Sleep Apnea and Daytime Sleepiness and Fatigue: Relation to Visceral Obesity, Insulin Resistance, and Hypercytokinemia

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ABSTRACT

Sleep apnea and associated daytime sleepiness and fatigue are common manifestations of mainly obese middle-aged men. The onset of sleep apnea peaks in middle age, and its morbidity and mortality sequelae include complications from accidents and cardiovascular events. The pathophysiology of sleep apnea remains obscure. The purpose of this study was to test three separate, albeit closely related, hypotheses. 1) Does sleep apnea contribute to the previously reported changes of plasma cytokine (tumor necrosis factor-α and interleukin-6) and leptin levels independently of obesity? 2) Among obese patients, is it generalized or visceral obesity that predisposes to sleep apnea? 3) Is apnea a factor independent from obesity in the development of insulin resistance? Obese middle-aged men with sleep apnea were first compared with nonapneic age- and body mass index (BMI)-matched obese and age-matched lean men. All subjects were monitored in the sleep laboratory for 4 consecutive nights. We obtained simultaneous indexes of sleep, sleep stages, and sleep apnea, including apnea/hypopnea index and percent minimum oxygen saturation. The sleep apneic men had higher plasma concentrations of the adipose tissue-derived hormone, leptin, and of the inflammatory, fatigue-causing, and insulin resistance-producing cytokines tumor necrosis factor-α and interleukin-6 than nonapneic obese men, who had intermediate values, or lean men, who had the lowest values. Because these findings suggested that sleep apneas might have a higher degree of insulin resistance than the BMI-matched controls, we studied groups of sleep-apneic obese and age- and BMI-matched nonapneic controls in whom we obtained computed tomographic scan measures of total, sc, and visceral abdominal fat, and additional biochemical indexes of insulin resistance, including fasting plasma glucose and insulin. The sleep apnea patients had a significantly greater amount of visceral fat compared to obese controls (<0.05) and indexes of sleep disordered breathing were positively correlated with visceral fat, but not with BMI or total or sc fat. Furthermore, the biochemical data confirmed a higher degree of insulin resistance in the group of apneics than in BMI-matched nonapneic controls. We conclude that there is a strong independent association among sleep apnea, visceral obesity, insulin resistance and hypercytokinemia, which may contribute to the pathological manifestations and somatic sequelae of this condition. (J Clin Endocrinol Metab 85: 1151–1158, 2000)

SLEEP APNEA is a common disorder associated with daytime sleepiness and fatigue, and significant morbidity and mortality due to accidents and cardiovascular events (1, 2). Male gender, advancing age, obesity, anatomical abnormalities (including small pharyngeal size due to fatty tissue of the neck), heredity, and instability of respiratory control during sleep, have been reported as risk factors for the development of sleep apnea. Two thirds of middle-aged apnic men suffer from obesity, particularly the android-central type, and one third have hypertension. Visceral obesity is associated with insulin resistance, and the so-called visceral fat or metabolic syndrome, i.e. dyslipidemia, dyscoagulation, hypertension, and diabetes mellitus type II, and their cardiovascular sequelae, mostly ischemic heart disease (3). Studies of the potential independent role of sleep apnea in the development of insulin resistance and/or vice versa have been inconsistent and inconclusive. Recent studies suggested that the effects of sleep apnea on insulin dynamics and effects could be accounted for completely by obesity (4, 5).

The pathophysiology of sleep apnea remains obscure, and most currently available treatments for this disorder are mechanical and associated with either variable response and/or poor compliance. Recently, we reported that the inflammatory cytokines tumor necrosis factor-α (TNFα) and interleukin-6 (IL-6), both of which produce sleepiness and fatigue, are elevated in sleep apnea and obesity and might play a role in the pathogenesis and pathological sequelae of both disorders (6). Like the adipostatic hormone leptin, these cytokines are released into the interstitial fluid of adipose tissue, and their circulating levels correlate positively with the body mass index (BMI) (7–9). TNFα correlates strongly with lipolysis, and this cytokine causes marked insulin resistance (7, 9, 10) and stimulates leptin secretion (11–13). Circulating concentrations of leptin are proportional not only to total body fat but also to the degree of insulin resistance (14). Chronic leptin administration has been associated with sym-
pathetic system activation and elevation of blood pressure (15), suggesting that it might play a role in the pathogenesis of manifestations that frequently accompany sleep apnea, namely hypertension and its sequelae.

