# Original Article

# Big Endothelin-1 and Nitric Oxide in Hypertensive Elderly Patients with and without Obstructive Sleep Apnea-Hypopnea Syndrome

*Iara Felicio Anunciato, Rômulo Rebouças Lobo, Eduardo Barbosa Coelho, Waldiceu Aparecido Verri Jr., Alan Luiz Eckeli, Paulo Roberto Barbosa Évora, Fernando Nobre, Júlio César Moriguti, Eduardo Ferriolli, Nereida Kilza da Costa Lima*
*Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo, Ribeirão Preto, SP – Brazil*

## Abstract

**Background:** The role of oxidative stress in hypertensive elderly patients with obstructive sleep apnea-hypopnea syndrome (OSAHS) is unknown.

**Objective:** The purpose was to evaluate the levels of big endothelin-1 (Big ET-1) and nitric oxide (NO) in elderly hypertensive patients with and without moderate to severe OSAHS.

**Methods:** Volunteers were hospitalized for 24 h. We obtained the following data: body mass index (BMI); 24-ambulatory blood pressure monitoring; and current medication. Arterial blood was collected at 7pm and 7am for determining plasma NO and Big ET-1 levels. Pulse oximetry was performed during sleep. Pearson's or Spearman's correlation and univariate analysis of variance were used for statistical analysis.

**Results:** We studied 25 subjects with OSAHS (group 1) and 12 without OSAHS (group 2) aged $67.0 \pm 6.5$ years and $67.8 \pm 6.8$ years, respectively. No significant differences were observed between the groups in BMI; number of hours of sleep; 24-h systolic and diastolic BPs; awake BP, sleep BP and medications to control BP between groups. No differences were detected in plasma Big ET-1 and NO levels at 19:00 h, but plasma Big ET-1 levels at 7:00 h were higher in group 1 ($p = 0.03$). In group 1, a negative correlation was also observed between the mean arterial oxyhemoglobin saturation level, 24-h systolic BP ($p = 0.03$, $r = -0.44$), and Big ET-1 ($p = 0.04$, $r = -0.41$).

**Conclusions:** On comparing elderly hypertensive patients with and without OSAHS having similar BP and BMI, we observed higher Big ET-1 levels After sleep in the OSAHS group. NO levels did not differ between the hypertensive patients with or without OSAHS. (Arq Bras Cardiol. 2013;101(4):344-351)

**Keywords:** Hypertension; Aged; Big endothelin-1; Obstructive sleep apnea-hypopnea syndrome.

## Introduction

The endothelium plays a fundamental role in the regulation of vascular tone and peripheral resistance through the synthesis of numerous vasoactive compounds, i.e., vasodilators such as nitric oxide (NO) and vasoconstrictors such as big endothelin-1 (Big ET-1), by endothelial cells[1-3]. Under pathological conditions such as systemic arterial hypertension, there is a disequilibrium of endothelium-derived factors with an attenuation of vasodilators and a predominance of vasoconstrictors[4]. The elimination rate of endothelin-1 (ET-1) is higher than that of its precursor Big ET-1. In addition, plasma Big ET-1 levels of humans, rabbits, and rats are higher than plasma ET-1 levels, with studies suggesting that the precursor is a more appropriate indicator for the quantification of release by endothelial cells[5].

NO has an important influence on the tone of peripheral blood vessels, and it is released in response to the shear stress produced by blood flow and by the activation of various of receptors[6]. Because NO is released continuously, systemic inhibition of its synthesis causes an elevation of blood pressure (BP). On this basis, oxidative stress plays an important role in hypertension pathogenesis[7].

Oxidative stress is also considered to be involved in the pathogenesis of arterial hypertension in obstructive sleep apnea-hypopnea syndrome (OSAHS)[8]. OSAHS is defined as a group of disorders characterized by periodic cessation of breathing, with consequent hypoxia, frequent awakenings, and sleep fragmentation during the night, triggering excessive daytime somnolence[9]. This syndrome is more prevalent among the elderly[10]. According to the apnea-hypopnea index (AHI/h), OSAHS is classified as mild (AHI/h, 5–15 events/h); moderate (AHI/h, 15–30 events/h); and severe (AHI/h, >30 events/h)[11]. Furthermore, mortality is significantly associated with high rates of respiratory changes [awakenings and microawakenings during sleep]; a body mass index (BMI) of >30 kg/m²; and male sex[12].

Few studies have assessed endothelial function in OSAHS, and the role of oxidative stress in elderly hypertensive patients with OSAHS is unknown.

Anunciato et al.
Big endothelin-1 and nitric oxide in OSAHS

**Original Article**

The objective of the present study was to evaluate Big ET-1 and NO before and after sleep in elderly hypertensive patients with and without OSAHS.

