Predictors of Elevated Nuclear Factor-κB–dependent Genes in Obstructive Sleep Apnea Syndrome

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Background: **Circulating nuclear factor-κB (NF-κB)–dependent genes, particularly tumor necrosis factor-α (TNF-α), are elevated in obstructive sleep apnea syndrome (OSAS) and likely contribute to cardiovascular disease. Furthermore, TNF-α is associated with excessive daytime sleepiness. We investigated the predictors of TNF-α and related genes in large, well-selected cohorts of subjects with OSAS and control subjects.**

Methods: **We performed a prospective study of 30 subjects who did not have OSAS (22 nonsleepy normal control subjects and 8 sleepy nonapneic subjects who snored), 36 subjects with mild to moderate OSAS, and 31 subjects with severe OSAS; all subjects were male. Groups were matched for age, body mass index, and other relevant variables. Subjects had no other disease and were not regularly taking medication. All had serum for TNF-α and related assays drawn after polysomnography. A total of 49 suitable subjects were treated with continuous positive airway pressure (CPAP); sleep studies together with serum assays were repeated 6 wk later.**

Results: **TNF-α levels were higher in subjects with OSAS than in subjects without OSAS ($p < 0.001$). In multivariate analysis, TNF-α was independently associated with the desaturation index ($r = 0.399$, $p < 0.001$), Epworth Sleepiness Score ($r = 0.243$, $p = 0.005$), and cholesterol ($r = 0.216$, $p = 0.018$). Furthermore, TNF-α levels were higher in sleepy nonapneic subjects who snored than in normal control subjects ($p = 0.002$) but lower than in subjects with OSAS ($p = 0.03$). CPAP therapy lowered TNF-α levels ($p = 0.004$). Another NF-κB–dependent cytokine, interleukin-8 (IL-8), showed similar differences between groups and after CPAP therapy, but a range of other mediators of inflammation, including IL-1, IL-6, IL-10, and IL-12, showed no differences.**

Conclusion: **Intermittent hypoxia is the strongest predictor of TNF-α levels, supporting a role for inflammation in the cardiovascular pathophysiology of OSAS. Furthermore, TNF-α levels are independently associated with excessive daytime sleepiness.**

Keywords: cardiovascular diseases; excessive daytime sleepiness; nuclear factor-κB; obstructive sleep apnea syndrome; tumor necrosis factor-α

Obstructive sleep apnea syndrome (OSAS) is a highly prevalent disorder affecting approximately 4% of adults (1) and is associated with repetitive episodes of transient oxygen desaturation during sleep. OSAS is associated with significant cardiovascular morbidity and mortality and is an independent risk factor for cardiovascular diseases, particularly systemic arterial hypertension (2, 3), but also coronary artery disease, congestive cardiac failure, and cerebrovascular events (4).

Inflammation is known to play an important role in the development of atherosclerosis, and various markers of inflammation are recognized cardiovascular risk factors, such as the proinflammatory cytokines tumor necrosis factor-α (TNF-α) and interleukin 6 (IL-6), chemokines such as interleukin 8 (IL-8), adhesion molecules such as soluble intercellular adhesion molecule 1, and the acute-phase factor C-reactive protein (5, 6).

Previous reports have selectively examined in individuals with OSAS the expression of inflammatory factors including IL-6, IL-8, and soluble intercellular adhesion molecule 1 (7–10). We, among others, have demonstrated elevated circulating TNF-α in subjects with OSAS in comparison with control subjects, independent of obesity, and a significant fall with effective continuous positive airway pressure (CPAP) therapy (9, 11–13). Moreover, we have demonstrated a selective activation of nuclear factor-κB (NF-κB)–dependent inflammatory pathways by intermittent hypoxia and reoxygenation in a cell culture model as a possible underlying mechanism (11). On the other hand, it has been proposed that TNF-α and IL-6 mediate sleepiness in disorders of excessive daytime sleepiness, and Vgontzas and coworkers have reported a significant decrease in sleepiness with the TNF-α receptor antagonist etanercept in a pilot study of eight subjects (7, 14).

The mechanisms increasing the level of TNF-α and other inflammatory markers in individuals with OSAS are unclear. A greater understanding of these mechanisms could provide insight into the development of cardiovascular complications of OSAS and possibly suggest novel therapeutic options. We hypothesized, based on our findings from the cell culture model, that the severity of intermittent hypoxia and reoxygenation correlates with TNF-α level. Thus, the purpose of the present study was to determine, with a particular focus on TNF-α, the independent clinical predictors of elevated proinflammatory markers in OSAS in a large, carefully selected patient population.

