Effect of Continuous Positive Airway Pressure Therapy on Hypothalamic-
Pituitary-Adrenal Axis function and 24-hour Blood Pressure profile in Obese Men 
with Obstructive Sleep Apnea Syndrome

Authors:

Gláucia Carneiro¹,
Sônia Maria Togeiro²,
Lílian F. Hayashi¹,
Fernando Flexa Ribeiro-Filho¹,
Artur Beltrame Ribeiro³,
Sérgio Tufik²,
Maria Teresa Zanella¹.

¹ Department of Medicine, Division of Endocrinology, Universidade Federal de Sao Paulo, Sao Paulo, Brazil.
² Department of Psychobiology, Sleep Disorders Center, Universidade Federal de Sao Paulo, Sao Paulo, Brazil.
³ Hospital do Rim e Hipertensão, Fundação Oswaldo Ramos, Brazil
Corresponding author

Name: Gláucia Carneiro

Current address: Rua Leandro Dupret, 365
                Vila Clementino – São Paulo – Brazil – CEP: 04025-011

Telephone: 55-11-59040400

fax number: 55-11-32871959

e-mail address: glauciacarneiro@uol.com.br

Reprint requests should be addressed to corresponding author.

Running head: HPA, 24h blood pressure profile, sleep apnea syndrome and nCPAP therapy
ABSTRACT

Obstructive sleep apnea syndrome (OSAS) increases the risk of cardiovascular events. Sympathetic nervous system and hypothalamic-pituitary-adrenal (HPA) axis activation may be the mechanism of this relationship. The aim of this study was to evaluate HPA axis and ambulatory blood pressure monitoring (ABPM) in obese men with and without OSAS and to determine whether nasal continuous positive airway pressure therapy (nCPAP) influenced responses. Twenty-four-hour ambulatory blood pressure monitoring and overnight cortisol suppression test with 0.25 mg of dexamethasone were performed in 16 obese men with OSAS and 13 obese men controls. Nine men with severe apnea were reevaluated three months after nCPAP therapy. Body mass index and blood pressure of OSAS patients and obese controls were similar. In OSAS patients, the percentage of fall in systolic blood pressure at night (p=0.027) and salivary cortisol suppression post DEX (p=0.038) were lower, while heart rate (p=0.022) was higher compared with obese controls. After nCPAP therapy, patients showed a reduction in heart rate (p=0.036) and a greater cortisol suppression after dexamethasone (p=0.001). No difference in arterial blood pressure (p=0.183) was observed after 3 months of nCPAP therapy. Improvement in cortisol suppression was positively correlated with an improvement in apnea-hypopnea index during nCPAP therapy (r= 0.799; p=0.010). In conclusion, men with OSAS present increased post dexamethasone cortisol levels and heart rate which were recovered by nCPAP.

Keywords: sleep disorders, low dose dexamethasone test, 24 hour Blood Pressure profile
INTRODUCTION

Obstructive Sleep Apnea Syndrome (OSAS) is receiving increased attention because it seems to be associated with a variety of long term consequences such as high rates of morbidity and mortality, mostly due to cardiovascular disease. Although obesity is the main risk factor for OSAS, it has been demonstrated that OSAS may increase the risk for hypertension, myocardial infarction, congestive heart failure and stroke independently of obesity. Continuous positive airway pressure (CPAP) therapy is the treatment of choice for patients with moderate-to-severe OSAS, since it is highly effective in improving nocturnal hypoxia and sleep fragmentation, enhancing the quality of life and reducing many cardiovascular complications related to OSAS. However, the lack of acceptance and inadequate adherence to CPAP therapy remain the major cause of sleep apnea treatment failure.

The mechanisms proposed to explain the increased cardiovascular disease in obstructive sleep apnea are under assessment. It is speculated that recurrent episodes of upper airways constriction, progressive hypoxemia and sleep fragmentation may result in neural and metabolic changes, including activation of peripheral sympathetic activity, inflammatory pathways and hypothalamic-pituitary-adrenal (HPA) axis, impairment of insulin sensitivity and generation of reactive oxygen species (ROS), which could predispose to vascular damage.

