

Arginase activity and nitric oxide levels in patients with obstructive sleep apnea syndrome

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OBJECTIVE: Obstructive sleep apnea syndrome is characterized by repetitive obstruction of the upper airways, and it is a risk factor for cardiovascular diseases. There have been several studies demonstrating low levels of nitric oxide in patients with obstructive sleep apnea syndrome compared with healthy controls. In this study, we hypothesized that reduced nitric oxide levels would result in high arginase activity. Arginase reacts with L-arginine and produces urea and L-ornithine, whereas L-arginine is a substrate for nitric oxide synthase, which produces nitric oxide.

METHODS: The study group consisted of 51 obstructive sleep apnea syndrome patients (M/F: 43/8; mean age 49 ± 10 years of age) and 15 healthy control subjects (M/F: 13/3; mean age 46 ± 14 years of age). Obstructive sleep apnea syndrome patients were divided into two subgroups based on the presence or absence of cardiovascular disease. Nitric oxide levels and arginase activity were measured via an enzyme-linked immunosorbent assay of serum samples.

RESULTS: Serum nitric oxide levels in the control subjects were higher than in the obstructive sleep apnea patients with and without cardiovascular diseases ($p < 0.05$). Arginase activity was significantly higher ($p < 0.01$) in obstructive sleep apnea syndrome patients without cardiovascular diseases compared with the control group. Obstructive sleep apnea syndrome patients with cardiovascular diseases had higher arginase activity than the controls ($p < 0.001$) and the obstructive sleep apnea syndrome patients without cardiovascular diseases ($p < 0.05$).

CONCLUSION: Low nitric oxide levels are associated with high arginase activity. The mechanism of nitric oxide depletion in sleep apnea patients suggests that increased arginase activity might reduce the substrate availability of nitric oxide synthase and thus could reduce nitric oxide levels.

KEYWORDS: Obstructive Sleep Apnea Syndrome; Nitric Oxide; Arginase Activity; Cardiovascular Diseases.

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INTRODUCTION

Obstructive sleep apnea syndrome (OSA) is characterized by repeated episodes of upper airway obstruction during sleep (1). Obstruction of the upper airway can lead to a decrease in blood oxygenation and fragmentation of the sleep cycle (2). Intermittent arterial oxygen desaturation is believed to be a major cause of cardiovascular alterations, which occur in patients with OSA. Therefore, OSA has been associated with cardiovascular disorders such as arterial hypertension, coronary artery disease, and cerebrovascular

diseases, and it is an independent etiologic factor for cardiovascular morbidity and mortality (1).

Nitric oxide (NO), which is synthesized from L-arginine via endothelial nitric oxide synthase (NOS), is a mediator that induces vasodilatation, inhibits platelet aggregation, and prevents adhesion of platelets to endothelial cells (3). Studies have indicated that a primary mechanism of NO action is the inhibition of smooth muscle cell contraction (2). Thus, long-term complications of OSA, including systemic and pulmonary hypertension, myocardial infarction, and stroke, might be influenced by NO dysregulation. Reports have shown that NO levels in OSA patients are lower than in healthy controls (4-8). Asymmetrical dimethyl arginine (ADMA), an endogenous competitive inhibitor of NOS, has been shown to be higher in the plasma of OSA patients, indicating the potential importance of NOS inhibition (9).

Arginase, an enzyme of the hepatic urea cycle, produces L-ornithine and urea from L-arginine (10,11). Two isoforms of arginase have been cloned: arginase I is mainly expressed

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in hepatic cell cytosol, and arginase II is expressed in the mitochondria of extrahepatic tissues, such as kidney and brain cells, monocytes, and macrophages (12,13). It is important to note that arginase can also be expressed in the immune system cells of humans and thus can lead to inflammation (11). It was demonstrated that arginase could be identified in human atherosclerotic plaques, mainly colocalized with macrophages around necrotic cores (14). Inflammatory processes can lead to endothelial dysfunction and result in cardiovascular diseases (15). Previous studies have shown that arginine administration could improve vascular reactivity and serve as an atheroprotective. Furthermore, NO production from arginine via endothelial NOS results in vasorelaxation and is generally regarded as atheroprotective because arginase can inhibit NO synthesis by competing with NOS for L-arginine, although the affinity of L-arginine is much greater for NOS than for arginase (10).