METHODS

Subjects

Males with suspected OSAS and no other medical disorder were considered for the study, which was approved by the St. Vincent's University Hospital Ethics Committee. Also recruited were males without OSAS matched for age and body mass index (BMI). All subjects gave written, informed consent, and no subject fitting the inclusion criteria refused participation. Each subject underwent clinical assessment and testing for full blood count, liver and kidney function, and cardiac enzymes, and was assessed for cardiovascular risk factors. Every subject also completed the Epworth Sleepiness Scale (ESS) (15). Supine blood pressure was measured three times while each subject was awake during the daytime. Overnight polysomnography (PSG) was performed as previously described (11). Apneas were defined as complete cessation of airflow for at least 10 s and hypopneas as reduction of respiratory signals for at least 10 s associated with oxygen desaturation of $\geq 4\%$ or arousal. The desaturation index (DI) was determined by the frequency of desaturations $\geq 4\%$ per hour. Sleep studies were performed

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in the sleep laboratory and supervised throughout by an experienced sleep technician.

CPAP Treatment

Fifty suitable subjects with moderate to severe OSAS, who had agreed to possible CPAP therapy before undergoing diagnostic sleep study, began nasal CPAP therapy within 1 wk after PSG. One subject dropped out due to intolerance of CPAP. The remaining 49 subjects underwent repeat sleep study after 6 wk of therapy. All were evaluated for symptoms and side effects, and objective compliance data were downloaded from the devices.

TNF-α ELISA

Serum samples were obtained from all subjects at the same time (7:00 A.M.) both after the initial PSG and from subjects with OSAS treated with CPAP after 6 wk of therapy. Samples were stored at –80°C. TNF-α levels were measured with an ultrasensitive ELISA kit (BioSource, Camarillo, CA).

Multiple Inflammatory Cytokine Assay

We utilized a novel ultrasensitive method to measure a panel of seven different serum inflammatory markers in parallel (Meso Scale Discovery, Gaithersburg, MD). Briefly, each well of a 96-well plate is captured with seven different antibodies. Analytes are detected with electrochemiluminescence detection technology using antibodies labeled with Sulfo-tag reagents that emit light on electrochemical stimulation.

Statistical Analysis

Subject baseline characteristics and serum ELISA results are expressed as mean ± standard deviation or median (interquartile range) depending on their distribution and compared using Student's *t* test or the Wilcoxon rank sum test for independent samples and the paired *t* test for paired samples. Categorical variables were compared using a χ^2 test. In addition, levels of TNF-α among the three groups were evaluated by one-way analysis of variance (ANOVA). To assess the correlations between TNF-α and baseline and PSG variables, we used the Pearson's correlation analysis. To assess the relative strength of the association, we used a stepwise multiple regression model with TNF-α as the dependent variable and age, BMI, smoking status, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, ESS, apnea–hypopnea index (AHI), DI, basal oxygen saturation (Sa_{O_2}), minimum Sa_{O_2}, and total sleep time < 90% as independent variables. Statistical analysis was performed using a commercial software package (SPSS version 11; SPSS, Inc., Chicago, IL).

RESULTS

Subjects

Baseline characteristics of the study population are described in Table 1, and detailed findings from PSG studies are given in Table E1 in the online supplement. Subjects were classified into three groups according to their apnea–hypopnea frequency as non-OSAS (AHI ⩽ 5), mild to moderate OSAS (AHI > 5 ⩽ 30), or severe OSAS (AHI > 30). The non-OSAS group consisted of 22 normal control subjects, recruited from the general population, who were not complaining of excessive daytime sleepiness (EDS; ESS 5 ± 2) and 8 individuals describing clinical features of OSAS including significant EDS (ESS 16 ± 3) and snoring but who demonstrated no objective findings of sleep-disordered breathing on PSG studies. The three groups were similar in demographics (age, BMI), smoking status, blood pressure, and lipid profile. No subject had clinical evidence of any other medical disorder, and each subject's detailed biochemical profile, including liver and renal function, cardiac enzymes, and resting electrocardiogram, was within normal limits. No subject was regularly taking any medication. AHI, oximetry data, and ESS were significantly different between the groups. After 6 wk of CPAP therapy in the 49 CPAP-treated subjects with OSAS, AHI fell from 37 (14;73) to 7 (4;11) ($p < 0.001$ compared with pre-CPAP levels) and all subjects showed Sa_{O_2} levels above 90% during sleep (% total sleep time < 90% 0% [0; 0]; minimum Sa_{O_2} 93% [91; 94]). ESS improved from 15 ± 5 to 7 ± 5 ($p < 0.001$) and daytime blood pressure from 134/84 ± 17/11 to 127/80 ± 15/10 ($p = 0.01$). Objective recordings from CPAP machines revealed a nightly compliance of 4.6 ± 1.3 h (mean ± SD).