Sympathetic nervous system has been well demonstrated to be activated in sleep apnea patients by investigating muscle sympathetic nerve activity, heart rate variability, blood and urinary catecholamine levels. In contrast, there are a limited number of studies which assess the effects of obstructive sleep apnea on cortisol.
secretion\textsuperscript{9,12,13,18,35}. Some studies show an elevation of cortisol levels\textsuperscript{13,18,35} in patients with OSAS while others do not\textsuperscript{9,12}.

The aim of the present study was to evaluate HPA axis, 24-hour heart rate and blood pressure values in severe obese patients with and without OSAS, and to assess if OSAS treatment with nasal CPAP influenced responses.

**MATERIALS AND METHODS**

Twenty-nine obese men, who were on the waiting list for bariatric surgery, were consecutive recruited from the Obesity Outpatient Clinic and Sleep Disorders Center of the Federal University of Sao Paulo. These patients, aged from 18 to 65 years and body mass index between 35-60 kg/m2 were submitted to polysomnography recordings (PSG) and classified according to their apnea/hypopnea index (AHI) in two different groups: AHI < 05 events/h: obese controls (n=13) or AHI \geq 10 events/h: OSAS patients (n= 16).

Exclusion criteria included history of smoking, sleep apnea treatment, cardiovascular disease, malignant tumors, thyroid disorders, severe depression, subjects with diabetes mellitus, chronic renal or hepatic failure, use of medication that could potentially affect sympathetic nervous system or steroid hormone secretion (alcohol, psychotropics, steroids, sympathomimetics, beta-blockers) and hepatic enzyme inducers such as carbamazepine, phenytoin, phenobarbitone, and rifampicin which reduce plasma dexamethasone concentrations\textsuperscript{17}. Antihypertensive medications remained unchanged during the study period.

A questionnaire included demographic data, sleep symptoms, medical history and medications in use was enrolled. Physical examinations and anthropometric
measurements were recorded including weight (in kilograms) and height (in meters). Body mass index (BMI) was calculated as the weight divided by the height squared.

Polysomnograms were recorded by the Sleep Analyser Computer (Alice 3 Diagnostics system) including one for OSAS diagnosis and other for positive airway pressure titration. An experienced sleep physician scored all sleep stages, arousals and respiratory events according to American Sleep Disorders Association criteria.

In healthy individuals, glucocorticoids synthesis and secretion follow a circadian rhythm with the highest levels in the morning and the nadir at around midnight. Overnight administration of dexamethasone (DEX), a potent exogenous glucocorticoid, suppresses the nocturnal surge in ACTH production and cortisol levels when measured the next morning. Dexamethasone test is the most commonly used method to evaluate the sensitivity of the HPA axis to negative feedback. However, the conventional dose of 1-mg completely suppresses cortisol secretion in normal people. For this reason, low-dose DEX test (< 1 mg) has been used by some authors to induce a more modest suppression enabling the detection of subtle differences in feedback sensitivity of glucocorticoid on the HPA axis.

The assessment of the HPA axis function in this study included low-dose (0.25 mg) dexamethasone suppression test and the circadian rhythm of cortisol secretion. Salivary cortisol measurement reflects the free fraction of cortisol in plasma. Advantages are the easy and noninvasive collection procedure and its stability at room temperature for at least 7 days. The subjects were given three Salivettes (Sarstedt, Rommelsdorf, Germany), which consist of a small cotton swab inside a centrifugation tube used to collect saliva, and a half tablet of 0.5 mg DEX (Decadron, Aché, Brazil). Salivary sample was obtained in the morning (8:00 am) and at bedtime around 11:00
h pm for all patients just before the administration of DEX. The next morning another salivary and blood samples were collected at 8:00 h am to measure cortisol and dexamethasone concentrations by RIA to confirm the ingestion of the drug. To analyse the results we used an index of percentage of salivary cortisol suppression (% cortisol suppression) calculated as the difference between the post DEX cortisol levels and baseline cortisol levels at 8:00 h am divided by baseline cortisol levels at 8:00 am.

