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Sleep Apnea, Sleepiness, Inflammation and Insulin Resistance in middle-aged Men and Women

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Abbreviated Title: OSA is associated with sleepiness, inflammation/insulin resistance in non-obese men, not improved by short-term CPAP treatment Corresponding author and reprint requests to:

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Abstract

In obese men Obstructive sleep apnea (OSA) is associated with inflammation/insulin resistance, however the findings are confounded by adipose tissue, a hormone and cytokine secreting organ. Our goal was to examine whether in a relatively non-obese population, OSA is associated with sleepiness and inflammation/insulin resistance and assess the effects of a 2-month placebo-controlled CPAP use.

Seventy seven subjects, 38 middle-aged men and postmenopausal women with OSA and 39 men and women controls were studied in the sleep laboratory for 4 nights. Sleepiness measures(objective and subjective), performance, serial 24hour blood samples for interleukin-6 (IL-6), Tumor Necrosis Factor Receptor 1 (TNFR1), leptin, adiponectin, and single samples for C-Reactive protein (hsCRP), fasting glucose and insulin levels were obtained.

Apneic men were significantly sleepier and had significantly higher hsCRP, IL-6, leptin and insulin resistance than controls. Apneic women had significantly higher hsCRP, however objective sleepiness, IL6, TNFR1, insulin resistance (HOMA index), leptin and adiponectin were similar to controls. CPAP improved subjective sleepiness but no changes were observed in any of the biomarkers.

In conclusion, OSA is associated with sleepiness, inflammation/insulin resistance even in non-obese men and this association is stronger in men than women. Short-term CPAP does not improve the inflammatory/metabolic aberrations in OSA.

Introduction

Obstructive sleep apnea (OSA) is the second most common sleep disorder and is associated with increased risk for cardiovascular morbidity and mortality and impairment in the quality of life [1]. We and others have shown that OSA in obese men is associated with elevated pro-inflammatory cytokines, i.e. interleukin 6 (IL-6) and Tumor Necrosis Factor (TNF-α) and insulin resistance, states that may mediate the association between OSA and cardiometabolic complications [2-8].

The vast majority of clinical/research studies on the association of OSA with inflammation/metabolic aberrations, including ours have focused primarily on obese men with apnea because the prevalence of OSA is higher in males and in overweight-obese populations [9, 10]. However, in these studies the findings were confounded by obesity, a major contributor to inflammation and insulin resistance. In addition, studies in non-obese apneic men are very few and their findings inconclusive [11-13] which in part has reinforced the belief among some researchers and clinicians that anatomic abnormalities are of primary importance [14]. Furthermore, studies exclusively on women or studies that have assessed gender differences are limited and have yielded inconclusive results [8, 15-19].

The effects of CPAP treatment on the above inflammatory/metabolic indices are inconsistent [20]. Notably, only a few of these previous studies, done almost exclusively in obese subjects, were placebo-controlled and the majority of them have shown no effect of short-term CPAP use on the inflammatory/metabolic profile [21-28].

To address these gaps in the literature we assessed in a comprehensive way in a predominantly non-obese population of apneic men and women and their respective controls and examined the independent and gender specific association of OSA with multiple biomarkers of inflammatory cardiometabolic morbidity. Despite our efforts, our sample of women consisted of slightly obese patients because a) in Central Pennsylvania women are heavier compared to national standards and b) women with OSA both in clinical and epidemiological samples are heavier than men [29]. A secondary goal was

to assess the effect of a placebo controlled (sham-CPAP) 2-month CPAP on the inflammatory and metabolic profile.

Subjects and methods

Subjects

Seventy-seven subjects, including sleep apnea patients and controls, participated in the study. We assessed the baseline and post CPAP/sham CPAP serial 24-hour plasma concentrations of IL-6, tumor necrosis factor receptor 1 (TNFR1), leptin and adiponectin. We also examined morning and evening levels of the high-sensitivity C-reactive protein (hsCRP) and single fasting morning levels of glucose and insulin. Based on data previously published for mean 24-hour IL-6 [30] as well as data from studies assessing the effects of anti-diabetic medications on fasting blood insulin levels [31] using a 2x2 crossover design, a sample size of 30 would have a power of at least 80% to detect a difference at the a=0.05 level.

