CPAP decreases plasma levels of soluble tumour necrosis factor- α receptor 1 in obstructive sleep apnoea

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ABSTRACT: There is increasing evidence that inflammation plays an important role in the development of cardiovascular complications in patients with obstructive sleep apnoea (OSA).

No previous works have studied levels of soluble tumour necrosis factor- α receptor (sTNFR)-1 in patients with OSA. The aims of the present study were to examine serum levels of sTNFR-1 and the effect of nasal continuous positive airway pressure (CPAP) in patients with OSA.

A prospective, randomised, placebo-controlled crossover study was performed. In total, 30 consecutive newly diagnosed OSA patients (apnoea/hypopnoea index 43.8 ± 27.0 events·h⁻¹) and 15 healthy obese patients were selected. Urinary levels of norepinephrine and epinephrine, as well as plasma sTNFR-1, tumour necrosis factor (TNF)- α , interleukin (IL)-6 and leukotriene (LT)B₄ levels were obtained at baseline and after 3 months of CPAP or sham CPAP.

Nocturnal urinary levels of norepinephrine, epinephrine and sTNFR-1 ($1,053 \pm 269$ versus 820 ± 166 pg·mL⁻¹) were significantly higher in OSA patients. There were no significant differences in plasma levels of IL-6, LTB₄, or TNF- α between the two study groups. There were no significant differences in blood pressure, urinary catecholamine levels, or plasma IL-6, LTB₄ and TNF- α levels after both treatment modalities. However, after 3 months of effective CPAP usage, sTNFR-1 levels were significantly reduced ($1,053 \pm 269$ versus 899 ± 254 pg·mL⁻¹).

Obstructive sleep apnoea patients have higher levels of soluble tumour necrosis factor- α receptor 1 than individuals without OSA; soluble tumour necrosis factor- α receptor 1 levels are lowered by continuous positive airway pressure therapy. These findings further corroborate a potential role of inflammation in the natural history of obstructive sleep apnoea.

KEYWORDS: Continuous positive airway pressure, inflammation, obstructive sleep apnoea

Obstructive sleep apnoea (OSA) is the most common form of sleep-disordered breathing, affecting 4% of middle-aged (30–60 yrs) males and 2% of middle-aged (30–60 yrs) females. OSA is characterised by repeated partial or complete closure of the upper airway during sleep leading to chronic intermittent hypoxia, sleep fragmentation and daytime sleepiness. This medical condition is associated with a great variety of cardiovascular disturbances and stroke, resulting in severe morbidity and mortality [1]. OSA has been associated with an increased risk of systemic hypertension, left and right ventricular dysfunction, ventricular hypertrophy, heart failure, metabolic dysregulation, pulmonary hypertension, coronary artery disease and cardiac arrhythmias. There are many risk factors for OSA, including sex (male), advanced age and obesity, which are the same for cardiovascular disease,

making it difficult to accurately determine the role of OSA as an independent risk factor.

Systemic inflammation is crucial in the pathogenesis of all stages of atherosclerosis. There is also increasing evidence that inflammation plays an important role in the development of cardiovascular complications in patients with OSA [2].

Conversely, several studies have demonstrated that tumour necrosis factor (TNF)- α and its soluble receptors, especially soluble TNF- α receptor (sTNFR)-1, are frequently elevated in patients with heart failure, providing useful prognostic information [3–5]. In addition, higher inflammatory marker levels may predict survival and development of overt heart failure in patients with myocardial infarction [6, 7] and may antedate new-onset heart failure even in patients with asymptomatic left ventricular systolic dysfunction

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STATEMENT OF INTEREST

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without prior myocardial infarction [8]. TNF- α soluble receptors have also been linked to disorders in glucose metabolism [9], and sTNFR-1 is probably an important regulator of sleep–wake cycles [10].

There are no data about the possible presence of elevated plasma sTNFR-1 levels in individuals with OSA. Moreover, previous studies evaluating the effects of continuous positive airway pressure (CPAP) therapy, the treatment of choice for OSA, on inflammatory marker levels were not examined with a randomised, placebo-controlled design [11–15]. The aims of the current study were: 1) to evaluate whether levels of sTNFR-1, as well as other pro-inflammatory cytokines, including TNF- α , interleukin (IL)-6, and leukotriene (LT) B_4 , are elevated in OSA and otherwise healthy obese patients compared with obese subjects without OSA; and 2) to determine whether treatment with CPAP decreases cytokine levels in a randomised, placebo-controlled crossover study design.