Twenty-four-hour ambulatory blood pressure monitoring (24h ABPM) was recorded with a SpaceLabs model 90202 Ambulatory BP Monitor (Redmond, WA, USA). An appropriate sized cuff was applied. Blood pressure was registered every 15 minutes during daytime (awake) and every 20 minutes during nighttime (asleep), based on the patient’s reports on their activities during day and night. The percentage of fall in systolic blood pressure at night (BP) was calculated by dividing the difference between mean daytime and mean nighttime systolic blood pressure by the mean daytime systolic blood pressure 24. Blood pressure was considered to be controlled in those patients with 24h- mean blood pressure values < 135/85 mmHg 6.

Six patients with mild or moderate OSAS immediately underwent bariatric surgery and ten patients with severe OSAS (AHI of more than 30 events per hour of sleep) were advised to follow nasal continuous positive airway pressure (nCPAP) therapy before bariatric surgery (mean nCPAP pressure of 11.2 ± 0.7 cm of H2O) in order to avoid surgery complications related with sleep apnea. One man who failed to use the device was excluded from the study before the follow-up analysis. Therefore, after three months of nCPAP therapy, nine patients with severe OSAS were reassessed and all measurements were repeated. The average nightly use of nCPAP was measured with a run-time course which ran when the patient is breathing through the machine and not just when the machine is switched on.
The study was approved by the UNIFESP Ethics Committee and written informed consent was obtained from all participants.

**Statistical Analysis**

Normally distributed variables are expressed as means ± SE or percentiles when appropriate. Continuous variables comparisons between OSAS and control obese groups were performed using unpaired Student’s t test. ANCOVA test were used to adjust comparisons for body mass index. To assess differences between categorical variables were used chi-square statistics. The results before and after nCPAP therapy were compared using paired t-test. Correlations between variables were assessed by Pearson coefficient. A p value of < 0.05 was considered statistically significant. Data analysis was performed using SPSS for Windows version 13.0.

**RESULTS**

As shown in Table 1, no differences were observed between groups for age, body mass index and prevalence of hypertension. In OSAS men, the mean 24-hour heart rate was higher (p= 0.022) and the percentage fall in blood pressure during sleep time was lower compared with obese men controls (p=0.027). Mean 24-hour systolic and diastolic blood pressure values were similar in the two groups.

Although basal salivary cortisol values at 08:00h am (p=0.715) and at bedtime (p=0.388) were not different between groups, a smaller cortisol suppression post DEX (% cortisol suppression) was evident in OSAS patients compared with obese controls (p=0.012) (Figure 1). As a consequence, salivary cortisol post DEX was significantly higher in OSAS patients than in obese control (p= 0.038). Comparisons between groups were adjusted for BMI (Table1).
All patients had detectable circulating plasma DEX level, indicating that all participants had ingested the DEX tablets (Table 1). DEX levels did not differ between OSAS patients and obese controls (84.5 ± 9.3 vs 101 ± 10.5; p=0.241) and in the total group, DEX levels did not correlate to plasma cortisol levels post DEX (r=-0.219; p=0.328).

Three months of nCPAP therapy was associated with a significant reduction in salivary cortisol after dexamethasone (p=0.009) and heart rate (p=0.036) compared with baseline. Also, a greater cortisol suppression (% cortisol suppression) post DEX was evident in apneic patients following the use of nCPAP (p=0.001). This was similar to the levels of obese controls (Figure 1). Average body mass index (p=0.913) did not change and no differences in sleep blood pressure fall and blood pressure values were observed after nCPAP therapy (Table 2). Six patients (67%) took anti-hypertensive drugs and blood pressure was controlled at entry.

Improvement of AHI in response to nCPAP were positively correlated with the improvement of cortisol suppression after oral dexamethasone (r=0.799; p=0.010) and negatively correlated with cortisol suppression before nCPAP (r=-0.883; p=0.002) (Figure 2).