The purpose of this study was to evaluate whether the biochemical indexes of chronic inflammation, including the proinflammatory cytokines TNFα and IL-6, and the adipose-derived tissue hormone, leptin, are elevated in sleep apnea independently of obesity; whether sleep apnea is an independent variable in the development of insulin resistance; and whether among obese individuals it is visceral, rather than generalized, obesity that predisposes to the development of sleep apnea.

Subjects and Methods

This study was completed in two phases. In the first phase, we recruited three groups, including men with obstructive sleep apnea, BMI-matched obese controls, and normal weight controls. In the second phase, participants included only men with sleep apnea and their BMI-matched obese controls.

Subjects

Fourteen male patients with obstructive sleep apnea and 11 obese and 12 normal weight male controls participated in the study. The subjects were recruited from the Sleep Disorders Clinic or through advertisement from the community. The mean ± SE ages of the apneics, obese controls, and normal weight controls were 46.6 ± 3.0, 40.2 ± 2.2, and 45.4 ± 2.8 yr, respectively (P = NS), whereas their BMIs were 38.4 ± 1.6, 36.2 ± 2.4, and 26.0 ± 0.8, respectively (P = NS between sleep apneics and obese controls; P < 0.01 between sleep apneics or obese controls and normal weight controls).

To qualify for the study, apneic patients had to have apnea of sufficient severity to warrant recommendation for treatment (16). These criteria included an apnea/hypopnea index (A/HI) of more that 20 events/h of sleep and clinical symptoms such as excessive daytime sleepiness and/or the presence of cardiovascular abnormalities, i.e., hypertension or cardiac arrhythmias. Control subjects who demonstrated an A/HI of more than 5 events/h of sleep were excluded from the study. Also apneics and control subjects with a
diagnosis of diabetes mellitus or who were receiving treatment with psychotropics, steroids, sympathomimetics, or sympatholytics, including β-blockers, were excluded from the study. All patients and controls were asked to abstain from nonsteroidal antiinflammatory medication for 1 week before the study. Five of the sleep apneics and none of the obese or the normal weight controls were treated for hypertension. Three of the apneics were treated with angiotensin-converting enzyme inhibitors, and two were treated with calcium channel blockers.

Procedures

Sleep laboratory. A thorough medical assessment, including physical examination, routine laboratory tests (including complete blood cell count, urinalysis, thyroid function tests, and electrocardiography), and sleep history, was completed for each patient and control subject. All potential participants in the study were screened in the sleep laboratory for 1 night for 8 h employing standard polysomnographic procedures (17). Throughout the night, respiration was monitored by thermocouples at the nose and mouth (model TCT1R, Grass Instrument Co., Quincy, MA) and thoracic strain gauges. All-night recordings of hemoglobin oxygen saturation (SaO2) were obtained using a cardiorespiratory oximeter (model 8800, Nonin Medical, Inc., Plymouth, MN). The intra- and interassay coefficients of variation ranged from 3.2–8.5% and 3.5–8.7% for IL-6. The lower detection limits for TNFα and IL-6 were 0.18, and 0.094 pg/mL, respectively. Leptin was measured by a commercially available RIA (Linco Research, Inc., St. Charles, MO). Samples were run in duplicate, and standards were run in triplicate. The intra- and interassay coefficients of variation were both below 5%. Plasma insulin was measured by specific RIA. The intra- and interassay coefficients of variation for insulin ranged from 3.5–4.6% and 4.5–7.0%, respectively.