TNF-α Levels

TNF-α levels were significantly different between the three groups (ANOVA, $p < 0.001$) and higher in the severe OSAS group (6.19 [4.90; 7.99] pg/ml) than in the non-OSAS (3.21 [1.91; 3.90], $p < 0.001$) and the mild to moderate OSAS group (4.15 [2.71; 6.05], $p < 0.001$). Levels were higher in the mild to moderate OSAS group than in the non-OSAS group but the difference did not reach statistical significance ($p = 0.11$; Figure 1).

Pearson's correlation analysis showed that TNF-α levels correlated positively with ESS ($r = 0.371$, $p < 0.001$), AHI ($r = 0.351$, $p < 0.001$), DI ($r = 0.344$, $p < 0.001$), and % total sleep

TABLE 1. BASELINE CHARACTERISTICS OF SUBJECTS GROUPED FOR OBSTRUCTIVE SLEEP APNEA SYNDROME

Characteristic	Non-OSAS	Mild to Moderate OSAS	Severe OSAS
n	30	35	31
Age, yr	41 ± 8	42 ± 8	43 ± 9
Body mass index, kg/m²	30.7 ± 3.1	32.9 ± 6.03	32.1 ± 3.5
Current smokers, n (%)	9 (30)	13 (37)	12 (39)
Total cholesterol, mmol/L	5.3 ± 0.8	5.2 ± 0.8	5.3 ± 0.8
HDL cholesterol, mmol/L	1.02 ± 0.25	1.03 ± 0.21	1.00 ± 0.18
LDL cholesterol, mmol/L	3.57 ± 0.72	3.29 ± 0.67	3.47 ± 0.73
Blood pressure, mm Hg	131/82 ± 18/9	128/81 ± 13/7	135/86 ± 18/12
Epworth Sleepiness Scale	8 ± 5	11 ± 5*	15 ± 5*†
Apnea–hypopnea index	1.2 ± 1.0	15.9 ± 7.7*	56.6 ± 20.9*†
Desaturation index	2 ± 3	15 ± 9*	52 ± 23*†
Basal Sa_{O_2}	94.0 ± 1.7	93.4 ± 1.7	92.4 ± 2.7†
Minimum Sa_{O_2}	88.7 ± 2.4	83.4 ± 5.0*	75.3 ± 9.8*†
% total sleep time < 90%	2 ± 5	7 ± 17*	26 ± 28*†

Definition of abbreviations: HDL = high-density lipoprotein; LDL = low-density lipoprotein; OSAS = obstructive sleep apnea syndrome.

* p value < 0.05 versus non-OSAS.

† p value < 0.05 versus mild to moderate OSAS.

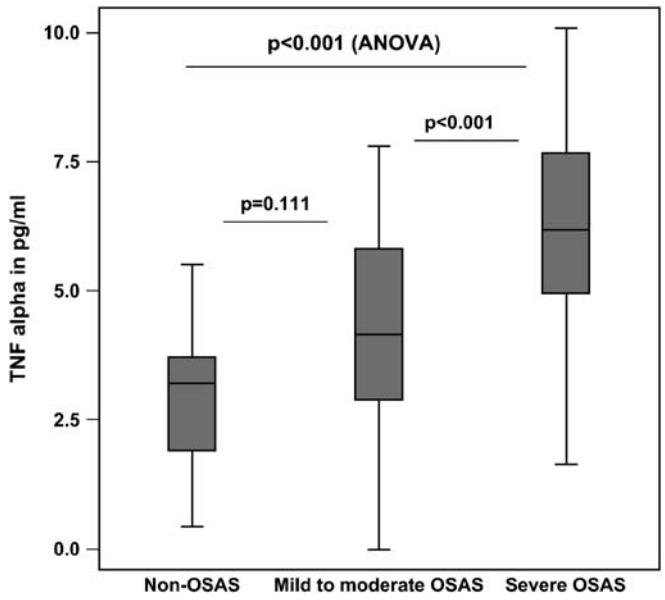

Figure 1. Serum level of tumor necrosis factor-α (TNF-α) in subjects without obstructive sleep apnea syndrome (OSAS), with mild to moderate OSAS, and with severe OSAS. *Boxes* represent values within the interquartile range; *whiskers*, the data range; and *lines across the boxes*, the median values.

time < 90% ($r = 0.201$, $p < 0.047$), and negatively with the minimum Sa_{O_2} ($r = -0.301$, $p = 0.001$). TNF-α level did not show any significant correlation with BMI, age, lipid levels, or smoking status.