MATERIALS AND METHODS

Study population

In total, 33 consecutive newly diagnosed OSA patients (apnoea/hypopnoea index (AHI) ≥ 10 events·h $^{-1}$ and excessive daytime sleepiness (Epworth sleepiness scale >11) and 15 obese control subjects (AHI <5 events·h $^{-1}$ and Epworth sleepiness scale <10) were selected to be studied from January 2004 to June 2005. None of the patients had ever received treatment for OSA. Control subjects were recruited from a list of healthy subjects from the present authors' Madrid metropolitan area of healthcare assigned to the Hospital La Paz (Madrid, Spain), who had undergone a routine health examination in the previous 3 months. A control subject similar in sex, age (± 2 yrs), weight (± 2 kg) and height (± 5 cm) with regard to the two preceding patients was randomly selected to be included in the study. Exclusion criteria for both study groups were: 1) unwillingness or inability to perform the testing procedure; 2) obstructive or restrictive lung disease demonstrated on pulmonary function testing; 3) current use of cardioactive drugs; 4) cardiac rhythm disturbances, including sinus bradycardia and sinus tachycardia; 5) known hypertension, or 24-h mean blood pressure of 135 systolic and/or 85 mmHg diastolic or more; 6) left ventricular ejection fraction $<50\%$; ischaemic or valvular heart disease; hypertrophic, restrictive or infiltrative cardiomyopathy; pericardial disease or stroke by history or physical examination, ECG, chest radiography, conventional stress testing and echocardiography; 7) diabetes mellitus, by history or two random blood glucose levels ≥ 126 mg·dL $^{-1}$; 8) morbid obesity (body mass index (BMI) >40 kg·m $^{-2}$); and 9) daytime hypoxaemia (arterial oxygen tension <70 mmHg (9.3 kPa)) or hypercapnia (arterial carbon dioxide tension >45 mmHg (6.0 kPa)). Withdrawal criteria were: 1) clinical exacerbation leading to a change in medication; 2) hospital admission for ≥ 10 days; and 3) average nightly CPAP usage <3.5 h. Three patients were deemed ineligible for inclusion after initial assessments (one had left ventricular systolic dysfunction secondary to ischaemic heart disease and two had unknown mitral stenosis); therefore, 30 OSA patients were finally included in the study.

The study was approved by the Institutional Ethics Committee at the Hospital Universitario La Paz (Madrid, Spain). All subjects gave their written informed consent prior to enrolment.

Study protocol

A single-centre, prospective, randomised, double-blind, placebo-controlled, crossover study was performed, in which OSA patients received effective CPAP (Aria LX CPAP device; Respironics, Murrysville, PA, USA) and sham CPAP therapy for two 12-week periods (fig. 1). The sham CPAP device consisted of a conventional CPAP device, in which the area of the exhalation port was amplified, thereby nearly cancelling nasal pressure (1–2 cmH $_2$ O (0.1–0.2 kPa)); an orifice resistor was connected between the tubing and the CPAP unit that loads the blower with the same airflow resistance as in effective CPAP.

OSA patients underwent a full-night CPAP titration study with an automated pressure setting device (Auto Set; ResMed, Sydney, Australia). Patients were given detailed instructions on using CPAP equipment, but they were not informed of the type of therapy they were receiving. Compliance data were measured with a run-time counter.

All subjects underwent baseline sleep study, 24-h ambulatory blood pressure monitoring (ABPM) and a transthoracic echocardiogram. Plasma levels of sTNFR-1, TNF- α , LT B_4 and IL-6 were measured. Urine specimens were also collected. OSA patients were randomised to receive either effective or sham CPAP therapy for 12 weeks. They were then readmitted to the hospital and the CPAP device was switched to the alternative mode of therapy for another 12 weeks. Echocardiogram, ABPM, plasma cytokines and catecholamines were measured just after each period with either effective or sham CPAP treatment.

Measurements

LT B_4 concentrations were measured by a forward sequential competitive enzyme immunoassay (R&D Systems, Minneapolis, MN, USA). The inter- and intra-assay variations between the samples were 10.5 and 9.3%, respectively, and the detection limit of the assay was 27.6 pg·mL $^{-1}$.

FIGURE 1. Protocol of the study. CPAP: continuous positive airway pressure.

Quantitative sandwich enzyme immunoassay kits (R&D Systems) were used to measure IL-6, TNF- α and sTNFR-1 concentrations in plasma. The inter- and intra-assay variations between the samples were as follows. IL-6: 3.3 and 1.6%; TNF- α : 4.6 and 4.2%; and sTNFR-1: 5.0 and 5.2%, respectively, *i.e.* the inter- and intra-assay variabilities were <10%. The detection limits of the assays were 0.7, 0.5 and 0.43 pg·mL⁻¹, respectively.