Computed tomographic (CT) scanning. The objective of these measurements was to assess and compare the distribution of abdominal fat (intraabdominal vs. sc fat) in sleep apneics and their obese controls. One of the obese controls did not complete this part of the study. Axial 8-mm CT sections were taken through the midvertebral bodies of L1, L3, L4, and L5 plus a fifth slice at the top of the femoral heads. No iv or oral contrast was used. The specific levels to be scanned were localized from an initial sagittal topogram. All scans were performed on a PQ5000 (130 kV; 200 mA, Picker International, Highland Heights, OH). Each image was segmented twice, once for intra-abdominal fat and once for sc fat. A CT range of −120 to −40 hounsfield units was used to encompass all fat. The total cross-sectional area was also calculated at each level so that the percentages of intraabdominal, sc, and total fat could be calculated. Subcutaneous fat was defined as fat between the skin surface and the outer margin of the back and abdominal wall musculature, and intraabdominal fat was defined as fat within the cavity formed by the back and abdominal wall musculature.

Statistical analyses

The results of parametric values are expressed as the mean ± se. For comparison of parametric values among the three groups we used MANOVA, which controlled for repeated measures. For comparisons between the two groups we employed the Student’s two-tailed t test. Relations among sleep variables, respiratory data, BMI, CT measures, and hormonal values were calculated using the Pearson product-moment correlation analysis and multiple regression analysis. The sleep variables were calculated based on the mean values from the 3 consecutive baseline nights (2–4). The critical statistical confidence level selected for all analyses was P < 0.05.

Results

Sleep, respiratory, and blood pressure data

The group of sleep apneics, compared to both obese and normal weight controls, demonstrated a significantly longer wake time after sleep onset, total wake time, and percentage of stage 1 sleep (P < 0.01; Table 1). The same patients demonstrated a significantly lower percentage of sleep time and stage 2 sleep (P < 0.05) than either control group. The AHI in sleep apneics was 48.7 ± 5.6, whereas in the obese and normal weight controls it was 1.3 ± 0.5
and 0.5 ± 0.3, respectively ($P < 0.01$). In addition, the apneics had a significantly lower minimum hemoglobin oxygen saturation (SaO₂) than the two control groups (74.6 ± 3.3 vs. 91.1 ± 1.6 vs. 94.4 ± 0.9; $P < 0.01$, respectively, for the comparisons between sleep apneics and either control group). Finally, systolic, diastolic, and mean arterial pressures were highest in the sleep apneic group (144.3 ± 7.0, 89.4 ± 3.6, and 107.7 ± 4.6, respectively), lowest in the normal weight controls (123.0 ± 1.6, 76.3 ± 1.4, and 91.9 ± 1.0), and intermediate in the obese controls (133.6 ± 2.8, 86.2 ± 3.4, and 102.0 ± 2.9), with differences between sleep apneics and normal weight controls being significant ($P < 0.01$) for all three blood pressure variables.

**Fig. 2.** Plasma TNFα, IL-6, or leptin levels (evening values), and BMI are positively correlated. ■, Sleep apneics; ○, obese controls; ▲, lean controls.
Phase I: effects of sleep disturbance vs. obesity on plasma TNFα, IL-6, and leptin

Plasma TNFα values were highest in the sleep apneic group and lowest in the normal weight controls for the average value ($P = 0.04$) as well as for the evening concentration ($P = 0.005$, Table 2 and Fig. 1). Plasma TNFα values were intermediate between the other two groups for the average as well as the evening values. Plasma IL-6 values were also highest in the sleep apneic group and lowest in the normal weight controls for the average ($P = 0.005$) as well as for the morning ($P = 0.04$) and evening concentrations ($P = 0.002$). Again, the values for the obese controls were midway between those for the other two groups. Mean plasma levels for leptin were highest in the sleep apneics and lowest in the normal weight controls ($P = 0.0001$). In addition, the obese controls had significantly higher values than the normal weight controls ($P = 0.05$) and lower values than the sleep apneics ($P = 0.02$). This general relationship among the three groups was present within both morning and evening measurements.