It is very important to determine the mechanism of NO reduction in OSA patients. We hypothesized that low NO levels in OSA patients would result in high arginase activity, which is an alternative pathway for L-arginine metabolism. To test this hypothesis, we determined the arginase activity and NO levels in patients with OSA. We also stratified our patient population according to the presence of cardiovascular disorders and compared these two subgroups, i.e., OSA patients with a proven cardiovascular disease (OSA CV(+)) and OSA patients without any cardiovascular disease (OSA CV(-)).

■ PATIENTS AND METHODS

Patient population

The study population consisted of 51 (M/F, 43/8) patients with OSA and 15 control subjects (M/F, 13/3), all of whom were examined using computerized polysomnography. All of the sleep studies were performed in the Sleep Disorders Laboratory of Süreyyapaşa Chest Diseases Hospital in Istanbul. All of the patients underwent a standard battery of examinations, including medical history, physical and cardiological examinations (blood pressure, heart rate, electrocardiography [ECG]), and laboratory screening tests. They were asked to complete an Epworth Sleepiness Scale and a questionnaire regarding sleep symptoms and medical history. Anthropometric measurements of body weight and height were obtained with the patient dressed only in underwear. BMI was calculated as body weight divided by the square of height. Diagnosis of OSA was established on the basis of clinical and polysomnographic criteria (16).

All of the patients had complaints of snoring, witnessed apnea, daytime sleepiness, and frequent night awakenings. The results of the sleep study were automatically recorded and were subsequently reviewed to ensure the accuracy of the data. OSA was diagnosed based on a review of all of the data.

An Apnea Hypopnea Index (AHI) >5 was diagnosed as OSA. The control group was recruited from healthy people who were free of medications and had no cardiovascular disease or respiratory disorders. All of the OSA patients and controls were free of diabetes mellitus type 2 and other known metabolic disorders, respiratory infections, and other respiratory disorders at the time of polysomnography. Routine ECG, echocardiography, and history revealed that 10 patients with OSA also had cardiovascular diseases, i.e., coronary artery disease (n = 5), myocardial infarction (n = 2),

and hypertensive heart disease (n = 3), with a mean duration time of 8 ± 4 years. Therefore, we divided the OSA patients into two subgroups: patients with cardiovascular diseases (OSA CV(+)) and without cardiovascular diseases (OSA CV(-)). The study protocol was approved by the local research ethics committee, and all of the subjects provided written informed consent.

Polysomnography

All of the patients underwent full-night PSG (Comet-Grass Technologies Astro-Med, West Warwick, Rhode Island, USA). Standard polysomnographic montages were used as follows: Four-channel electroencephalography (EEG, C4-A2, C3-A1, O2-A1, O1-A2); left and right electro-oculography (EOG); submental electromyography (EMG), a nasal cannula to record nasal pressure and a thermistor to monitor nasal and oral airflow; left and right anterior tibialis movement sensors; respiratory effort by thoraco-abdominal belts; ECG; finger pulse oximetry; a neck microphone for recording snoring; and a sensor on the thoracic belt to record the body posture. PSG recordings were continued for at least 6 hours.

Sleep stage and respiratory event scorings have been described previously (17,18). Briefly, apnea was defined as complete cessation of airflow lasting for at least 10 seconds, whereas hypopnea was defined as a decrease in airflow, if it was associated with either arousal (defined as the appearance for 3 seconds of an alpha rhythm on EEG channels or an increase in the submental EMG signal) or oxygen desaturation of $\geq 3\%$. AHI was calculated by dividing the total number of apnea and hypopnea events by the total sleep time. The minimal oxygen saturation was determined as the lowest saturation value associated with a respiratory event. The Oxygen Desaturation Index (ODI) was the number of times per hour of sleep that the blood's oxygen level decreased by 3% or more from baseline. The average numbers of episodes of apnea and hypopnea per hour of sleep were calculated and expressed as the AHI. In addition to clinical symptoms, an AHI of >30 was also used as an inclusion criterion for the patient population, and an AHI of <5 was used for the nonapneic healthy controls. On the morning after PSG, blood pressure was measured with a pneumoelectric micro-processor-controlled instrument and was recorded. Additionally, fasting blood samples were obtained in the morning after the sleep study.