During the same night that urine specimens were collected, respiratory polygraphy was performed in OSA patients and control subjects. A previously validated portable respiratory recording device was used (Sibel Home-300; Sibel S.A., Barcelona, Spain) [16], which records oronasal airflow (using a thermistor and nasal cannula prongs), chest wall impedance, oxygen saturation, snoring and body position. Oximetry data were averaged over 2-s sampling intervals. Artefacts were removed by eliminating all changes of oxygen saturation between consecutive sampling intervals of >4%·s⁻¹, and any oxygen saturation <20%.

Respiratory events were classified as either obstructive or central on the basis of presence or absence of respiratory effort. Respiratory events were scored as apnoea when there was a cessation of oronasal airflow lasting ≥ 10 s. Hypopnoea was defined as a decrease of 50% in oronasal airflow lasting >10 s, associated with a fall in arterial oxygen saturation (S_{a,O_2}) of >4% of the preceding baseline level. Mean night-time S_{a,O_2} , minimum S_{a,O_2} (lowest values recorded during sleep), oxygen desaturation index and percentage of time with S_{a,O_2} <90% on nocturnal oximetry were computed as indices of nocturnal oxygen saturation.

Spirometry, echocardiogram and 24-h ABPM were performed on each patient as previously described [17, 18]. Urinary excretion of norepinephrine and epinephrine were also determined as previously described [17].

Statistical analyses

Values are expressed as mean $\pm$ SD or percentage. All statistical tests were two-sided. The comparisons between the groups were performed using the Wilcoxon rank-sum test, while the Chi-squared test was used for evaluating frequencies. Relationships between variables were determined by the Pearson's correlation analysis. Comparisons of effects of the treatments over time were made with repeated-measures ANOVA, using treatment as a within-subject factor and order as a between-subject factor. When ANOVA results showed significant differences between treatment conditions, *post hoc* multiple comparisons were performed with the Bonferroni test. A value of $p < 0.05$ was considered statistically significant.

RESULTS

All individuals in both groups were male. Characteristics of the studied population are shown in table 1. There were no differences in either demographic, blood pressure or lung function data between the two study groups. Nocturnal levels of norepinephrine and epinephrine were higher in patients with OSA. Nocturnal epinephrine dip was higher in control subjects. Although the values were within the normal limits, OSA patients had higher values of left ventricular posterior

TABLE 1 Baseline characteristics in obstructive sleep apnoea (OSA) patients and control subjects

