

STUDY OF METABOLIC CHANGES IN PATIENTS WITH OBSTRUCTIVE SLEEP APNEA SYNDROME BEFORE AND AFTER USE OF CONTINUOUS POSITIVE AIRWAY PRESSURE

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ABSTRACT

Background and objective: Obstructive Sleep Apnea Syndrome (OSAS) is the most common sleep-disordered breathing (SDB) syndrome and is characterized by repetitive, total, or partial pharyngeal collapse during sleep. These symptoms induce both hypoxemia and brief arousals from sleep. As a result, daytime sleepiness, reduction in cognitive performance, an increase in the incidence of cardiovascular diseases and traffic accidents have been observed. To date, the most effective treatment for OSAS has been the use of Continuous Positive Airway Pressure (CPAP). Studies confirming the reversibility of alterations such as hypertension, hyperleptinemia, and an increase in inflammatory cytokines after therapy with CPAP in patients with OSAS are controversial. The purpose of this study was to evaluate the metabolic markers present in the blood of patients with OSAS before and after use of nasal CPAP treatment.

Methods: Thirteen patients with moderate to severe OSAS were selected for the current study. After submitting their informed consent to participate in the study, the selected patients answered a sleep questionnaire and were submitted to a physical examination. They were also asked to perform a polysomnography throughout an entire night for diagnosis, CPAP pressure titration, and blood collection. All patients were submitted to treatment with CPAP for six months, after which all evaluations were repeated.

Results: When we compared patients with OSAS with healthy control individuals, we found increased levels of ghrelin and triglycerides and reduced levels of HDL in patients with OSAS ($p < 0.05$). In addition, no difference in leptin levels was observed. After a six-month treatment period with CPAP, a significant drop in ghrelin levels could be observed ($p < 0.05$). No changes in the patients' body weight was observed during the treatment period (29 ± 4 x 29 ± 3 Kg/m²).

Conclusion: We thus concluded that OSAS can be considered an independent factor for increasing ghrelin levels and that a reduction in the levels of this hormone occurs after six months of CPAP treatment.

Keywords: Obstructive Sleep Apnea Syndrome, CPAP, leptin, ghrelin, obesity.

INTRODUCTION

Obstructive Sleep Apnea Syndrome (OSAS) is the most common chronic sleep-disordered breathing (SDB) syndrome among the adult population. Approximately 4% to 24% of male adults and 1% to 9% of female adults in the general population suffer from OSAS (1,2).

OSAS is characterized by repetitive episodes of partial upper airway obstruction (hypopnea) or total upper airway obstruction (apnea), causing oxyhemoglobin desaturation and, in persistent cases, hypercapnia, which are frequently reverted by arousals, thus inducing sleep fragmentation (3).

Recent prospective and controlled studies with prolonged follow-ups demonstrated that OSAS is an independent risk factor for hypertension (4). In addition, OSAS contributes to other comorbidities, such as cardiac arrhythmias (5), coronary insufficiency (6), heart failure (7), and stroke (8).

Currently, researchers are searching for physiopathogenic explanations that concur to the increase in cardiovascular abnormalities associated with OSAS. Two main lines of research that aim at assessing the two major OSAS consequences, hypoxia-hypercapnia and sleep fragmentation (9-11), have been established.

It has been suggested that obesity (proinflammatory syndrome), which is frequently associated with OSAS, would be a determinant or predisposing agent for increasing cardiac risk in this syndrome (12). In early studies in the field, it was hypothesized that resistance to insulin in patients with OSAS was thoroughly dependent on obesity (13). Nevertheless, in 2000, Vgontzas and collaborators detected higher resistance to insulin and hypercytokinemia in obese patients with OSAS when compared with obese patients without OSAS, and patients of normal weight, thus suggesting that such alterations might contribute to a high cardiovascular risk when this syndrome is present (14). Another study found higher levels of leptin, insulin, and triglycerides in obese patients with OSAS compared with obese patients without OSAS (15). After treatment with CPAP, a reduction in leptin and triglyceride levels was observed, but not in serum insulin levels. This evidence suggests that resistance to insulin may be involved in the genesis of OSAS (15).