Blood sampling

Peripheral venous blood samples were obtained in the morning after the diagnostic study night. The blood samples, centrifuged at $4,000 \times g$ for 10 min, and serum samples were aliquoted and stored at -20°C for analysis. Additionally, liver function tests, renal function tests, and thyroid function tests were performed to determine comorbid disorders. Circulating total cholesterol, triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), glucose, and hsCRP measurements were also obtained by enzymatic methods, using commercial kits for standard evaluations.

Measurement of nitric oxide levels and arginase activity

NO rapidly degrades into nitrate and nitrite. Spectrophotometric quantitation of nitrite using Griess reagent is sensitive,



but it does not measure nitrate. With a commercial kit, the reduction of nitrate to nitrite was performed using NADH-dependent enzyme nitrate reductase, followed by spectrophotometric analysis of total nitrite using Griess reagent (OxisResearch, Portland, OR, USA).

The serum activity of arginase I was quantified according to the manufacturer's instructions and guidelines for using the enzyme-linked immunosorbent assay (ELISA) kit, based on the sandwich principle specific for humans (Hycult Biotech, Uden, the Netherlands).

All of the biochemical parameters were measured in duplicate, and the mean value was used in the analyses.

Data analysis

Statistical analyses were performed with the GraphPad InStat for Windows (San Diego, CA, USA) computer program. Values are expressed as means \pm SDs. Comparisons of two groups were performed using Student's *t* test; comparisons of more than two independent groups were undertaken by analysis of variance (ANOVA) with Tukey's post hoc test. Additionally, Spearman's rank correlation analysis and multiple linear regression analysis were applied to the data. Significance was determined as $p < 0.05$.

RESULTS

The demographic and anthropometric parameters, medications, cardiovascular findings, sleep tests, and biochemical analysis results for the control subjects and OSA patients are shown in Table 1.

Comparisons showed significantly higher weight and BMI values in the OSA patients compared with the control group ($p < 0.05$). AHI, ODI, and hsCRP levels OSA were significantly higher in the OSA patients than in the control subjects ($p < 0.001$ for both), but the two study groups were similar in terms of serum lipid parameters and fasting glucose level. NO levels in the OSA patients and control subjects were $28.1 \pm 24.1 \mu\text{M}$ and $43.4 \pm 32.1 \mu\text{M}$, respectively ($p < 0.05$). Arginase activity was significantly higher in the OSA group compared with the control group ($54.7 \pm 27.0 \text{ ng/ml}$ vs. $24.6 \pm 27.6 \text{ ng/ml}$; $p < 0.001$).

As shown in Figure 1, the NO levels in the OSA CV(-) patients were lower than in the healthy controls, but the difference failed to reach statistical significance. However, the OSA CV(+) patients had significantly lower ($p < 0.05$) NO levels than the controls (Figure 1). Arginase activity was significantly increased ($p < 0.01$) in the OSA CV(-) patients compared with the healthy group. The OSA CV(+) patients had significantly higher arginase activity than the OSA CV(-) patients ($p < 0.05$) and the control subjects ($p < 0.001$; Figure 2).

There were positive correlations between hsCRP and NO levels ($r = 0.311$, $p = 0.026$) and between hsCRP and AHI ($r = 0.415$, $p = 0.002$) in the OSA patients with and without cardiovascular diseases. hsCRP and arginase activity were not correlated in the OSA patients ($r = -0.104$; $p = 0.466$). As expected, a negative correlation between arginase activity and NO levels was determined in the OSA patients ($r = -0.384$, $p < 0.005$). A multiple regression analysis between NO levels, arginase activity, and BMI in all of the OSA patients yielded significant results ($r^2 = 0.1579$, $p = 0.0162$). Whereas arginase activity made a significant contribution, the *p* value of which was 0.0092, the NO level did not, with

a *p*-value of 0.9854. There were no contributions in the control subjects ($p = 0.6590$).

