

Effect of continuous positive airway pressure on inflammatory, antioxidant, and depression biomarkers in women with obstructive sleep apnea: A randomized controlled trial.

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Abstract

Study objectives. The effect of continuous positive airway pressure (CPAP) on mediators of cardiovascular disease and depression in women with obstructive sleep apnea (OSA) is unknown. We aimed to assess the effect of CPAP therapy on a variety of biomarkers of inflammation, antioxidant activity and depression in women with OSA.

Methods. We conducted a multicenter, randomized controlled trial in 247 women diagnosed with moderate-to-severe OSA (apnea-hypopnea index [AHI] ≥ 15). Women were randomized to CPAP (n=120) or conservative treatment (n=127) for 12 weeks. Changes in tumor necrosis factor α (TNF α), interleukin 6 (IL-6), C-reactive protein (CRP), intercellular adhesion molecule 1 (ICAM-1), catalase (CAT), superoxide dismutase (SOD), and brain-derived neurotrophic factor (BDNF) were assessed. Additional analyses were conducted in subgroups of clinical interest.

Results. Women had a median (25th-75th percentiles) age of 58 (51-65) years, body mass index 33.5 (29.0-38.3) Kg/m² and AHI 33.3 (22.8-49.3). No differences were found between groups in the baseline levels of the biomarkers.

After 12 weeks of follow-up, there were no changes between groups in any of the biomarkers assessed. These results did not change when the analyses were restricted to sleepy women or to those with severe OSA. In women with CPAP use ≥ 5 hours/night, only TNF α levels decreased compared to the control group (-0.29 ± 1.1 vs -0.06 ± 0.53 , intergroup difference -0.23 [95%CI -0.03 to -0.50]; $p=0.043$).

Conclusions. Twelve weeks of CPAP therapy does not improve biomarkers of inflammation, antioxidant activity or depression compared to conservative treatment in women with moderate-to-severe OSA.

Key words: obstructive sleep apnea, continuous positive airway pressure, women, biomarkers, inflammation, depression, oxidative stress.

Clinical trial registration: Effect of CPAP Treatment in Women With Moderate-to-severe OSA, <http://www.clinicaltrials.gov>, NCT02047071

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Statement of significance

Obstructive sleep apnea (OSA) is associated with systemic inflammation and oxidative stress, however, the effect of continuous positive airway pressure (CPAP) therapy on inflammatory or antioxidant biomarkers has not yet been assessed in women. We have conducted the first randomized controlled trial to investigate the effect of CPAP on a wide variety of inflammation, antioxidant activity and depression biomarkers in female population. Compared with the control group, 12 weeks of CPAP therapy did not achieve any significant improvement in any of the biomarkers assessed. Only in women with CPAP use ≥ 5 hours/night, TNF α levels decreased compared to the control group. These results do not support the treatment of OSA based on an expectancy of improvement of this outcome.

INTRODUCTION

Obstructive sleep apnea (OSA) has been recognized as a risk factor for hypertension, cardiovascular disease, quality of life (QoL) impairment and depression¹⁻³. OSA is characterized by recurrent upper airway obstruction episodes that provoke intrathoracic pressure surges, sleep fragmentation, and hypoxia-reoxygenation cycles, which trigger a variety of intermediate mechanisms that mediate these clinical outcomes^{1,4}. More specifically, intermittent hypoxia (IH) results in increased reactive oxygen species leading, to oxidative stress and systemic inflammation. Both these conditions have been well documented in patients with OSA, and they are also known to be mechanistic facilitators of cardiovascular diseases and other disorders such as depression^{1,5-7}. Thus, the study of these intermediate pathways may help us to understand the different clinical consequences of OSA, and they could be used as surrogate outcome measures in the cardiovascular and mood disorder fields.

Several studies have analyzed the effect of continuous positive airway pressure therapy (CPAP) on a wide variety of biomarkers of oxidative stress and inflammation, but most of them either used an observational design or enrolled a relatively small sample⁸⁻¹². The few randomized-controlled trials (RCT) with larger sample sizes, however, are restricted to men or do not adequately analyze a possible gender effect, and none of them assessed biomarkers associated with mood disorders such as depression¹³⁻¹⁶. Given that OSA differs between men and women in terms of prevalence, pathophysiology, severity, clinical presentation, and association with mood disorders¹⁷, a differing response can be expected in the molecular signatures of OSA in both genders. In fact, some data suggest that oxidative stress may be more associated with OSA in women than in men¹⁸.