Stepwise multiple linear regression analysis identified DI, ESS, and total cholesterol as independent predictors of TNF-α level (Table 2).

Relationship between TNF-α Level and Subjective Sleepiness

Having identified DI and ESS as independent predictors of the TNF-α level in the total populations, we next sought to evaluate the impact of sleepiness on TNF-α level. For this purpose, we separated the non-OSAS group into the normal control population and the group of subjects who were sleepy, snored, and had no evidence of OSAS or other sleep-related disease from PSG studies. None of these subjects described symptoms of narcolepsy, periodic limb movements, or any other sleep disorder associated with EDS so upper airway resistance syndrome may represent the underlying diagnosis. We compared these two groups to the group of subjects with severe OSAS from which we excluded three subjects who did not report significant EDS (ESS ⩽ 9; Table 3 and Table E2). The three groups did not differ in any demographic or clinical variables. As shown in Figure 2, TNF-α levels were significantly different (ANOVA, $p < 0.001$). Levels were higher in the nonapneic, sleepy, snoring group (4.49 [3.35; 6.94] pg/ml) than in the control group (2.46 [1.66; 3.40] pg/ml, $p = 0.002$) but lower than in the OSAS group (6.32 [5.70; 8.17] pg/ml, $p = 0.03$).

TABLE 2. STEPWISE MULTIPLE REGRESSION ANALYSIS OF THE RELATIONSHIP BETWEEN TUMOR NECROSIS FACTOR-α LEVEL AND INDEPENDENT VARIABLES

Variable	r*	p Value
Desaturation index	0.399	< 0.001
Epworth Sleepiness Scale	0.243	0.005
Total cholesterol	0.216	0.018
Apnea–hypopnea index	0.068	0.517
Basal Sa_{O_2}	0.063	0.548
Minimum Sa_{O_2}	−0.039	0.712
% total sleep time < 90%	−0.139	0.181
Body mass index	−0.159	0.125
Age	0.132	0.206
Smoking	0.102	0.329
LDL cholesterol	0.174	0.094
HDL cholesterol	−0.141	0.175

Definition of abbreviations: HDL = high-density lipoprotein; LDL = low-density lipoprotein.

* r = partial correlation coefficient.

Effect of CPAP Therapy on TNF-α Level

Subjects with OSAS, who were started on nasal CPAP therapy after their diagnostic PSG, were reevaluated 6 wk later. There was no change in BMI, no other disease was diagnosed, and no other medications were introduced. Treatment with nasal CPAP significantly decreased TNF-α levels from 5.56 ± 2.10 to 4.13 ± 2.99 pg/ml ($p = 0.004$; Figure 3).

Multiple Inflammatory Cytokine Assay

Having identified the independent correlation of TNF-α with intermittent hypoxia and subjective sleepiness, we next investigated if these findings also apply to other proinflammatory markers. Therefore, using the same serum samples as for the TNF-α assay, we measured simultaneously the levels of interleukin-1 (IL-1), IL-12, IL-6, IL-8, IL-10, IFN-γ, and TNF-α by an ultrasensitive multiplex electrochemiluminescence assay. Detection limits were as follows: IL-1, 0.099 pg/ml; IL-12, 1.008 pg/ml; IL-6, 0.090 pg/ml; IL-8, 0.032 pg/ml; IL-10, 0.307 pg/ml; IFN-γ, 0.301 pg/ml; and TNF-α, 0.058 pg/ml. Levels of TNF-α correlated well with these obtained from the ELISA assay ($r = 0.713$, $p < 0.001$). However, values were lower than these obtained by the ELISA method due to the use of different standards, which accounts for interassay variability. The results of the measured markers are summarized in Table 4. As indicated, the only other marker elevated in subjects with severe OSAS was IL-8. Multivariate analysis revealed basal Sa_{O_2} as the strongest and ESS as the further independent predictor of IL-8 level (Table 5). Again, there was a statistical difference between the levels in subjects with sleepiness but without OSAS and subjects without either sleepiness or OSAS. Levels in the severe OSAS group were higher than in the sleepy non-OSAS group, however, although the difference did not reach statistical significance (Figure 4). CPAP therapy received over 6 wk led to a significant decrease in IL-8 levels (from 5.48 ± 1.53 to 4.68 ± 1.56 pg/ml, $p = 0.002$). This same course of CPAP therapy had no significant effect on values of the other inflammatory markers (IL-1: from 0.29 ± 0.26 to 0.23 ± 0.24 pg/ml, $p = 0.215$; IL-12: from 3.05 ± 7.44 to 2.98 ± 6.42 pg/ml, $p = 0.801$; IL-6: from 5.13 ± 22.65 to 4.46 ± 16.39 pg/ml, $p = 0.103$; IFN-γ: from 0.85 ± 1.98 to 0.69 ± 1.34, $p = 0.597$; IL-10: from 1.31 ± 0.49 to 1.08 ± 0.60, $p = 0.052$). TNF-α levels detected by the multiplex assay, similar to the ELISA measurements, were significantly lower after 6 wk of CPAP therapy (2.04 ± 0.60 vs. 1.45 ± 0.93 pg/ml, $p = 0.001$).