The subjects were recruited from the Sleep Disorders Clinic and through advertisements from the community. Inclusion and exclusion criteria for apneic and control subjects have been described elsewhere [29]. Sleep Apnea patients that had used CPAP previously were excluded from the study.

Current smoking and the use of lipid-lowering medication (i.e. statins, fibrates), factors known to affect inflammatory markers, were also recorded. Hypertension was defined as a systolic blood pressure >=140 mm Hg and/or diastolic blood pressure >= 90 mm Hg or as the use of anti-hypertensive medications.

The study was approved by the University Institutional Review Board and all participants provided a written informed consent.

Procedures

Sleep Laboratory

A thorough medical assessment, including history, physical examination, routine laboratory tests and sleep history was completed for each participant [4]. Anthropometric parameters were obtained and BMI was calculated based on height and weight measured as part of the physical examination. All potential participants were screened in the sleep laboratory for 1 night for 8 hours and the subjects who met the inclusion criteria were monitored in the sleep laboratory for 4 consecutive nights (1 adaptation and 3 baseline nights) [29].

The study design included also two consecutive 2-month periods of CPAP and sham-CPAP treatment for OSA patients in a random counterbalanced order as previously described [29]. Patients were blinded to the treatment they were getting and they were reassessed with the same protocol as previously described at the end of each 2-month period. The sleep records were scored independently of any knowledge of the experimental conditions according to standardized criteria [29].

Daytime sleepiness and performance

Epworth Sleepiness Scale (ESS)

Subjective sleepiness was assessed with the ESS the first day at the sleep lab [4].

Multiple Sleep Latency Test (MSLT) and Psychomotor Vigilance Test (PVT)

During the fourth day (day of blood sampling) the subjects' levels of sleepiness and alertness were evaluated using MSLT and PVT as described before [4].

Anxiety and Depression symptoms

Anxiety and depression symptoms were assessed with the Beck Anxiety (BAI) and Beck Depression Inventory-II (BDI-II) respectively.

Twenty-four-hour blood sampling

The blood was drawn during the fourth day and night in the sleep laboratory at baseline and was repeated in sleep apnea patients at the end of both CPAP and sham-CPAP treatment periods (for more details see supplement).

Assaying

Blood collected from the indwelling catheter was collected in EDTA-containing tube and refrigerated until centrifugation (within 3 hours). Blood was stored in a -80 C freezer until assay. Concentrations of IL-6, TNFR1 were measured every 60 minutes, leptin and adiponectin were measured every 120 minutes throughout the 24-hour period, hsCRP was measured twice (morning and evening), while single blood samples for the measurement of fasting blood glucose and insulin were drawn the morning following the overnight sleep recording. All samples were processed in the same manner (for more details see supplement).

CPAP and sham-CPAP usage

All patients with sleep apnea underwent consecutively CPAP and sham-CPAP treatment in a random order. The methodology is described in detail in the supplement. To better explore the effect of CPAP on variables of interest, we further conducted sensitivity analyses by creating two groups; 'low adherence' and 'high adherence' group according to hours of daily CPAP use (Supplementary Section S4). The design of the study also included an intervening 1 week wash out period between the two treatment phases. Patients, investigators, respiratory therapists were all blinded to treatment phase.

Statistical analysis

For comparisons of the groups' anthropometric baseline characteristics, the independent-samples Student *t-test* was used. The Kolmogorov-Smirnov test was used to confirm normality. Data that were not normally distributed were logarithmically transformed before the analyses. Sleep variables were

calculated based on the mean values from nights 2 and 3 (night 4 was not included due to potential blood draw-induced sleep disturbance). Comparisons of the inflammatory markers levels between control and apneic men were conducted using Linear Mixed Models after including lipid lowering medication use and smoking status as covariates and between apneic and control women after controlling for BMI, age, lipid-lowering medication, hypertension and smoking status, since all these factors are well known to affect inflammatory markers' levels. Since apneic women were significantly heavier than controls, the same comparisons were repeated within a subgroup matched for BMI (sensitivity analysis) [29].

Subjective sleepiness data in men were compared with the independent samples Student *t-test* and in women subjective sleepiness data were compared with ANCOVA after adjustment for BMI, age and race. Objective sleepiness/performance data were compared after controlling for total sleep time (average nights 2 and 3) for men and for BMI, age and total sleep time for women.