Our group has recently published the results of a RCT that investigated the effect of 12 weeks of CPAP on different clinical outcomes in women with moderate-to-severe OSA^{19,20}. In the current study, we address the effect of CPAP on the levels of inflammatory, antioxidant activity, and depression biomarkers in this cohort of women with OSA.

METHODS

Design, settings and patients

This multicenter, open-label, randomized-controlled trial of parallel groups with final blind evaluation, addressed the effect of 12 weeks of CPAP on a variety of clinical outcomes, including QoL, mood disorders, and BP in women with moderate-to-severe OSA (NCT02047071). The study design and population has previously been described in detail^{19,20}. Briefly, women between 18-75 years referred for OSA suspicion and diagnosed with moderate-to-severe OSA (apnea-hypopnea index [AHI] ≥ 15) in 19 Spanish Sleep Units were eligible for the study. Women were excluded if they had respiratory failure, heart failure grade III-IV NYHA, unstable disorders, pregnancy, severe daytime sleepiness (Epworth sleepiness score [ESS] > 18), prior diagnosis of OSA or CPAP treatment or central sleep apnea. The study was approved by the Ethics Committee of each participating center. All the participants provided informed signed consent.

The current study includes those women in whom blood samples were obtained and available for analysis at baseline and after 12 weeks of follow-up.

Procedures

Sleep study and initial visit

Every woman underwent a home respiratory polygraphy with a device previously validated against polysomnography. Every sleep study was manually scored by skilled staff. All the studies included recording of the oro-nasal flow and pressure, respiratory movements, and oxyhemoglobin saturation (SaO₂). Apnea was defined as complete cessation of oro-nasal flow for ≥ 10 seconds and was classified as either obstructive or central, based on the presence or absence of respiratory efforts. Hypopnea was defined as a 30-90% reduction in oro-nasal flow for ≥ 10 seconds followed by a $\geq 3\%$ decrease in SaO₂. The AHI was defined as the number of apnea plus hypopnea per hour of recording. A sleep study was considered valid if at least 4 hours of recording and more than 3 hours of subjective sleep were reported.

After OSA diagnosis, the women completed a standardized protocol that included general and anthropometric data, menopausal status, history of cardiovascular diseases, subjective sleep duration, subjective sleepiness measured by the Epworth Sleepiness Scale (ESS)²¹, and clinical history related to OSA.

Blood sampling

After OSA diagnosis and prior to randomization, blood samples were collected after an overnight fast (between 07:00 and 09:00 hours). Tubes were centrifuged and plasma/serum samples were aliquoted and stored at -80°C . In this study, we assessed the following biomarkers of inflammation, antioxidant activity and depression: tumor necrosis factor α (TNF α), interleukin 6 (IL-6), C-reactive protein (CRP), intercellular adhesion molecule 1 (ICAM-1), catalase activity (CAT), superoxide dismutase activity (SOD), and brain-derived neurotrophic factor (BDNF). All the biomarkers were assayed

at the Instituto de Biomedicina de Sevilla (IBiS). Detailed data from these analyses are provided in the **supplementary file and supplementary Table 1**.

Randomization and Intervention

Women with an AHI ≥ 15 were randomized to either CPAP or conservative treatment by using a computer-generated list of random numbers in the coordinating center and stratified by center. The results were mailed in numbered, opaque, sealed envelopes.

Women randomized to the conservative treatment group received dietary and sleep hygiene counseling, whereas those allocated to the treatment group received dietary and sleep hygiene counseling plus CPAP. The participants were advised not to change their cardiovascular medication throughout the follow-up, unless dictated by clinical needs.

For those women randomized to CPAP therapy, the optimal pressure was titrated on a second night, using an auto CPAP device, according to a previous validation by the Spanish Sleep Network²². The optimal pressure was determined in a centralized manner, by two blinded expert researchers, based on the visual evaluation of the raw data recording from the night study, with no significant leaks (less than 0.40 L/s). This fixed pressure was maintained throughout the study.

Study endpoints

The endpoint of the present study was the changes in the biomarkers of inflammation, antioxidant activity and depression at 12 weeks compared to baseline, in the CPAP versus the control group.