## Methods

The hypertensive patients were selected from the outpatient services of the following centers of the Ribeirao Preto School of Medicine, São Paulo University: the Hypertension Center of the Clinical Hospital; the Geriatric Clinic of the School Health Center; and the Center for Family Health –V. All hypertensive volunteers with moderate to severe OSAHS had previous diagnoses confirmed by polysomnography; control hypertensive subjects were also subjected to further examination to exclude OSAHS. The hypertensive patients diagnosed with moderate or severe OSAHS were diagnosed by the Laboratory of Clinical Neurophysiology (Clinical Hospital – Ribeirao Preto School of Medicine) and selected following the exclusion criteria described below.

Exclusion criteria were based on the following factors that could affect the evaluation of plasma Big ET-1 and NO levels: age <60 years, diabetes mellitus, dyslipidemia, heart and lung diseases, smoking, past history of smoking, drinking, use of continuous positive pressure airway ventilation, cardiac arrhythmia, and use of medications that might interfere with sleep (anxiolytics, antidepressants, and neuroleptics). Patients who did not consent to participate were also excluded.

The volunteers were admitted to the Clinical Research Unit of HCFMRP-USP. Upon admission, weight and height were measured using a digital scale (Filizola), and the device for the ambulatory blood pressure monitoring (ABPM) was installed (SAPCELABS 90207, Redmond, USA. The ABPM device was left in place for 24 h, divided into a probable wakefulness period (7:00–23:00 h) and a probable sleep period (23:00–7:00 h). The device was programmed to take readings at 15 minute intervals during the probable wakefulness period and at 20 minute intervals during the probable sleep period. Before each blood collection for the determination of Big ET-1 and plasma NO, three BP measurements were made with the subject in the sitting position, using a mercury column device (auscultatory method) on the upper limb contralateral to that wearing the cuff for ABPM.

A 4.5-ml arterial blood sample was collected at 19:00 h on the day of admission and 7:00 h the following morning for the determination of plasma NO levels ($\mu$M). Each sample was divided into three aliquots, and 1.5 ml arterial blood was stored in an Eppendorf tube containing 0.08 ml heparin and centrifuged (Eppendorf AG, model 5418R) for 10 min at 5000 rpm and a standard temperature of 4°C. The supernatant (300 $\mu$l) of each Eppendorf tube was removed and transferred to a new tube, which was stored in a freezer at −70°C for later determination by chemiluminescence (specificity of 93.9%). Each arterial blood sample was also processed for the determination of plasma Big ET-1 levels (pg/ml). The collected sample was immediately divided into three

aliquots in Eppendorf tubes. Each tube contained 0.1 ml EDTA/1.5 ml blood and 0.1 ml aprotinin/1.5 ml blood each. To reach this value, 0.39 mg EDTA was diluted in 350 ml 0.9% saline solution and 0.39 mg aprotinin was diluted in 350 ml 0.9% saline solution. The sample was then centrifuged in the same apparatus as that used for the sample used for quantifying NO levels for 15 min but at 1600 rpm and a temperature of 0°C. The supernatant (300 ml) of each Eppendorf tube was then removed, transferred to a new tube, and stored in a freezer at -70°C for later determination by ELISA (sensitivity of 0.30 pg/ml and specificity of 100%). The sensitivity for this kit was determined using the guidelines provided by the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols. (National Committee for Clinical Laboratory Standards Evaluation Protocols, SC1, (1989) Villanova, PA, USA: NCCLS).

Pulse oximetry (DIXTAL, model DX 2022) was recorded from 7pm to 7am for all volunteers. A finger sensor placed on the index finger contralateral to the arm used for BP measurement by ABPM device was used in order to obtain the level of arterial oxyhemoglobin saturation (O2 Sat).

The polysomnography study was performed in the Clinical Neurophysiology Laboratory of HCFMRP-USP using the Biologic Sleepscan Vision PSG Polygraph (NatusBiologic Systems, San Carlos, CA, USA) to obtain the AHI/h, as previously described, the total number of apnea and hypopnea (NAH) episodes during sleep, and the number of NAM.

The mean and standard deviation was calculated for data with a normal distribution (parametric), and the median was calculated for non-parametric data (those regarding Big ET-1 and NO). The SAS®9.0 software was used for these analyses. Fisher's exact test was used to describe the frequencies according to gender. To determine possible correlations between the variables, Pearson's correlation coefficient was calculated for parametric data and Spearman's correlation coefficient was calculated for non-parametric data using R software. For the study of Big ET-1 and NO, we corrected group comparison including BP, BMI and NAH in the analysis since these are factors that may influence the final result (univariate analysis of variance).The study was submitted to the Ribeirao Preto School of Medicine's Ethics Committee and approved in accordance with protocol number 14103. All participants received detailed information about the goals and procedures of the study, and they signed an informed consent form in compliance with Resolution 196/96 of the National Health Council.

## Results

Table 1 shows the participant characteristics obtained at the initial clinical evaluation. No difference was observed between the groups regarding the distribution of antihypertensive medications used. Between group 1 and group 2, the respective use was as follows: diuretics, 68% and 66.6%; angiotensin-converting enzyme inhibitors or angiotensin receptor antagonists, 68% and 58.3%; calcium channel antagonists, 20% and 25%; and beta-blockers, 32% and 50%.