Furthermore, since it has been shown that IL-6 blood levels are affected by the continuous blood-drawing technique, i.e. IL-6 values are higher at the end of the 24-hour blood draw compared to the beginning we proceded with a 'detrended' analysis [4]. Insulin resistance was expressed with the HOMA index, according to the following formula (fasting plasma glucose * fasting serum insulin) / 405.

For comparisons between baseline and post CPAP and sham-CPAP phases, linear mixed-effects models were used for the repeated measures analyses or ANOVA for the repeated measures where appropriate. The specified covariance structure was AR (1) and pairwise comparisons using Bonferroni correction were performed. Group (baseline, sham-CPAP, CPAP), gender and time as well as their interactions were treated as fixed effects. Effects of order of intervention were also assessed by a 2x2 ANOVA. The level $p<0.05$ was used to determine statistical significance. All analyses were conducted using SPSS 17.0 (SPSS Inc., Chicago, IL).

Results

Demographic, emotional distress, sleep, respiratory and sleepiness data

Participants' demographic and sleep characteristics are presented in Table 1. Participants were middle-aged (age range: 41.7-66.3 years) and all women were postmenopausal not on hormone replacement therapy.

Apneic men compared to controls did not differ significantly in any of the measured demographic characteristics apart from a trend for higher waist circumference. Controls and apneics did not differ significantly in terms of total scores in both BAI and BDI-II (p=0.31 for BAI and p=0.94 for BDI-II). As expected, apneic men were significantly different than controls in several sleep and breathing related variables (Table 1).

In terms of daytime sleepiness, apneic men were both subjectively and objectively sleepier than controls (mean ESS total score in apneics vs. controls: 10.85 ± 5.44 vs. 7.42 ± 5.00, p=0.04, mean MSLT in apneics vs. controls: 10.25 ± 0.99 min vs. 13.54 ± 1.08 min, p=0.04). No significant difference in lapses or median RT was observed (apneics vs. controls: lapses: 2.33 ± 0.66 vs. 2.79 ± 0.66, p=0.27; median RT: 230.93 ± 6.30 msec vs. 237.79 ± 6.86 msec, p=0.49).

Apneic women compared to their respective controls had significantly higher systolic blood pressure and used lipid-lowering medication more frequently (Blood pressure data are presented in Table 1 before adjusting for confounders). They also had significantly higher BDI-II scores (p=0.02) but their BAI scores were not significantly different (p=0.34). In terms of daytime sleepiness, apneic women were subjectively (mean ESS total score in apneics vs. controls: 11.12 ± 1.06 vs. 7.52 ±0.97, p=0.02) but not objectively sleepier than controls (mean MSLT in apneics vs. controls: 12.43 ± 1.06 min vs. 10.69 ± 0.99 min, p=0.28). Finally, no difference in PVT lapses or median RT was observed (apneics vs. controls: lapses: 2.99 ± 2.38 vs. 6.59 ± 2.18, p=0.30; median RT: 251.78 ± 15.22 msec vs. 263.43 ± 13.99 msec, p=0.59).

Inflammatory markers (hsCRP, TNFR1, IL-6) and indices of insulin resistance (HOMA, leptin and adiponectin blood levels)

Men

Apneic subjects had in general an impaired metabolic/inflammatory profile compared to controls (Table 3). More specifically apneic men had higher morning, evening and mean 24-h hs-CRP values. Average IL-6 concentrations were also significantly higher in the apneic group than controls and apneic individuals demonstrated higher IL-6 values at each specific time point (Figure 1). However, no significant difference was observed in TNFR1 values (Table 3). Apneic men demonstrated also higher levels of leptin and insulin resistance but adiponectin levels were not different between the 2 groups.

Women

Apneic women had significantly higher hs-CRP and early morning IL-6 levels, while there was a non-significant increase in average 24-h IL-6 levels compared to controls. No significant difference in TNF-R1, HOMA index, leptin or adiponectin was detected (Table 3).

We also performed analyses in a subgroup of women matched for BMI. Demographic, sleep and respiratory data are presented in Table 2. Results in this subgroup were similar (Table 3).

