 29 events/hour and the median oxygen desaturation during apneic events was $4.6 \pm 1.8\%$. All patients exhibited high serum levels of C-reactive protein, regardless of the severity of apnea, whereas liver enzymes were normal. Oxygen desaturation greater than 4.6% was associated with a 1.5-fold increase in insulin resistance, according to the homeostasis model assessment index. Histopathology data suggested that significant nocturnal desaturation might predispose to hepatic inflammation, hepatocyte ballooning, and liver fibrosis. Fasting blood glucose levels and steatosis scores were not affected by nocturnal hypoxia. There was no relationship between the respiratory disturbance index and insulin resistance or liver histopathology.

Conclusions: Hypoxic stress of sleep apnea may be implicated in the development of insulin resistance and steatohepatitis in severe obesity.

Keywords: hypoxemia; fatty liver disease; metabolic syndrome; sleep-disordered breathing; liver injury

Obstructive sleep apnea (OSA) is a complex disorder consisting of upper airway obstruction, chronic intermittent hypoxia (CIH), and sleep fragmentation (1). Epidemiologic studies have demonstrated that OSA is associated with insulin resistance and glucose intolerance, independent of obesity (2–4). Clinical investigations have also shown that OSA results in low-grade systemic inflammation (5, 6). Both insulin resistance and systemic inflammation may contribute to the increased cardiovascular risk in patients with OSA (7–9). Insulin resistance, systemic

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Sleep apnea predisposes to metabolic dysfunction and systemic inflammation and may lead to liver injury, but its effect in severe obesity is unknown.

What This Study Adds to the Field

Nocturnal oxygen desaturation is associated with insulin resistance and may predispose to nonalcoholic fatty liver disease in severe obesity, but does not affect serum C-reactive protein levels.

inflammation, and OSA are particularly prevalent in patients with severe obesity defined as a body mass index (BMI) exceeding 40 kg/m^2 (2, 10–12). While obesity causes systemic inflammation, insulin resistance, and sleep apnea (12–15), sleep apnea may further exacerbate the inflammatory and metabolic disturbances (2, 3, 6). Nevertheless, it is not known whether concomitant OSA is implicated in metabolic dysregulation and systemic inflammation in severe obesity.

One of the consequences of obesity and insulin resistance is nonalcoholic fatty liver disease (NAFLD). Work suggests that OSA may also contribute to the progression of NAFLD (16–19). NAFLD includes a spectrum of disease severity, ranging from steatosis without inflammation to nonalcoholic steatohepatitis (NASH) and liver cirrhosis (20–22). The reliable diagnosis and staging of NAFLD from steatosis to cirrhosis is possible only by liver biopsy (23). Day and James (21) proposed a “two-hit” model to explain the evolution of NAFLD. The “first hit” involves the accumulation of triglyceride in hepatocytes, and has been specifically attributed to obesity and insulin resistance. The “second hit” induces progression of hepatic steatosis to NASH (21). Obesity, age over 45 years, diabetes, hypertriglyceridemia, and hypertension have been identified as risk factors for the progression of NAFLD (24), but causes for NAFLD progression, which occurs in approximately 25–30% of patients with hepatic steatosis, remain unknown (25).

We have previously used a mouse model of CIH and have shown that CIH induces insulin resistance in genetically obese mice and converts diet-induced hepatic steatosis to NASH (26, 27). Although obesity and OSA have been implicated in the development of systemic inflammation, insulin resistance, and NAFLD (2–4, 10–21, 24, 28), the inflammatory, metabolic, and hepatic profiles of severe obesity with and without concomitant sleep apnea have not been elucidated. Patients presenting for bariatric surgery offer distinct advantages in elucidating the link between OSA and NAFLD severity, because of an increased susceptibility to both disorders in severe obesity, unbiased access to liver tissue during surgery, and large numbers of

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Correspondence and requests for reprints should be addressed to Vsevolod Y. Polotsky, M.D., Ph.D., Johns Hopkins Asthma and Allergy Center, 5501 Hopkins Bayview Circle, Baltimore, MD 21224. E-mail: vpolots1@jhmi.edu

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well-characterized obese patients. We hypothesized that CIH of OSA exerts an independent effect on markers of systemic inflammation, insulin resistance, and NAFLD in severely obese individuals.

METHODS

Participants

Ninety patients were recruited from the Johns Hopkins Bayview Medical Center Bariatric Surgery Clinic (Baltimore, MD). Men and women were recruited in proportion to their representation in the bariatric clinic population (~75% women, ~25% men). Patient inclusion criteria included age greater than 21 years and a body mass index (BMI) greater than 35 kg/m². Exclusion criteria included known diagnosis of OSA, use of nasal continuous positive airway pressure (CPAP) within the prior 3 months, recent weight loss of 10% or more, diabetes mellitus (defined by prior clinical diagnosis or use of hypoglycemic agents or documented fasting glucose > 126 mg/dl), current or past history of alcohol abuse, liver disease, history of HIV disease, current systemic use of steroids, unstable cardiovascular disease, and supplemental oxygen use. Informed consent was obtained from all subjects, and the Johns Hopkins University Institutional Review Board approved the protocol.

