Sleep apnoea, sleepiness, inflammation and insulin resistance in middle-aged males and females

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ABSTRACT In obese males obstructive sleep apnoea (OSA) is associated with inflammation and insulin resistance; however, findings are confounded by adipose tissue, a hormone- and cytokine-secreting organ. Our goal was to examine whether in a relatively nonobese population, OSA is associated with sleepiness and inflammation/insulin resistance, and to assess the effects of a 2-month placebo-controlled continuous positive airway pressure (CPAP) use.

77 subjects, 38 middle-aged males and post-menopausal females with OSA and 39 male and female controls, were studied in the sleep laboratory for 4 nights. Measures of sleepiness (objective and subjective), performance, serial 24-h blood samples for interleukin (IL)-6, tumour necrosis factor receptor (TNFR)-1, leptin and adiponectin, and single samples for high-sensitivity C-reactive protein (hsCRP), fasting glucose and insulin levels were obtained.

Apnoeic males were significantly sleepier and had significantly higher hsCRP, IL-6, leptin and insulin resistance than controls. Apnoeic females had significantly higher hsCRP; however, objective sleepiness, IL-6, TNFR-1, insulin resistance (Homeostatic Model Assessment 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 and insulin resistance, even in nonobese males, and this association is stronger in males than in females. Short-term CPAP does not improve the inflammatory/metabolic aberrations in OSA.

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OSA is associated with sleepiness and inflammation/insulin resistance in nonobese males and females http://ow.ly/qesMA

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Introduction

Obstructive sleep apnoea (OSA) is the second most common sleep disorder and is associated with increased risk of cardiovascular morbidity and mortality and impairment in quality of life [1]. We and others have shown that OSA in obese males is associated with elevated pro-inflammatory cytokines, *i.e.* interleukin (IL)-6 and tumour 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 males with apnoea 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 nonobese apnoeic males 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 of females exclusively or studies that have assessed sex differences are limited and have yielded inconclusive results [8, 15–19].

The effects of continuous positive airway pressure (CPAP) treatment on the above inflammatory/metabolic indices are inconsistent [20]. Notably, only a few of these previous studies, performed almost exclusively on 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 a predominantly nonobese population of apnoeic males and females and their controls and examined the independent and sex-specific association of OSA with multiple biomarkers of inflammatory cardiometabolic morbidity. Despite our efforts, our sample of females consisted of slightly obese patients because 1) in Central Pennsylvania females are heavier compared to national standards and 2) females with OSA both in clinical and epidemiological samples are heavier than males [29]. A secondary goal was to assess the effect of a placebo-controlled (sham-CPAP) 2-month CPAP treatment on the inflammatory and metabolic profile.

Subjects and methods

Subjects

77 subjects, including sleep apnoea patients and controls, participated in the study. We assessed the baseline and post-CPAP/-sham-CPAP serial 24-h plasma concentrations of IL-6, TNF receptor (TNFR)-1, 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-h IL-6 [30] as well as data from studies assessing the effects of antidiabetic medications on fasting blood insulin levels [31] using a 2×2 crossover design, a sample size of 30 would have a power of $\geqslant 80\%$ to detect a difference at the $\alpha = 0.05$ level.

The subjects were recruited from the sleep research and treatment centre at Penn State Milton S. Hershey Medical Center (Hershey, PA, USA) and, through advertisements, from the community. All participants provided written informed consent. Inclusion and exclusion criteria for apnoeic and control subjects have been described elsewhere [29]. Sleep apnoea patients that had used CPAP previously were excluded from the study.

Current smoking and the use of lipid-lowering medication (*i.e.* statins and fibrates), factors known to affect inflammatory markers, were also recorded. Hypertension was defined as a systolic blood pressure $\geqslant 140$ mmHg and/or diastolic blood pressure $\geqslant 90$ mmHg or as the use of antihypertensive medication.