CPAP effect on the inflammatory/metabolic indices and sleepiness

Gender and group effects were not observed so the effect of CPAP/sham-CPAP on the variables of interest was studied in the entire sample (Table 4). No significant effect of order of the intervention on any variable of interest was observed either (supplement table S1). The average daily Sham-CPAP use was 5.26 ± 1.24 hours and the average daily CPAP use was 6.07 ± 1.21 hours. Adherence to CPAP did not depend on whether it was given first or second, while there was a trend for patients that used the sham CPAP after CPAP to adhere less (p=0.19). The majority of our participants, apart from two, were regular users.

CPAP improved significantly the respiratory and sleep variables but no significant changes were observed in hsCRP, IL6, TNFR1, leptin, adiponectin or HOMA index (Table 5) compared to either baseline or sham-CPAP phase.

Epworth sleepiness scale total score decreased significantly during CPAP phase in comparison to both baseline and sham-CPAP phases (baseline vs. CPAP vs. sham-CPAP: 10.40 ± 0.90 vs. 7.46 ± 0.59 vs. 9.66 ± 0.76, all $p<0.01$) while during sham-CPAP phase was similar to baseline. Average MSLT values did not increase compared to baseline during the CPAP phase (baseline vs. CPAP: 12.18 ± 0.63 min vs. 12.70 ± 0.63 min, p=1.00) while they became worse during the sham-CPAP phase (baseline vs. sham-CPAP: 12.18 ± 0.63 min vs. 10.37 ±0.63 min, p=0.09).

Number of lapses did not improve after CPAP compared to baseline while they became worse after sham-CPAP (baseline vs. CPAP: 2.50 ± 0.53 vs. 2.79 ± 0.68, p=1.00; baseline vs. sham-CPAP: 2.50 ± 0.53 vs. 4.13 ± 0.91, p=0.08). A similar pattern was observed for median RT (baseline vs. CPAP: 236.67 ± 6.18 msec vs. 239.20 ± 5.88 msec, p=1.00; baseline vs. sham-CPAP: 236.67 ± 6.18 msec vs. 246.67 ± 6.53 msec, p=0.04).

Discussion

The two primary findings of this study are (1) sleep apnea is significantly associated with sleepiness, inflammation and insulin resistance even in non obese men and (2) in men, apnea is associated with a worse inflammatory/metabolic profile than women. A secondary finding is that a two-month CPAP treatment period does not improve insulin resistance and low-grade inflammation despite a significant improvement of sleep and respiratory variables.

Our results expand our previous findings on obese men and demonstrate a significant association of sleep apnea with low-grade inflammation/metabolic dysregulation in non-obese men as well. Results from previous studies on overweight men with OSA have shown higher insulin resistance than controls [18], whereas one study that reported higher TNFα levels did not control for BMI effect [12]. Our study is the first to examine multiple inflammatory/metabolic factors, including leptin and adiponectin in non-obese

men in a comprehensive way using serial 24-hour blood sampling. These results suggest that similarly to obese patients with sleep apnea, metabolic abnormalities and chronic low-grade inflammation are also present in non-obese individuals, a group of people in whom OSA has been traditionally associated with the absence of metabolic derangement [14, 32]. Furthermore, these findings, in combination with the recent finding that sleep apnea in non-obese men is associated with visceral adiposity [29] suggest that sleep apnea in these individuals is a manifestation of an underlying metabolic syndrome with the primary culprits being visceral obesity, inflammation and insulin resistance, determined by both genetic/constitutional and environmental factors.

In our study the inflammatory/metabolic aberrations in men with sleep apnea were more severe than in women. This is consistent with our earlier study that showed that in non-obese men with apnea, visceral fat is the predominant fat problem, which is more strongly associated with inflammation/insulin resistance than subcutaneous fat. In contrast, in women it was total fat that correlated with inflammatory and cardiometabolic indices. Previous findings on the association of inflammation/metabolic aberrations in women are weak and/or inconclusive. For example, one study reported an independent association of OSA with CRP but another one failed to replicate such a finding [8, 15]. Furthermore, whereas one study found no gender differences in the association of OSA with insulin resistance, two other studies reported that after controlling for BMI the association of OSA with insulin resistance/glucose intolerance was lost in women [16-18]. Finally, a recently published study concluded that sleep disordered breathing assessed with in-home polysomnography is significantly associated with the metabolic syndrome in midlife women [19].