Study Protocol

Demographic and anthropometric characteristics were assessed. One to 2 months before surgery, patients underwent a full-night sleep study to characterize their sleep and breathing patterns. Standard criteria were employed to evaluate the breathing patterns during sleep. All respiratory events were defined as previously described (29, 30). Apnea was defined as a drop in the peak thermal sensor excursion by at least 90% of baseline for at least 10 seconds. Hypopnea was defined as a decrease in the nasal pressure signal excursions for at least 10 seconds accompanied by desaturation of 4% or more from pre-event baseline or an arousal from sleep. The respiratory disturbance index (RDI) was calculated as a sum of apneas and hypopneas. Obstructive sleep apnea was diagnosed as an RDI of at least 5/hour. Nocturnal oxyhemoglobin desaturation was characterized as the mean fall in oxyhemoglobin saturation from baseline to nadir during sleep-disordered breathing episodes (ΔSaO_2). At the termination of the sleep study at 7 A.M., patients had fasting blood sampled for liver enzymes, glucose, insulin, and C-reactive protein and arterial blood gas (ABG) was measured. Patients and their treating physicians were provided with results of the sleep study and were alerted to the presence and severity of the subject's sleep apnea. During the ensuing bariatric surgery, a liver biopsy was performed for histopathologic analysis.

Liver Biopsy

Liver biopsies are considered the standard of care for bariatric surgery patients (31) and routinely obtained intraoperatively at the Johns Hopkins Bayview Medical Center. We analyzed liver biopsy prospectively in a subset of 20 consecutive patients recruited for the present study. Liver tissue was fixed in buffered 10% formalin and analyzed in paraffin sections stained with hematoxylin-eosin and Masson trichrome. Histologic evaluation was performed by a liver pathologist (M.S.T.) in a blinded fashion according to the NASH Clinical Research Network Scoring System based on the NAFLD activity score (NAS) (32). Briefly, steatosis was scored according to the number of affected hepatocytes as 0 (<5%), 1 (6–33%), 2 (34–66%), or 3 (> 67%). Lobular inflammation was scored according to the number of infiltrates in the $\times 20$ field as 0 (none), 1 (1), 2 (2–4), or 3 (> 4). Ballooning was scored according to the number of affected hepatocytes as 0 (none), 1 (few), or 2 (many). Fibrosis was scored as 0 (none), 1 (mild pericellular), 1.5 (moderate pericellular), 2 (portal plus pericellular), or 3 (bridging). NAS was calculated as a sum of steatosis, lobular inflammation, and ballooning (32).

Blood Work

ELISA was used for measurements of serum insulin (Linco Research, Inc., St. Charles, MO) and C-reactive protein (CRP; R&D Systems,

Inc., Minneapolis, MN). Serum glucose levels and liver tests including alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total and direct bilirubin, total protein, and albumin were performed in the Johns Hopkins Bayview Medical Center Clinical Laboratory. The homeostasis model assessment (HOMA) index was calculated as fasting serum insulin ($\mu\text{U/ml}$) $\times$ fasting blood glucose (mmol/L)/22.5 (33).

Statistical Analyses

All values are reported in tables as mean $\pm$ standard deviation of the mean (SD) and in figures as mean $\pm$ standard error of the mean (SEM). Statistical comparisons between clinical parameters in various groups of patients were performed using unpaired *t* tests or chi square tests where appropriate. Relationships between measurements of obesity, sleep apnea severity, the severity of oxyhemoglobin desaturation, and markers of inflammation and metabolic dysfunction were ascertained using simple and multivariable linear regression and the Pearson product moment correlation. Statistical comparisons of histologic parameters were performed using general linear model analysis of variance with the Tukey *post hoc* test for multiple comparisons.