Previous studies demonstrated that in both OSAS and in obesity, in addition to resistance to insulin, patients showed an independent increase in inflammatory cytokines levels (TNF- α and IL-6). In 1997, Vgontzas and collaborators reported an increase of tumor necrosis factor-alpha (TNF- α) and in interleukin-6 (both related to sleepiness), not only in the presence of OSAS, but also in obese patients. These results were independent of each other. Nevertheless, it still remains to be established whether the increase in such cytokines and resistance to insulin are primary OSAS events or events secondary to OSAS (16,17).

The vast majority of obese people show high levels of circulating leptin. The strong correlation between obesity and OSAS in humans and the establishment of leptin as a ventilatory stimulant and appetite suppressor in rats has raised the possibility that apnea is associated with a leptin deficiency. However, recent studies demonstrate that individuals with OSAS show an increase in leptin levels, thus suggesting that there may be a relative resistance

to circulating leptin (15).

Phipps and collaborators have recently reported hyperleptinemia associated with a collapse of hypercapnic breathing in obese people. In such cases, treatment with leptin might reverse the hypoventilatory syndrome in obese patients who show a ventilatory response to hypercapnia (18).

Shimizu and collaborators have recently evaluated 21 patients with an apnea/hypopnea index (AHI) above 20 after one-day of treatment with CPAP. They detected reduction in serum leptin levels and in cardiac sympathetic activation. It still remains to be elucidated whether the reduction in leptin levels after treatment with CPAP has influenced sympathetic activity or whether the reduction in sympathetic activity has contributed to the reduction in leptin levels (19).

Ghrelin is an appetite-stimulating peptide derived predominantly in the stomach. Ghrelin levels increase before meals and decrease after meals. The mechanism of this hormone can be considered opposed to that of the leptin hormone, which is produced in fatty tissues and controls appetite (20). In obese people, plasma ghrelin concentrations are lower than those found in people with average weights. (21). Yildiz and collaborators have demonstrated that, in average weight subjects, ghrelin concentrations show an increase during the night, which exceeds the increase after meals, although this type of increase was not observed in obese subjects (22).

Most studies conducted with patients who have OSAS and measurements of metabolic hormones are controversial, as obesity represents a confounding factor. Obese patients with OSAS compared with obese patients who do not have OSAS showed high levels of ghrelin and leptin, which decreased after treatment with CPAP. No changes in the body mass index (BMI) of such patients could be observed (23).

We could thus conclude that leptin and ghrelin hormones play key roles in the metabolism of patients who have OSAS, regardless of obesity.

The purpose of this study was to verify whether obesity in the presence of OSAS determines metabolic alterations or aggravates them and to verify the clinical response and the metabolic markers before and after the use of CPAP equipment.

MATERIALS AND METHODS

Casuistics

Thirteen patients who underwent ambulatory treatment for breathing disorders at the Universidade Federal de São Paulo (UNIFESP) in 2004 and who were clinically and polysomnographically diagnosed as having OSAS, as per the American Academy of Sleep Medicine (24) task force, were selected for study. After receiving orientation regarding the study, patients agreed to participate in the study and signed an informed consent term.

The study was approved by UNIFESP Ethics Research Committee (number 1266/03).

Inclusion Criteria

- Patients who met the polysomnographic criteria for OSAS

proposed by American Academy of Sleep Medicine in 2005 included:

- Adults between 30 and 60 years of age.
- Both male and female.
- BMI < 35 Kg/m².
- AHI higher or equal to 15 events per hour (OSAS from mild to severe)
- Normal hemogram, hepatic function, renal function and electrocardiogram.
- Patient's consent after explanation of the protocol and signature of the informed consent term.
- Availability for answering questionnaires, visiting the sleep laboratory for undergoing polysomnography (for diagnosis, CPAP titration, and control after a six-month treatment and use of CPAP equipment for a minimum period of 6 months).

Exclusion Criteria

- Endocrine disorders, such as hypothyroidism and diabetes.
- Lung diseases.
- Cardiovascular diseases.
- Infectious diseases.
- Neurological and psychiatric diseases.
- Other sleep disorders.
- Individuals using sleep inductors, neuroleptic drugs, or beta blockers.
- Tobacco smokers, alcohol addicts and shift workers.
- Patients who have already undergone previous OSAS treatment.