	OSA group	Control group	p-value [#]
Subjects n	30	15	
Age yrs	52 $\pm$ 13	48 $\pm$ 10	0.292
Height cm	171 $\pm$ 7	173 $\pm$ 5	0.264
Weight kg	89 $\pm$ 14	86 $\pm$ 16	0.546
BMI kg·m⁻²	30.5 $\pm$ 4.0	28.7 $\pm$ 4.7	0.188
Current smokers %	37	27	0.374
Smoking history pack-yrs	20 $\pm$ 12	20 $\pm$ 7	0.965
AHI events·h⁻¹	43.8 $\pm$ 27.0	3.7 $\pm$ 3.3	0.000
AI events·h⁻¹	29.4 $\pm$ 26.4	1.0 $\pm$ 0.8	0.005
Obstructive events %	96 $\pm$ 6	66 $\pm$ 31	0.000
Total sleep study time <90% S_{a,O_2} %	18.41 $\pm$ 23.39	0.04 $\pm$ 0.05	0.000
ODI events·h⁻¹	44.6 $\pm$ 26.7	4.7 $\pm$ 3.8	0.000
Mean nocturnal S_{a,O_2} %	91 $\pm$ 6	94 $\pm$ 2	0.024
Minimum S_{a,O_2} %	72 $\pm$ 15	85 $\pm$ 5	0.016
FVC % pred	105 $\pm$ 17	99 $\pm$ 16	0.313
FEV₁ % pred	111 $\pm$ 15	108 $\pm$ 16	0.500
FEV₁/FVC %	85.8 $\pm$ 7.2	88.2 $\pm$ 3.6	0.156
Daytime SBP mmHg	126 $\pm$ 10	122 $\pm$ 9	0.154
Daytime DBP mmHg	79 $\pm$ 6	78 $\pm$ 5	0.529
Daytime MBP mmHg	95 $\pm$ 7	92 $\pm$ 6	0.208
Night-time SBP mmHg	117 $\pm$ 11	110 $\pm$ 10	0.059
Night-time DBP mmHg	70 $\pm$ 7	67 $\pm$ 6	0.127
Night-time MBP mmHg	86 $\pm$ 8	82 $\pm$ 8	0.058
Diurnal norepine μg·g⁻¹	34.9 $\pm$ 13.1	29.9 $\pm$ 11.6	0.211
Diurnal epine μg·g⁻¹	7.0 $\pm$ 4.9	7.9 $\pm$ 4.5	0.571
Nocturnal norepine μg·g⁻¹	21.9 $\pm$ 12.1	12.9 $\pm$ 6.2	0.002
Nocturnal epine μg·g⁻¹	6.5 $\pm$ 5.3	4.2 $\pm$ 2.4	0.049
Norepine nocturnal dip %	36 $\pm$ 27	48 $\pm$ 24	0.182
Epine nocturnal dip %	16 $\pm$ 10	32 $\pm$ 46	0.039
Heart rate beats·min⁻¹	76 $\pm$ 13	78 $\pm$ 11	0.640
LVDD mm	52.1 $\pm$ 2.8	50.7 $\pm$ 5.8	0.399
LVSD mm	32.5 $\pm$ 2.5	30.3 $\pm$ 4.8	0.060
Posterior wall mm	10.6 $\pm$ 1.2	9.3 $\pm$ 1.4	0.004
Interventricular septum mm	10.7 $\pm$ 1.1	9.5 $\pm$ 1.5	0.009
Left ventricular mass g	213.4 $\pm$ 38.0	178.3 $\pm$ 39.5	0.007
Left ventricular mass index g·m⁻²	107.0 $\pm$ 19.6	89.5 $\pm$ 17.6	0.007
LVSF %	37.6 $\pm$ 3.1	40.1 $\pm$ 4.8	0.076
LVEF %	67.1 $\pm$ 3.7	70.1 $\pm$ 5.9	0.084
Left atrial diameter mm	37.6 $\pm$ 3.6	36.1 $\pm$ 5.6	0.363

Data are expressed as mean $\pm$ SD, unless otherwise stated. BMI: body mass index; AHI: apnoea/hypopnoea index; AI: apnoea index; S_{a,O_2} : arterial oxygen saturation; ODI: oxygen desaturation index; FVC: forced vital capacity; % pred: % predicted; FEV₁: forced expiratory volume in one second; SBP: systolic blood pressure; DBP: diastolic blood pressure; MBP: mean blood pressure; norepine: norepinephrine; epine: epinephrine; LVDD: left ventricular diastolic diameter; LVSD: left ventricular systolic diameter; LVSF: left ventricular shortening fraction; LVEF: left ventricular ejection fraction. [#]: Wilcoxon test.

FIGURE 2. Serum soluble tumour necrosis factor- α receptor (sTNFR)-1 levels in obstructive sleep apnoea (OSA) patients and control subjects. Box and whisker plots represent median, interquartile range and range. #: $p=0.032$ by Wilcoxon test.

wall, interventricular septum thickness, left ventricular mass, and left ventricular mass index.

Baseline levels of sTNFR-1 were higher in OSA patients compared with control obese subjects (fig. 2). There was no significant difference in TNF- α , IL-6 or LTB₄ levels between the two study groups (table 2).

Several associations between baseline variables and cytokine levels were revealed. There were no significant relationships between plasma levels of TNF- α or LTB₄ and any studied variable. Significant positive relationships were found between weight and sTNFR-1 ($r=0.446$, $p=0.033$) and IL-6 ($r=0.736$, $p=0.003$) levels. Also, BMI was associated with high sTNFR-1 ($r=0.414$, $p=0.049$) and IL-6 ($r=0.693$, $p=0.006$) levels. Low epinephrine nocturnal dip was associated with higher sTNFR-1 ($r=-0.484$, $p=0.019$) levels. In addition, levels of IL-6 were positively correlated with diurnal norepinephrine levels ($r=0.667$, $p=0.009$) and negatively correlated with forced vital capacity % predicted ($r=-0.636$, $p=0.015$) and forced expiratory volume in one second % pred ($r=-0.583$, $p=0.029$).