Sample size

The initial sample size was calculated for the primary endpoint of the original study (QoL), but also for enough power to analyze other secondary endpoints. In the current study, the sample size consists of 247 women (120 allocated to CPAP and 127 to conservative treatment). This sample size would enable us to detect, with an α error of 0.05 and a power of 90%, a change of at least 4 ng/mL with an SD of 7 ng/mL in BDNF levels between groups (at least 70 participants would be needed in each group)²³, as well as to detect a change of at least 1 mg/L with an SD of 2 mg/L in CRP levels between groups (at least 84 participants would be needed in each group)⁸.

Statistical analysis

Results are expressed as mean (SD) or median (25th-75th percentiles) for continuous variables and number of patients (%) for categorical variables. Baseline continuous variables were compared using the Mann-Whitney test, and categorical variables were compared using Chi-square or Fisher exact tests, as appropriate.

The inter-group comparison of the changes in the biomarker levels were assessed by analysis of covariance with adjustments for baseline values and age. The analyses were performed on an intention-to-treat basis. An additional per-protocol analysis was conducted, including in the CPAP group only those patients who used the device for at least 5 hours/night in average.

We conducted sensitivity analyses to investigate the effect of CPAP therapy on specific subgroups of clinical interest, such as those with severe OSA ($AHI \geq 30$) and those with daytime sleepiness ($ESS > 10$).

Spearman correlation and scatterplots were used to investigate whether there were any relationships between the change (final-baseline) in biomarker levels and CPAP adherence measured by the hours of CPAP use, or the change in ESS, systolic and diastolic BP.

Two-tailed p-values of <0.05 were considered significant. IBM SPSS 23.0 statistical package (IBM, Armonk, NY, USA) was used for data processing and analysis.

RESULTS

Of the 571 women initially assessed, 307 were randomized in the original study, and 247 (120 allocated to CPAP and 127 to conservative treatment) had blood samples available at baseline and 12 weeks, and were finally included in the current study (**Figure 1**). The reason for the absence of blood samples in 60 women is that some centers did not have the means to centrifuge the blood samples and store them at -80°C , so they did not participate in this secondary analysis. There were no differences in terms of age, BMI, ESS, menopausal status, AHI, T90, ODI3, or minimum SaO₂ ($p>0.05$ for all comparisons) between the group of 60 women without blood samples and the group of 247 with blood samples.

Women had a median (25th-75th percentiles) age of 58 (51-65) years (77.3% were postmenopausal), body mass index (BMI) 33.5 (29.0-38.3) Kg/m² (71.2% had a BMI \geq 30), waist-to-hip ratio 0.90 (0.86-0.94), ESS 10 (6-13) and AHI 33.3 (22.8-49.3).

The CPAP and control groups were comparable, except for age; women in the CPAP group were older than those in the control group (**Table 1**). The baseline levels of the different biomarkers were similar between groups. No significant change in weight was observed in the CPAP and control groups over the course of the study.

In the treated group, the median CPAP pressure was 10 (8-12) cmH₂O, the average residual AHI after titration was 2.0 (0.7-4.1), the mean (SD) adherence to the device after 12 weeks was 5.0 (2.2) hours/night, and 76 women (63.3%) had adherence of at least 5 hours or higher.

Intention-to-treat analysis

In both the CPAP and control groups, there was a significant improvement in TNF α levels from baseline to 12 weeks. CRP levels also decreased in the CPAP group, but not in the control group (**Table 2**). However, there was no significant change in the levels of the inflammatory (IL-6, CRP, TNF α , ICAM-1), antioxidant activity (CAT, SOD) and depression (BDNF) biomarkers between the CPAP and control groups at the end of the follow-up (**Table 2**).

Per-protocol analysis

After 12 weeks of follow-up, there was no significant change in the levels of the IL-6, CRP, ICAM-1, CAT, SOD, and BDNF between those women with CPAP use ≥ 5 hours/night and the control group (**Table 3**). However, a significant improvement was observed in the TNF α levels in the CPAP ≥ 5 hours/night group, compared to the control group (-0.29 ± 1.1 vs -0.06 ± 0.53 , non-adjusted intergroup difference -0.23 [95%CI -0.03 to -0.50]; $p=0.043$). Further adjustment for baseline values and age did not change these results.

Sensitivity analyses

In the subgroup of women with an $AHI \geq 30$, we did not find any changes in the biomarker levels between groups, at the end of the follow-up, compared to baseline (**Table 3**). Similarly, no changes were observed in the subgroup of women with an $ESS > 10$ (**Table 3**).