DISCUSSION

This report presents the largest published study to date of proinflammatory mediators in subjects with OSAS and demonstrates significant relationships of the NF-κB–dependent genes TNF-α and IL-8 with sleep-related oxygen desaturation and also, independently, with subjective sleepiness. This study has a number of strengths compared with some previous studies. First, we took great care in selecting subjects to exclude anyone with a

TABLE 3. BASELINE CHARACTERISTICS OF SUBJECTS GROUPED FOR OBSTRUCTIVE SLEEP APNEA SYNDROME AND SLEEPINESS

	Non-OSAS, Nonsleepy	Non-OSAS, Sleepy	Severe OSAS, Sleepy
n	22	8	28
Age, yr	40 ± 6	44 ± 7	43 ± 6
Body mass index, kg/m^2	31.2 ± 3.2	29.9 ± 2.4	32.0 ± 3.7
Current smokers, n (%)	7 (32)	2 (25)	12 (32)
Total cholesterol, mmol/L	5.3 ± 0.9	5.0 ± 0.8	5.3 ± 0.9
HDL cholesterol, mmol/L	1.02 ± 0.25	1.04 ± 0.28	0.99 ± 0.18
LDL cholesterol, mmol/L	3.62 ± 0.74	3.46 ± 0.67	3.46 ± 0.71
Blood pressure, mm Hg	131/81 ± 15/8	131/83 ± 23/12	135/86 ± 18/12
Epworth Sleepiness Scale	5 ± 3	16 ± 3*	16 ± 4*
Apnea–hypopnea index	1.2 ± 1.0	1.2 ± 0.8	57.9 ± 21.5*†
Desaturation index	2 ± 3	2 ± 1	52 ± 23*†
Basal Sa_{O_2}	94.0 ± 1.8	93.8 ± 1.5	92.2 ± 2.8*
Minimum Sa_{O_2}	88.9 ± 2.6	88.4 ± 2.1	75.4 ± 8.8*†
% total sleep time < 90%	2 ± 6	1 ± 1	28 ± 29*†

For definition of abbreviations, *see* Table 1.
* p value < 0.05 versus non-OSAS.
† p value < 0.05 versus mild to moderate OSAS.

cardiovascular or other medical disorder or who was taking any medication. Thus, the study population allows the evaluation of potential relationships between proinflammatory markers and OSAS without the potential influence of confounding factors. Second, the patient and control groups were similar in important variables such as age, BMI, smoking status, blood pressure, and lipid profile. Finally, the relatively large sample size allowed us to determine predictors of increased inflammation in OSAS to suggest underlying mechanisms.

OSAS is widely recognized as an independent risk factor for cardiovascular diseases, particularly systemic arterial hypertension, even after adjustment for potential confounding factors (2, 3). For example, a recent study has found occult OSAS in 83% of patients with drug resistant hypertension (16). Moreover, the U.S. Sleep Heart Health Study of more than 6,000 subjects identified OSAS as an independent risk factor for cardiovascular diseases including coronary artery disease, congestive cardiac failure, and stroke (4). CPAP therapy prevents apneas and associated oxygen desaturations, and there is growing evidence of long-term benefit from CPAP therapy to cardiovascular morbidity and mortality (17, 18).