Pearson's correlation coefficients between levels of sTNFR-1, TNF- α , IL-6 and LTB₄ and sleep parameters in patients with OSA are shown in table 3. There was no significant relationship

TABLE 2 Plasma levels of pro-inflammatory cytokines in obstructive sleep apnoea (OSA) patients and control subjects			
	OSA group [#]	Control group [†]	p-value [‡]
IL-6 pg·mL ⁻¹	4.2±3.4	3.6±1.8	0.523
LTB ₄ ng·mL ⁻¹	5972±8239	7650±18033	0.780
TNF- α pg·mL ⁻¹	18.5±13.4	11.4±12.2	0.526
sTNFR-1 pg·mL ⁻¹	1053±269	820±166	0.032
Data are expressed as mean±SD, unless otherwise stated. IL: interleukin; LT: leukotriene; TNF: tumour necrosis factor; sTNFR: soluble TNF- α receptor. #: n=30; †: n=15; ‡: Wilcoxon test.			

between any of the sleep parameters and circulating cytokine levels.

Five OSA patients failed to complete the study, two because of an average nightly usage of CPAP of <3.5 h, and three were lost from follow-up. Complete measurements were available in 25 patients. Mean CPAP pressure was 10±2 cmH₂O (0.98±0.20 kPa), and the average nightly usage was similar on sham CPAP and effective CPAP (6.3±1.6 versus 6.2±1.1 h; $p=0.6636$). There were no differences in heart rate, weight, blood pressure recordings or urinary catecholamines at baseline and after sham or effective CPAP in OSA patients (table 4). There was no randomisation of patients in the cross-over design. After 12 weeks on effective CPAP, a significant reduction in sTNFR-1 levels was observed (table 5 and fig. 3). After both modes of treatment, no significant differences were observed in either systolic function or ventricular structure parameters.

DISCUSSION

In the present study, levels of sTNFR-1 were significantly higher in obese OSA and otherwise healthy patients compared with obese control subjects. In addition, circulating TNF- α , IL-6 and LTB₄ levels were not different between the two study groups. Interestingly, after 3 months of CPAP treatment, circulating sTNFR-1 levels were lower in OSA patients.

It has been shown that elevated sTNFR-1 levels predict the incidence of fatal and nonfatal cardiovascular events in several conditions, such as heart failure and acute coronary syndrome [3–7]. In that sense, in patients with acute myocardial infarction sTNFR-1 levels have been shown to be a major predictor of mortality and heart failure [6, 7]. Increasing circulating levels of sTNFR-1 are also associated with increased mortality in a wide range of heart failure patients [3–5]. Reported levels of sTNFR-1 in both heart failure and ischaemic patients were higher than those observed in the present OSA patient population, probably reflecting the fact that the present

TABLE 3 Correlation between plasma cytokine levels and sleep parameters in obstructive sleep apnoea patients				
	IL-6	LTB ₄	TNF	sTNFR-1
AHI				
Correlation coefficient r	0.237	-0.203	0.123	0.104
p-values	0.414	0.506	0.478	0.638
ODI				
Correlation coefficient r	0.396	-0.101	0.241	0.321
p-values	0.203	0.754	0.124	0.156
Mean nocturnal Sa,O₂				
Correlation coefficient r	-0.491	0.211	0.024	0.014
p-values	0.075	0.489	0.781	0.811
Minimum Sa,O₂				
Correlation coefficient r	-0.209	0.140	0.011	0.053
p-values	0.473	0.649	0.825	0.811
IL: interleukin; LT: leukotriene; TNF: tumour necrosis factor; sTNFR: soluble tumour necrosis factor- α receptor; AHI: apnoea/hypopnoea index; ODI: oxygen desaturation index; Sa,O ₂ : arterial oxygen saturation.				

TABLE 4 Weight, blood pressure data, and urinary catecholamines in 25 obstructive sleep apnoea patients at baseline and after both sham continuous positive airway pressure (CPAP) and effective CPAP

	Baseline	Sham CPAP	CPAP
Weight kg	89 ± 14	89 ± 15	89 ± 16
BMI kg·m ⁻²	30.4 ± 4.0	30.3 ± 4.1	30.2 ± 4.4
Heart rate beats·min ⁻¹	76 ± 13	72 ± 11	79 ± 20
Daytime SBP mmHg	126 ± 10	126 ± 12	127 ± 9
Daytime DBP mmHg	79 ± 6	77 ± 6	78 ± 5
Daytime MBP mmHg	95 ± 7	94 ± 8	95 ± 7
Night-time SBP mmHg	118 ± 12	117 ± 12	117 ± 17
Night-time DBP mmHg	70 ± 8	70 ± 6	69 ± 9
Night-time MBP mmHg	86 ± 9	86 ± 7	86 ± 12
Diurnal norepine µg·g ⁻¹	36.7 ± 13.8	34.2 ± 13.1	31.3 ± 12.0
Diurnal epine µg·g ⁻¹	7.4 ± 5.8	7.7 ± 3.4	7.8 ± 4.4
Nocturnal norepine µg·g ⁻¹	23.7 ± 12.8	22.6 ± 12.7	20.1 ± 10.0
Nocturnal epine µg·g ⁻¹	6.8 ± 5.9	6.1 ± 5.3	6.3 ± 4.3

Data are presented as mean ± SD. BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; MBP: mean blood pressure; norepine: norepinephrine; epine: epinephrine. There were no significant differences at any time point (repeated-measures ANOVA). There was not a randomisation of patients in the crossover design.