No correlations were found between the changes in the biomarkers at 12 weeks compared to baseline and the changes during this period in the ESS score, systolic BP and diastolic BP (**supplementary Table 2**), or between the changes in the biomarkers and the average hours of CPAP use (**supplementary Table 3**).

DISCUSSION

To the best of our knowledge, this is the first RCT to investigate the effect of CPAP on a wide range of inflammatory and antioxidant activity biomarkers in a population specifically composed of OSA women, as well being the first to analyze markers of depression in OSA patients. Our results do not support any effect of CPAP therapy on biomarkers of inflammation, antioxidant activity or depression in women with moderate-to-severe OSA, except in the $TNF\alpha$ levels of those who had above-average CPAP adherence.

Although repetitive cycles of IH have been associated with a rise in different markers of inflammation and oxidative stress in OSA patients, few studies have specifically analyzed this effect in women^{24,25}. Svensson et al studied 398 women from the general population and found that CRP and IL6 levels were increased in women with OSA, defined as an $AHI \geq 15$, compared to those without OSA (1.9 vs 1.3 mg/L, $p=0.003$; and 1.0 vs 0.8 ng/L, $p=0.04$, respectively), whereas TNF levels were similar in both groups (2.0 vs 2.0 pg/mL, $p=0.7$)²⁶. Another study observed a relationship between OSA and

oxidative stress in elderly women, but not in elderly men¹⁸. A study in a population-based cohort of 295 midlife women showed that lower sleep efficiency was associated with higher circulating levels of IL-6²⁷. Given that CPAP has consistently been shown to prevent IH associated with OSA, there is a pathophysiological rationale to expect a beneficial effect from CPAP therapy on these biomarkers of inflammation and oxidative stress.

OSA is a risk factor for mood disorders, particularly depression³. There are gender differences in this association, with a prevalence of depression of 35% in OSA women, compared to 12-25% in men^{28,29}. To our knowledge, no study has previously investigated the possible effect of OSA therapy on biomarkers of depression, but since there is now evidence to suggest that inflammation and oxidative stress may play a role in the pathophysiology of major depressive disorders⁵⁻⁷, and since the levels of some markers such as BDNF have been reported to decrease in patients with depression and increase with antidepressant therapy²³, there would also be a rationale for expecting an improvement in these biomarkers in OSA women.

Our results, however, do not support these hypotheses as we did not find any improvement in different biomarkers of inflammation, antioxidant activity or depression in our sample. Although observational studies and some small RCTs have reported a controversial effect of CPAP^{8-10,30,31}, our results concur with those of recent larger RCTs which did not observe any beneficial effect of CPAP therapy on a wide range of biomarkers, including IL-6, IL-8, IL-10, CRP, TNF α , F2-isoprostanes, and myeloperoxidase¹³⁻¹⁶. These studies predominantly featured men, however, or they failed to investigate any potentially different outcome in women.

The lack of effect of CPAP on diverse biomarkers observed in our study cannot be attributable to a lack of effect of CPAP in women, given that we have previously

shown, using this same cohort, that 12 weeks of CPAP therapy significantly improved different aspects of QoL, daytime sleepiness, mood state and depression symptoms, as well as BP measurements, compared to conservative treatment^{19,20}, so other reasons should be sought to explain our results.

Given that there is a relationship between certain cytokines and excessive daytime sleepiness (EDS)^{32,33}, it has been argued that the effect of CPAP may be reduced in non-sleepy OSA patients¹⁶. The median ESS in our sample was 10, but when we specifically studied a subgroup of women with EDS (ESS>10), the results did not change. Some researchers have noted, however, that inflammation may be associated with objective rather than with subjective sleepiness³⁴.

Other studies have attributed their negative results to the enrolment of non-severe OSA patients. Paz y Mar et al argued that in their trial, the mean AHI of 20/h and 2% sleep time <90% oxygen saturation (T90) may have not been sufficient to result in oxidative stress up-regulation¹⁵. In our study, the median AHI was 33.3/h, which is in the range of severe OSA, and the median T90% was 8.4% (i.e., four times higher than in the Paz y Mar study). Furthermore, even when we analyzed a subgroup of women with an AHI \geq 30, no differences were observed between groups.

Another possibility is that the lack of effect of CPAP therapy may have been due to the relatively short duration of treatment. In this respect, however, 12 weeks of CPAP were enough to detect an improvement in clinical outcomes in this same cohort, as previously stated. Although it cannot be ruled out that a longer treatment period may have achieved discernible changes, another trial showed no improvement in inflammatory biomarkers after 1 year of follow-up¹⁶.