TABLE 1. CHARACTERISTICS OF STUDY COHORT

	Total (n = 90)	Women (n = 75)	Men (n = 15)
Age, yr	41.1 $\pm$ 9.5	41.3 $\pm$ 9.7	40.0 $\pm$ 9.7
Race			
White, n (%)	61 (67.8)	50 (66.7)	11 (73.3)
African-American, n (%)	29 (32.2)	25 (33.3)	4 (26.7)
Height, cm	167 $\pm$ 8	165 $\pm$ 6	179 $\pm$ 7*
Weight, kg	137 $\pm$ 25	132 $\pm$ 20	166 $\pm$ 25*
Neck, cm	41.6 $\pm$ 4.2	40.7 $\pm$ 3.4	46.4 $\pm$ 5.0*
Waist, cm	131 $\pm$ 17	128 $\pm$ 15	149 $\pm$ 16*
Waist/hip	0.94 $\pm$ 0.23	0.93 $\pm$ 0.25	0.99 $\pm$ 0.09
Body mass index, kg/m ²	49.0 $\pm$ 7.9	48.5 $\pm$ 7.9	51.6 $\pm$ 7.7
Comorbidity			
Hypertension, n (%)	35 (38.8)	27 (36)	8 (53)
Coronary artery disease, n (%)	2 (2.2)	1 (1.3)	1 (6.7)
Asthma, n (%)	18 (20)	18 (24)	0 [†]
COPD, n (%)	0	0	0
Hypothyroidism, n (%)	12 (13.3)	12 (16)	0
Arterial blood gas			
pH	7.41 $\pm$ 0.03	7.41 $\pm$ 0.03	7.41 $\pm$ 0.03
PO ₂ , mm Hg	83.2 $\pm$ 11.2	85.7 $\pm$ 10.5	74.7 $\pm$ 10.8*
PCO ₂ , mm Hg	39.8 $\pm$ 4.3	38.2 $\pm$ 4.4	42.8 $\pm$ 4.1 [‡]
Prevalence of OSA, n (%)	73 (81.1)	59 (78.7)	14 (93.3)
Mild (RDI, 5–15/h), n (%)	28 (31.1)	26 (34.7)	2 (13.3)
Moderate (RDI, 16–30/h), n (%)	21 (23.3)	18 (24)	3 (20)
Severe (RDI, >30/h), n (%)	24 (26.7)	15 (20)	9 (60) [‡]
Sleep characteristics			
Total sleep time, min	388 $\pm$ 72.6	398 $\pm$ 60.3	342 $\pm$ 107.2
Sleep efficiency, %	85.3 $\pm$ 11.2	86.3 $\pm$ 10.0	80.6 $\pm$ 15.4
Stage N1, %	13.8 $\pm$ 12.2	12.1 $\pm$ 10.5	22.4 $\pm$ 16.4 [†]
Stage N2, %	60.1 $\pm$ 11.0	60.5 $\pm$ 10.3	58.3 $\pm$ 14.4
Stage N3, %	10.7 $\pm$ 9.0	11.8 $\pm$ 8.9	5.1 $\pm$ 7.5 [‡]
Stage REM, %	15.3 $\pm$ 7.3	15.5 $\pm$ 6.6	14.1 $\pm$ 10.2
N-REM RDI, events/h	23.5 $\pm$ 30.7	19.7 $\pm$ 29.8	42.5 $\pm$ 29.0 [†]
REM RDI, events/h	39.4 $\pm$ 27.6	36.3 $\pm$ 26.5	57.4 $\pm$ 28.5
Total RDI, events/h	26.2 $\pm$ 29.2	22.8 $\pm$ 28.6	43.6 $\pm$ 27.1 [†]
N-REM baseline SaO ₂	95.8 $\pm$ 1.7	95.9 $\pm$ 1.6	92.9 $\pm$ 6.9
REM baseline SaO ₂	95.0 $\pm$ 3.4	95.3 $\pm$ 2.3	92.9 $\pm$ 7.0
Total baseline SaO ₂	95.6 $\pm$ 1.9	95.8 $\pm$ 1.6	95.3 $\pm$ 2.9
N-REM ΔSaO_2	4.6 $\pm$ 1.8	4.4 $\pm$ 1.6	5.6 $\pm$ 2.5
REM ΔSaO_2	6.2 $\pm$ 2.8	5.7 $\pm$ 2.4	8.6 $\pm$ 4.1 [†]
Total ΔSaO_2	5.2 $\pm$ 1.8	5.0 $\pm$ 1.6	6.0 $\pm$ 2.5

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; N-REM = non-rapid eye movement; OSA = obstructive sleep apnea; RDI = respiratory disturbance index; ΔSaO_2 = mean fall in oxyhemoglobin saturation from baseline to nadir during sleep-disordered breathing episodes.

Values are reported as means $\pm$ standard deviation of the mean unless indicated otherwise.

* $P < 0.001$; [†] $P < 0.05$; and [‡] $P < 0.01$, for comparison between sexes.

Analyses were conducted with STATA 9.0 (College Station, TX) or Mini-tab 13.1 (State College, PA).