To assess the potential effects of antihypertensive medication on cytokine levels in apneics, we further analyzed the cytokine data by comparing apneics taking medication to those not taking medication. The sleep apneics who were taking medication for hypertension demonstrated lower cytokine and leptin values than those not taking medication (TNFα, $3.0 \pm 0.2$ vs. $3.6 \pm 0.2$ and $2.6 \pm 0.2$ vs. $3.2 \pm 0.2$ for morning and evening, respectively; IL-6, $1.8 \pm 0.4$ vs. $2.6 \pm 0.9$ and $2.7 \pm 0.3$ vs. $4.3 \pm 0.8$ for morning and evening, respectively; leptin, $24.3 \pm 5.5$ vs. $26.3 \pm 3.9$ and $22.7 \pm 4.2$ vs. $31.3 \pm 4.6$ for morning and evening, respectively; all comparisons were nonsignificant).

The ratio of morning/evening secretion of TNFα, IL-6, and leptin was significantly lower in sleep apneics and obese controls than in normal weight controls, indicating that not only the amount but also the daily pattern of secretion of cytokines is altered in obese subjects (Table 2).

Mean (the average of morning and evening concentrations) IL-6 and leptin values and evening TNFα values were positively correlated with BMI ($r_{xy} = 0.74$, $P < 0.01$; $r_{xy} = 0.82$, $P < 0.01$; and $r_{xy} = 0.40$, $P < 0.05$, respectively; Fig. 2). Mean TNFα, IL-6, and leptin values were significantly correlated with indexes of sleep apnea ($r_{xy} = 0.32$, $P < 0.05$; $r_{xy} = 0.38$, $P < 0.05$; and $r_{xy} = 0.55$, $P < 0.01$, respectively, for A/HI and $r_{xy} = -0.45$, $r_{xy} = -0.52$, and $r_{xy} = -0.44$, all $P < 0.01$, respectively, for minimum SaO2). Mean IL-6 values were positively correlated with mean leptin ($r_{xy} = 0.64$; $P < 0.001$) and TNFα values ($r_{xy} = 0.39$; $P < 0.05$). Evening plasma TNFα values were positively correlated with the corresponding leptin levels ($r_{xy} = 0.28$; $P < 0.1$).

Phase II: visceral vs. generalized obesity and sleep apnea and biochemical indexes of insulin resistance

There were no differences between the two groups in terms of total body fat or sc fat at all five levels. In contrast, sleep apneics compared to obese controls had a significantly greater amount of visceral fat at L1, L3, L4, and L5 levels (all $P < 0.05$; numerical data at the L3 level are shown in Table 3). BMI correlated significantly with total body fat (measured at L3: $r_{xy} = 0.83$; $P < 0.01$) and sc fat ($r_{xy} = 0.88$; $P < 0.01$), but not with visceral fat. Visceral, but not sc, fat was significantly correlated with indexes of sleep apnea ($r_{xy} = 0.70$; $P < 0.01$ for A/HI and $r_{xy} = -0.60$; $P < 0.01$ for minimum SaO2; Fig. 3).

Mean fasting blood glucose levels were higher in the apneics than in obese controls (106.2 ± 4.1 vs. 85.4 ± 4.4; $P < 0.01$; Table 4). Mean plasma insulin levels were also higher in sleep apneics than in obese controls (25.7 ± 4.2 vs. 14.6 ± 2.5; $P < 0.05$; Fig. 4). The sleep apneics who were taking medication for hypertension compared to those who were not taking medication demonstrated lower insulin levels, which, however, were not different from control values (16.9 ± 4.2 vs. 29.8 ± 5.0; $P = NS$).

Plasma insulin levels were positively correlated to leptin levels ($r_{xy} = 0.48$; $P < 0.05$) and tended to correlate to IL-6 levels ($r_{xy} = 0.37$; $P < 0.1$).