Other possibilities cannot be excluded. For instance, many of the biomarkers assessed in this study are also associated with obesity, which was present in 71.2% of our sample, and did not change during the follow-up. If these biomarkers were more closely related to obesity than to OSA, CPAP would play only a minor role in improving their levels^{35,36}. Although we have used some of the most widely studied biomarkers related to OSA, we cannot confirm that they are the most accurate surrogates for measuring the CPAP response to inflammation and oxidative stress associated with OSA^{11,12,24}. Finally, it is unclear whether OSA is associated with higher cardiovascular risk in the elderly³⁷, although our sample had a median age of 58 years and therefore cannot be considered elderly. Nevertheless, to account for this possibility, and given that there were baseline age differences between groups, we specifically analyzed the subgroup of women under 58 years, but the results did not change (data not shown).

Adherence has been highlighted as a key reason to explain the negative effect of CPAP on cardiovascular outcomes in recent RCTs^{38,39}. This was not the case in our RCT. The average adherence in our study was 5.0 (2.2) hours/night, which can be considered adequate. Furthermore, when we selected a subgroup of women with adherence above the average, the results did not change, except for TNF α , which significantly decreased compared to conservative treatment. Whether this finding means that this biomarker is more sensitive to the effect of CPAP or better reflects the inflammatory process associated with OSA should be addressed in future studies.

Our study has limitations. First, OSA was diagnosed by means of a respiratory polygraphy, rather than by a conventional polysomnography, but it seems unlikely that the diagnostic method influenced the final findings. Second, sleepiness was not objectively assessed, and since inflammation seems to be associated with objective sleepiness³⁴, we cannot rule out our sample being predominantly non-sleepy, which

may have precluded any beneficial effect of CPAP on, at least, some of the biomarkers. Third, although we previously found an improvement in QoL, mood state and depression in this cohort ¹⁹, only 20.2% of the women could be defined as having depression, according to the Hospital Anxiety and Depression questionnaire score, so the small proportion of depressive women may have prevented us from detecting a beneficial effect in some markers, such as the BDNF. Finally, the lack of observed effect may be due to the fact that biomarker measures at baseline were within the normal range. Unfortunately, we do not have a control group of non-OSA women to compare. However, the CRP and IL6 measures for women in our cohort were higher than those observed in women with OSA in Svensson's study (3.6 vs 1.9 mg/L, and 2.7 vs 1.0 pg/mL, respectively), and more than three times higher compared to non-OSA women (3.6 vs 1.3 mg/L, and 2.7 vs 0.8 pg/mL, respectively). Thus, at least for these biomarkers, the baseline levels were above normal values and, therefore, a "floor effect" would not explain our negative results. Nevertheless, in order to account for this limitation, we analyzed different subgroups of clinical interest in which these CV biomarkers would be expected to be increased, such as women with more severe OSA and women with excessive daytime somnolence. Furthermore, we have run an exploratory analysis to investigate the effects of CPAP on those women who were in the upper tertiles of each biomarker at baseline, so that only those with the highest levels were analyzed. This analysis showed no differences between the CPAP and control groups in any of the biomarkers assessed (data not shown), which supports the main findings of our study.

In conclusion, 12 weeks of CPAP therapy did not change the circulating levels of a wide range of inflammatory, antioxidant activity and depression biomarkers in women

with moderate-to-severe OSA, compared to a non-treated control group. Given that CPAP showed a beneficial clinical effect in this same cohort, it is possible that other intermediate pathways not assessed in this study play a greater role in the outcomes of OSA in women.

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ACKNOWLEDGEMENTS

The authors would like to thank Dr Veronica Ines Vargas, UEMAC, Hospital Universitario Virgen Macarena, Sevilla, Spain, who contributed to the interpretation of the data for some of the biomarkers.