RESULTS

General and Sleep Characteristics of the Bariatric Cohort

The characteristics of our 90 research subjects are presented in Table 1. The patient age varied between 22 and 68 years, but 76 subjects (84.4%) were between 30 and 55 years of age. The majority of patients were white women, which represents the typical population in our bariatric clinic. The BMI ranged from 35 to 86.7 kg/m², and 53 patients (58.8%) had a BMI between 45 and 55 kg/m². Both sexes reported hypertension, whereas bronchial asthma was common in women than in men. The majority of patients had normal ABG during wakefulness. Men had lower daytime Po₂ and higher Pco₂ than women (Table 1), which can be attributed to the higher propensity of obese men to hypoventilate (34). As expected, OSA was diagnosed in the majority of research subjects (Table 1). Analysis of the sleep studies revealed increased stage N1 sleep and a spectrum of sleep-disordered breathing, which was more severe in REM sleep. Men had higher body weight, larger neck, and waist circumferences than women. Men also had more severe OSA at the same level of BMI. Consequently, men demonstrated worse

quality of sleep than women with increased stage N1 sleep and decreased slow wave N3 sleep (Table 1).

To compare the clinical and sleep characteristics between the upper and lower ends of RDI and Δ SaO₂ spectrum (Table 2), we determined median values for RDI of 15 events/hour and for Δ SaO₂ of 4.6% in our group. Patients with RDI greater than 15 events/hour and a Δ SaO₂ of 4.6% or more were older and had a larger neck size, but their BMI was similar to patients with an RDI less than 15/hour and a Δ SaO₂ less than 4.6%. The prevalence of hypertension was higher in subjects with more severe OSA. Daytime ABG values were within the normal range, regardless of the severity of OSA and nocturnal oxyhemoglobin desaturation (Table 2). Patients with an RDI greater than 15/hour and a Δ SaO₂ of 4.6% or more had lower daytime Po₂ than patients with RDI less than 15/hour and Δ SaO₂ less than 4.6%, whereas Pco₂ did not differ. As expected, individuals with a high RDI showed increased stage N1 sleep and more severe nocturnal oxyhemoglobin desaturations, whereas individuals with more severe nocturnal oxyhemoglobin desaturations had higher RDI (Table 2).

Indices of Systemic Inflammation, Insulin Resistance, and Liver Injury

The serum concentration of CRP, a major marker of systemic inflammation (35), was markedly elevated for the entire group (Table 3), and the severity of obesity (BMI) was positively associated with serum CRP (β = 0.15 mg/dl for a 1-kg/m² increase in BMI; P = 0.02; see Figure E1A in the online supplement), whereas waist circumference, a measure of fat distribution, was of borderline statistical significance (β = 0.06 mg/dl for a 1-cm increase in waist circumference; P = 0.054; Figure E1B). In multiple regression analyses, BMI no longer predicted CRP levels after adjustments for waist circumference (β = 0.11 mg/dl for a 1-kg/m² increase in BMI; P = 0.20). Of note, CRP did not differ significantly between those with and without OSA, nor did it differ in groups defined by the level of desaturation (Table 3).

All patients showed high normal levels of fasting blood glucose and elevated levels of fasting serum insulin and the HOMA index (Figure 1). The severity of obesity was not associated with elevations in fasting serum insulin levels (β = 0.01 μ U/ml for a 1-kg/m² increase in BMI; P = 0.95) or with the degree of insulin resistance as assessed by the HOMA index (β = 0.009 HOMA index unit for a 1-kg/m² increase in BMI; P = 0.84; Figure E1C). There was no difference in serum glucose, insulin, and HOMA index levels in individuals with RDI less than 15/hour and greater than 15/hour (Figure 1A). There was no relationship observed between baseline oxygen saturation and level of insulin resistance as assessed by HOMA index. In contrast, insulin resistance was associated with the severity of nocturnal oxyhemoglobin desaturation (β = 0.38 units of HOMA index per 1% of Δ SaO₂, P = 0.03; Figure E1D). After adjusting for waist circumference and BMI, the association between HOMA index and Δ SaO₂ remained significant (β = 0.40, P = 0.03). The HOMA index was also associated with the average SaO₂ during hypoxic events (unadjusted β = -0.28, P = 0.01), independent of BMI and waist (adjusted β = -0.30, P = 0.008). Subjects with severe nocturnal oxyhemoglobin desaturation (Δ SaO₂ $\geq$ 4.6%) demonstrated a greater than 1.5-fold increase in the HOMA index (HOMA index elevated by 1.61 units; P = 0.01) and a trend to higher fasting serum insulin levels (insulin elevated by 4.4 μ U/ml; P = 0.09), compared with the subjects with mild desaturation (Figure 1B). Moreover, adjusting for BMI and waist circumference did not alter the relationship between the severity of nocturnal oxyhemoglobin desaturation and the HOMA index (HOMA index elevated by

