Choroidal Blood Flow Regulation after Posture Change or Isometric Exercise in Men with Obstructive Sleep Apnea Syndrome

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PURPOSE. Obstructive sleep apnea (OSA) syndrome generates hypertension, atherosclerosis, and endothelial and autonomic dysfunction, which may mutually interact with ocular vascular regulation. Exercise and posture changes can be used to manipulate blood pressure, ocular perfusion pressure (OPP), or both. It was hypothesized that choroidal vascular reactivity in response to isometric exercise and posture changes could be altered in OSA patients.

METHODS. Healthy men were matched 1:1 for body mass index, sex, and age with patients with newly diagnosed OSA without cardiovascular comorbidities. All subjects underwent sleep studies and cardiovascular phenotyping (24-hour blood pressure monitoring, arterial stiffness measurements, and cardiac and carotid echography). Choroidal reactivity was assessed by laser Doppler flowmetry, which measured subfoveal choroidal blood flow.

RESULTS. During exercise, blood pressure parameters increased significantly within the same range, with a similar profile over time in OSA patients and control subjects. A significant linear relationship (P = 0.0003) was noted between choroidal vascular resistance and the OPP changes during exercise in OSA patients and control subjects. From the sitting to the supine position, a significant decrease in mean arterial pressure occurred in both groups (10.9%–13.4%; P < 0.001). In both populations, no significant change in choroidal blood flow or vascular resistance was found during the posture change. Choroidal blood flow responses to exercise and posture changes were unchanged after 6 to 9 months of continuous positive airway pressure treatment.

CONCLUSIONS. This study strongly suggests that the regulation of choroidal blood flow, which depends on the orthosympathetic and parasympathetic systems, is unaltered in men with OSA who have no comorbidities. (ClinicalTrials.gov number, NCT00874913.) (Invest Ophthalmol Vis Sci. 2011; 52:9489–9496) DOI:10.1167/iovs.11-7936
tients without associated comorbidities and in matched healthy control subjects. This investigation was also carried out after continuous positive airway pressure (nCPAP), which may improve vascular reactivity in OSA patients.24

MATERIALS AND METHODS

Study Population

OSA Patients. Twenty-one patients with newly diagnosed OSA and no associated comorbidities were included in this prospective study. Fourteen patients participated in the isometric exercise experiment and 15 in the posture experiment. The study was conducted in accordance with the Declaration of Helsinki for research involving human subjects and adhered to Good Clinical Practice guidelines. Informed consent was obtained from the subjects after explanation of the study. The study protocol was approved by the local institutional review board (IRB 6705) and was registered on ClinicalTrials.gov (NCT00874913). This study encompasses data not previously reported but acquired from subjects who completed the protocol previously described.30

Inclusion criteria were presence of OSA, defined by an apnea-hypopnea index (AHI) greater than 15/hour (number of episodes of partial [hypopnea] or complete [apnea] upper airway obstruction); age 18 to 80 years; and affiliation with the health care system. Exclusion criteria were ocular disease (including cataract or retinal disease, ametropia greater than 3 diopters, optic neuropathy), diabetes, cardiovascular treatment (vasoconstrictors, vasodilators, beta and alpha agonists or antagonists, NO-derived medication), corticosteroids, theophylline, sildenafil, immunosuppressors, neuroleptics, nonsteroidal anti-inflammatory agents, estrogen plus progestin treatment, hypnotics (benzodiazepines), and local treatment for ocular hypertension or glaucoma. CPAP compliance was considered acceptable if the device was used for at least 4 hours per night.29

Controls. Control subjects, matched 1:1 with OSA patients for body mass index (BMI), sex, and age, were assessed by a complete overnight polysomnographic study to rule out OSA and then were included. At the screening visit, each subject underwent a general overnight polysomnographic study to rule out OSA and then were included. Each subject completed the protocol previously described.30

Polysomnography

Continuous recordings were taken with electrode positions C3/A2-C4/A1-C2z/01 of the International 10-20 System of Electrode Placement, eye movements, chin electromyogram, and EEG with modified V2 lead. Sleep was scored manually according to standard criteria.26 Air flow was measured with nasal pressure associated with the sum of buccal and nasal thermistor signals. Respiratory effort was monitored with abdominal and thoracic bands. An additional indicator of respiratory effort (pulse transit time) was recorded concurrently. Oxygen saturation was measured using a pulse oximeter (Biox-Ohmeda 3700; Ohmeda, Liberty Corner, NJ). Respiratory events were scored in line with clinical research recommendations.26