Controls

Thirteen healthy volunteers matched for BMI, age, and sex were selected and studied using the same clinical, polysomnographic, and laboratorial methods.

Study Design

Anamnesis, a physical examination, and filling out the Epworth Sleepiness Scale (ESS) were performed both at the beginning and the end of the research period (25). Patients were also submitted to routine lab tests for selection and exclusion of other pathologies not related to the study.

Three polysomnography PSGs were accomplished during the research period. The first aimed at assigning a diagnosis, the second was for CPAP titration, and the third was performed six months after the use of CPAP equipment. During the research period, the subjects under evaluation visited the lab once a month in order to clarify any possible doubt regarding diagnosis and treatment for the syndrome. They also visited for analysis of adherence to treatment through the CPAP built-in compliance meter. Blood collection for measuring any metabolic changes was performed at the beginning and end of the treatment. At the end of treatment with CPAP equipment, the clinical tests and ESS were also repeated.

Study development

Clinical analyses of the patients

Anamnesis and a detailed physical test including ectoscopy, oropharyngeal evaluation, arterial pressure, pulse, weight, and

height, as well as neck, hip and waist circumferences were accomplished. ESS evaluates eight routine situations, ranging from those which do not require great attention to those which require a great level of attention. The Epworth Sleepiness Scale ranges from 0 to 3 (0, 1, 2, 3), where 0 corresponds to no possibility of sleeping, 1 to a slight possibility of sleeping, 2 to a relative possibility of sleeping, and 4 to a great possibility of sleeping (25).

Lab Analysis

Blood samples for the assessment of metabolic changes were collected at two independent times (at the beginning of the study and six months after treatment with CPAP). On the evening before blood was to be collected for detection of any metabolic change, patients were admitted in the Sleep Institute and were instructed to fast from 08:00 pm to 06:00 am the following day; blood was collected early in the morning. Serum ghrelin and leptin levels, as well as blood glucose levels at fasting, insulin, total cholesterol, triglycerides, HDL, LDL, VLDL, fibrinogen and CRP (C-reactive protein) were tested.

Polysomnographic Study

PSG equipment used for data processing, collection, analysis, and compilation of the elements necessary for a polysomnographic study was utilized. This equipment was contained in a Sonolab computerized system, version 2003-A.

The study utilized fifteen channels, including three for electroencephalography (EEG - C3/A2, C4/A1, O1/A2, O2/A1), two for right and left electrooculography (EOG), one for submentonian electromyography (EMG), one for anterior tibial electromyography, one for electrocardiography (ECG), one for airflow (thermistor) and nasal pressure cannula, two for thoracic-abdominal movements, one for registering tracheal vibration, one for measuring oxyhemoglobin saturation by pulse oximeter (Oxycap, Ohmeda, Denver, CO or Model N1000, Nelcor Inc, Hayward CA), and one for registering corporeal position.

All polysomnographic tests were performed at night, in a dark and quiet room specifically designed for this procedure. Patients arrived at the lab approximately two hours before their usual bedtime to become familiar with the space and prepare to sleep. After that, the polysomnographic equipment was assembled. On average, it took nine hours to accomplish all tests (10:00 pm to 07:00 am). Sleep staging followed patterns established by Rechtschaffen and Kales (1968) (26). For breathing events, the analysis followed AASM patterns (1999) (27), and, for arousals, it followed ASDA patterns (1992) (28). For lower limb movements, ASDA patterns were followed (1993) (29).

Statistical Analysis

The parametric results were expressed through mean and standard deviation. For comparison of parametric variables between groups, we applied the t test for independent samples.

RESULTS

Ages and BMIs for the control group and the OSAS group were

similar to each other (Table 1). When comparing data referring to the control group and the OSAS group, ESS scores showed an increase in the group with OSAS ($p < 0.001$). In addition, increases in AHI and arousal index ($p < 0.01$), and a lower SpO_2 when compared with the control group ($p < 0.001$) were observed (Table 1).

Table 1: General data and PSG data from controls and patients with OSAS.