Multivariate analysis among plasma cytokine concentrations, types of fat, and sleep apnea indexes

The multiple regression analysis for IL-6 indicated that in terms of the fat types, sc fat was making the strongest contribution for both groups ($P = 0.0003$). Yet, IL-6 correlated with visceral fat within the obese control group, but not within the sleep apnea group. After adjustment for fat type, A/HI continued to make an independent contribution to the IL-6 levels ($P = 0.01$). For TNFα, there was a significant association with sc fat within the obese control group, but not within the sleep apnea group. As with IL-6, when fat types were adjusted for, A/HI made a significant contribution to TNFα concentrations ($P = 0.05$). As expected, multiple regression analysis for leptin indicated that both sc and visceral fat made significant contributions ($P < 0.0001$), with visceral fat being the stronger of the two. Unlike its effect on plasma cytokine concentrations, A/HI did not make an additional contribution to leptin levels.

Discussion

Sleep apnea was associated with plasma TNFα and IL-6 elevations independently from obesity; among obese patients, it was the visceral rather than the sc or total body fat that predisposed to the development of sleep apnea, and sleep apnea contributed to the development of fasting hyperinsulinemia independently from obesity.

Indeed, both TNFα and IL-6 were highest in the sleep apneics, whereas in the BMI-matched obese controls, the cytokine values were intermediate between those of normal

| TABLE 3. Abdominal fat distribution in sleep apneics and obese controls |
|-------------------------|-------------------------|
|                        | Sleep apneics (n = 14)   | Obese controls (n = 10) |
|                        | BMI (cm²)                |                          |
| Total body fat area     | 38.4 ± 1.6               | 36.4 ± 2.7               |
| Visceral fat area       | 978.8 ± 46.6             | 589.0 ± 67.8             |
| sc fat area (cm²)       | 360.7 ± 40.7             | 368.5 ± 55.9             |
| Data are presented as the mean ± SE. | | |

"a P < 0.01."
weight controls and apneics. In a previous study we demonstrated that plasma levels of TNFα and IL-6 were elevated in sleep apnea, with a positive correlation to BMI (6). This study confirms our earlier findings and suggests that the elevation of both cytokines in sleep apnea has an additional component that is independent of obesity. Although the mean plasma IL-6 levels of obese controls were not significantly different from the sleep apnea or the normal weight control groups, they were approximately equidistant from those of the other two groups, both in absolute terms and as estimated differences generated by the MANOVA. In addition, the TNFα values for the obese controls were actually closer to the normal weight control values than to values in the sleep apnea group. These data support a typical dose-response pattern. The fact that the sleep apneic and nonsleep apneic obese patients had similar BMIs and body weights

![Graph showing correlation between sleep apnea and cytokines](image)

**Fig. 3.** Visceral fat significantly correlated with indexes of sleep apnea. ■, Sleep apneics, ○, obese controls.

**TABLE 4.** Plasma glucose and insulin levels in sleep apneics and obese controls

<table>
<thead>
<tr>
<th></th>
<th>Sleep apneics (n = 14)</th>
<th>Obese controls (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (μg/dL)</td>
<td>106.2 ± 4.1a</td>
<td>85.4 ± 4.4</td>
</tr>
<tr>
<td>Insulin (μg/mL)</td>
<td>25.70 ± 4.22b</td>
<td>14.55 ± 2.49</td>
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Data are presented as the mean ± SE.

a P < 0.01.
b P < 0.05.
further supports the conclusion that sleep apnea represents an additional independent factor leading to elevations of the inflammatory cytokines. One possible reason why the plasma cytokine concentrations of obese controls were not significantly different from those of the two extreme groups could have been the sample size.

We previously proposed that cytokines, particularly TNF\(\alpha\) and IL-6, may mediate daytime sleepiness associated with disorders of excessive daytime sleepiness (EDS), such as sleep apnea or obesity (6). An important question is whether the observed peripheral elevation of cytokines in the patients with EDS is of any relevance to phenomena generated in the brain, e.g. sleep and sleepiness. It is possible that the observed increases in inflammatory cytokines in the plasma of patients with EDS may, in fact, reflect much higher elevations in the production and/or target sites of these cytokines, in this case the central nervous system. Furthermore, there are several ways that peripheral cytokines can communicate and signal the brain to elicit central nervous system manifestations, e.g. sleepiness, including acting in areas outside the blood-brain barrier (periventricular organs), crossing the blood-brain barrier (18, 19), and through peripheral autonomic afferent nerves (20). Indeed, even small doses of IL-1 administered peripherally in rats can produce sleep (20).