Cardiovascular Phenotype of OSA Patients and Control Subjects

Ambulatory blood pressure monitoring (ABPM) was carried out with a lightweight monitor (Diasons Integra; Novacor SA, Rue-Malmaison, France) every 15 minutes during daytime and every 30 minutes during nighttime. The following ABPM parameters were studied: mean systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), and heart rate over 24 hours and throughout the daytime (7 am–10 pm) and nighttime (10 pm–7 am).27 Hypertension and normal nocturnal dipping were defined as previously reported.28 Echocardiography, as well as carotid intima-media thickness and stiffness, were assessed as previously described by our group.29

Choroidal Blood Flow Measurements

The LDF instrument used in this study to measure subfoveal choroidal blood flow (ChBF) has been described previously.30 The blood flow

| Table 1. General Characteristics of OSA Patients and Control Subjects |
|-----------------------------|-----------------------------|-----------------------------|
|                             | OSA Patients (n = 14)       | Healthy Controls (n = 14)   | P   |
|                             |                             |                             |     |
| Anthropometrics             |                             |                             |     |
| Age, y                      | 49.6 ± 2.4                  | 50.5 ± 2.6                  | 0.95 |
| Body mass index, kg/m²      | 26.3 ± 0.5                  | 25.2 ± 0.4                  | 0.04 |
| Sleep studies               |                             |                             |     |
| AHI, hours of sleep         | 38.9 ± 4.2                  | 4.1 ± 0.6                   | <0.001 |
| Mean nocturnal saturation, %| 95.9 ± 0.4                  | 94.3 ± 0.3                  | 0.51 |
| Time spent at SaO₂ < 90%, min | 28.8 ± 9.1                  | 1.1 ± 0.6                   | 0.001 |
| Cardiovascular phenotype    |                             |                             |     |
| Systolic blood pressure, mm Hg | 123.8 ± 2.8                  | 124.9 ± 1.6                  | 0.73 |
| Diastolic blood pressure, mm Hg | 82.1 ± 1.7                  | 79.5 ± 1.7                  | 0.16 |
| Mean blood pressure, mm Hg  | 95.0 ± 1.9                  | 94.6 ± 1.6                  | 0.86 |
| Nocturnal dipping, no. patients (%) | 7 (50)                      | 5 (36)                      | 0.99 |
| Arterial stiffness, m/s     | 9.8 ± 0.5                   | 9.1 ± 0.6                   | 0.49 |
| IMT right carotid, μm       | 0.65 ± 0.04                 | 0.58 ± 0.02                 | 0.44 |
| IMT left carotid, μm        | 0.64 ± 0.04                 | 0.63 ± 0.03                 | 0.65 |
| Carotid plaque, no. patients (%) | 2 (14)                      | 0                          |     |
| Left ventricular ejection fraction, % | 67.4 ± 1.1                  | 67.0 ± 1.2                  | 0.97 |
| Dysthlelipemia, no. patients (%) | 1 (7)                       | 1 (7)                       | 0.99 |

IMT, intima-media thickness.
measurement is obtained from the choriocapillaris layer behind the fovea, the superficial layer of the choroid with a dense network of capillaries. The instrument uses a coherent near-infrared probing beam (785 nm, 90 μW at the cornea) that conforms to the American National Standards Institute standard Z 156.1 for laser safety. The beam is focused at the fovea, and the subject is asked to look directly at the beam. Light backscattered by the tissue in the sampled volume is collected by a bundle of optic fibers and guided to an avalanche photodiode. The output photocurrent is sampled at a frequency of 240 kHz with a 16-byte resolution and processed with graphical programming software (LabVIEW; National Instruments, Austin, TX) to ascertain the ChBF parameters in real time at a rate of 17 Hz using an algorithm based on photon diffusion and probabilistic theory. These parameters are choroidal velocity (ChBVel [kHz]), choroidal volume (ChBVol, in arbitrary units [AU]), and relative flow (ChBF = ChBVol × ChBVel [AU]) of the red blood cells within the sampled tissue region. The software automatically rejects signals for which the light intensity (direct current [DC]) is not within ±10% of its most frequent value or the volume is suddenly too large because of microsaccades, for example. Care was taken to keep the DC signal as constant as possible during recording. Two or more continuous 30-second recordings of the choroidal LDF parameters were obtained for each measurement, and a minimum 12-second valid measurement in each eye was analyzed.