DISCUSSION

In the present study, we demonstrated that OSA patients had significantly higher arginase activity and lower NO levels than healthy controls. In addition, these differences might have been related to the presence of cardiovascular disorders. Indeed, in further analysis, we divided OSA patients into two subgroups regarding the presence of cardiovascular disorders, and we found that CV-positive OSA patients showed the highest levels of arginase activity, whereas healthy controls showed the lowest levels of this enzyme. The mean arginase activity of the CV-negative OSA patients was higher than that of the healthy controls and lower than that of the CV-positive group. To our knowledge, this was the first study that measured arginase activity in OSA patients. Arginase activity in OSA patients was correlated with the presence of cardiovascular disorders.

There have been several studies regarding increased arginase activity in humans (19) and in experimental animal models (20,21). Jung et al. reported that arginase activity was upregulated during ischemia-reperfusion in mouse myocardial tissue. Inhibition of arginase by nor-NOHA, an arginase inhibitor, protected against myocardial infarction (20). In another study, inhibition of arginase activity in vascular smooth muscle cells by the same drug resulted in a significantly decreased rate of cell proliferation (21). In parallel with these findings, increased arginase activity was also shown to contribute to vascular endothelial dysfunction in diabetes (22). Moreover, it was demonstrated that serum arginase I activity was significantly increased and that serum arginine levels were depleted in humans after myocardial infarction (19). These authors demonstrated that arginase expression and activity were upregulated in ischemic myocardium and coronary arterioles following ischemia-reperfusion (19). All of these previous reports suggested that a limitation in NO bioavailability could lead to dysregulation of vascular function and could contribute to the development of cardiac disorders. In the present study, arginase activity was significantly higher in the OSA patients with cardiovascular disorders compared with the OSA patients without cardiovascular disease. This finding could have some implications. First, OSA might cause an increase in arginase activity, which in turn reduces NO levels through a decrease in arginine availability to nitric oxide synthase. Reduced NO levels constitute a well-known pathophysiologic mechanism for the development of cardiac disorders. Second, increased arginase activity could be a common pathophysiologic mechanism for the development of both sleep apnea and heart diseases. However, the relationship of arginase activity with sleep apnea remains to be elucidated. Arginase, which metabolizes L-arginine into urea and ornithine, competes directly with NOS for L-arginine. Hence, increases in arginase activity could decrease tissue and cellular arginine levels, reducing arginine's availability to eNOS. This process could lead to decreased NO production and increased production of superoxide by eNOS (22).

A significant amount of evidence has shown NO reduction in patients with OSA, which could be reversed by nasal continuous positive airway pressure (nCPAP)



Table 1 - Comparison of demographic, anthropometric, polysomnographic, and biochemical parameters between controls and obstructive sleep apnea syndrome patients.