The milder profile of inflammatory/metabolic aberrations in women than that of men raises an important question. If, indeed, women with sleep apnea have a more favorable inflammatory/metabolic profile compared to men we would have expected that women would suffer less cardiometabolic abnormalities, for example hypertension, diabetes, stroke etc. The information on this issue is inconclusive. For example some studies have shown a stronger effect of OSA on cardiovascular events in men [33-35], while others

have not found a gender effect [36, 37]. Future studies need to address the possible gender effect in terms of the association of OSA with cardiometabolic disorders in women.

In our study, men with sleep apnea were sleepier both subjectively and objectively than controls. In contrast, women with OSA were subjectively but not objectively sleepier compared to their respective controls. This is in agreement with other investigators that have also reported higher levels of sleepiness in men with apnea versus women [38]. It has been shown that objective sleepiness is associated with physiological changes, whereas subjective sleepiness is associated mainly with anxiety and depression [39]. In this study women with apnea in contrast to men were more anxious/ depressed compared to their controls. Previous studies have also shown that women with apnea are more depressed compared to men [39, 40]. Furthermore, we have previously postulated that pro-inflammatory cytokines are mediators of objective sleepiness [2]. The stronger elevation of inflammatory markers in men than in women is consistent with the greater degree of sleepiness observed in men. Finally PVT performance was not different between the two groups. This lack of difference, in contrast to MSLT values, may be secondary to the fact that PVT sensitivity is affected by factors such as motivation and duration [4].

In the current study, CPAP did not improve low-grade inflammation and insulin sensitivity compared to baseline and sham-CPAP. This is consistent with previous literature in that most placebo controlled studies have not reported a beneficial CPAP effect on inflammation/metabolic profile [21-28]. One of them reported an improvement in TNFR1 values, but failed to show improvement in any other inflammatory markers [22] and two of them observed an improvement of insulin sensitivity in obese [28] and in those with severe apnea and impaired glucose tolerance [27] compared to sham-CPAP. In one of these two studies, insulin sensitivity was measured with repeated sampling of insulin and glucose (Short insulin tolerance test, SITT) whereas in the other CPAP did not improve glucose tolerance compared to placebo. Finally a recent controlled study on patients with Obesity Hypoventilation Syndrome reported no effect of a 1-month non-invasive ventilation treatment on inflammatory, metabolic and cardiovascular markers [41].

We have shown that both obese and non-obese men with apnea have increased visceral adiposity that CPAP did not correct [4, 29]. Thus, the lack of CPAP effect on these markers may be explained by the fact that CPAP did not decrease visceral fat which is the main source of inflammation in these patients. Several placebo controlled studies on obese apneic men have reported similar findings, i.e. lack of CPAP effect on visceral adiposity and/or inflammatory/metabolic aberrations [24, 25, 27, 42]. In one of these studies insulin resistance improved after 6 months [25] and in the other study improvement was noted only in the more severe apneic group after 2 months of CPAP treatment [27]. It appears that further studies with a longer duration, focusing on more severe apneics and controlling for the confounding effect of visceral obesity are needed to examine further the potential effect of CPAP on inflammatory and metabolic parameters.

The current study has some limitations. The most significant limitation is our inability to match apneic and control women in terms of BMI. However, the weight of our women participants lies at the lower end of the BMI of women with OSA typically evaluated in a sleep disorders clinic or those detected in epidemiological samples. Furthermore in addition to controlling for BMI difference, we also conducted sensitivity analysis in a subgroup of women matched for BMI and the results remained unchanged. However, one limitation of this approach is the inadequate power. Another limitation is that our study is primarily of a cross-sectional nature and cannot determine the causality of the associations studied. Finally, a potential limitation of our study is that the duration of the treatment was only 2 months which might have been too short to reverse the inflammation/insulin resistance abnormalities. However, studies that assess the effectiveness of drug treatment on insulin resistance usually show improvement in 3 months, so some trend for improvement, even not significant should have been detected [43]. Moreover, we did not observe a significant effect in any of these markers even when we examined the effect on the 'high compliance' group suggesting that the degree of compliance cannot explain our negative findings.