**DISCUSSION**

In the present study, we demonstrated a blunted response of cortisol suppression after dexamethasone and a higher 24-hour heart rate in obese men with obstructive sleep apnea syndrome compared with obese male controls. These findings may reflect activation of sympathetic nervous and stress system in apneic patients, which could be due to nocturnal hypoxia and sleep fragmentation with several awakening and arousal episodes. In addition, we showed a significant reduction in heart
rate and a marked improvement in dexamethasone induced salivary cortisol suppression in patients with OSAS after 3 months of nCPAP therapy. The reduction of apnea-hypopnea-index after nCPAP therapy was positively correlated with cortisol suppression in response to low dose oral dexamethasone.

Stress related disorders such as depression, anorexia, alcoholism, excessive exercising, malnutrition, premenstrual tension syndrome may be associated with increase CRH activity and ACTH secretion, resulting in chronic exposure to circulating cortisol levels and loss of the normal negative feedback of the HPA axis by glucocorticoids. In accordance with this hypothesis, it was expected that OSAS would be associated with an activation of HPA axis in response to stress caused by recurrent intermittent hypoxia, sleep fragmentation and frequent cerebral arousals during apneic events. However, only a few studies have assessed the relationship between sleep apnea and HPA axis and results are still controversial. In the majority of these studies, the HPA axis was assessed by a single morning plasma cortisol measurement, which might not reflect the episodic nature of cortisol secretion and its appropriate elevations during the hypoxemia stress. Two recent studies have evaluated the 24-hour circadian secretory pattern of cortisol in obese patients with and without sleep apnea. In agreement with our results, both studies have demonstrated that salivary and plasma cortisol secretion was circadian in OSAS patients and obese control. However, Dadoun et al. failed to find any significant differences for overnight cortisol secretion between obese patients with or without sleep apnea syndrome. Our results match those who have demonstrated that sleep apnea in obese men is associated with increased cortisol level during the nighttime period (11pm-07am) compared to obese controls, which is recovered after the use of nCPAP for 3 months. Hence, we hypothesize that
obstructive sleep apnea should be recognized by clinician and corrected before further clinical investigations for endocrine causes of hypercortisolemia.

Long-term mild activation of HPA axis has been shown to altering important functions in patients with some evidence of hypercortisolism, i.e., adrenal incidentalomas, depression or alcoholism, increasing the risk for chronic conditions such weight gain, lethargy, weakness, loss of libido, diabetes mellitus, hypertension and osteoporosis 22. Thus, further prospective studies should explore these clinical manifestations in patients with OSAS and increased cortisol levels.

A positive and well established relationship between sleep apnea and the prevalence and severity of hypertension has been reported 4,14,26,38. Nevertheless, we did not find any differences in 24-hour blood pressure values between patients with or without sleep apnea. Blood pressure fall during sleep, however, was smaller in OSAS patients than in obese controls. The reasons for these discrepancies might be the fact that, in our study, more than half of patients were on hypertensive medication, although none received beta blocker therapy.

Therapy with nCPAP is the most effective treatment for sleep apnea, preventing recurrent occlusion of upper airway during sleep 32. However, the effects of nCPAP on blood pressure have shown conflicting results 1,3,21. We did not find significant changes in blood pressure levels and blood pressure fall during sleep after nCPAP therapy in severe obese patients with OSAS. As our patients were under antihypertensive therapy and blood pressure levels were controlled at baseline, this may be the reason for negative results. Consistent with our findings Campos-Rodriguez et al 3 have reported that 24 months of therapeutic nCPAP reduced 24-hour ambulatory blood pressure measurements only in a subgroup of patients with incompletely
controlled hypertension at baseline. They suggest that in a group of controlled hypertensive patients nCPAP therapy failed to reduce blood pressure.

Some limitations of the current study include the small sample size and lack of a post nCPAP treatment control group. However at the time of the study, sham-CPAP machines capable of use in a double-blinded setting were not available. Moreover, ethical approval to leave patients with severe symptomatic OSAS untreated before bariatric surgery was not forthcoming from university ethics committee.