The study was approved by the institutional review boards of Penn State Milton S. Hershey Medical Center and Pennsylvania State University College of Medicine (Hershey, PA, USA).

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 body mass index (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 h and the subjects who met the inclusion criteria were monitored in the sleep laboratory for four consecutive nights (one adaptation and three 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 their treatment and they were reassessed with the same protocol as previously described at the end of each

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2-month period. The sleep records were scored independently of any knowledge of the experimental conditions according to standardised criteria [29].

Daytime sleepiness and performance

Epworth Sleepiness Scale

Subjective sleepiness was assessed using the Epworth Sleepiness Scale (ESS) on the first day at the sleep laboratory [4].

Multiple Sleep Latency Test and Psychomotor Vigilance Test

During the fourth day (day of blood sampling) the subjects' levels of sleepiness and alertness were evaluated using the Multiple Sleep Latency Test (MSLT) and the Psychomotor Vigilance Test (PVT), as previously described [4].

Anxiety and depression symptoms

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

24-h blood sampling

Blood was drawn during the fourth day and night in the sleep laboratory at baseline and was repeated in sleep apnoea patients at the end of both CPAP and sham-CPAP treatment periods (for more details see the online supplementary material).

Assays

Blood collected from an indwelling catheter was collected in EDTA-containing tubes and refrigerated until centrifugation (within 3 h). Blood was stored at -80 °C until assay. Concentrations of IL-6 and TNFR-1 were measured every 60 min, leptin and adiponectin were measured every 120 min throughout the 24-h 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 the online supplementary material).

CPAP and sham-CPAP usage

All patients with sleep apnoea consecutively underwent CPAP and sham-CPAP treatment in a random order. The methodology is described in detail in the online supplementary material. 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'', according to hours of daily CPAP use (online supplementary material S4). The design of the study also included an intervening 1-week washout period between the two treatment phases. Patients, investigators and respiratory therapists were all blinded to treatment phases.

Statistical analysis

For comparisons of the groups' anthropometric baseline characteristics, the independent-samples 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 apnoeic males were conducted using linear mixed models after including lipid-lowering medication use and smoking status as covariates, and between apnoeic and control females after controlling for BMI, age, lipid-lowering medication, hypertension and smoking status, since all these factors are well known to affect levels of inflammatory markers. Since apnoeic females were significantly heavier than controls, the same comparisons were repeated within a subgroup matched for BMI (sensitivity analysis) [29].

Subjective sleepiness data in males were compared using the independent samples t-test and in females subjective sleepiness data were compared by 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 males and for BMI, age and total sleep time for females.

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-h blood draw compared to the beginning, we proceeded with a ''detrended'' analysis [4]. Insulin resistance was expressed with the Homeostatic Model Assessment (HOMA) index, according to the 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

TABLE 1 Demographic and sleep characteristics of the study population

	Males			Females		
	Controls	Apnoeic patients	p-value	Controls	Apnoeic patients	p-value
Subjects n	18	20		21	18	
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
Apnoea/hypopnoea index	3.03±1.98	42.42±22.51	<0.01	1.63±1.52	32.14±18.47	<0.01
Minimum oxygen saturation %	89.00±5.03	80.80±8.08	<0.01	91.19±3.77	83.11±3.80	<0.01
Waist circumference 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 mmHg						
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
Hypertension[#] %	22.2	25.0	0.84	23.8	44.4	0.15
Smoking current %	22.2	5.0	0.12	9.5	0.0	0.28
Lipid-lowering medication[¶] %	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, unless otherwise stated. BMI: body mass index; WASO: wake after sleep onset; TST: total sleep time; N1: stage 1 sleep; N2: stage 2 sleep; SWS: slow-wave sleep; REM: rapid eye movement sleep; ESS: Epworth Sleepiness Scale. [#]: defined as systolic blood pressure ⩾140 mmHg and/or diastolic blood pressure ⩾90 mmHg or as the use of antihypertensive medication; [¶]: defined as use of statins and/or fibrates; [+]: data are presented as mean±SE.