TABLE 2. RELATIONSHIPS BETWEEN CLINICAL AND SLEEP CHARACTERISTICS OF OBESE INDIVIDUALS AND SEVERITY OF SLEEP-DISORDERED BREATHING

	Respiratory Disturbance Index		Δ SaO ₂	
	<15/h (n = 45)	>15/h (n = 45)	<4.6% (n = 45)	$\geq$ 4.6% (n = 45)
Sex				
Female, n (%)	42 (93.3)	33 (73.3)	39 (87.7)	36 (80.0)
Male, n (%)	3 (6.7)	12 (26.7)	6 (13.3)	9 (20.0)
Age, yr	38.8 $\pm$ 8.1	43.3 $\pm$ 10.2*	38.3 $\pm$ 8.4	43.8 $\pm$ 9.7†
Body mass index, kg/m ²	48.1 $\pm$ 7.9	49.9 $\pm$ 7.9	47.8 $\pm$ 6.3	50.3 $\pm$ 9.2
Neck, cm	40.3 $\pm$ 3.9	43.0 $\pm$ 4.2†	40.6 $\pm$ 3.8	42.8 $\pm$ 4.4*
Waist, cm	128 $\pm$ 17	135 $\pm$ 18	129 $\pm$ 18	134 $\pm$ 17
Waist/hip	0.91 $\pm$ 0.10	0.97 $\pm$ 0.31	0.92 $\pm$ 0.10	0.96 $\pm$ 0.31
Prevalence of hypertension, n (%)	12 (26.7)	23 (51.1)*	15 (44.4)	20 (50)
Arterial blood gas				
pH	7.41 $\pm$ 0.03	7.41 $\pm$ 0.03	7.41 $\pm$ 0.03	7.41 $\pm$ 0.03
Po ₂ , mm Hg	86.8 $\pm$ 8.8	80.8 $\pm$ 12.1†	86.9 $\pm$ 9.8	80.8 $\pm$ 11.3†
Pco ₂ , mm Hg	39.4 $\pm$ 4.0	40.1 $\pm$ 4.5	39.0 $\pm$ 4.1	40.5 $\pm$ 4.2
Sleep characteristics				
Total sleep time, min	395 $\pm$ 72	381 $\pm$ 73	394 $\pm$ 73	384 $\pm$ 73
Sleep efficiency, %	87.4 $\pm$ 9.5	83.3 $\pm$ 12.4	85.7 $\pm$ 11.1	85.1 $\pm$ 11.4
Stage N1, %	11.0 $\pm$ 10.2	16.7 $\pm$ 13.5*	13.0 $\pm$ 11.8	14.7 $\pm$ 12.7
Stage N2, %	58.3 $\pm$ 9.4	61.8 $\pm$ 12.2	59.9 $\pm$ 9.1	60.4 $\pm$ 12.7
Stage N3, %	14.7 $\pm$ 9.3	6.7 $\pm$ 6.7	12.0 $\pm$ 9.5	9.4 $\pm$ 8.4
Stage REM, %	15.9 $\pm$ 6.9	14.7 $\pm$ 7.8	15.1 $\pm$ 7.5	15.5 $\pm$ 7.2
N-REM RDI, events/h	5.1 $\pm$ 3.8	42.0 $\pm$ 34.6†	12.9 $\pm$ 15.5	34.1 $\pm$ 38.0†
REM RDI, events/h	22.6 $\pm$ 16.8	57.4 $\pm$ 25.6†	26.2 $\pm$ 22.3	52.8 $\pm$ 26.2†
Total RDI, events/h	7.6 $\pm$ 4.3	44.9 $\pm$ 31.7†	14.7 $\pm$ 14.7	37.8 $\pm$ 35.2†
N-REM baseline SaO ₂	96.2 $\pm$ 1.4	95.6 $\pm$ 2.0	96.2 $\pm$ 1.4	95.5 $\pm$ 2.0*
REM baseline SaO ₂	96.1 $\pm$ 1.4	93.8 $\pm$ 4.4†	96.2 $\pm$ 1.4	93.8 $\pm$ 4.4†
Total baseline SaO ₂	96.1 $\pm$ 1.4	95.3 $\pm$ 2.2*	96.3 $\pm$ 1.3	95.1 $\pm$ 2.2*
N-REM Δ SaO ₂	3.9 $\pm$ 1.2	5.3 $\pm$ 2.0‡	3.5 $\pm$ 0.8	5.6 $\pm$ 2.0‡
REM Δ SaO ₂	4.8 $\pm$ 1.2	7.6 $\pm$ 3.3‡	4.5 $\pm$ 1.2	7.8 $\pm$ 3.1‡
Total Δ SaO ₂	4.3 $\pm$ 1.1	6.0 $\pm$ 2.1‡	3.9 $\pm$ 0.8	6.5 $\pm$ 1.7‡

Values are reported as means $\pm$ standard deviation of the mean unless indicated otherwise.