## Original Article

Table 1 - Anthropometric and clinical characteristics of group 1 (hypertensive patients with obstructive sleep apnea-hypopnea syndrome) and group 2 (hypertensive patients without obstructive sleep apnea-hypopnea syndrome)

| Characteristics | Group 1 (25) | Group 2 (12) |
|---|---|---|
| Age | 67.0 ± 6.5 | 67.8 ± 6.8 |
| Weight (G) | 78.8 ± 15.1 | 75.8 ± 13.1 |
| Height (M) | 1.60 ± 0.11 | 1.60 ± 0.09 |
| BMI (kg/m²) | 30.3 ± 4.8 | 29.0 ± 5.0 |
| Number of antihypertensive medications | 1.9 ± 0.9 | 2.3 ± 0.9 |
| Hours of sleep | 5.3 ± 0.8 | 5.1 ± 1.6 |

*Values are reported as mean ± standard deviation (unpaired Student t-test, p > 0.05); BMI: Body mass index*

Table 2 lists the BP values obtained by ABPM and by the auscultatory method.; no significant differences existed between the groups ($p > 0.05$). The decrease in BP during the sleep period compared with that during the wakefulness period was as follows: $8.2 \pm 9.5$ mmHg for systolic BP (SBP) and $7.4 \pm 6.1$ mmHg for diastolic BP (DBP) in group 1 (percentage decrease: $6.6 \pm 7.5\%$ mmHg for SBP and $9.8 \pm 8.2\%$ for DBP); $6.4 \pm 9.5$ mmHg for SBP and $5.8 \pm 6.5$ mmHg for DBP in group 2 (percentage decrease: $4.6 \pm 7.8\%$ for SBP and $6.7 \pm 8.5\%$ mmHg for DBP).

Evaluation of the BP measurements obtained for the volunteers as a whole revealed a good statistical correlation between the measurements obtained by auscultation and 24-h ABPM (SBP: $p < 0.001$, $r = 0.68$; DBP: $p < 0.001$, $r = 0.61$).

Table 3 presents the values of NAH, AHI, and NAM, as well as the minimum and mean $O_2$ saturation levels recorded during the sleep period ($p < 0.001$).

Plasma NO levels determined at 19:00 h and 7:00 h (Figure 1) did not differ between the groups ($p > 0.05$). However, plasma Big ET-1 levels differed between the groups at 7am only, when it increased in the OSAHS group following sleep (Figure 2).

When group 1 and group 2 were analyzed jointly, no correlations were found between BP and several variables, including Big ET-1, NO, $O_2$ saturation, AHI, and NAM. However, a correlation was observed between BMI and the mean $O_2$ saturation level ($r = -0.43$, $p = 0.008$) and between BMI and NAM ($r = 0.51$, $p = 0.002$) (Figure 3).

Considering only the group with OSAHS, we detected significant correlations between *auscultatory* SBP and Big ET-1 ($r = 0.42$, $p = 0.04$), 24-hour systolic ABPM and mean $O_2$ Sat ($r = -0.44$, $p = 0.03$), and 24-hour DBP and NAM ($r = 0.50$, $p = 0.01$).

The $O_2$ saturation levels were negatively correlated with Big ET-1 ($r = -0.41$, $p = 0.04$).

Considering only the group without OSAHS, we observed that sleep SBP recorded by ABPM was correlated with NAH ($r = 0.71$ $p = 0.01$) and AHI ($r = 0.67$, $p = 0.02$). Inverse correlation was observed between the $O_2$ saturation level and NAM ($r = -0.61$, $p = 0.05$) and the $O_2$ saturation level and BMI ($r = -0.58$, $p = 0.05$).

## Discussion

Today, sleep changes are highly prevalent in the general population, in particular the elderly. Epidemiological studies have pointed out that BP elevation is associated with sleep disorders[13,14] due to the high AHI[9], resulting in daytime hypersomnolence and cardiorespiratory changes[14]. Furthermore, Mary et al[8] stated that BP elevation occurs at the end of each episode of obstructive apnea.

When evaluating BP by means of ABPM, we did not observe differences in systolic (S) BP or diastolic (D) BP between subjects with and without OSAHS during sleep, wakefulness and during a 24-hour period. This result differs from that reported by Martinez Garcia et al[9] who observed higher SBP and DBP in patients with OSAHS, but agrees with the report of Davies et al[15] who did not detect a difference in wakefulness or 24-hour SBP between groups with and without OSAHS, and also with an Oxford study cited by Davies et al[15] which, when comparing SBP and DBP between the same groups, did not detect a difference during sleep or wakefulness. In our study, the BP decrease during the sleep period was the same between the groups with and without OSAHS, and both groups showed an attenuation of the decline in BP, with values <10%. It is important to point out that the two groups studied were composed of controlled hypertensive subjects continuously taking antihypertensive medications, with no differences in the number of medications taken. However, the small number of volunteers may have limited the power for detecting differences in BP between the groups.