specified covariance structure was autoregressive (1) and pairwise comparisons using Bonferroni correction were performed. Group (baseline, sham-CPAP, CPAP), sex and time, as well as their interactions, were treated as fixed effects. Effects of the order of intervention were also assessed by a 2×2 ANOVA. $p<0.05$

TABLE 2 Inflammatory and metabolic indices in patients with sleep apnoea *versus* controls

	Males			Females		
	Controls	Apnoeic patients	p-value	Controls	Apnoeic patients	p-value
Subjects n	18	20		21	18	
log hsCRP ng·mL^{-1}	-0.18±0.08	0.09±0.08	0.02	-0.04±0.08	0.45±0.08	0.01
				(0.04±0.10)	(0.47±0.09)	(0.01)
log IL-6 pg·mL^{-1}	0.37±0.03	0.48±0.03	0.02	0.44±0.04	0.53±0.04	0.10
				(0.43±0.05)	(0.55±0.04)	(0.11)
TNFR-1 pg·mL^{-1}	881.25±34.23	929.57±32.45	0.31	1092.77±44.07	994.24±47.00	0.17
				(1144.24±63.25)	(1033.06±53.05)	(0.23)
log leptin ng·mL^{-1}	0.62±0.06	0.78±0.05	0.05	1.36±0.05	1.43±0.06	0.25
				(1.47±0.04)	(1.57±0.04)	(0.11)
Adiponectin ng·mL^{-1}	4.89±0.60	4.75±0.54	0.87	12.68±1.44	10.84±1.57	0.43
				(11.63±1.71)	(8.96±1.52)	(0.28)
HOMA index	2.37±0.40	4.00±0.37	0.01	3.59±0.37	3.35±0.40	0.69
				(3.81±0.44)	(3.89±0.39)	(0.90)

Data are presented as mean±SE, unless otherwise stated. Data for males are presented after controlling for lipid-lowering medication and smoking status. Data for females are presented after controlling for age, body mass index (BMI), lipid-lowering medication, hypertension and smoking status. Data in parentheses are from the subgroup of females matched for BMI after controlling for age, lipid-lowering medication, hypertension and smoking status (n=13 controls and n=16 apnoeics). hsCRP: high-sensitivity C-reactive protein; IL: interleukin; TNFR: tumour necrosis factor receptor; HOMA: Homeostatic Model Assessment.

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was used to determine statistical significance. All analyses were conducted using SPSS 17.0 (SPSS Inc., Chicago, IL, USA).

Results

Demographic, emotional distress, sleep, respiratory and sleepiness data

Participants' demographic and sleep characteristics are presented in table 1. Participants were middle-aged (range 41.7–66.3 years) and all females were post-menopausal and not undergoing hormone replacement therapy.

Apnoeic males compared to controls did not differ significantly in any of the measured demographic characteristics, apart from a trend for higher waist circumference. Controls and apnoeics 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, apnoeic males were significantly different to controls in several sleep- and breathing-related variables (table 1).

In terms of daytime sleepiness, apnoeic males were both subjectively and objectively sleepier than controls (mean ESS total score in apnoeics *versus* controls 10.85±5.44 *versus* 7.42±5.00, p=0.04; mean MSLT in apnoeics *versus* controls 10.25±0.99 min *versus* 13.54±1.08 min, p=0.04). No significant difference in number of lapses or median reaction time was observed between apnoeics and controls (lapses 2.33±0.66 *versus* 2.79±0.66, p=0.27; median reaction time 230.93±6.30 ms *versus* 237.79±6.86 ms, p=0.49).