OSA patients were free of any overt cardiovascular disease. Whether higher levels of sTNFR-1 are present in a nonselected population of OSA subjects needs to be determined in future studies. Heart failure and coronary artery disease share multiple cardiovascular pathophysiological mechanisms with OSA, including endothelial dysfunction, systemic inflammation, impaired baroreflex and sympathetic overactivity.

Elevated sTNFR-1 levels are also reported to be associated with type-2 diabetes mellitus, regardless of obesity [9]. It is conceivable that the association of OSA and high sTNFR-1 levels may raise the rate of cardiovascular events in this patient population. Increased plasma levels of pro-inflammatory cytokines may be linked not only to poor cardiovascular outcomes in OSA but also to excessive daytime sleepiness, which is a typical feature of OSA [19]. Moreover, elevated circulating cytokine levels may be the result of the enhanced cellular release of such cytokines in response to intermittent apnoea-related hypoxia, supporting a direct role for inflammation as a potential mechanism of atherogenesis in OSA [2, 20, 21]. In this regard, OSA and chronic intermittent hypoxaemia are associated with the activation of nuclear factor κB, which is involved in the transcription of numerous genes involved in the inflammatory cascade; its downstream products (including the TNF-α system) may be key factors in the link between OSA and cardiovascular diseases. However, further studies are needed in order to evaluate whether increased sTNFR-1 levels can play a role in predicting subsequent prognosis during long-term follow-up in the entire spectrum of individuals with OSA due to initial inflammatory responses, or whether, on the contrary, they are an epiphenomenon secondary to sleep deprivation.

TABLE 5 Plasma levels of pro-inflammatory cytokines in 25 obstructive sleep apnoea patients at baseline and after both sham continuous positive airway pressure (CPAP) and effective CPAP

	Baseline	Sham CPAP	CPAP
IL-6 pg·mL ⁻¹	4.3 ± 3.5	4.6 ± 2.4	4.7 ± 2.0
LTB ₄ ng·mL ⁻¹	4961 ± 7433	4456 ± 11818	4583 ± 1243
TNF-α pg·mL ⁻¹	18.2 ± 12.6	17.9 ± 11.4	15.7 ± 10.9
sTNFR-1 pg·mL ⁻¹	1103 ± 269	1132 ± 347	899 ± 254*

Data are expressed as mean ± SD. IL: interleukin; LT: leukotriene; TNF: tumour necrosis factor; sTNFR: soluble TNF-α receptor. #: p < 0.05 versus baseline and p < 0.05 versus sham CPAP (repeated-measures ANOVA).

Previous studies have reported that patients with OSA develop systemic inflammation with increased levels of circulating pro-inflammatory cytokines, such as TNF-α, IL-6 and others, as compared with healthy subjects, and CPAP therapy reduces the levels of OSA-induced inflammatory cytokines [11–13, 15, 22]. Importantly, the increased levels of cytokines correlated with OSA severity, regardless of obesity [23]. Conversely, some authors have reported no increased levels of inflammatory mediators in individuals with OSA when compared with subjects without OSA [14, 24, 25]. In the present study, only sTNFR-1 levels were higher in OSA patients than in control subjects, but no differences were found for the other pro-inflammatory markers studied. These opposing results may be due to differences in the duration of the disease in the study samples, variable presence of comorbid conditions or the measurement techniques used. Cellular actions of TNF-α are mediated by TNF receptors. Circulating levels of TNF-α are frequently undetectable or highly variable in humans and sTNFR-1 levels may be a better marker for the biological effects and activation status of the TNF-α system.

FIGURE 3. Individual serum soluble tumour necrosis factor-α receptor (sTNFR)-1 levels at baseline, after sham continuous positive airway pressure (CPAP) and CPAP therapy in 25 obstructive sleep apnoea patients. *: p < 0.05. ●: mean values.