The result of conventional BP measurement performed at 19:00 h and 7:00 h by auscultatory method by a trained investigator correlated well with the 24-h BP obtained by ABPM. This suggests that where skills exist, auscultatory method can be used in clinical practice when ABPM is not readily available, or at times when periodic reevaluation is necessary. However, it is important to note that ABPM is the gold standard for BP evaluation[16], and that obtaining data in this manner allowed the study to take be conducted during sleep and wakefulness.

An important result of the present study was the difference in minimum and mean $O_2$ saturation levels observed during sleep, with the OSAHS group showing significant hypoxemia, as expected. Lima et al[17] observed the same hypoxic effect in patients with sleep apnea compared to healthy individuals, and similar data were obtained by Peled et al[12].

**Table 2 -** Systolic blood pressure (SBP) and diastolic blood pressure (DBP) during ambulatory blood pressure monitoring (ABPM) and blood pressure measurement by the auscultatory method in group 1 (hypertensive patients with sleep apnea-hypopnea syndrome) and group 2 (hypertensive patients without sleep apnea-hypopnea syndrome)

| BP | Mean | BP | Group 1 (25) | Group 2 (12) |
|---|---|---|---|---|
| ABPM | 24-Hours | SBP | 122 ± 12 | 127 ± 13 |
| | | DBP | 72 ± 11 | 74 ± 10 |
| | Sleep | SBP | 116 ± 15 | 122 ± 13 |
| | | DBP | 68 ± 12 | 70 ± 8 |
| | Wakefulness | SBP | 123 ± 13 | 127 ± 15 |
| | | DBP | 75 ± 10 | 76 ± 11 |
| Auscultatory | At 19:00 Hours | SBP | 130 ± 22 | 132 ± 12 |
| | | DBP | 79 ± 13 | 78 ± 9 |
| | At 7:00 Hours | SBP | 129 ± 17 | 144 ± 22 |
| | | DBP | 80 ± 11 | 85 ± 11 |

Values are reported as mean ± standard deviation (unpaired Student t-test, $p > 0.05$ group 1 vs. group 2). BP: Blood pressure.

**Table 3 -** Number of awakenings and microawakenings (NAM), number of apnea-hypopnea (NAH) episodes and apnea-hypopnea index (AHI) obtained from polysomnography, and minimum oxygen saturation (min $O_2$ saturation) and mean oxygen saturation (mean $O_2$ saturation) in group 1 (hypertensive patients with sleep apnea-hypopnea syndrome) and group 2 (hypertensive patients without sleep apnea-hypopnea syndrome)

| Variables | Group 1 (25) | Group 2 (12) |
|---|---|---|
| NAM | 259.7 ± 133.9 | 140.2 ± 66.0* |
| NAH | 140.2 ± 66.0 | 17.3 ± 11.0* |
| AHI | 29.0 ± 13.7 | 3.1 ± 1.6* |
| Minimum $O_2$ Saturation | 80.7 ± 7.1 | 92.3 ± 4.5* |
| Mean $O_2$ Saturation | 91.1 ± 3.8 | 94.6 ± 2.4* |

Values are reported as mean ± standard deviation (unpaired Student t-test, *$p < 0.001$ vs. group 1).
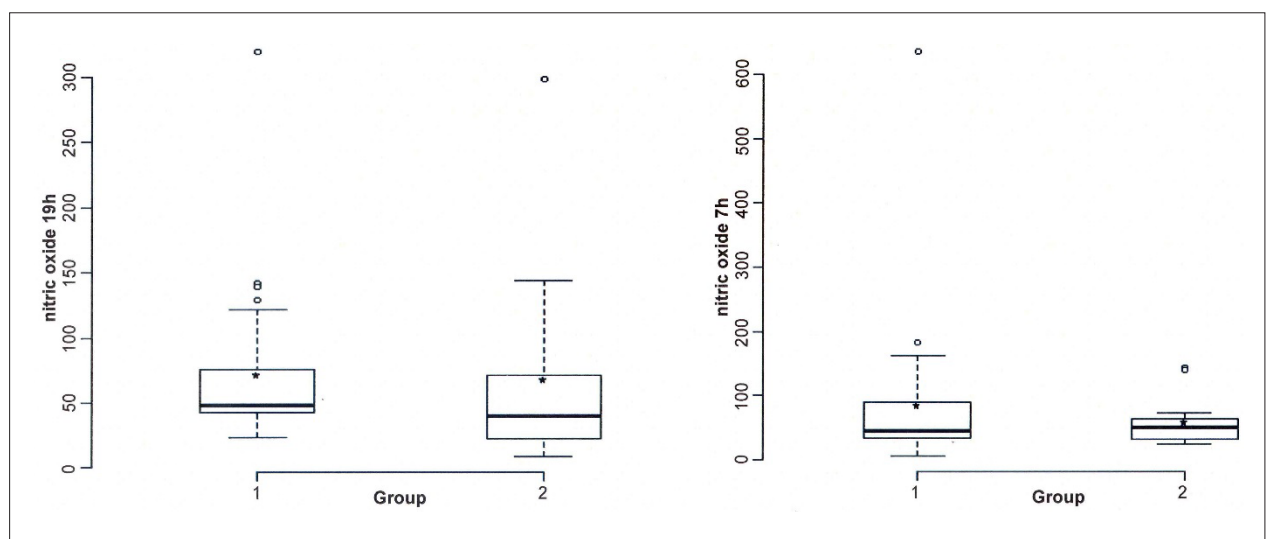