The role of inflammation in the development of atherosclerosis is well established. Once activated by various stimuli (e.g., low-density lipoprotein cholesterol, injury, or infection), macrophages, lymphocytes, and other inflammatory cells release cytokines, chemokines, and growth factors (5). The proinflammatory cytokine TNF-α is recognized as a critical factor in this process, inducing the expression of cellular adhesion molecules, which mediate adhesion of leucocytes to the vascular endothelium (19, 20). Circulating levels of TNF-α have been shown to correlate

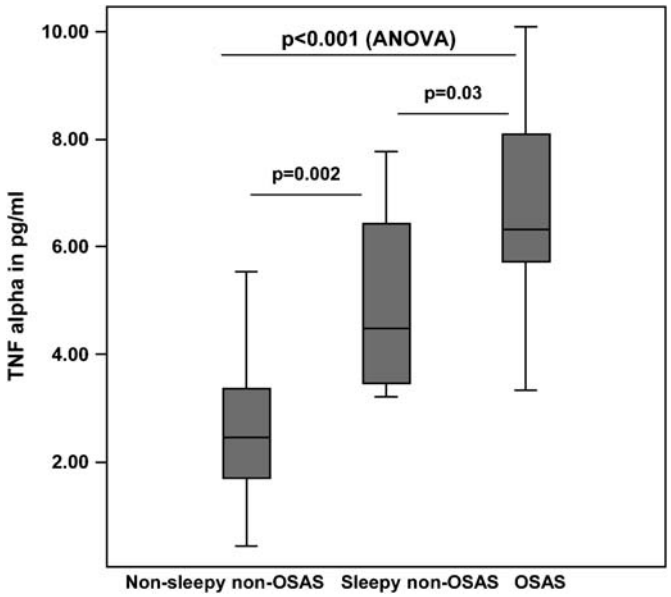

Figure 2. Serum level of TNF-α in nonsleepy subjects without OSAS, sleepy subjects without OSAS, and subjects with OSAS. *Boxes* represent values within the interquartile range; *whiskers*, the data range; and *lines across the boxes*, the median values.

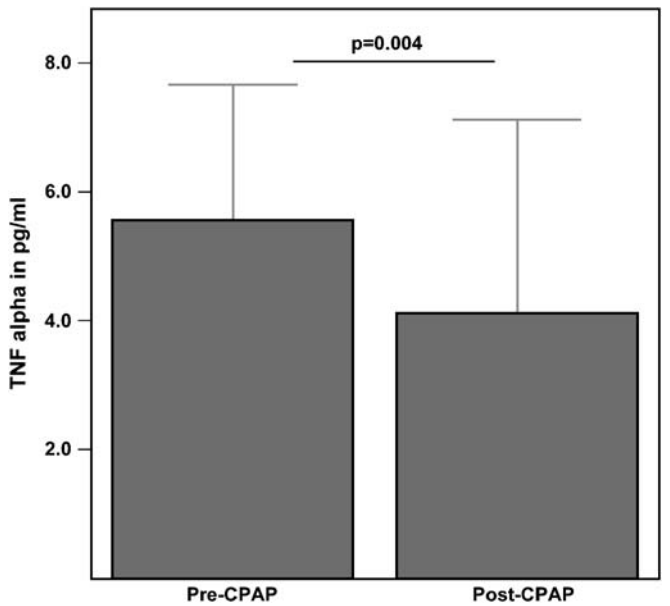

Figure 3. Impact of 6 wk of continuous pressure airway pressure (CPAP) therapy on serum levels of TNF-α in subjects with OSAS. *Boxes* represent mean values and *error bars*, standard deviation of the mean.

TABLE 4. SERUM LEVELS OF ADDITIONAL PROINFLAMMATORY MARKERS IN SUBJECTS GROUPED FOR OBSTRUCTIVE SLEEP APNEA SYNDROME

	Non-OSAS	Mild to Moderate OSAS	Severe OSAS	ANOVA‡
TNF-α, pg/ml	1.15 (0.95, 1.24)	1.55 (1.25, 1.60)	2.13 (1.88, 2.46)*†	< **0.001**
IL-1, pg/ml	0.37 (0.00, 0.43)	0.36 (0.00, 0.43)	0.39 (0.00, 0.44)	0.883
IL-12p70, pg/ml	0.59 (0.00, 2.24)	0.00 (0.00, 2.16)	0.00 (0.00, 2.76)	0.331
IFN-γ, pg/ml	0.78 (0.00, 1.04)	0.00 (0.00, 1.04)	0.00 (0.00, 0.98)	0.291
IL-6, pg/ml	0.87 (0.61, 1.25)	1.13 (0.85, 1.67)	1.07 (0.80, 1.35)	0.351
IL-8, pg/ml	5.05 (4.15, 5.70)	5.01 (4.00, 5.74)*	5.59 (4.65, 7.54)*†	**0.003**
IL-10, pg/ml	1.20 (0.93, 1.45)	1.06 (0.97, 1.52)	1.04 (0.91, 1.46)	0.284

Definition of abbreviation: ANOVA = one-way analysis of variance; IL = interleukin; OSAS = obstructive sleep apnea syndrome.
* p value < 0.05 versus non-OSAS.
† p value < 0.05 versus mild to moderate OSAS.
‡ Values in boldface are statistically significant.

with signs of early atherosclerosis among healthy middle-aged men (21) and are predictive of coronary heart disease and congestive cardiac failure (22). Moreover, persistent increased levels of TNF-α after myocardial infarction are predictive of future coronary events (23). Several studies have identified increased TNF- α levels in subjects with OSAS and both T cells and monocytes have been suggested as potential sources (9, 11–13). However, subject numbers in most of the published studies were too small or populations were not sufficiently matched and selected to allow identification of predictors of this inflammatory response.