In this study we also demonstrated that leptin was significantly increased in sleep apneics compared to levels in both BMI-matched obese and normal weight subjects. Leptin is elevated in obese subjects in proportion to their BMI or percent body fat (12), and its secretion is further modulated by the stress system and cytokines (9, 21). Our study suggests that the increase in leptin levels in sleep apnea is possibly related to the higher amount of visceral fat and/or cytokines in this group. We also demonstrated that not only the amount but also the daily patterns of TNF\(\alpha\), IL-6, and leptin secretion were disturbed in sleep apneics and obese controls. The relative increase in the evening levels of these hormones in apneics and obese subjects may contribute to pathological manifestations in the cardiovascular system, including nighttime elevation of blood pressure (22, 23).

Our sleep apneics had significantly higher fasting plasma insulin levels than BMI-matched obese controls. Previous studies reported inconsistent results in terms of an association between sleep apnea and insulin resistance. A large study showed a modest relation \(r^2 = 0.10\) between the A/HI and fasting insulin levels, but not fasting blood glucose levels (24). Two other studies showed an association between severity of sleep apnea and indexes of insulin resistance (25) and that sleep apnea occurred commonly in obese patients with diabetes type II who had excessive daytime sleepiness or heavy snoring (26). In contrast, two other controlled studies suggested that the relation between sleep apnea and plasma insulin levels (4) or insulin resistance (5) reflected the known effects of obesity. However, in one of these studies the apneics were otherwise healthy normotensive individuals (5), whereas in the second one they were lean and less symptomatic than our patients (4).

Our study demonstrated that in obese patients with significant sleep apnea (diagnosed on the basis of both clinical and laboratory criteria), nocturnal sleep and respiratory disturbances were strong independent risk factors for hyperinsulinemia. The independent effects of these disturbances could be explained by microawakening- and/or hypoxia-related nocturnal increases in sympathetic system and hypothalamic-pituitary-adrenal axis activities. The fact that two previous studies showed no effect of treatment of apnea on insulin levels may be due...
either to the short term assessment of these effects (27, 28) or to the fact that the currently available treatments for sleep apnea are not effective in reversing the metabolic manifestations of this disorder, particularly when the disorder has existed for several years and has led to an adverse and probably irreversible redistribution of fat.

Previous studies have shown that obesity is a significant risk factor for sleep apnea (1, 2, 16, 29). Our study suggests that among obese individuals, it is visceral fat, rather than generalized obesity, that predisposes to the development of sleep apnea. Our sleep apneics had a significantly higher amount of visceral fat compared to our obese control subjects, whereas there were no differences between the two groups in terms of BMI or amount of sc or total body fat. Central obesity with increased visceral fat is closely associated with insulin resistance, dyslipidemia, hypertension, diabetes mellitus type II, and their cardiovascular sequelae (3). Our study suggests that visceral fat may also play a significant role in the development of sleep apnea.

Our overall findings indicate that sleep apnea in obese middle-aged men is associated with visceral obesity, inflammatory cytokine elevations, hyperleptinemia, and hyperinsulinemia. It appears that visceral obesity/insulin resistance determined by both genetic/constitutional and environmental factors may be the principal culprits, progressively leading to worsening metabolic syndrome manifestations and sleep apnea. Progressive deterioration of sleep apnea may then accelerate the worsening of visceral obesity and the metabolic syndrome by providing a stress stimulus and causing nocturnal elevations of hormones, such as cortisol and insulin, that promote visceral adiposity, metabolic abnormalities, and cardiovascular complications (30, 31).

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