In conclusion, our findings demonstrate that obstructive sleep apnea syndrome is associated with increased 24-hour heart rate, decreased percentage of fall in systolic blood pressure at night and lower cortisol suppression after low dose dexamethasone, suggesting that there is an activation of sympathetic nervous system and hypothalamic-pituitary-adrenal axis. Our results also show the beneficial effects of nCPAP on 24-hour heart rate and salivary cortisol levels after low dose dexamethasone test, which may contribute to reducing cardiovascular and metabolic complications related to OSAS.

ACKNOWLEDGMENTS

We would like to thank Eveli Truksinas for skillful technical assistance in follow-up assessment of nCPAP therapy.

GRANTS

The manuscript was supported by grants from Associação Fundo de Incentivo à Psicofarmacologia (AFIP), Hospital do Rim e Hipertensão - Fundação Oswaldo Ramos, Coordenação de Aperfeiçoamento de Pessoal de nível Superior (CAPES) and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP)-CEPID.
DISCLOSURES

No financial or other potential conflicts of interest exist for all the authors.

REFERENCES


show a peculiar alteration of the corticotroph but not of the thyrotroph and lactotroph function. Clin Endocrinol (Oxf) 60: 41-8, 2004.


TABLES LEGENDS

Table 1: Clinical and laboratorial characteristics of obese men with and without sleep apnea.

Abbreviations: AHI, apnea-hypopnea index; BMI, Body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; ΔBP, percentage of fall in systolic blood pressure at night; S Cortisol, salivary cortisol; % cortisol suppression, cortisol post DEX minus cortisol basal at 8:00 am/ cortisol basal at 8:00 am. Data are expressed by mean ± SE or n (%). p*: significance after adjustment for BMI.

Table 2: Characteristics of men with sleep apnea before and after three months of nCPAP therapy.

Abbreviations: AHI, apnea-hypopnea index; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; ΔBP, percentage of fall in systolic blood pressure at night; S Cortisol, salivary cortisol; % cortisol suppression, cortisol post DEX minus cortisol basal at 8:00 am / cortisol basal at 8:00 am . Data are expressed by mean ± SE.
FIGURES LEGENDS

Figure 1: Salivary cortisol response to 0.25 mg dexamethasone and % cortisol suppression in obese men with and without OSAS.
Abbreviations: DEX, Dexamethasone; OSAS, obstructive sleep apnea syndrome; CPAP, continuous positive airway pressure. Data are expressed by mean ± SE.

Figure 2: Correlations among cortisol suppression at baseline, changes (Δ) in cortisol suppression and changes (Δ) in apnea-hypopnea index (AHI) following nCPAP therapy.
**Table 1:** Clinical and laboratorial characteristics of obese men with and without sleep apnea.

<table>
<thead>
<tr>
<th></th>
<th>OSAS</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No (n=13)</td>
<td>Yes (n=16)</td>
</tr>
<tr>
<td>AHI, events/hr</td>
<td>3.2 ± 0.5</td>
<td>65.7 ± 9.9</td>
</tr>
<tr>
<td>Age, years</td>
<td>38.8 ± 3.3</td>
<td>40.1 ± 2.8</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>42.8 ± 1.3</td>
<td>46.9 ± 2.0</td>
</tr>
<tr>
<td>24h SBP, mmHg</td>
<td>127.6 ± 2.3</td>
<td>133.1 ± 3.3</td>
</tr>
<tr>
<td>24h DBP, mmHg</td>
<td>76.1 ± 1.9</td>
<td>79.6 ± 2.3</td>
</tr>
<tr>
<td>24h Heart rate, beats/min</td>
<td>76.3 ± 2.8</td>
<td>89.3 ± 3.4</td>
</tr>
<tr>
<td>Δ BP, %</td>
<td>11.5 ± 1.8</td>
<td>5.5 ± 1.2</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>07 (53.8)</td>
<td>11 (68.8)</td>
</tr>
<tr>
<td>S Cortisol basal, ng/dL</td>
<td>483.9 ± 77.5</td>
<td>442.2 ± 57.5</td>
</tr>
<tr>
<td>S Cortisol 11h pm, ng/dL</td>
<td>177.8 ± 53.5</td>
<td>233.1 ± 56.3</td>
</tr>
<tr>
<td>S Cortisol post DEX, ng/dL</td>
<td>233.2 ± 65.6</td>
<td>445.4 ± 61.6</td>
</tr>
<tr>
<td>% cortisol suppression</td>
<td>-48.8 ± 10.5</td>
<td>-16.6 ± 6.1</td>
</tr>
<tr>
<td>Dexamethasone, ng/dL</td>
<td>101 ± 10.5</td>
<td>84.5 ± 9.3</td>
</tr>
<tr>
<td>Plasma cortisol, µg/dL</td>
<td>3.6 ± 1.0</td>
<td>6.9 ± 1.0</td>
</tr>
</tbody>
</table>