Study Protocol

Patients were asked to abstain from alcohol and caffeine for at least 12 hours before the trial. LDF was systematically performed on the right eye. Systolic and diastolic blood pressure measurements were obtained (Dinamap, Critikon, Tampa, FL) during LDF measurements. The IOP of the fellow eye was then immediately measured using a tonometer (Tonopen XL; Reichert Technologies, Depew, NY). Mean ocular perfusion pressure (OPP) was calculated according to the following formula:

\[
\text{OPP}_{\text{satting position}} = (0.74 \times \text{MAP}) - \text{IOP} \quad \text{and} \quad \text{OPP}_{\text{supine position}} = (0.84 \times \text{MAP}) - \text{IOP}, \]

where MAP was calculated as: MAP = DBP + \(1/3\)(SBP – DBP).

The study was conducted after a randomized, double-blind, three-way crossover design. Two types of experiment were conducted: isometric exercise consisting in squatting (for 2 minutes) and a change in body posture from the sitting to the supine position (for 10 minutes; Fig. 1). These experiments took place on a different day than the gas experiments reported in another paper. Scheduled resting periods for these experiments took place on a different day than the gas experiments reported in another paper. Scheduled resting periods for these experiments took place on a different day than the gas experiments reported in another paper. Scheduled resting periods for these experiments took place on a different day than the gas experiments reported in another paper. Scheduled resting periods for these experiments took place on a different day than the gas experiments reported in another paper. Scheduled resting periods for these experiments took place on a different day than the gas experiments reported in another paper. Scheduled resting periods for these experiments took place on a different day than the gas experiments reported in another paper. Scheduled resting periods for these experiments took place on a different day than the gas experiments reported in another paper. Scheduled resting periods for these experiments took place on a different day than the gas experiments reported in another paper. Scheduled resting periods for these experiments took place on a different day than the gas experiments reported in another paper. Scheduled resting periods for these experiments took place on a different day than the gas experiments reported in another paper. Scheduled resting periods for these experiments took place on a different day than the gas experiments reported in another paper. Scheduled resting periods for these experiments took place on a different day than the gas experiments reported in another paper. Scheduled resting periods for these experiments took place on a different day than the gas experiments reported in another paper. The two groups were also compared at baseline for general parameters (Table 1; paired t-test or Wilcoxon test according to the normality for quantitative data, McNemar test for qualitative data). Statistical Analysis

Data are presented as mean ± SEM. Normalized data during the experiment were calculated according to baseline data. Data analysis was conducted with statistical analysis and graphics software (NCSS 97 [NCSS, Kaysville, UT]; SAS 9.1.3 [SAS Institute, Cary, NC]). Normality was assessed using skewness and kurtosis tests. To check the ANOVA assumptions, variance equality was also tested using the Modified Levene Equal Variance Test. The changes within each group were analyzed by a one-way repeated ANOVA measure (repeated measure factor was time). Paired t-tests were then used for post hoc analysis. The P value was modified using Bonferroni correction. The corrected F value for post hoc analysis was 0.008. A repeated-model ANOVA was also used with two repeated factors, the factor group (first model: OSA versus healthy; second model: before versus after CPAP) and the factor time. We analyzed group and time effects as well as group-time interaction. The two groups were also compared at baseline for general parameters (Table 1; paired t-test or Wilcoxon test according to the normality for quantitative data, McNemar test for qualitative data). The relationships between OPP and blood flow and OPP and vascular resistance were studied using the generalized estimating equation.

Sensitivity (the minimum statistically significant change in LDF parameters that could be detected) was calculated using the formula:

\[
S = (t \times SD)(\sqrt{n} \times P_{\text{mean}}) \times 100, \]

where \(S\) is the mean value of all measurements, \(SD\) is the SD of the difference between the paired measurement for all subjects, and \(t\) is the two-tailed value of the \(t\) distribution at a 0.05 significance level for the \(n - 1\) degrees of freedom. In the present experiment, sensitivity for ChBF in OSA patients and control subjects was 9% and 7%, respectively.

As described previously, when using LDF to detect a 15% difference in flow with 80% power by means of a paired test, seven subjects are needed to evaluate changes within one session.

For the exercise experiment, to define the pressure-flow relationship, OPP data were divided into five groups of eight values. The mean values from these groups were used to determine the OPP at which the ChBF significantly deviated from baseline.

RESULTS

Study Protocol

Table 1. As a whole group, OSA patients were middle-aged, lean, and otherwise healthy except for OSA with a
limited amount of oxygen desaturation at night. As cardiovascular consequences, OSA patients exhibited at most subclinical lesions of the cardiovascular system, stage 1 hypertension, or both. No patient had diabetes mellitus. The OSA group consisted of 14 patients assessed for exercise and 15 patients for posture. In both groups, BMI was within the normal range, with a statistically but not clinically significant difference between control subjects and OSA patients.