**Figure 1 -** *Plasma levels of Nitric oxide (µM) in group 1 (OSAHS) and group 2 (no OSAHS).*
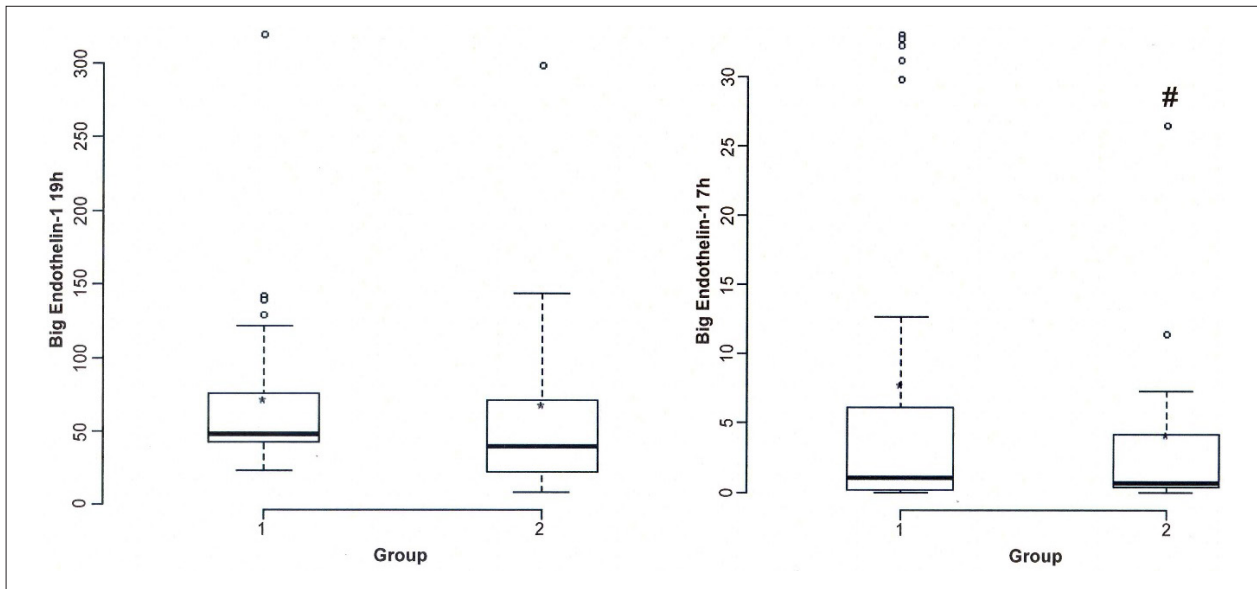
# Original Article



**Figure 2 -** *Plasma levels of Big-Endothelin-1 (pg/ml) in group 1 (OSAHS) and group 2 (no OSAHS).*
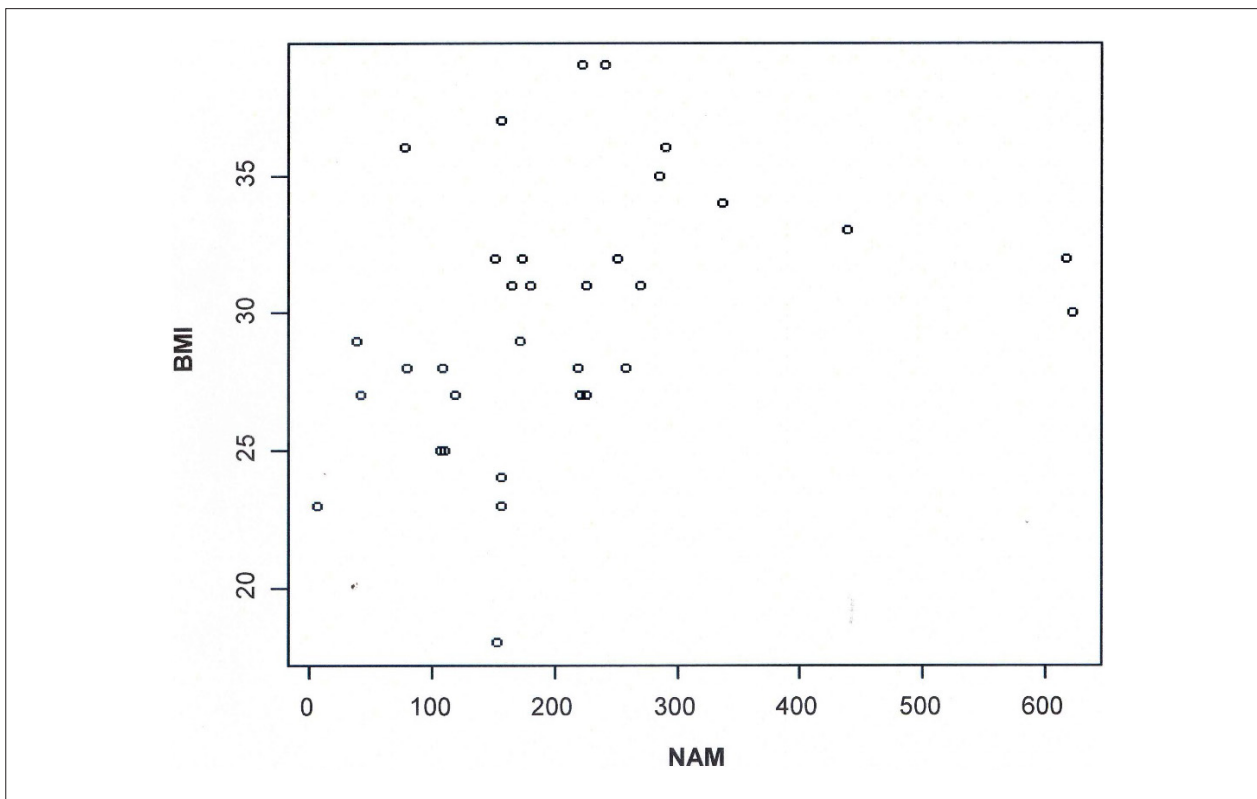