Abbreviations: AHI, apnea-hypopnea index; BMI, Body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; Δ BP, percentage of fall in systolic blood pressure at night; S Cortisol, salivary cortisol; % cortisol suppression, cortisol post DEX minus cortisol basal at 8:00 am / cortisol basal at 8:00 am. Data are expressed by mean ± SE or n (%). p*: significance after adjustment for BMI.
Table 2. Characteristics of men with sleep apnea before and after three months of nCPAP therapy

<table>
<thead>
<tr>
<th></th>
<th>CPAP</th>
<th>change from baseline</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre (n=09)</td>
<td>Post (n=09)</td>
<td></td>
</tr>
<tr>
<td>AHI, events/hr</td>
<td>92 ± 7.6</td>
<td>21.1 ± 9.7</td>
<td>-70.8 ± 5.3</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>44.3 ± 2.4</td>
<td>44.4 ± 2.5</td>
<td>0.07 ± 0.70</td>
</tr>
<tr>
<td>24h SBP, mmHg</td>
<td>131.5 ± 3.7</td>
<td>125.7 ± 1.9</td>
<td>-5.7 ± 3.9</td>
</tr>
<tr>
<td>24h DBP, mmHg</td>
<td>77.3 ± 2.5</td>
<td>76.0 ± 2.7</td>
<td>-1.3 ± 2.9</td>
</tr>
<tr>
<td>24h Heart rate, beats/min</td>
<td>82.1 ± 3.8</td>
<td>74.3 ± 3.5</td>
<td>-7.7 ± 3.08</td>
</tr>
<tr>
<td>Δ BP, %</td>
<td>6.2 ± 1.9</td>
<td>7.3 ± 1.5</td>
<td>1.2 ± 2.1</td>
</tr>
<tr>
<td>S Cortisol basal, ng/dl</td>
<td>501.1 ± 89.0</td>
<td>521.2 ± 58.6</td>
<td>20.1 ± 89</td>
</tr>
<tr>
<td>S Cortisol post DEX, ng/dl</td>
<td>438.6 ± 70.4</td>
<td>168.1 ± 41.4</td>
<td>-270 ± 78.4</td>
</tr>
<tr>
<td>% cortisol suppression</td>
<td>-21.8 ± 8.5</td>
<td>-69.4 ± 6.2</td>
<td>-47.5 ± 8.6</td>
</tr>
</tbody>
</table>

Abbreviations: AHI, apnea-hypopnea index; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; Δ BP, percentage of fall in systolic blood pressure at night; S Cortisol, salivary cortisol; % cortisol suppression, cortisol post DEX minus cortisol basal at 8 h / cortisol basal at 8 h. Data are expressed by mean ± SE
Salivary Cortisol (ng/dL)

- Basal
- Post 0.25 DEX

OBES (n=13)

Base-Post CPAP

OSAS

Baseline Post CPAP Control

% cortisol suppression

* p<0.05 vs baseline
\begin{align*}
\Delta \text{AHI (events/h)} &\quad r = 0.799; p = 0.010 \\
\Delta \% \text{cortisol suppression} &\quad r = -0.883; p = 0.002
\end{align*}