	Control n= 15	OSA (all) n= 51	OSA CV(-) n= 41	OSA CV(+) n= 10
Demographic				
Mean age, year	46 ± 14	49 ± 10	48 ± 10	53 ± 6
Male/female, n	13/3	43/8	37/4	6/4
Anthropometric				
Height, cm	172 ± 10	171 ± 9	172 ± 8	165 ± 10 ^d
Weight, kg	81.8 ± 8.2	90.5 ± 13.2 ^a	91.3 ± 13.5	87.5 ± 11.5
BMI, kg/m ²	27.7 ± 3.9	31.0 ± 5.4 ^a	30.9 ± 4.9	31.5 ± 7.1
Medications				
Beta blockers		7		7
ACE inhibitors		4		4
Ca ²⁺ channel blockers		2		2
Salicylic acid	2	12	5	7
Fibrates	2	12	4	8
Statins	3	21	11	10
Cardiovascular Findings				
Heart rate (beat/min)	79 ± 4	79 ± 8	80 ± 8	78 ± 7
SBP (mm Hg)	130 ± 4	131 ± 5	130 ± 4	136 ± 4 ^{c,e}
DBP (mm Hg)	76 ± 4	79 ± 7	78 ± 7	82 ± 5
Sleep Test				
AHI, n/h	1.5 ± 1.7	55.1 ± 17.2 ^b	54.7 ± 18.6 ^b	52.5 ± 16.5 ^b
Min O ₂ , %	89.3 ± 1.6	73.1 ± 8.6 ^b	73.6 ± 7.7 ^b	71.1 ± 11.8 ^b
Mean O ₂ , %	92.2 ± 0.9	90.3 ± 4.0	90.2 ± 4.3	90.6 ± 3.5
ODI, n/h	6.3 ± 2.3	55.8 ± 18.5 ^b	56.3 ± 18.7 ^b	54.1 ± 18.8 ^b
Biochemistry				
Glucose, mg/dl	94.7 ± 4.7	109.6 ± 38.6	107.3 ± 33.3	119.1 ± 56.7
Triglyceride, mg/dl	210.4 ± 42.2	214.2 ± 40.6	211.5 ± 44.9	211.6 ± 42.8
Total C, mg/dl	203.7 ± 138.0	197.6 ± 121.2	198.8 ± 104.9	187.5 ± 154.1
HDL-C, mg/dl	44.9 ± 13.5	41.0 ± 7.4	41.2 ± 7.7	39.8 ± 5.7
LDL-C, mg/dl	118.1 ± 35.2	131.5 ± 33.1	130.9 ± 32.2	133.7 ± 36.6
VLDL-C, mg/dl	42.1 ± 27.6	37.9 ± 20.9	39.8 ± 20.9	37.4 ± 31.0
hsCRP, mg/dl	1.0 ± 0.7	5.9 ± 3.9 ^b	6.0 ± 3.6 ^b	6.2 ± 3.9 ^b

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; AHI, apnea-hypopnea index; Min O₂, minimum oxygen level; Mean O₂, mean oxygen level; ODI, oxygen desaturation index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very low-density lipoprotein cholesterol; hsCRP, high-sensitivity C-reactive protein; OSA, obstructive sleep apnea syndrome.

Comparison between the control and OSA patient (all) groups was performed using Student's t test; all of the other comparisons were performed using Tukey's post hoc test.

^ap<0.05 compared with the control group.

^bp<0.001 compared with the control group.

^cp<0.01 compared with the control group.

^dp<0.05 compared with the OSA CV(-) group.

^ep<0.01 compared with the OSA CV(-) group.

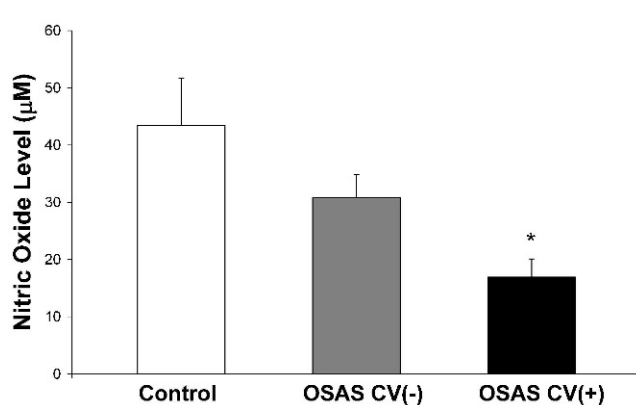


Figure 1 - Comparison of nitric oxide levels in control subjects and in obstructive sleep apnea patients without (obstructive sleep apnea syndrome cardiovascular (-)) and with cardiovascular diseases [obstructive sleep apnea syndrome cardiovascular (+)] *p<0.05 compared with the control group.

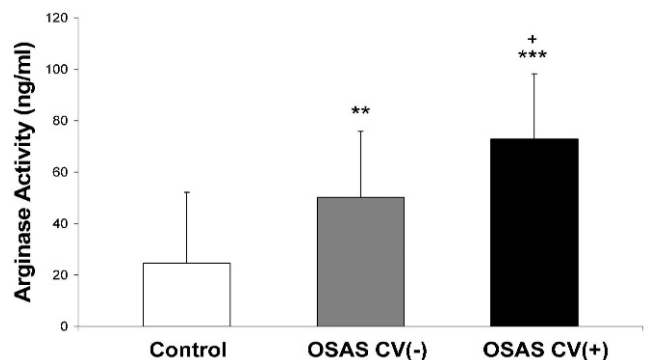


Figure 2 - Arginase activity in control subjects and in obstructive sleep apnea patients without (obstructive sleep apnea syndrome cardiovascular (-)) and with cardiovascular diseases (obstructive sleep apnea syndrome cardiovascular (+)) **p<0.01 and ***p<0.001 compared with the control group; †p<0.05 compared with the obstructive sleep apnea syndrome cardiovascular (-) group.