In the present study, two groups of obese individuals were studied. A positive correlation was found between sTNFR-1, IL-6 levels and BMI in OSA patients, but there were no significant relationships between sleep parameters and plasma cytokine levels. However, BMI did not differ between the two study groups. Obesity is very common among OSA patients and it may also be associated with inflammation. In view of the current results, the present authors speculate that at the initial stages of the disease, when clinical cardiovascular disturbances have not yet developed, the enhanced sympathetic activity secondary to repetitive nocturnal obstructive respiratory events may be the predominant mechanism responsible for the rise in sTNFR-1 levels. An ongoing prospective randomised clinical trial is studying whether the increased risk of cardiovascular complications in individuals with OSA is caused primarily by the inflammatory effects of OSA or obesity [26].

The present study has several limitations. All individuals were males. Therefore, the findings may not be readily extrapolated to female patients. In addition, the present study included a limited number of patients. That is explained by the fact that the process of including only newly diagnosed middle-aged OSA and otherwise healthy patients to achieve the purpose of the present study was very difficult. As is well known, in the global population of patients with OSA the presence of coexistent comorbid conditions is very common. The present data do not allow an analysis of whether the arousal index is related to TNF- α and sTNFR-1, since the patients were diagnosed by means of polygraphy. Since the principal target of the present study was to value the effect of the CPAP on serum levels of sTNFR-1 in OSA patients, neither the design nor the sample size are suitable for the evaluation of the possible determinants of the levels of sTNFR-1 in these patients. However, the present authors speculate that the impact of sleep fragmentation on sTNFR-1 levels in OSA patients might be considered to be discrete. Total sleep deprivation increases the plasma levels of sTNFR-1 and IL-6, but there have not been any identified changes associated with a partial sleep deprivation, which corresponds better to the situation of the OSA patients. Finally, the present study did not use polysomnography, but a portable respiratory recording device, to diagnose OSA. Although conventional polysomnography is considered the gold standard for the diagnosis of OSA, it has been previously demonstrated that an accurate diagnosis of OSA can be made without using neurological variables, even in an unattended setting, suggesting that the portable respiratory recording device is therefore suitable for use in epidemiological studies [27].

In conclusion, high circulating soluble tumour necrosis factor- α receptor-1 levels may be observed in obese obstructive sleep apnoea patients with no associated comorbid conditions. Further studies will determine whether it could serve as an early marker of ongoing systemic inflammation in obstructive sleep apnoea, increasing the risk for eventual future cardiovascular adverse events. Chronic continuous positive airway pressure application might be useful in reducing the potential cardiovascular risks of elevated circulating plasma soluble tumour necrosis factor- α receptor-1 and other pro-inflammatory cytokines in patients with obstructive sleep apnoea.

REFERENCES

- 1 Marin JM, Carrizo SJ, Vicente E, Agusti AG. Long-term cardiovascular outcomes in men with obstructive sleep apnoea-hypopnoea with or without treatment with continuous positive airway pressure: an observational study. *Lancet* 2005; 365: 1046–1053.
- 2 Ryan S, Taylor CT, McNicholas WT. Selective activation of inflammatory pathways by intermittent hypoxia in obstructive sleep apnea syndrome. *Circulation* 2005; 112: 2660–2667.
- 3 Deswal A, Petersen NJ, Feldman AM, Young JB, White BG, Mann DL. Cytokines and cytokine receptors in advanced heart failure: an analysis of the cytokine database from the Vesnarinone trial (VEST). *Circulation* 2001; 103: 2055–2059.
- 4 Ferrari R, Bachetti T, Confortini R, *et al.* Tumor necrosis factor soluble receptors in patients with various degrees of congestive heart failure. *Circulation* 1995; 92: 1479–1486.
- 5 Rauchhaus M, Doehner W, Francis DP, *et al.* Plasma cytokine parameters and mortality in patients with chronic heart failure. *Circulation* 2000; 102: 3060–3067.
- 6 Ueland T, Kjekshus J, Froland SS, *et al.* Plasma levels of soluble tumor necrosis factor receptor type I during the acute phase following complicated myocardial infarction predicts survival in high-risk patients. *J Am Coll Cardiol* 2005; 46: 2018–2021.
- 7 Valgimigli M, Ceconi C, Malagutti P, *et al.* Tumor necrosis factor- α receptor 1 is a major predictor of mortality and new-onset heart failure in patients with acute myocardial infarction: the Cytokine-Activation and Long-Term Prognosis in Myocardial Infarction (C-ALPHA) study. *Circulation* 2005; 111: 863–870.
- 8 Vasan RS, Sullivan LM, Roubenoff R, *et al.* Inflammatory markers and risk of heart failure in elderly subjects without prior myocardial infarction: the Framingham Heart Study. *Circulation* 2003; 107: 1486–1491.
- 9 Bullo M, Garcia-Lorda P, Salas-Salvado J. Plasma soluble tumor necrosis factor- α receptors and leptin levels in normal-weight and obese women: effect of adiposity and diabetes. *Eur J Endocrinol* 2002; 146: 325–331.
- 10 Fang J, Wang Y, Krueger JM. Mice lacking the TNF 55 kDa receptor fail to sleep more after TNF- α treatment. *J Neurosci* 1997; 17: 5949–5955.
- 11 Minoguchi K, Tazaki T, Yokoe T, *et al.* Elevated production of tumor necrosis factor- α by monocytes in patients with obstructive sleep apnea syndrome. *Chest* 2004; 126: 1473–1479.
- 12 Ohga E, Tomita T, Wada H, Yamamoto H, Nagase T, Ouchi Y. Effects of obstructive sleep apnea on circulating ICAM-1, IL-8, and MCP-1. *J Appl Physiol* 2003; 94: 179–184.
- 13 Ryan S, Taylor CT, McNicholas WT. Predictors of elevated nuclear factor- κ B-dependent genes in obstructive sleep apnea syndrome. *Am J Respir Crit Care Med* 2006; 174: 824–830.
- 14 Ryan S, Nolan G, Hannigan E, Cunningham S, Taylor CT, McNicholas WT. Cardiovascular risk markers in obstructive sleep apnoea syndrome and correlation with obesity. *Thorax* 2007; 62: 509–514.
- 15 Yokoe T, Minoguchi K, Matsuo H, *et al.* Elevated levels of C-reactive protein and interleukin-6 in patients with obstructive sleep apnea syndrome are decreased by nasal continuous positive airway pressure. *Circulation* 2003; 107: 1129–1134.