Apnoeic females compared to their controls had significantly higher systolic blood pressure and used lipid-lowering medication more frequently (blood pressure data before adjustment for confounders are presented in table 1). 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, apnoeic females were subjectively (mean ESS total score in apnoeics *versus* controls 11.12±1.06 *versus* 7.52 ±0.97, p=0.02), but not objectively, sleepier than controls (mean MSLT in apnoeics *versus* controls 12.43±1.06 min *versus* 10.69±0.99 min, p=0.28). Finally, no difference in PVT lapses or median reaction time was observed between apnoeics and controls (lapses 2.99±2.38 *versus* 6.59±2.18, respectively, p=0.30; and median reaction time 251.78±15.22 ms *versus* 263.43±13.99 ms, respectively, p=0.59).

Inflammatory markers (hsCRP, TNFR-1 and IL-6) and indices of insulin resistance (HOMA, leptin and adiponectin blood levels)

Males

Apnoeic subjects in general had an impaired metabolic/inflammatory profile compared to controls (table 2). More specifically, apnoeic males had higher morning, evening and mean 24-h hs-CRP values. Mean IL-6 concentrations were also significantly higher in the apnoeic group than controls and apnoeic individuals demonstrated higher IL-6 values at each specific timepoint (fig. 1). However, no significant difference was observed in TNFR-1 values (table 2). Apnoeic males also demonstrated higher levels of leptin and insulin resistance, but adiponectin levels were not different between the two groups.

Females

Apnoeic females had significantly higher hs-CRP and early morning IL-6 levels, while there was a nonsignificant increase in average 24-h IL-6 levels compared to female controls (fig. 2). No significant difference in TNFR-1, HOMA index, leptin or adiponectin was detected (table 2).

We also performed analyses in a subgroup of females matched for BMI. Demographic, sleep and respiratory data are presented in table 3. Inflammatory and metabolic results in this subgroup were similar (table 2).

Effect of CPAP on inflammatory and metabolic indices and sleepiness

Sex 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 (online supplementary table S5). The mean±SD daily sham-CPAP use was 5.26±1.24 h and the daily CPAP use was 6.07±1.21 h. Adherence to CPAP did not depend on whether it was given first or second, but there was a trend for patients who used the sham-CPAP after CPAP to adhere less (p=0.19). The majority of participants, apart from two, were regular users of CPAP.

CPAP improved significantly the respiratory and sleep variables, but no significant changes were observed in hsCRP, IL-6, TNFR-1, leptin, adiponectin or HOMA index (table 5) compared to either baseline or sham-CPAP phase.

ESS total score decreased significantly during the CPAP phase in comparison to both baseline and sham-CPAP phases (mean±SE baseline *versus* CPAP *versus* sham-CPAP 10.40±0.90 *versus* 7.46±0.59 *versus* 9.66±0.76, respectively, all p<0.01), while ESS scores during the sham-CPAP phase were similar to