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- Biomarkers assay: E. A-O, V. S-L
- Statistical analysis of the data: J. C-G
- Drafting of manuscript or critical revision of major intellectual content: All authors.
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Abbreviation list

AHI: apnea-hypopnea index

BDNF: brain-derived neurotrophic factor

BMI: body mass index

BP: blood pressure

CAT: catalase

CPAP: continuous positive airway pressure

CRP: C-reactive protein

EDS: excessive daytime sleepiness

ESS: Epworth sleepiness score

ICAM-1: intercellular adhesion molecule 1

IL-6: interleukin 6

IH: intermittent hypoxia

OSA: obstructive sleep apnea

QoL: quality of life

RCT: randomized-controlled trials

SaO₂: oxyhemoglobin saturation

SOD: superoxide dismutase

TNF α : tumor necrosis factor α

T90: percent sleep time <90% oxygen saturation

DISCLOSURE STATEMENT

None of the authors have any financial disclosure to declare regarding the current manuscript

None of the authors have any non-financial disclosure to declare regarding the current manuscript

FUNDING

This study was supported by a grant from the Consejería de Salud de la Junta de Andalucía (PI-0056-2016), and a grant from the Sociedad Española de Neumología y Cirugía Torácica SEPAR (19/2016). None of these institutions played any role in the objectives, methods, data analysis, or conclusions of the study.

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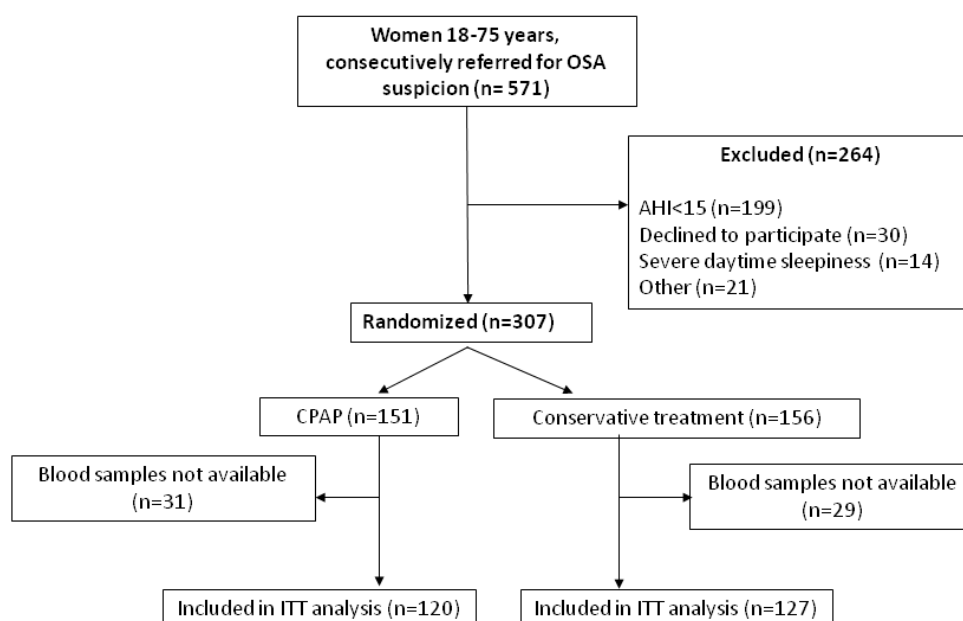
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Figure legends

Figure 1. Flow chart of the study



Accepted

Table 1. Baseline characteristics of the sample.

Variables	Whole group (n= 247)	Control Group (n= 127)	CPAP Group (n= 120)	p-value
Age (years)	58.0 (51.0 – 65.0)	56.0 (50.0 – 63.0)	60.0 (53.0 – 67.0)	0.006
BMI (kg/m ²)	33.5 (29.0 – 38.3)	33.5 (29.7 – 39.4)	33.5 (28.8 – 37.1)	0.183
Neck circumference (cm)	37.0 (35.0 – 40.0)	38.0 (35.0 – 40.0)	37.0 (35.0 – 40.0)	0.174
Waist-to-hip ratio	0.90 (0.86 – 0.94)	0.90 (0.86 – 0.95)	0.90 (0.85 – 0.94)	0.150
Post-menopausal	191 (77.3%)	95 (74.8%)	96 (80.0%)	0.364
Physical activity (< 30 min/day)	122 (49.4%)	65 (51.1%)	57 (47.5%)	0.407
Sleep duration (hours/day)	7.5 (6.0 – 8.3)	7.5 (6.0 – 8.3)	7.0 (6.0 – 8.3)	0.466
Systolic BP (mm Hg)	129.0 (119.5 – 142.5)	127.5 (117.5 – 141.0)	130.0 (120.0 – 144.0)	0.304