On the other hand, Vgontzas and coworkers found increased TNF-α levels in subjects with disorders associated with EDS including OSAS, narcolepsy, and idiopathic hypersomnia, and concluded that TNF-α might promote sleepiness (7). However, in this study, levels were highest among subjects with OSAS, and a pathophysiologic role of sleep disturbance and hypoxia in this condition was suggested. We have identified in a cell culture model a selective and dose-dependent activation of inflammatory NF-κB–dependent pathways over adaptive hypoxia-inducible factor (HIF)-1–mediated pathways by intermittent hypoxia and reoxygenation, which supports a specific role for the OSAS-associated typical pattern of hypoxia and reoxygenation in the pathophysiology of cardiovascular complications in OSAS (11). In the present study, we also found levels of IL-8 to correlate with OSAS severity, in keeping with findings by Ohga and colleagues (10), and greatly supporting the present TNF-α findings. IL-8 is a chemoattractant and plays an important role in the development of atherosclerosis by mediating adhesion of neutrophils and monocytes to the vascular endothelium (24). Both TNF-α and IL-8 are predominantly under the control of NF-κB (25, 26), supporting our central hypothesis of a primary activation of this transcription factor by OSAS leading to an inflammatory response that may contribute to the development of cardiovascular complications in this disorder. None of the other proinflammatory markers assayed in our cohorts were related to the severity of OSAS. Although we recognize that elevated levels of IL-6 have been reported elsewhere (7–9), some of these reports may have been limited by smaller numbers, lack of adequately matched normal control populations, particularly in terms of BMI, and the inclusion of subjects with established cardiovascular or metabolic diseases. However, Vgontzas and coworkers reported an independent association of OSAS with IL-6 even after adjustment for obesity (27). The impact of CPAP therapy on IL-6 levels is largely unknown and our data do not indicate any significant reduction with CPAP. We recognize that the multiple inflammatory cytokine assay used in the current study is novel and therefore we cannot be confident about its accuracy. Nonetheless, the strong correlation between TNF-α levels as measured by ELISA and multiplex assay suggests a high quality assay.

TABLE 5. STEPWISE MULTIPLE REGRESSION ANALYSIS OF THE RELATIONSHIP BETWEEN INTERLEUKIN-8 LEVEL AND INDEPENDENT VARIABLES

Variable	*r**	p Value
Basal Sa_{O_2}	−0.309	**0.002**
Epworth Sleepiness Scale	0.211	**0.042**
Desaturation index	0.115	0.266
Apnea–hypopnea index	0.073	0.479
Total cholesterol	−0.012	0.911
Minimum Sa_{O_2}	−0.016	0.877
% total sleep time < 90%	−0.018	0.866
Body mass index	−0.123	0.237
Age	0.061	0.556
Smoking	0.017	0.873
HDL cholesterol	0.202	0.051
LDL cholesterol	−0.063	0.545

For definition of abbreviations, *see* Table 2.
* *r* = partial correlation coefficient.

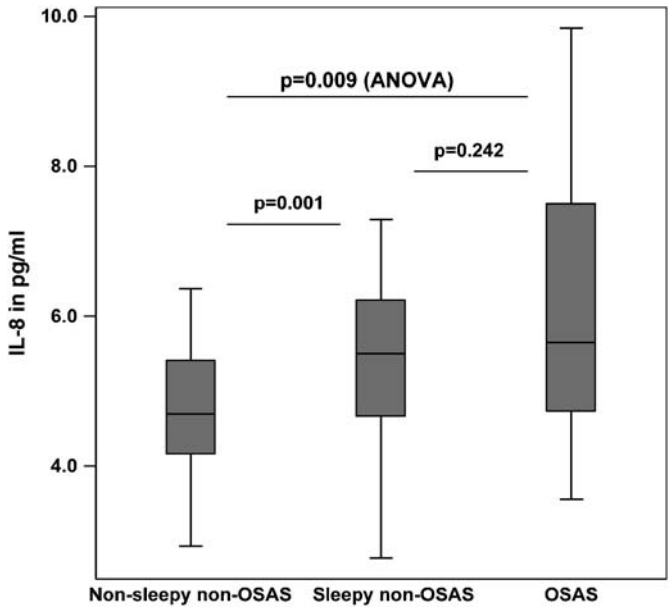

Figure 4. Serum level of interleukin 8 (IL-8) in subjects without sleepiness or OSAS, subjects with sleepiness but without OSAS, and subjects with OSAS. *Boxes* represent values within the interquartile; *whiskers*, the data range; and *lines across the boxes*, the median values.