**Figure 3 -** *Correlation between body mass index (BMI) and number of awakenings and microawakenings (NAM) in hypertensive patients with and without OSAHS (Spearman, r = 0.51, p = 0.002).*

Anunciato et al.
Big endothelin-1 and nitric oxide in OSAHS

**Original Article**

When we evaluated the respiratory events by polysomnography we observed significant differences between groups regarding NAM, NAH and AHI, in agreement with published reports, since these are relevant data for the characterization of individuals. These data agree with those reported byr Lima et al[17] and Ventura et al[18].

The clinical importance of NO regarding BP has been well defined since this powerful vasodilator directly affects the tonus of peripheral blood vessels and its inhibition generates hypertension [7]. Basal NO activity is known to be reduced in hypertensive patients[19]. Ip et al[8] stated that endothelial NO can also play an important role in BP regulation in individuals with OSAHS. However, literature reports have shown that NO deficiency can be reversed in hypertensive individuals by the administration of antihypertensive medications[3,20]. A study on hypertensive mice demonstrated that, after the use of captopril, no inhibition of NO synthesis was observed, with consequent BP regulation. Another antihypertensive agent that results in BP attenuation and improved NO-mediated vasodilation, is spironolactone[20]. As already mentioned, basal NO levels in the present study did not differ between the hypertensive individuals with and without OSAHS. It should be pointed out that both groups consisted of hypertensive subjects controlled with antihypertensive medications *with controlled BP*. Based on these data, we can suggest that NO activity is equilibrated in cases of treated hypertension.

Studies conducted on elderly persons have demonstrated an increase in plasma ET-1 levels compared with young individuals[4]. Zamarron-Sanz et al[21] confirmed a significant increase in ET-1 levels in individuals with OSAHS compared to healthy subjects. Jordan et al[22] detected increased plasma Big ET-1 levels in a group of patients with OSAHS not receiving clinical or surgical treatment. In the present study there was a higher Big ET-1 concentration in individuals with OSAHS at 7am, after the sleep period.

In general, plasma ET-1 levels tend to be low and there is no consensus on a definitive protocol that would facilitate the reproducible determination of ET-1 or Big ET-1 levels in different biological fluids. There are many variations in the extraction protocols above basal values, ranging from 0.5 to 50 pg/ml, with comparison of the results obtained often being difficult[23]. In the present study, Big ET-1 determinations showed wide variability although identical processing and storage techniques were used.

Evaluation of the variables in the OSAHS group revealed a negative correlation between SBP and $O_2$ saturation levels, i.e., SBP increased with decreasing arterial oxygenation. Moreover, increased Big ET-1 levels were correlated with higher $O_2$ saturation levels, and SBP was positively correlated with Big ET-1 levels; thus, the greater the vasoconstriction caused by Big ET-1, the higher the SBP. Further, we observed a positive correlation between NAH, AHI, and plasma Big ET-1 levels, suggesting a possible increase in vasoconstriction associated with increasing NAH episodes. No matter how small the number of awakenings among individuals with the syndrome, an increase in sympathetic tonus will occur, with a marked elevation not only of BP, but also of heart rate. Depending on the intensity

of sleep apnea, this process may occur hundreds of times during the night[24]. Thus, NAM during sleep contributes to autonomic hyperactivity as one of the mechanisms that explains hypertension associated with OSAHS[25]. In the present study, a positive correlation was observed between NAM and DBP (during sleep, wakefulness, and on 24-h ABPM), with the increase in DBP being related to the greater sleep fragmentation during the total sleep time. We did not observe a significant correlation between NAM and SBP ($p > 0.05$).