**Exercise Experiment**

At baseline, MAP and OPP were similar in both groups (n = 14 OSA patients, n = 14 control subjects), whereas IOP was higher in the healthy group (13.6 ± 0.6 mm Hg in OSA patients; 16.2 ± 0.8 mm Hg in control subjects; P = 0.02) but within the normal range. MAP increased during exercise: 27% and 35% at 1 and 2 minutes in OSA patients (P < 0.001 from baseline) and 23% and 35% in control subjects (P < 0.001 from baseline), respectively. MAP variations were similar in both groups (P = 0.27). A significant increase of approximately 25% to 30% in both SBP and DBP occurred during exercise within a similar range in both groups. There was no significant difference between groups for SBP (P = 0.16) or DBP (P = 0.57). OPP increased during exercise: 33% and 41% at 1 and 2 minutes in OSA patients (P = 0.004 from baseline) and 28% and 45% at 1 and 2 minutes in control subjects (P < 0.001 from baseline), respectively (no difference between groups; P = 0.32).

The relationship between the OPP and ChBF during the squatting period is shown in Figures 2A and 2B. No statistical correlation was found between ChBF and OPP (P = 0.28) in OSA and control subjects. A significant linear relationship was noted between vascular resistance (R) and the OPP changes (OSA subjects, R = 0.85 × OPP + 8.50; control subjects, R = 0.67 × OPP + 20.77) during exercise (P = 0.0003), with a similar slope in OSA and control subjects (P = 0.3). There was no statistical interaction (P = 0.82) of the relation between OPP and ChBF and the group (OSA or healthy).

**Posture Change Experiment**

OSA patients (n = 15) differed from control subjects (n = 15) in that they had a lower mean IOP (P = 0.003; Table 2). In OSA patients and control subjects, a significant decrease (P < 0.001) in MAP (Fig. 3) was noted at 5 minutes (respectively, 10.3%, and 10.3%) and 10 minutes (respectively, 10.9%, and 13.4%) during the supine position. There was no significant difference between groups (P = 0.82). In both populations, MAP regained baseline values at the end of the experiment. A significant increase in IOP was noted in the OSA group (P = 0.02). OPP remained stable throughout the experiment (Fig. 3).

In both populations, no significant change in ChBVol, ChBF, and choroidal vascular resistance was found during the posture change for both groups. A significant increase in ChBVel was noted in both groups (20.4% vs. 9% in control subjects; P = 0.18).
After 6 to 9 months of CPAP treatment, the significant relationship between choroidal vascular resistance and OPP increase ($P = 0.0001$, exercise experiment) was similar ($P = 0.8$) to that observed before treatment.

Reactivity to the change in posture showed a greater MAP decrease in OSA patients after CPAP than before CPAP ($P = 0.04$): $-18.8 \pm 1.6$ mm Hg at 10 minutes ($-10.8 \pm 2.5$ mm Hg before CPAP). The LDF blood flow parameter changes (ChBF, ChVol, ChVel) during the posture experiment after CPAP treatment were not statistically different from the variations obtained before treatment ($P = 0.18$, $P = 0.98$, $P = 0.52$, respectively).

**DISCUSSION**

This study showed for the first time that the response of subfoveal choroidal blood flow to isometric exercise and posture changes is not altered significantly in otherwise healthy OSA patients. We demonstrated that choroidal blood flow is appropriately regulated in OSA patients when OPP increases.
Most vascular changes associated with OSA have been studied with regard to macrovasculature, with demonstrations of abnormal vascular reactivity in cerebral and forearm circulation. On the contrary, we previously reported that OSA without comorbidities does not alter the choroidal vascular response to O₂ or CO₂. These data did not preclude that vascular reactivity in OSA patients may be impaired for other stimuli, involving different mechanisms of local microvascular regulation. Vasoreactivity dependent mainly on the adrenergic system and myogenic properties has been previously described during isometric exercise, whereas the autonomic nervous system is the main factor for vascular regulation during posture changes.

Near-infrared LDF has already demonstrated its capability to quantify the response of subfoveal choroidal blood flow to various stimuli. Because the LDF signal depends partly on the structural properties of the tissue measured, which was the submacular (subfoveal) choroid in this study, the absolute values of blood flow parameters in the two groups of patients at a given time cannot be compared. In contrast, comparisons of relative changes of blood flow parameters are appropriate when the subjects' values are normalized according to baseline values. The reproducibility of the measurements of this experiment was similar to that described previously with a similar LDF (7.4%), with 9% sensitivity of blood flow measurements in OSA patients and 7% in control subjects, based on 11 subjects.