Multivariate analysis identified DI, which is the principal marker in OSAS of the severity of intermittent hypoxia and reoxygenation, and the basal interapnea oxygen saturation as the strongest predictor for TNF-α and IL-8 levels, respectively. These findings support our previous cell culture findings (11). Because levels did not relate to AHI in the present study, the findings suggest that apnea or hypopnea without oxygen desaturation has less influence on cytokine production. The findings also underline the fact that different measures of hypoxia might significantly differ in predicting a proinflammatory response. There is growing evidence that sustained and intermittent hypoxia lead to significantly different molecular responses and different patterns of transcription factor activation (11, 28, 29). Thus, markers such as DI that specifically identify the severity of intermittent hypoxia are most relevant to the evaluation of our central hypothesis, namely that intermittent hypoxia and reoxygenation is particularly important in the proinflammatory response associated with OSAS. Other markers such as the percent total sleep time $< 90\%$ Sa_{O_2} or the mean oxygen saturation are less suitable to distinguish between sustained and intermittent hypoxia and might therefore be less suitable as variables to evaluate this proinflammatory response and presumed subsequent cardiovascular risk.

A further finding of the present study is the potential role of sleepiness in mediating the inflammatory process. We found a significant difference in TNF-α and IL-8 levels between nonsleepy and sleepy nonapneic groups, and ESS remained an independent predictor in the multivariate analysis. However, the data do not allow us to determine if the sleepiness is a cause or effect of the elevated cytokine levels. Vgontzas and colleagues suggested that sleepiness is mediated by TNF-α (7). Sleepiness was not the primary outcome variable of our study and the sleepy nonapneic group is relatively small to allow more detailed analysis. Although upper airway resistance syndrome is possible as an underlying condition in these subjects, other conditions often associated with OSAS such as obesity, metabolic syndrome, and depression are known to independently contribute to sleepiness (30, 31). Based on our findings, future studies should be undertaken to specifically investigate this relationship in comparing larger groups of subjects with sleepiness but without apnea who snore with groups of subjects suffering from other conditions associated with daytime sleepiness. Because nasal CPAP ameliorates the frequent oxygen desaturation in OSAS and associated daytime sleepiness (32, 33), it should not be surprising that this treatment lowers TNF-α and IL-8 levels, as demonstrated by the present and other reports. In our cohort, TNF-α level after 6 wk of therapy did not reach that of the control group. However, the duration of treatment was relatively short and optimal compliance level was not reached in all subjects. Moreover, the ESS after CPAP was 7 ± 5, suggesting that some subjects had residual hypersomnolence. Thus, some residual OSAS during the 6-wk period of domiciliary CPAP together with associated residual hypersomnolence likely accounts for the failure to reach control levels.

A potential limitation of the present study is the use of the ESS as the tool to evaluate daytime sleepiness because a subjective assessment of sleepiness may not be the most reliable indicator. However, the ESS is well validated (34, 35), and objective tools such as the multiple sleep latency test are labor intensive and day-to-day variability can be high (36, 37). We included only male subjects to avoid sex differences and, given our sample size, we believe that the ESS represents a reasonable indicator of sleepiness for the purposes of the present study. However, future studies should evaluate objective measures of sleepiness in the detailed evaluation of the role of inflammatory mediators in the sleepiness associated with OSAS.

We did not identify an independent correlation of BMI with TNF-α, which is a recognized adipokine (38). However, we took great care to match all groups in BMI to establish a role of OSAS in predicting TNF-α levels. Thus, the range in BMI throughout our cohort was small, which might explain the lack of relationship. In addition, studies on humans suggest a predominant release of IL-6 over TNF-α from adipose tissue, which could have contributed to our findings (39).

In conclusion, the present data provide evidence for a specific role for intermittent hypoxia and reoxygenation and, separately, daytime sleepiness in increased circulating TNF-α and IL-8 levels in OSAS and suggest mechanisms for the cardiovascular pathophysiology in this condition. Furthermore, CPAP therapy is an effective treatment to lower this inflammatory response.

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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