In conclusion, our findings suggest that sleep apnea is a manifestation of the metabolic syndrome even in non-obese men. In mildly obese women, the inflammation-metabolic abnormalities are milder than in

men, which may reflect the gender differences in terms of visceral adiposity and sleep apnea. From a practical standpoint, this difference suggests the need for gender-specific therapeutic strategies, such as reduction of visceral fat and inflammation through exercise or pharmacological treatment in men [44, 45] and weight loss in women [46].

Finally, CPAP at least in the short-term does not improve the inflammatory/metabolic dysregulation in subjects with OSA and its use should be combined with methods that improve these aberrations, i.e. exercise, insulin sensitizing and anti-inflammatory agents.

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Table 1. Demographic and sleep characteristics of the study population

	Men (n=38)			Women (N=39)		
	Controls N=18	**Apneics N=20**	**P**	**Controls N=21**	**Apneics N=18**	**P**
Age, years	52.39 ± 6.23	53.87 ± 6.76	0.49	54.93 ± 6.07	57.83 ± 5.89	0.14
BMI, kg/m^2	26.60 ± 2.65	27.09 ± 2.60	0.57	27.95 ± 4.12	30.54 ± 3.19	0.04
Apnea/Hypopnea Index	3.03 ± 1.98	42.42 ± 22.51	<0.01	1.63 ± 1.52	32.14 ± 18.47	<0.01
Minimum O_2 Saturation	89.00 ± 5.03	80.80 ± 8.08	<0.01	91.19 ± 3.77	83.11 ± 3.80	<0.01
Waist (cm)	96.40 ± 7.47	99.50 ± 6.71	0.18	90.45 ± 12.18	100.50 ± 8.28	0.01
Sleep latency (min)	15.94 ± 12.46	11.58 ± 7.60	0.21	15.54 ± 8.13	20.74 ± 11.27	0.10
WASO (min)	66.75 ± 29.94	110.00 ± 49.59	<0.01	59.98 ± 38.56	87.63 ± 52.21	0.06
TST (min)	397.65 ± 34.21	356.27 ± 49.82	<0.01	405.93 ± 40.49	372.81 ± 53.91	0.03
TST (%)	82.56 ± 7.16	74.16 ± 10.61	<0.01	84.32 ± 8.59	77.48 ± 11.18	0.03
N1 %	14.11 ± 3.56	21.98 ± 9.11	<0.01	12.07 ± 6.33	14.29 ± 5.39	0.25
N2 %	64.71 ± 9.61	55.47 ± 13.53	0.02	59.59 ± 7.39	54.20 ± 10.49	0.07
SWS %	6.14 ± 7.11	7.25 ± 7.52	0.64	15.73 ± 9.73	18.69 ± 9.56	0.35
REM %	15.05 ± 4.91	15.30 ± 7.17	0.90	12.59 ± 6.31	12.80 ± 6.59	0.92
REM latency (min)	124.95 ± 69.71	139.57 ± 68.66	0.51	134.29 ± 81.14	141.73 ± 69.98	0.76
Blood pressure (mm Hg)						
Systolic	126.06 ± 13.82	127.60 ± 16.21	0.76	120.05 ± 12.34	131.22 ± 15.33	0.02

Diastolic	76.06 ± 7.04	78.95 ± 8.19	0.26	73.52 ± 7.06	73.17 ± 13.31	0.91
HTN						
Yes, %	22.2	25.0	0.84	23.8	44.4	0.15
Smoking (current)						
Yes, %	22.2	5.0	0.12	9.5	0.0	0.28
Lipid-lowering medication use						
Yes, %	11.1	30.0	0.15	0.0	22.2	0.04
ESS	7.42 ± 5.00	10.85 ± 5.44	0.04	7.52 ±0.97	11.12 ± 1.06	0.02

Data are presented as mean ± SD apart from ESS that are presented as mean ± SE. WASO: Wake after Sleep onset, TST: total sleep time, N1: stage 1 sleep, N2: stage 2 sleep, SWS: Slow Wave Sleep, HTN: hypertension defined as Systolic blood pressure >=140 mmHg and/or diastolic blood pressure >=90 mmHg or as the use of antihypertensive medication, lipid lowering medication: use of statins and/or fibrates

Table 2. Demographic and sleep characteristics in the subgroup of women matched for BMI