Vasoregulation during Exercise-Related Increase in OPP

Recent studies showed that OSA may impair cardiovascular, ventilatory, and metabolic responses to exercise. Alteration of maximal oxygen consumption, blunted heart rate response, and abnormal blood pressure (BP) profile are attributed primarily to chronic sympathetic activation. Isometric exercise was chosen in this experiment because it allows a rapid increase in BP, irrespective of muscle mass. In OSA patients in the present study, MAP and OPP increased similarly to the increases observed in healthy humans, which contrasts with previous studies addressing maximal exercise capacity in obese severe OSA patients with comorbid conditions. This discrepancy is consistent with the reported correlation between the severity of comorbid OSA and the increased blood pressure response to exercise.

The OPP-ChBF relationship in our healthy control subjects confirmed previous studies showing that the choroid is capable of maintaining its perfusion level over a wide range of OPPs, up to 67% above the baseline levels. For example, in these studies, a 60% to 70% increase in OPP resulted in a 12% to 15% increase in ChBF, similar to what we observed in OSA patients and their control subjects in this study.

The mechanism to maintain stable perfusion within the eye has been shown to be ocular vasoconstriction, as suggested in this study by increased vascular resistance, which was comparable in the two groups studied. The adaptation of choroidal vascular resistance is achieved through the sympathetic nervous system by way of rich choroid innervation, including a key role played by vasoconstrictive α₁-receptor. The short posterior ciliary arteries, with vascular branches to the choroid and the optic nerve, could also have participated in this regulation because they have α₁ adrenoceptors and 5-HT receptors and exhibit a myogenic tone. The release of vasoconstricting substances by the vascular endothelium, including endothelin-1, also participated. Experiments in control subjects demonstrated the role of NO and endothelin-1 in choroidal blood flow regulation during isometric exercise. Our patients exhibited a limited amount of desaturation and were not obese. In these conditions, sympathetic activation and endothelin system stimulation were probably less pronounced. This might have accounted for our results.

Posture

The posture experiment induced different physiological changes, including IOP, MAP, and OPP changes, and then possible regulation at the level of the choroid or in the vascular system upstream from the eye. The moderate decrease in BP during the posture experiment was similar in the two groups of subjects (OSA patients and control subjects), suggesting that baroreflex sensitivity was not grossly impaired in our OSA population. In our study including OSA patients without comorbidities, chronic intermittent hypoxia remained the main stimulus, potentially inducing cardiovascular autonomic imbalance. The IOP increase after tilting to the supine position was significantly higher in the OSA group, with a small difference between the two groups (+15% vs. +7%, respectively) and had little impact on OPP (because of the OPP formula, with a major contribution of BP). Consistent with previous studies, the increase in IOP caused by the posture change was correlated with the magnitude of tilting. We did not find a significant change in choroidal blood flow from the sitting to the supine position, whereas an increase in velocity occurred. This increase in velocity was compensated by a volume decrease, suggesting a choroid vasoconstriction. Our results are in accordance with those demonstrating a 6.3% decrease in choroidal blood flow (within the range of measurement sensitivity) in a healthy population during 30 minutes in the supine position.

A buffering system located in the posterior ciliary arteries or at the level of the internal and common carotid arteries eliminates most of the increase in OPP induced by tilting from the upright to the supine position. One distinctive feature of our study was the rigorous screening for comorbidities that commonly accompany OSA (obesity, diabetes, coronary disease, and high blood pressure). Indeed our OSA population had carotid intima-media thicknesses and carotid-femoral pulse wave velocities similar to those of control subjects, suggesting that the carotids of our OSA patients were not extensively impaired and permitted physiological vasoregulation. This regulation was also maintained after CPAP treatment. Finally, this posture experiment suggested that mechanisms of ocular blood flow at the site of the carotid and ciliary arteries or within the eye are maintained in otherwise healthy OSA patients.

In conclusion, abnormal macrovascular reactivity is admitted in OSA and can secondarily lead to chronic cardiovascular disease. Our results showed that choroidal vascular responses to changes in both blood pressure (isometric exercise) and posture were similar in otherwise healthy OSA patients and healthy control subjects. In the absence of comorbidities associated with OSA, our results strongly suggest that the regulation of ocular blood flow, which partially depends on the orthosympathetic and parasympathetic systems, is not altered in the early course of OSA.

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References