	Controls (n = 13) Mean ± SD	Patients with OSAS (n = 13) Mean ± SD	p
Age	36 ± 6	37 ± 7	NS
BMI (Kg/m ²)	27 ± 3	29 ± 3	NS
ESS	5 ± 4	15 ± 4	0.001
AHI	2 ± 1	41 ± 29	0.001
SpO ₂ min	89 ± 3	77 ± 13	0.001
SE	87 ± 8	88 ± 9	NS
S1	3 ± 2	7 ± 5	0.04
S2	59 ± 6	64 ± 12	NS
S3+4	15 ± 4	11 ± 7	NS
REM	22 ± 3	18 ± 9	NS
ARI	8 ± 3	38 ± 37	0.01

BMI: Body Mass Index, ESS: Epworth Sleepiness Scale; AHI: Apnea/Hypopnea Index; SpO₂ min: minimal oxygen saturation; SE: Sleep Efficiency; ARI: Arousal Index.

During their basal evaluations, patients with OSAS showed increased ghrelin and triglyceride levels and reduced HDL levels when compared with the control group. In addition, no significant difference in leptin levels could be detected (Table 2).

Table 2: Metabolic parameters of to controls and patients with OSAS.

	Controls (n = 13) Mean ± SD	Patients with OSAS (n = 13) Mean ± SD	p
Ghrelin (pg/ml)	1010 ± 262	1642 ± 942	0.04
Leptin (ng/ml)	15 ± 6	20 ± 11	NS
Glucose (mg/dl)	90.5 ± 7.5	93.63 ± 8.95	NS
Insulin (μIU/ml)	6.28 ± 4.85	9.11 ± 4.65	NS
Triglycerides (mg/dl)	120.5 ± 55.6	164 ± 47	0.04
Total cholesterol (mg/dl)	203 ± 43	213 ± 42.84	NS
LDL (mg/dl)	123 ± 33	137 ± 39	NS
VLDL (mg/dl)	24.5 ± 11	33 ± 9	NS
HDL (mg/dl)	56.5 ± 10.44	44 ± 11	0.01
CRP (mg/dl)	0.13 ± 0.1	1.6 ± 5	NS
Fibrinogen (g/L)	3.2 ± 0.7	3.6 ± 1.3	NS

LDL: low-density lipoprotein; VLDL: very low-density lipoprotein; HDL: high-density lipoprotein; CRP: C-Reactive Protein.

When comparing overweight and average weight patients (BMI between 24 and 29.9 Kg/m²) with Class I obese patients (BMI between 30 and 35 Kg/m²), no difference was observed in either ghrelin or leptin levels (Table 3).

Table 3: Ghrelin and leptin values in patients with OSAS: normal weight/overweight versus obesity Class I.

	Normal Weight/ Overweight (n=7) Mean ± SD	Obesity Class I (n=6) Mean ± SD	p
BMI (Kg/m ²)	26 ± 2	32 ± 1	0.0002
Ghrelin (pg/ml)	1597 ± 919	1705 ± 1078	NS
Leptin (ng/ml)	17 ± 8	27 ± 12	NS

BMI: Body Mass Index.

After treatment with CPAP, a significant drop in ghrelin levels was observed, while no difference could be observed in the leptin levels of patients with OSAS (Table 4). No change in patients' weight during the treatment period was observed (29 ± 4 x 29 ± 3 Kg/m²). In addition, no significant difference in the parameters evaluated was observed after the treatment period.

Table 4: Basal data from patients with OSAS after 6 months using CPAP equipment.

	Basal (n = 13) Mean ± SD	After CPAP (n = 13) Mean ± SD	p
BMI (Kg/m ²)	29 ± 4	29 ± 3	NS
AHI	41 ± 29	4 ± 2	0.0001
Ghrelin (pg/ml)	1642 ± 942	1032 ± 444	0.04
Leptin (ng/ml)	20 ± 11	22 ± 10	NS
Glucose (mg/dl)	93.63 ± 8.95	94.63 ± 9.90	NS
Insulin (μIU/ml)	9.11 ± 4.65	13.11 ± 4.69	NS
Triglycerides (mg/dl)	164 ± 47	168 ± 69	NS
Total Cholesterol (mg/dl)	213 ± 42.84	220.45 ± 43.89	NS
LDL (mg/dl)	137 ± 39	142 ± 40	NS
VLDL (mg/dl)	33 ± 9	34 ± 14	NS
HDL (mg/dl)	44 ± 11	44 ± 8	NS
CRP (mg/dl)	1.6 ± 5	0.3 ± 0.2	NS
Fibrinogen (g/L)	3.6 ± 1.3	3.6 ± 0.6	NS

BMI: Body Mass Index; AHI: Apnea/Hypopnea Index; LDL: low-density lipoprotein; VLDL: very low-density lipoprotein; HDL: high-density lipoprotein; CRP: C-Reactive Protein.