Diastolic BP (mm Hg)	80.0 (72.5 – 90.0)	80.0 (72.5 – 89.0)	80.0 (70.0 – 90.0)	0.723
Prior cardiovascular Events	20 (8.1%)	11 (8.7%)	9 (8.1%)	0.935
Apnea-Hypopnea index	33.3 (22.8 – 49.3)	31.4 (20.3 – 47.2)	35.9 (24.2 – 50.1)	0.168
Oxygen desaturation index (3%)	32.9 (22.1 – 49.7)	30.4 (21.1 – 50.0)	34.6 (23.3 – 49.7)	0.381
Minimum SaO2	77.0 (70.0 – 83.0)	78.0 (72.0 – 83.0)	77.0 (70.0 – 83.0)	0.662
T90%	8.4 (2.3 – 29.1)	10.0 (2.3 – 29.1)	7.8 (2.4 – 29.1)	0.887
Epworth score	10.0 (6.0 – 13.0)	10.0 (6.0 – 13.0)	10.0 (7.0 – 13.0)	0.229
BDNF (ng/mL)	33.257 (25.416- 40.202)	33.286 (241.69-42.214)	33.229 (25.556-39.147)	0.640
IL6 (pg/mL)	2.79 (1.76 – 4.31)	2.79 (1.77 – 4.31)	2.77 (1.75 – 4.34)	0.854
TNFα (pg/mL)	0.90 (0.69 – 1.21)	0.91 (0.69 – 1.25)	0.89 (0.71 – 1.15)	0.984
CRP (mg/L)	3.625 (2.162 – 5.456)	3.579 (2.224 – 3.579)	3.717 (1.946 – 5.913)	0.865

ICAM-1 (pg/mL)	254.0 (197.0 – 325.0)	265.0 (201.0 – 348.0)	251.0 (190.3 – 300.3)	0.091
CAT (nmol/min/mL)	31.2 (24.1 – 40.0)	31.8 (25.4 – 41.9)	30.9 (23.2 – 37.4)	0.131
SOD (U/ml)	0.25 (0.06 – 0.61)	0.23 (0.03 – 0.60)	0.26 (0.07 – 0.62)	0.584

Results are expressed as mean \pm SD or median (25th-75th percentiles), or N° (%)

BMI: body mass index. BP: blood pressure. T90%: % night-time spent with oxygen saturation below 90%. BDNF: brain-derived neurotrophic factor. IL-6: interleukin 6. TNF α : tumor necrosis factor α . CRP: C-reactive protein. ICAM-1: intercellular adhesion molecule 1. CAT: catalase. SOD: superoxide dismutase.

Table 2. Effect of CPAP treatment on biomarkers of depression, inflammation and oxidative stress. Results of the intention-to-treat analysis.

Biomarkers	CPAP group				Control group				Non-adjusted Inter-group differences (95%CI) *, **	p-value
	12 weeks	Baseline	Intra-group differences	p-value	12 weeks	Baseline	Intra-group differences	p-value		
BDNF	32.09 ± 11.48	31.73 ± 14.10	0.35 ± 13.35	0.771	32.55 ± 11.00	32.34 ± 13.67	0.20 ± 13.37	0.862	0.14 (-3.21 to 3.51)	0.931
IL6	3.5 ± 2.6	3.6 ± 2.8	-0.15 ± 2.5	0.500	3.6 ± 2.5	3.8 ± 5.0	-0.23 ± 5.2	0.622	0.07 (-0.95 to 1.1)	0.394
TNF α	0.95 ± 0.36	1.1 ± 0.78	-0.14 ± 0.70	0.032	1.06 ± 1.46	1.35 ± 1.72	-0.29 ± 1.1	0.004	0.15 (-0.08 to 0.38)	0.198
CRP	3.58 ± 2.09	3.86 ± 2.18	-0.28 ± 1.38	0.033	3.69 ± 1.99	3.87 ± 2.12	-0.18 ± 1.39	0.155	-0.10 (-0.46 to 0.25)	0.563
ICAM-1	259.2 ± 96.5	264.1 ± 114.8	-4.9 ± 87.4	0.540	280.4 ± 109.7	290.4 ± 125.1	-10.0 ± 69.4	0.106	5.1 (-14.6 to 24.8)	0.610
CAT	33.3 ± 17.6	32.6 ± 16.1	0.70 ± 18.4	0.680	34.8 ± 18.0	35.8 ± 17.3	-0.99 ± 20.0	0.577	1.7 (-3.2 to 6.6)	0.492
SOD	0.44 ± 0.54	0.43 ± 0.54	0.01 ± 0.25	0.574	0.51 ± 0.70	0.41 ± 0.51	0.10 ± 0.51	0.057	-0.09 (-0.19 to 0.01)	0.085

* The inter-group differences are expressed as the effect of continuous positive airway pressure treatment versus conservative treatment, at the end of the follow-up compared with baseline.