* P < 0.05; † P < 0.01; and ‡ P < 0.001, for the difference between patients with RDI less than 15/hour and greater than 15/hour and between patients with Δ SaO₂ less than 4.6% or at least 4.6% or more.

TABLE 3. RELATIONSHIPS BETWEEN SEVERITY OF SLEEP-DISORDERED BREATHING AND SERUM LEVELS OF LIVER ENZYMES AND C-REACTIVE PROTEIN IN OBESE INDIVIDUALS

	Respiratory Disturbance Index		ΔSaO_2	
	<15/h	>15/h	<4.6%	≥ 4.6
	(n = 45)	(n = 45)	(n = 45)	(n = 45)
ALT, U/L	11.1 $\pm$ 6.1	12.3 $\pm$ 6.2	11.2 $\pm$ 6.4	12.4 $\pm$ 5.9
AST, U/L	17.5 $\pm$ 6.6	17.8 $\pm$ 5.9	17.1 $\pm$ 6.5	18.3 $\pm$ 5.9
Alkaline phosphatase, U/L	64.2 $\pm$ 20.3	66.0 $\pm$ 20.6	63.7 $\pm$ 21.7	66.8 $\pm$ 18.9
Total bilirubin, mg/dl	0.29 $\pm$ 0.25	0.23 $\pm$ 0.14	0.25 $\pm$ 0.23	0.26 $\pm$ 0.16
Direct bilirubin, mg/dl	0.10 $\pm$ 0.08	0.09 $\pm$ 0.05	0.08 $\pm$ 0.07	0.10 $\pm$ 0.05
Total protein, g/dl	6.63 $\pm$ 0.29	6.80 $\pm$ 0.29	6.65 $\pm$ 0.29	6.81 $\pm$ 0.29
Albumin, g/dl	4.09 $\pm$ 0.27	4.16 $\pm$ 0.27	4.08 $\pm$ 0.27	4.19 $\pm$ 0.25
C-reactive protein, mg/L	8.44 $\pm$ 4.78	9.05 $\pm$ 4.91	8.19 $\pm$ 4.89	9.34 $\pm$ 4.74

Definition of abbreviations: ALT = alanine aminotransferase; AST = aspartate aminotransferase.

1.65 units; $P = 0.02$), suggesting an independent effect of CIH on insulin resistance. All 90 patients exhibited normal serum levels of liver enzymes, bilirubin, total protein, and albumin, regardless of the severity of obesity or sleep-disordered breathing (Table 3).

Liver pathology was assessed in 20 consecutive patients, whose anthropometric, clinical, sleep characteristic, daytime ABG, and indices of systemic inflammation and insulin resistance did not differ from the entire group (Tables E1 and E2). Semiquantitative histopathologic indices of liver injury and inflammation were compared in four groups of patients: (1) subjects who displayed mild nocturnal oxyhemoglobin desaturation ($\Delta\text{SaO}_2 \leq 4.6\%$) and low RDI ($<15/\text{h}$), $n = 7$; (2) patients who showed mild oxyhemoglobin desaturation ($\Delta\text{SaO}_2 \leq 4.6\%$) and high RDI ($>15/\text{h}$), $n = 3$; (3) individuals who demonstrated severe oxyhemoglobin desaturation ($\Delta\text{SaO}_2 > 4.6\%$) and low

RDI, $n = 6$; and (4) patients who exhibited severe oxyhemoglobin desaturation and high RDI, $n = 4$. Evidence of hepatic steatosis was found in 14 patients, regardless of OSA status (Figure 2). Patients with moderate to severe OSA (RDI $> 15/\text{h}$) and severe hypoxemia exhibited significantly more severe hepatic lobular inflammation than did patients with an RDI less than 15/hour and mild hypoxemia (Figures 2 and 3A). Severe nocturnal hypoxemia was associated with ballooning of hepatocytes, high NAS, and pericellular fibrosis in the liver (Figure 2 and Figures 3B–3D). In contrast, patients with high RDI without hypoxemia did not exhibit hepatocyte ballooning and liver fibrosis and had low NAS. Daytime PCO_2 was within the normal range in all patients, regardless of the severity of OSA or liver histopathology (*see* Table E1). The BMI was similar in all patient groups (Figure 3E), suggesting that OSA had an independent detrimental effect on the liver.