	Women		
	Controls (n=13)	**Apneics (n=16)**	**p**
Age, years	54.21 ± 6.61	57.28 ± 6.00	0.20
BMI, kg/m^2	30.36 ± 2.75	31.52 ± 1.54	0.19
Apnea/ Hypopnea Index	1.69 ± 1.61	33.94 ± 18.78	<0.01
Minimum O_2 Saturation	91.07 ± 4.00	82.62 ± 3.76	<0.01
Waist (cm)	96.67 ± 10.13	102.06 ± 6.40	0.12
Sleep latency (min)	13.54 ± 7.30	21.54 ± 11.63	0.04
WASO (min)	55.78 ± 33.77	90.67 ± 53.35	0.05
TST (min)	411.64 ± 37.19	368.77 ± 54.69	0.02
TST (%)	85.61 ± 8.02	76.69± 11.43	0.02
N1 %	11.64 ± 7.71	14.60 ± 5.60	0.26
N2 %	60.49 ± 6.77	52.95 ± 10.46	0.03
SWS %	15.53 ± 10.05	19.03 ± 10.02	0.34
REM %	12.33 ± 5.56	13.39 ± 6.64	0.65
REM latency (min)	154.34 ± 89.29	135.14 ± 71.05	0.53
Blood pressure (mm Hg)			
Systolic	123.62 ± 10.60	130.69 ± 16.21	0.19
Diastolic	76.15 ± 5.39	75.00 ± 12.87	0.76
HTN			
Yes, %	30.8	50.0	0.29
Smoking (current)			
Yes, %	7.7	0.0	0.25

Lipid-lowering medication use			
Yes, %	0.0	25.0	0.08
ESS	8.90 ± 3.59	10.81 ± 5.34	0.28

WASO: Wake after Sleep onset, TST: total sleep time, N1: stage 1 sleep, N2: stage 2 sleep, SWS: Slow Wave Sleep, HTN: hypertension defined as Systolic blood pressure >=140 mmHg and/or diastolic blood pressure >=90 mmHg or as the use of antihypertensive medication, lipid lowering medication: use of statins and/or fibrates

Table 3. Inflammatory and metabolic indices in patients with sleep apnea *vs.* controls

	Men			Women		
	Controls (n=18)	**Apneics (n=20)**	**P**	**Controls (n=21)**	**Apneics (n=18)**	**P**
Log hsCRP (ng/ml)	-0.18 ± 0.08	0.09 ± 0.08	0.02	-0.04 ± 0.08 (0.04± 0.10)	0.45 ± 0.08 (0.47 ± 0.09)	0.01 (0.01)
log IL-6 (pg/ml)	0.37 ± 0.03	0.48 ± 0.03	0.02	0.44 ± 0.04 (0.43 ± 0.05)	0.53 ± 0.04 (0.55 ± 0.04)	0.10 (0.11)
TNF-R1 (pg/ml)	881.25 ± 34.23	929.57 ± 32.45	0.31	1092.77 ± 44.07 (1144.24 ± 63.25)	994.24 ± 47.00 (1033.06 ± 53.05)	0.17 (0.23)
logLeptin (ng/ml)	0.62 ± 0.06	0.78 ± 0.05	0.05	1.36 ± 0.05 (1.47 ± 0.04)	1.43 ± 0.06 (1.57 ± 0.04)	0.25 (0.11)
Adiponectin (ng/ml)	4.89 ± 0.60	4.75 ± 0.54	0.87	12.68 ± 1.44 (11.63 ± 1.71)	10.84 ± 1.57 (8.96 ± 1.52)	0.43 (0.28)
HOMA	2.37± 0.40	4.00 ± 0.37	0.01	3.59 ± 0.37 (3.81± 0.44)	3.35± 0.40 (3.89 ± 0.39)	0.69 (0.90)

Men data are presented as mean values ± SE after controlling for lipid-lowering medication and smoking status. Women data are presented as mean values ± SE after controlling for age, BMI, lipid-lowering medication, hypertension and smoking status. In parentheses the data from the subgroup of women matched for BMI after controlling for age, lipid-lowering medication, hypertension and smoking status (n=13 controls and n=16 apneics)

Table 4. Sleep and respiratory data in men and women with sleep apnea at baseline and after Sham-CPAP and CPAP treatment phases