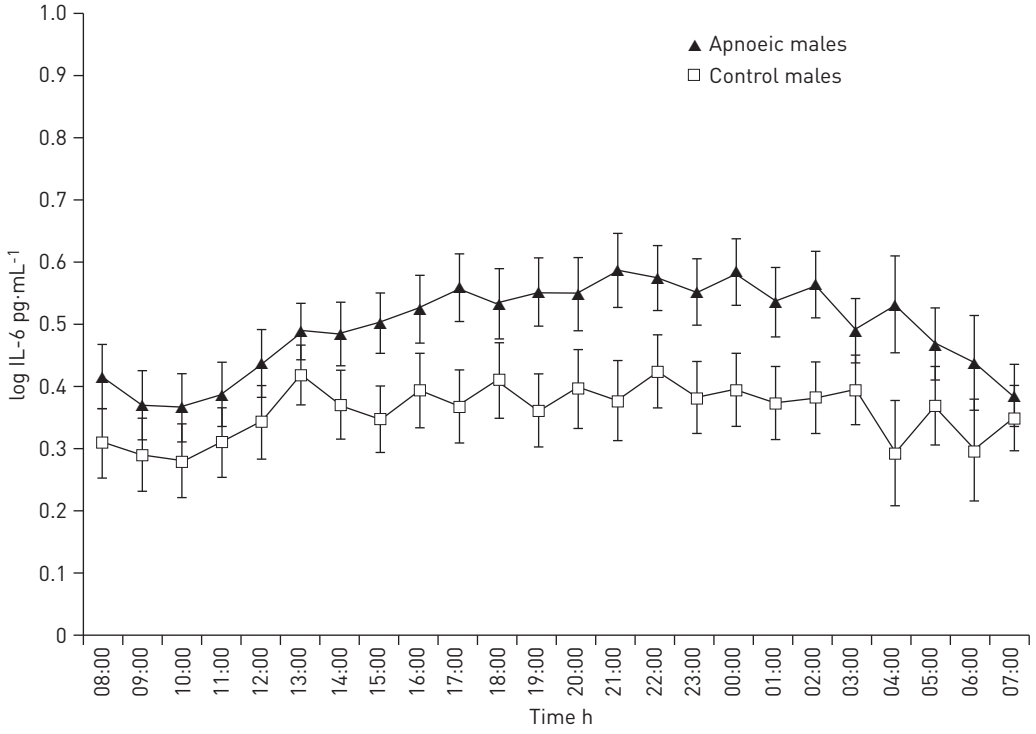

FIGURE 1 24-h log-transformed interleukin (IL)-6 values in control *versus* apnoeic males at baseline.

baseline. Mean±SE MSLT values did not increase compared to baseline during the CPAP phase (baseline *versus* CPAP 12.18±0.63 min *versus* 12.70±0.63 min, p=1.00), while they became worse during the sham-CPAP phase (baseline *versus* sham-CPAP 12.18±0.63 min *versus* 10.37±0.63 min, p=0.09).

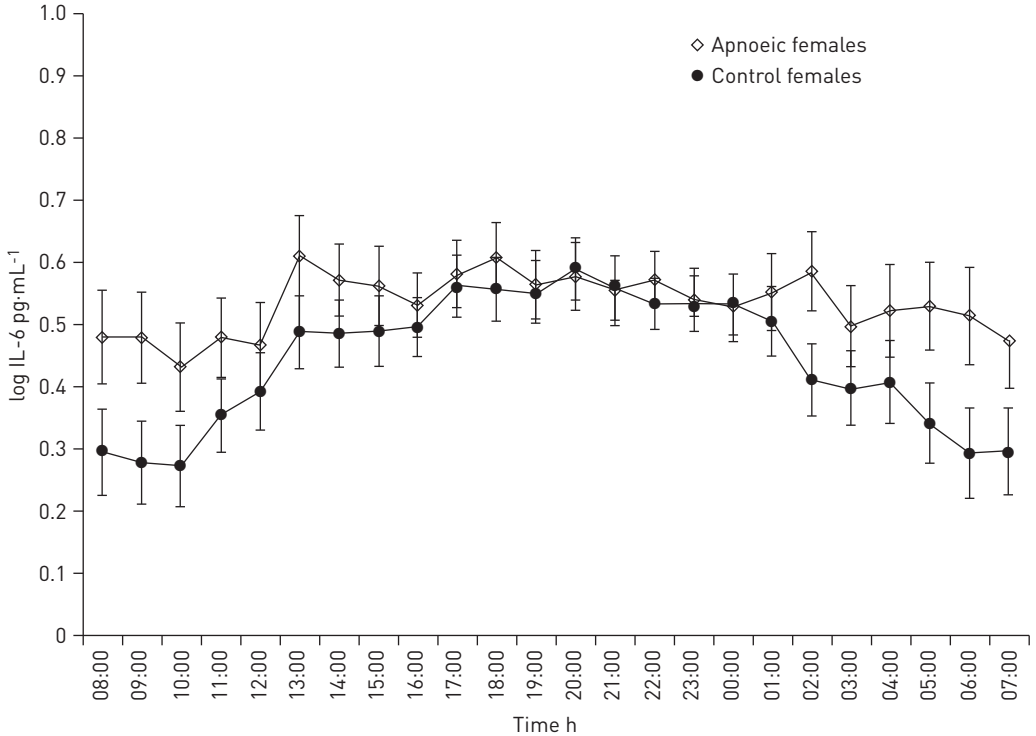

FIGURE 2 24-h log-transformed interleukin (IL)-6 values in the entire sample of control *versus* apnoeic females at baseline.