Figure 1. Fasting serum glucose, serum insulin, and the homeostasis model assessment (HOMA) index in severely obese individuals with obstructive sleep apnea (OSA). The HOMA index was calculated as fasting serum insulin ($\mu\text{U}/\text{ml}$) $\times$ fasting blood glucose (mmol/L)/22.5 (33). (A) Comparison of patients with the respiratory disturbance index (RDI) less than 15 per hour and greater than 15 per hour ($n = 45$ per group). (B) Comparison of patients with average fall in oxyhemoglobin saturation during apneic events (ΔSaO_2) less than 4.6% and at least 4.6% ($n = 45$ per group).

Figure 2. (A) Representative image of liver without inflammation in an individual without OSA. Macrovesicular hepatic steatosis is evident, but inflammation is absent (hematoxylin-eosin; original magnification, $\times 100$). (B) Representative image of liver in an individual with OSA and severe nocturnal oxyhemoglobin desaturation. Macrovesicular hepatic steatosis is evident, and lobular inflammation is present (arrows) (hematoxylin-eosin; original magnification, $\times 100$). (C) Representative image of liver without pericellular fibrosis in an individual without OSA (Masson trichrome; original magnification, $\times 100$). (D) Representative image of liver in an individual with OSA and severe nocturnal oxyhemoglobin desaturation. Prominent pericellular perisinusoidal fibrosis is present. Collagen depositions are stained blue and have a chicken-wire appearance (Masson trichrome; original magnification, $\times 100$).

DISCUSSION

The main purpose of this study was to explore relationships between OSA, nocturnal intermittent hypoxemia, insulin resistance, systemic inflammation, and NAFLD in an unselected cohort of patients with severe obesity. We used a rigorous approach excluding all individuals with previously diagnosed and treated OSA, nocturnal hypoxemia, diabetes mellitus, and liver disease. Our study has demonstrated that the severity of nocturnal oxyhemoglobin desaturation, but not the RDI, predicted the severity of insulin resistance and might be implicated in the development of NASH. In contrast, severe obesity was associated with high levels of serum CRP, but there was no relationship between the severity of OSA, nocturnal hypoxemia, and serum CRP. Our findings suggest that obesity and OSA have distinct metabolic, inflammatory and hepatic profiles.

OSA, Systemic Inflammation, and Metabolic Dysfunction in Severe Obesity

Obesity and OSA have been independently associated with metabolic dysfunction and systemic inflammation (6, 12, 35). We demonstrated a greater than 80% prevalence of OSA in severe obesity, which is consistent with previous reports (10, 11, 36). Thus, in severe obesity metabolic dysfunction can be related not only to adiposity per se but also to concomitant OSA. In this study, we have segregated inflammatory and metabolic biomarkers specific for severe obesity and OSA.

Severe obesity leads to chronic inflammation, which has been implicated in poor cardiovascular outcomes (13). OSA causes low-grade inflammation and hypercytokinemia (6). C-reactive protein, one of the markers of systemic inflammation, was found to be an important predictor of the severity of atherosclerosis and poor

Figure 3. Relationships between hepatic histopathology and the severity of OSA as measured by respiratory disturbance index (RDI < 15 events/h or ≥ 15 events/h) and average fall in oxyhemoglobin saturation during apneic events ($\Delta SaO_2 < 4.6\%$ and $\geq 4.6\%$). (A) Hepatic lobular inflammation score; (B) hepatocyte ballooning score; (C) nonalcoholic fatty liver disease (NAFLD) activity score defined as the sum of steatosis, lobular inflammation, and ballooning scores (32); (D) hepatic fibrosis score; and (E) body mass index (BMI). * $P < 0.05$ between the group of patients with RDI less than 15 events/hour, ΔSaO_2 less than 4.6% and the group of patients with RDI greater than 15 events/hour, ΔSaO_2 at least 4.6% or more.

cardiovascular outcomes (35, 37, 38). Our findings confirm those of previous studies demonstrating similar marked elevations of serum CRP levels in severely obese individuals (12, 15). The role of OSA in CRP elevation is controversial; some studies report an independent effect (5) whereas others attribute high levels of CRP to concurrent obesity (39). Our findings indicate that elevations in serum CRP levels are related to obesity and not to concomitant OSA. Alternatively, excessive adiposity may overwhelm any possible proinflammatory effects of intermittent hypoxemia or sleep fragmentation in the severely obese population.

Severe obesity leads to insulin resistance (14). OSA is also associated with insulin resistance, glucose intolerance, and high levels of hemoglobin A1c, independent of BMI (3, 4, 40), and insulin resistance improves with CPAP treatment (2). Resolution of OSA with weight loss may contribute to weight loss-induced improvement in insulin resistance (41). Murine data indicate that intermittent hypoxia (IH) mediates the effect of OSA on insulin resistance (26, 42). The impact of IH is further highlighted by our present findings, which demonstrate that insulin resistance is linked specifically to IH rather than to apnea periodicity (RDI). Thus, the hypoxic stress of OSA may be implicated in the progression of insulin resistance in severe obesity.