therapy (6,7,9,23). Plasma or serum NO levels or NO metabolites are lower in OSA patients (5,24). Some authors have reported that reduced NO levels were associated with ≥ 20 episodes of AHI (8) or mild/severe OSA (4,7). It was also concluded that short- and long-term CPAP therapy could increase NO levels (6,25), reduce oxidative stress, and improve quality of life (26) in OSA patients. Several explanatory mechanisms have been suggested for NO depletion in OSA. First, oxygen is a co-substrate of NOS, and nocturnal oxyhemoglobin desaturation might result in depressed NO synthesis (5,9). Second, oxidative stress is increased in OSA, and the superoxide anion, which is produced during oxidative phosphorylation, might react with NO to produce peroxynitrite. NO consumption in this manner could be responsible for NO depletion (27). Third, NOS inhibitors could increase in OSA and suppress NO synthesis. In fact, ADMA, a NOS inhibitor, was shown to be higher in the plasma samples of OSA patients (28). As a fourth mechanism for NO depletion in sleep apnea patients, we suggest the arginase pathway. Increased arginase activity could reduce the substrate availability of NOS and thus reduce NO levels.

Inflammation plays a critical role in cardiovascular disease pathology. It has been reported that hsCRP, an acute-phase response protein, is higher in OSA patients (15,29,30). In our study, we demonstrated that hsCRP levels were higher in OSA patients regardless of the presence of cardiovascular disease. Ogino et al. showed that arginase I activity had a significant correlation with hsCRP levels in healthy humans (13). This correlation was confounded via RBC or WBC variables. It was concluded that in healthy humans, the contribution of hepatocyte-derived arginase I to serum arginase I might be small compared with that to WBCs and RBCs. In our study, a correlation between hsCRP levels and arginase activity was not demonstrated in OSA patients. Several studies have shown that arginase activity is positively modulated by inflammatory molecules in rat vascular smooth muscle cells (31,32). In human umbilical vein endothelial cells, inflammatory molecules upregulated arginase activity but decreased the cell proliferation rate (12). In another study of human aortic endothelial cells, CRP uncoupled with endothelial NOS and decreased the production of NO (33). With reference to these articles, in our study, higher hsCRP levels and arginase activity resulted in lower NO levels in OSA patients. There was a positive correlation between hsCRP and NO levels and a negative correlation between NO levels and arginase activity.

This study had several limitations that deserve comment. The measurement of arginine levels in plasma samples would be beneficial because reduced arginine levels might support our hypothesis. By the same token, decreases in arginase and arginine lead to the formation of L-ornithine and urea. Thus, L-ornithine measurements would indicate arginine consumption via the arginase pathway. Second, duration of disease could be a significant factor determining arginase activity levels. Controlling for disease duration would have increased the validity of our results, but it was not practically possible to determine the exact time of disease state because admission time can vary from patient to patient. Some of the patients might have been admitted to the sleep laboratory in early stages of the disease and so were diagnosed early, whereas some other patients might

have been admitted in later stages of the disease and thus were diagnosed later.

In conclusion, arginase activity was increased in patients with OSA. Increased arginase activity and reduced NO levels in OSA patients might be related to the presence of cardiovascular disorders. Here, we suggest a putative mechanism regarding NO depletion in OSA, which stresses increased arginase activity.

■ AUTHOR CONTRIBUTIONS

Yüksel M designed the experiments, collected the data, and prepared the manuscript. Kuzu-Okur H and Pelin Z collected the data and performed the statistical analysis. Veliöglu-Ögünç A performed the experiments on the serum samples. Öztürk L wrote and criticized the manuscript.

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