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TABLE 3 Demographic and sleep characteristics in the subgroup of females matched for body mass index (BMI)

	Controls	Apnoeic patients	p-value
Subjects n	13	16	
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
Apnoea/hypopnoea index	1.69±1.61	33.94±18.78	<0.01
Minimum oxygen saturation %	91.07±4.00	82.62±3.76	<0.01
Waist circumference 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 mmHg			
Systolic	123.62±10.60	130.69±16.21	0.19
Diastolic	76.15±5.39	75.00±12.87	0.76
Hypertension[#] %	30.8	50.0	0.29
Smoking (current) %	7.7	0.0	0.25
Lipid-lowering medication[¶] %	0.0	25.0	0.08
ESS	8.90±3.59	10.81±5.34	0.28

Data are presented as mean±SD, unless otherwise stated. WASO: wake after sleep onset; TST: total sleep time; N1: stage 1 sleep; N2: stage 2 sleep; SWS: slow-wave sleep; REM: rapid eye movement sleep; ESS: Epworth Sleepiness Scale. [#]: defined as systolic blood pressure ⩾140 mmHg and/or diastolic blood pressure ⩾90 mmHg or as the use of antihypertensive medication; [¶]: defined as use of statins and/or fibrates.

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

Discussion

The two primary findings of this study are: 1) sleep apnoea is significantly associated with sleepiness, inflammation and insulin resistance even in nonobese males and 2) in males, apnoea is associated with a worse inflammatory/metabolic profile than females. A secondary finding is that a 2-month CPAP treatment

TABLE 4 Sleep and respiratory data in males and females with sleep apnoea at baseline and after sham-continuous positive airway pressure (CPAP) and CPAP treatment phases

	Baseline	Post-CPAP	Post-sham-CPAP
Subjects n	35	35	35
Apnoea/hypopnoea index	38.49±3.66*	2.53±0.75	31.84±4.91[#]
Minimum oxygen 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. WASO: wake after sleep onset; TST: total sleep time; N1: stage 1 sleep; N2: stage 2 sleep; SWS: slow-wave sleep; REM: rapid eye movement sleep. *: p<0.05 baseline *versus* CPAP; [#]: p<0.05 CPAP *versus* sham-CPAP.

TABLE 5 Inflammatory and metabolic indices in the entire group of patients with sleep apnoea

	Baseline	CPAP	Sham-CPAP	p-value#	p-value¶	p-value+
BMI kg·m^{-2}	28.55±0.57	29.02±0.57	28.67±0.57	0.11	1.00	0.12
Apnoea/hypopnoea index	38.49±3.66	2.53±0.76	31.84±4.91	<0.01	0.35	<0.01
Minimum oxygen saturation	82.11±1.12	91.69±0.76	83.26±1.16	<0.01	0.41	<0.01
log hsCRP ng·mL^{-1}	0.21±0.06	0.21±0.06	0.15±0.06	1.00	0.41	0.44
log IL-6 pg·mL^{-1}	0.47±0.03	0.47±0.03	0.45±0.03	1.00	1.00	1.00
TNFR-1 pg·mL^{-1}	974.33±26.47	1020.36±26.47	1007.11±26.47	0.67	0.99	1.00
log leptin ng·mL^{-1}	1.14±0.03	1.14±0.03	1.13±0.03	1.00	1.00	1.00
Adiponectin ng·mL^{-1}	7.32±0.63	6.71±0.63	6.88±0.63	1.00	1.00	1.00
HOMA index	3.82±0.34	3.46±0.24	3.64±0.43	1.00	1.00	1.00