OSA and NASH in Severe Obesity

Although insulin resistance is a recognized cause of hepatic steatosis, the progression of steatosis to NASH is not well understood. Our study extends previously published reports exploring relationships between OSA, liver injury, and NASH. Tanne and colleagues studied moderately obese patients who presented with abnormalities of serum liver enzymes for liver biopsy and found that severe OSA with an apnea-hypopnea index greater than 50 events/hour was a risk factor for steatohepatitis, independent of body weight (16). Other studies also reported an independent association between OSA, liver injury, and NASH in moderately obese and overweight subjects, based on liver enzymes or imaging techniques (17, 18, 28), although liver biopsy was not performed. Jouet and colleagues (19) reported an unusually high prevalence of elevated liver enzymes in the bariatric population (46.6%), but found no association between OSA and NASH diagnosed by liver biopsy. In the latter study, OSA was characterized only by apnea-hypopnea index, whereas relationships between nocturnal hypoxemia and NASH were not examined. In another bariatric case series, Kallwitz and colleagues (43) reported a higher prevalence of serum alanine aminotransferase elevation in patients with OSA and a trend toward a higher prevalence of OSA in patients with hepatic fibrosis. Nevertheless, the retrospective nature of this study made it difficult to obtain liver tissue from an unbiased sample of patients, and concomitant comorbidities and hypoxic indices could not be assessed. In contrast, our study addressed these concerns with a prospective, unbiased sampling of liver tissue in well-characterized bariatric patients.

Our data revealed that nocturnal CIH was associated with lobular inflammation, hepatocyte ballooning, and liver fibrosis, but not with hepatic steatosis. We hypothesize that severe obesity per se acts as “the first hit” in the progression of NAFLD inducing hepatic steatosis, whereas the presence of CIH of OSA acts as the “second hit” (21), inducing progression of hepatic steatosis to NASH. Our previous experiments in the mouse model showed that CIH increases lipid peroxidation and activates a redox-dependent transcription factor, NF- κ B, in the liver (44, 45). We have also shown that, in mice with diet-induced hepatic steatosis, but not in mice with normal livers, CIH increases liver levels of proinflammatory cytokines IL-1 β , IL-6, macrophage inflammatory protein-2, and tumor necrosis factor- α as well as α 1(I) collagen and indices of lobular inflammation and

fibrosis (27, 45, 46). Our present report and previous experimental data suggest that the hypoxic stress of OSA may induce oxidative stress in the livers of patients with severe obesity, leading to inflammation and fibrosis, which culminate in NASH.

Strengths and Weaknesses

Our data are novel in several respects. First, unlike previous reports, we examined relationships between nocturnal hypoxemia and liver histopathology. We found that severe hypoxemia predicts the presence of NASH. Second, we employed rigorous exclusion criteria and eliminated patients with diabetes type 2 and taking diabetes medications. Diabetes type 2 may accelerate (24), and diabetes medications (such as metformin and troglitazones) may inhibit, the progression of NAFLD (47–49). Third, none of our patients had any liver enzyme elevation or clinical manifestations of liver disease. Given that the biopsy is the most sensitive and specific test for diagnosis of NASH (23), our data suggest that hypoxic stress rather than RDI per se predicts early or occult NASH in severely obese patients. Thus, we were able to establish that hypoxic stress in OSA and NASH were independently associated in well-characterized severely obese patients who were subjected to rigorous, unbiased selection criteria and a thorough, blinded analysis of liver biopsies using the established, standardized NASH Clinical Research Network Scoring System (32). Nevertheless, we acknowledge limitations of our study including its cross-sectional nature, which precludes establishing that intermittent hypoxemia causes insulin resistance and NASH. In addition, although the HOMA index is a well-validated measure of insulin resistance (33), it is still inferior to the “gold standard” euglycemic hyperinsulinemic clamp (50). Finally, we recognize that the number of subjects from whom liver biopsies were available was small, restricting the size of subgroups in our analysis and limiting the generalizability of our findings.

CONCLUSIONS

We have shown that the hypoxic stress of OSA predicts the severity of insulin resistance and may impact on the progression of NAFLD to NASH in patients with severe obesity. In contrast, a traditional index of OSA severity, the RDI, had no predictive value for metabolic dysfunction or liver disease in the bariatric population. Severe obesity was associated with systemic inflammation with high serum CRP levels, but there was no relationship between sleep-disordered breathing and serum CRP. Future prospective studies and interventional studies with CPAP are needed to elucidate causal relationships between OSA, insulin resistance, and NASH in severe obesity.

Conflict of Interest Statement: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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