Martinez-Garcia et al[9] found a more significant correlation between NAM and DBP (during sleep and wakefulness). In contrast, Logan et al[26] observed a greater correlation between sleep fragmentation and SBP.

On comparing BMI with NAM, we observed that a higher BMI was correlated with an increase in NAM, suggesting that BMI may interfere with the sleep of individuals with OSAHS. According to Ware, McBrayer and Scott, apnea events are highly sensitive to changes in BMI, i.e., the greater the weight, the higher the probability of apneic events[27]. As described earlier, obesity is an established, important pathogenic factor for OSAHS[14].

Approximately 70% of individuals diagnosed with sleep apnea are considered to be obese according to their BMI, with this being the only significant risk factor that is reversible[10]. In the present group without OSAHS, an increased number of NAH and an increased AHI were correlated with SBP elevation. Grote et al[28] in studying the influence of AHI on BP, observed that the probability of uncontrolled hypertension increases by 2% with each unit increase in AHI.

Antczak et al.[29] reported that there is evidence that obesity, even in the absence of respiratory sleep disorders, negatively affects the quality of sleep, increasing daytime somnolence. Furthermore, studies have observed that obese patients without OSAHS had a higher frequency of awakenings during sleep compared with patients of normal weight[30,31]. On this basis, we believe that, regardless of whether an individual has OSAHS or not, obesity is an extremely important factor regarding the quality of sleep. In the present study, groups did not differ in terms of the prevalence of obesity.

In addition, we observed that obesity negatively influences $O_2$ saturation levels, with an increased BMI causing greater desaturation. When correlating NAM with $O_2$ saturation levels, we observed a decline in saturation with increasing sleep fragmentation.

In conclusion, elderly hypertensive subjects with OSAHS presented a greater Big-ET-1 level after the sleep period regardless of BP, since their BP was controlled and similar to the SBP of hypertensive elderly subjects without OSAHS. In patients with OSAHS, a correlation was observed between Big ET-1 and SBP; Big ET-1 and NAH; and $O_2$ saturation and SBP. In both groups, a higher BMI was correlated with greater desaturation and NAM. NO levels did not differ between the hypertensive patients with or without OSAHS. It is noteworthy that both groups consisted of hypertensive patients controlled with antihypertensive medications.

## Original Article

## References

1. Stankevičius E, Kėvelaitis E, Vainorius E, Simonsen U. [Role of nitric oxide and other endothelium-derived factors]. Medicina (Kaunas). 2003;39(4):333-41.

2. Lytsy P, Lind L, Sundström J. Endothelial function and risk of hypertension and blood pressure progression: the prospective investigation of the vasculature in Uppsala seniors. J Hypertens. 2013;31(5):936-9.

3. Virdis A, Ghiadoni L, Taddei S. Effects of antihypertensive treatment on endothelial function. Curr Hypertens Rep. 2011;13(4):276-81.

4. Thijssen DH, Rongen GA, van Dijk A, Smits P, Hopman MT. Enhanced endothelin-1-mediated leg vascular tone in healthy older subjects. J Appl Physiol. 2007;103(3):852-7.

5. Gratton JP, Rae GA, Bkaily G, D'Orléans-Juste P. ET(B) receptor blockade potentiates the pressor response to big endothelin-1 but not big endothelin-2 in the anesthetized rabbit. Hypertension. 2000;35(3):726-31.

6. Vessières E, Freidja ML, Loufrani L, Fassot C, Henrion D. Flow (shear stress)-mediated remodeling of resistance arteries in diabetes. Vascul Pharmacol. 2012;57(5-6):173-8.

7. Spieker LE, Noll G, Ruschitzka FT, Maier W, Luscher TF. Working under pressure: the vascular endothelium in arterial hypertension. J Hum Hypertens. 2000;14(10-11):617-30.

8. Ip MS, Lam B, Chan LY, Zheng L, Tsang KW, Fung PC, et al. Circulating nitric oxide is suppressed in obstructive sleep apnea and is reversed by nasal continuous positive airway pressure. Am J Respir Crit Care Med. 2000;162(6):2166-71.

9. Martinez-Garcia MA, Gomes-Aldaravi R, Gil-Martinez T, Soler-Cataluna JJ, Bernacer-Alpera B, Roman-Sanches P. [Sleep-disordered breathing in patients with difficult-to-control hypertension]. Arch Bronconeumol. 2006;42(1):14-20.

10. Martins AB, Tufik S, Moura SM. Physiopathology of obstructive sleep apnea-hypopnea syndrome. J Bras Pneumol. 2007;33(1):93-100.

11. Oztürk E, Dursunoğlu N, Dursunoğlu D, Ozkurt S, Rota S. [Evaluation of serum adiponectin levels in patients with obstructive sleep apnea syndrome]. Turk Kardiyol Dern Ars. 2012;40(6):505-12.

12. Peled N, Shitrit D, Bendayan D, Peled E, Kramer MR. Association of elevated levels of vascular endothelial growth factor in obstructive sleep apnea syndrome with patient age rather than with obstructive sleep apnea syndrome severity. Respiration. 2007;74(1):50-5.