	Baseline (n=35)	**Post CPAP (n=35)**	**Post Sham-CPAP (n=35)**
Apnea/ Hypopnea Index	38.49 ± 3.66*	2.53 ± 0.75	31.84 ± 4.91†
Minimum O_2 Saturation	82.11 ± 1.11*	91.69 ± 0.76*	83.26 ± 1.15†
Sleep latency (min)	17.43 ± 1.16	14.71 ± 1.83	15.43 ± 2.22
WASO (min)	97.82 ± 8.96*	69.35 ± 7.63	89.81 ± 8.86†
TST (min)	365.75 ± 9.06*	395.85 ± 9.24	374.85 ± 8.41†
TST (%)	76.06 ± 1.90*	82.45 ± 1.70	78.14 ± 1.92†
N1 %	18.29 ± 1.48*	12.57 ± 0.93	16.77 ± 1.36†
N2 %	54.99 ± 2.15	59.10 ± 1.88	59.02 ± 1.88
SWS %	12.56 ± 1.72	12.34 ± 1.79	12.00 ± 1.66
REM %	14.15 ± 1.19	14.02 ± 0.95	12.20 ± 1.04
REM latency (min)	120.58 ± 10.92	120.66 ± 12.88	136.16 ± 11.43

Data are presented as mean ± SE. * $p<0.05$ baseline vs. CPAP † $p<0.05$ CPAP vs. sham-CPAP

Table 5. Inflammatory and metabolic indices in the entire group of patients with sleep apnea (n=35)

	Baseline	CPAP	sham CPAP	p1	p2	p3
BMI (kg/m^2)	28.55 ± 0.57	29.02 ± 0.57	28.67 ± 0.57	0.11	1.00	0.12
AHI	38.49 ± 3.66	2.53 ± 0.76	31.84 ± 4.91	<0.01	0.35	<0.01
Minimum O_2 Saturation	82.11 ± 1.12	91.69 ± 0.76	83.26 ± 1.16	<0.01	0.41	<0.01
Log hsCRP (ng/ml)	0.21 ± 0.06	0.21 ± 0.06	0.15 ± 0.06	1.00	0.41	0.44
log IL-6 (pg/ml)	0.47 ± 0.03	0.47 ± 0.03	0.45 ± 0.03	1.00	1.00	1.00
TNF-R1 (pg/ml)	974.33 ± 26.47	1020.36 ± 26.47	1007.11 ± 26.47	0.67	0.99	1.00
logLeptin (ng/ml)	1.14 ± 0.03	1.14 ± 0.03	1.13 ± 0.03	1.00	1.00	1.00
Adiponectin (ng/ml)	7.32 ± 0.63	6.71 ± 0.63	6.88 ± 0.63	1.00	1.00	1.00
HOMA	3.82 ± 0.34	3.46 ± 0.24	3.64 ± 0.43	1.00	1.00	1.00

Data are presented as mean values ± SE

p1: comparison between baseline-CPAP

p2: comparison between baseline-sham CPAP

p3: comparison between CPAP-sham CPAP

Legends to the figures

Figure 1. Twenty-four hour log-transformed Interleukin-6 values in control (■) vs. apneic men (○) at baseline

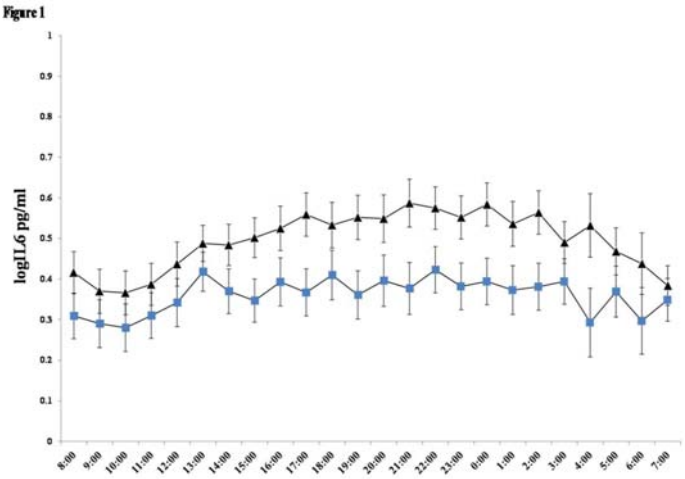

Figure 2. Twenty-four hour log-transformed Interleukin-6 values in the entire sample of control (●) vs. apneic women (◊) at baseline