Data are presented as mean±SD, unless otherwise stated. n=35. CPAP: continuous positive airway pressure; BMI: body mass index; hsCRP: high-sensitivity C-reactive protein; IL: interleukin; TNFR: tumour necrosis factor receptors; HOMA: Homeostatic Model Assessment. #: comparison between baseline and CPAP; ¶: comparison between baseline and sham-CPAP; +: comparison between CPAP and sham-CPAP.

period does not improve insulin resistance and low-grade inflammation despite a significant improvement in sleep and respiratory variables.

Our results expand our previous findings on obese males and demonstrate a significant association of sleep apnoea with low-grade inflammation/metabolic dysregulation in nonobese males as well. Results from previous studies on overweight males with OSA have shown higher insulin resistance than controls [18], whereas one study that reported higher TNF-α levels did not control for BMI effect [12]. Our study is the first to examine multiple inflammatory/metabolic factors, including leptin and adiponectin, in nonobese males in a comprehensive way using serial 24-h blood sampling. These results suggest that, similarly to obese patients with sleep apnoea, metabolic abnormalities and chronic low-grade inflammation are also present in nonobese 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 apnoea in nonobese males is associated with visceral adiposity [29], suggest that sleep apnoea 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 males with sleep apnoea were more severe than in females. This is consistent with our earlier study which showed that in nonobese males with apnoea, visceral fat is the predominant fat problem, which is more strongly associated with inflammation/insulin resistance than subcutaneous fat. In contrast, in females it was total fat that correlated with inflammatory and cardiometabolic indices. Previous findings on the association of inflammation/metabolic aberrations in females are weak and/or inconclusive. For example, one study reported an independent association of OSA with CRP but another failed to replicate such a finding [8, 15]. Furthermore, whereas one study found no sex 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 females [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 females [19].

The milder profile of inflammatory/metabolic aberrations in females than that of males raises an important question. If, indeed, females with sleep apnoea have a more favorable inflammatory/metabolic profile compared to males we would have expected that females 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 males [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 females.

In our study, males with sleep apnoea were sleepier both subjectively and objectively than controls. In contrast, females 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 males with apnoea *versus* females [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 females with apnoea in contrast to males were more anxious/depressed compared to their controls. Previous studies have also shown that females with apnoea are more depressed compared with males [39, 40].

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Furthermore, we have previously postulated that pro-inflammatory cytokines are mediators of objective sleepiness [2]. The stronger elevation of inflammatory markers in males than in females is consistent with the greater degree of sleepiness observed in males. 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 effect of CPAP on inflammation/metabolic profile [21–28]. One study reported an improvement in TNFR-1 values, but failed to show improvement in any other inflammatory markers [22] and two studies observed an improvement of insulin sensitivity in obese subjects [28] and in those with severe apnoea 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), 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 noninvasive ventilation treatment on inflammatory, metabolic and cardiovascular markers [41].

We have shown that both obese and nonobese males with apnoea have increased visceral adiposity not corrected by CPAP [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 apnoeic males 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 apnoeic group after 2 months of CPAP treatment [27]. It appears that further studies with a longer duration, focusing on more severe apnoeic subjects 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 apnoeic and control females in terms of BMI. However, the weight of the female participants in this study lies at the lower end of the BMI of females 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 females matched for BMI, and the results remained unchanged. However, one limitation of this approach is 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 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 if 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 apnoea is a manifestation of the metabolic syndrome even in nonobese males. In mildly obese females, the inflammation/metabolic abnormalities are milder than in males, which may reflect sex differences in terms of visceral adiposity and sleep apnoea. From a practical standpoint, this difference suggests the need for sex-specific therapeutic strategies, such as reduction of visceral fat and inflammation through exercise or pharmacological treatment in males [44, 45] and weight loss in females [46].

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

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