13. Vijayan VK. Morbidities associated with obstructive sleep apnea. Expert Rev Respir Med. 2012;6(5):557-66.

14. Zhang W, Si LY. Obstructive sleep apnea syndrome (OSAS) and hypertension: pathogenic mechanisms and possible therapeutic approaches. Ups J Med Sci. 2012;117:370-382.

15. Davies CW, Crosby JH, Mullins RL, Barbour C, Davies RJ, Stradling JR. Case-control study of 24 hour ambulatory blood pressure in patients with obstructive sleep apnoea and normal matched control subjects. Thorax. 2000;55(9):736-40.

16. Marui FR, Bombig MT, Francisco YA, Thalenberg JM, Fonseca AH, Souza Dd, et al. [Assessment of resistant hypertension with home blood pressure monitoring]. Arq Bras Cardiol. 2010;95(4):536-40.

17. Lima AM, Franco CM, Castro CM, Bezerra Ade A, Ataíde LJ, Halpern A. [Obstructive sleep apnea contribution to oxidative stress in obesity]. Arq Bras Endocrinol Metabol. 2008;52(4):668-76.

18. Ventura C, Oliveira AS, Dias R, Teixeira J, Canhão C, Santos O, et al. The role of nocturnal oximetry in obstructive sleep apnoea-hypopnoea syndrome screening. Rev Port Pneumol. 2007;13(4):525-51.

19. Ruschitzka F, Quaschning T, Noll G, deGottardi A, Rossier MF, Enseleit F, et al. Endothelin 1 type A receptor antagonism prevents vascular dysfunction and hypertension induced by 11beta-hydroxysteroid dehydrogenase inhibition: role of nitric oxide. Circulation. 2001;103(25):3129-35. Erratum in Circulation. 2001;104(10):1208.

20. Torok J. Participation of nitric oxide in different models of experimental hypertension. Physiol Res. 2008;57(6):813-25.

21. Zamarrón-Sanz C, Ricoy-Galbaldon J, Gude-Sampedro F, Riveiro-Riveiro A. Plasma levels of vascular endothelial markers in obstructive sleep apnea. Arch Med Res. 2006;37(4):552-5.

22. Jordan W, Reinbacher A, Cohrs S, Grunewald RW, Mayer G, Ruther E, et al. Obstructive sleep apnea: plasma endothelin-1 precursor but not endothelin-1 levels are elevated and decline with nasal continuous positive airway pressure. Peptides. 2005;26(9):1654-60.

23. Shaw SG, Schmid M, Casty A. Critical factors in the radioimmunoassay of endothelin-1, endothelin-3, and big endothelin-1 in human plasma. Anal Biochem. 2000;278(2):143-9.

24. Somers VK, White DP, Amin R, Abraham WT, Costa F, Culebras A, et al; American Heart Association Council for High Blood Pressure Research Professional Education Committee, Council on Clinical Cardiology; American Heart Association Stroke Council; American Heart Association Council on Cardiovascular Nursing; American College of Cardiology Foundation. Sleep apnea and cardiovascular disease: an American Heart Association/american College Of Cardiology Foundation Scientific Statement from the American Heart Association Council for High Blood Pressure Research Professional Education Committee, Council on Clinical Cardiology, Stroke Council, and Council On Cardiovascular Nursing. In collaboration with the National Heart, Lung, and Blood Institute National Center on Sleep Disorders Research (National Institutes of Health. Circulation. 2008;118(10):1080-111. Erratum in: Circulation. 2009;119(12):e380.

25. Bursztyn M, Ben-Dov IZ. Diabetes mellitus and 24-hour ambulatory blood pressure monitoring: broadening horizons of risk assessment. Hypertension. 2009;53(2):110-1.

26. Logan AG, Perlikowski SM, Mente A, Tisler A, Tkacova R, Niroumand M, et al. High prevalence of unrecognized sleep apnoea in drug resistant hypertension. J Hypertens. 2001;19(12):2271-7.

27. Ware JC, McBrayer RH, Scott JA. Influence of sex and age on duration and frequency of sleep apnea events. Sleep. 2000;23(2):165-70.

28. Grote L, Hedner J, Peter JH. Sleep-related breathing disorder is an independent risk factor for uncontrolled hypertension. J Hypertens. 2000;18(6):679-85.

29. Antczak J, Horn B, Richter A, Jernajczyk W, Bodenschatz R, W Schmidt EW. The influence of obesity on sleep quality in male sleep apnea patients before and during therapy. J Physiol Pharmacol. 2008;59 Suppl 6:123-34.

30. Resta O, Foschino Barbaro MP, Bonfitto P, Giliberti T, Depalo A, Pannacciulli N, et al. Low sleep quality and daytime sleepiness in obese patients without obstructive sleep apnoea syndrome. J Intern Med. 2003;253(5):536-43.

31. Vgontzas AN, Bixler EO, Tan TL, Kantner D, Martin LF, Kales A. Obesity without sleep apnea is associated with daytime sleepiness. Arch Intern Med. 1998;158(12):1333-7.