** Further adjustment for baseline values and age did not change the results.

CPAP: continuous positive airway pressure. BDNF: brain-derived neurotrophic factor. IL-6: interleukin 6. TNF α : tumour necrosis factor α . CRP:

C-reactive protein. ICAM-1: intercellular adhesion molecule 1. CAT: catalase. SOD: superoxide dismutase.

Table 3. Effect of continuous positive airway pressure therapy on biomarkers of depression, inflammation and oxidative stress in the subgroups of women with good adherence to CPAP (defined as an average use of at least 5 hours/night), excessive daytime sleepiness (defined as an Epworth score >10), and severe OSA (defined as an AHI \geq 30).

Biomarkers	CPAP group	Control group	Non-adjusted Inter-group differences (95%CI) *,**	p-value
	Intra-group differences	Intra-group differences		
CPAP use \geq5 hours/night	n=75	n=127		
BDNF	1.564 \pm 14.840	0.208 \pm 13.377	1.356 (-2.652 to 5.365)	0.845
IL6	-0.49 \pm 2.1	-0.23 \pm 5.2	0.27 (1.49 to 0.96)	0.392
TNF α	-0.29 \pm 1.1	-0.06 \pm 0.53	-0.23 (-0.03 to -0.50)	0.043
CRP	-0.337 \pm 1.312	-0.181 \pm 1.394	0.155 (0.562 to 0.250)	0.450
ICAM-1	6.0 \pm 78.9	-10.0 \pm 69.4	16.0 (-4.9 to 36.9)	0.132

CAT	-2.5 ± 16.7	-0.99 ± 20.0	-1.5 (-6.9 to 3.9)	0.582
SOD	0.03 ± 0.26	0.10 ± 0.51	-0.07 (-0.2 to 0.05)	0.255
Epworth >10	n=57	n=54		
BDNF	1.104 ± 13.170	-1.368 ± 14.738	2.473 (-2.777 to 7.723)	0.353
IL6	0.15 ± 3.0	0.61 ± 2.4	0.46 (1.49 to 0.57)	0.448
TNFα	-0.18 ± 0.8	-0.29 ± 1.3	0.12 (-0.29 to 0.53)	0.397
CRP	-0.170 ± 1.383	-0.070 ± 1.221	0.100 (0.609 to 0.409)	0.454
ICAM-1	-8.5 ± 87.7	-5.4 ± 67.2	3.1 (32.5 to 26.3)	0.500
CAT	0.85 ± 21.2	-3.9 ± 15.8	4.8 (-2.3 to 11.9)	0.181
SOD	0.01 ± 0.25	0.15 ± 0.71	-0.15 (-0.35 to 0.05)	0.154
AHI ≥ 30	n=72	n=77		
BDNF	2.666 ± 13.023	2.590 ± 13.367	0.076 (-4.368 to 4.521)	0.973
IL6	-0.03 ± 3.0	0.01 ± 6.6	-0.04 (-1.7 to 1.6)	0.960
TNFα	-0.16 ± 0.8	-0.40 ± 1.1	0.24 (-0.07 to 0.55)	0.135
CRP	-0.139 ± 1.408	-0.333 ± 1.546	0.193 (-0.328 to 0.715)	0.464

ICAM-1	1.4 ± 99.8	-9.3 ± 75.6	10.6 (-19.2 to 40.5)	0.482
CAT	-0.1 ± 16.0	1.11 ± 19.7	-1.2 (-7.3 to 4.9)	0.702
SOD	0.02 ± 0.28	0.07 ± 0.37	-0.05 (-0.16 to 0.06)	0.374

* The inter-group differences are expressed as the effect of continuous positive airway pressure treatment versus conservative treatment, at the end of the follow-up compared with baseline.

** Further adjustment for baseline values did not change the results.

CPAP: continuous positive airway pressure. AHI: apnea-hypopnea index. BDNF: brain-derived neurotrophic factor. IL-6: interleukin 6. TNF α : tumor necrosis factor α . CRP: C-reactive protein. ICAM-1: intercellular adhesion molecule 1.. CAT: catalase. SOD: superoxide dismutase.