- 16 Ballester E, Solans M, Vila X, *et al.* Evaluation of a portable respiratory recording device for detecting apnoeas and hypopnoeas in subjects from a general population. *Eur Respir J* 2000; 16: 123–127.
- 17 Arias MA, Garcia-Rio F, Alonso-Fernandez A, Mediano O, Martinez I, Villamor J. Obstructive sleep apnea syndrome affects left ventricular diastolic function: effects of nasal continuous positive airway pressure in men. *Circulation* 2005; 112: 375–383.
- 18 Garcia-Rio F, Pino JM, Alonso A, *et al.* White coat hypertension in patients with obstructive sleep apnea-hypopnea syndrome. *Chest* 2004; 125: 817–822.
- 19 Krueger JM, Obal FJ, Fang J, Kubota T, Taishi P. The role of cytokines in physiological sleep regulation. *Ann N Y Acad Sci* 2001; 933: 211–221.
- 20 Minoguchi K, Yokoe T, Tazaki T, *et al.* Increased carotid intima-media thickness and serum inflammatory markers in obstructive sleep apnea. *Am J Respir Crit Care Med* 2005; 172: 625–630.
- 21 Savransky V, Nanayakkara A, Li J, *et al.* Chronic intermittent hypoxia induces atherosclerosis. *Am J Respir Crit Care Med* 2007; 175: 1290–1297.
- 22 Shamsuzzaman AS, Winnicki M, Lanfranchi P, *et al.* Elevated C-reactive protein in patients with obstructive sleep apnea. *Circulation* 2002; 105: 2462–2464.
- 23 Ciftci TU, Kokturk O, Bukan N, Bilgihan A. The relationship between serum cytokine levels with obesity and obstructive sleep apnea syndrome. *Cytokine* 2004; 28: 87–91.
- 24 Guilleminault C, Kirisoglu C, Ohayon MM. C-reactive protein and sleep-disordered breathing. *Sleep* 2004; 27: 1507–1511.
- 25 Imagawa S, Yamaguchi Y, Ogawa K, *et al.* Interleukin-6 and tumor necrosis factor- α in patients with obstructive sleep apnea-hypopnea syndrome. *Respiration* 2004; 71: 24–29.
- 26 Samaha FF, Giesler JC. Inflammatory response to sleep apnea in obese subjects: the Cardiovascular Effects of Obstructive Sleep Apnea (COSA) Study. www.clinicaltrials.gov Identifier NCT00371293.
- 27 Collop NA, Anderson WM, Boehlecke B, *et al.* Clinical guidelines for the use of unattended portable monitors in the diagnosis of obstructive sleep apnea in adult patients. Portable Monitoring Task Force of the American Academy of Sleep Medicine. *J Clin Sleep Med* 2007; 3: 737–747.