DISCUSSION

Our study has established that patients with OSAS show increased ghrelin and triglycerides levels in addition to reduced HDL levels. We also established that these patients did not show any increase in leptin and glucose levels or resistance to insulin when compared with control subjects. When we compared average weight and overweight OSAS patients with the more obese OSAS patients, we could not find any difference in metabolic measurements among these patients. This leads to the conclusion that obesity is not a determining factor for the results obtained from this study. The use of CPAP for reverting ghrelin levels reinforces the role of OSAS as the responsible agent for this alteration.

The age and the absence of other comorbidities associated with OSAS in our sample justify the mild metabolic alterations in patients with OSAS who participated. The time elapsed from illness onset, which was not estimated in this study, may be another determining factor.

Ulukavak and collaborators analyzed 30 obese subjects with moderate to severe OSAS and 22 obese control subjects without OSAS. They reported a significant increase in leptin levels in the group of obese subjects with OSAS when compared with the group of obese subjects without OSAS. In view of these results, Ulukavak and collaborators suggest that leptin is a hormonal factor affected by OSAS and is not determined by obesity alone (30). Our results differed, perhaps due to ethnic or environmental differences existing in the sample population studied.

We observed that, in our sample, regardless of obesity, patients with OSAS showed increased levels of ghrelin, which decreased after six months of CPAP treatment. However, patients did not show any difference in leptin levels. The fact that we have selected patients without severe obesity ($BMI > 35 \text{ Kg/m}^2$) may have interfered with our results. Interesting enough, we observed that, when comparing our data with data collected by Ulukavak and collaborators (2005), the leptin results we found in our subjects were quite similar to those found in their groups of obese people without OSAS. This finding points to the possibility that differences in leptin levels in patients with OSAS occur only when such patients are severely obese.

In a recent study, 30 patients with severe OSAS were selected. In this study, leptin and basal ghrelin levels were evaluated two days and two months after treatment with CPAP. Patients with OSAS showed high ghrelin levels in the basal analysis when compared with the control group. In most cases, patients with OSAS showed a decrease in these levels after a two-day treatment with CPAP. Leptin values did not show a significant difference in the two first days of treatment, although patients with OSAS showed higher values than those in the control group. After a two-month treatment period, leptin levels were significantly reduced and BMI levels showed no alteration. The most significant drop in this study was observed in patients with a $BMI < 30 \text{ Kg/m}^2$ (23).

In addition, patients with OSAS, although they had fragmented sleep in view of arousals, usually maintained their total sleep period. Recent studies have demonstrated that a reduction in sleep period is associated with a reduction in leptin levels, an increase in ghrelin levels, and an increase in weight (31,32).

As for insulin, a previous study demonstrated that patients with moderate to severe OSAS showed an increase in insulin sensitivity after a two-day treatment period with CPAP and remained stable for three months after treatment. The improvement in insulin sensitivity after two days was much greater in patients with a body mass index less than 30 Kg/m^2 than in more obese patients. These results may reflect a reduction in sympathetic activity, indicating that OSAS is an independent risk factor for increasing insulin resistance (33).

OSAS contributes to unbalanced ghrelin levels, even in patients without severe obesity. Thus, our data suggest that OSAS has an isolated effect for determining increases in the levels of this hormone. In view of this, CPAP therapy has resulted in a significant reduction in the levels of this hormone without any association with a reduction in weight, confirming an OSAS-independent effect. Despite the limited size of the sample utilized in this study, our data point to the importance of metabolic alterations for the development of comorbidities associated with OSAS. In addition, our results illustrate the importance of controlling these alterations via adequate treatment of the syndrome.

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