Effects of Isometric Exercise on Subfoveal Choroidal Blood Flow in Smokers and Nonsmokers

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PURPOSE. Little is known about potential effects of smoking on ocular blood flow regulation. In the present study, the hypothesis was that choroidal blood flow (CBF) changes during an increase in ocular perfusion pressure induced by isometric exercise are altered in chronic smokers.

METHODS. The study was performed in 24 (12 smokers and 12 nonsmokers) healthy male volunteers in an observer-masked, two-cohort study design. The difference in CBF regulation between smokers and nonsmokers was tested during isometric exercise over a period of 6 minutes. CBF was assessed with laser Doppler flowmetry (LDF), and ocular perfusion pressure (OPP) was calculated from mean arterial pressure (MAP) and intraocular pressure (IOP).

RESULTS. Six minutes of isometric exercise induced a significant increase in MAP, pulse rate (PR), OPP, and CBF in smokers and nonsmokers (each P < 0.001). The increase in CBF was significantly higher in the smoking group (P < 0.001) than in the healthy control group, whereas a comparable increase in MAP (P = 0.18), PR (P = 0.18), and OPP (P = 0.43) occurred in smokers and nonsmokers. IOP remained unchanged during isometric exercise in both groups. Moreover, in smokers, CBF started to increase at OPPs more than 49% above baseline, whereas CBF in nonsmokers remained stable until an increase in OPP of 74% over baseline. This difference between the two groups was significant (P < 0.001).

CONCLUSIONS. These data indicate abnormal CBF regulation in chronic smokers compared with age-matched nonsmoking subjects during isometric exercise. The pathways responsible for this abnormal blood flow response remain to be elucidated.

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Habitual smoking is associated with an increased risk of coronary artery disease and cerebral and peripheral vascular disease,1,2 including such ocular diseases as age-related macular degeneration (AMD) and hypertensive or diabetic retinopathy.5 Several adverse effects of smoking on the endothelium have been reported, including increased oxidative stress,4 decreased levels of the antioxidant vitamin C,4,5 and abnormalities in endothelial nitric oxide (NO) activity.6,7 However, the exact pathophysiological mechanisms responsible for smoking-related vascular damage are unclear.

Autoregulation represents an important factor for the physiological integrity of the ocular circulation. It is the ability of a vascular bed to maintain a constant blood flow despite changes in perfusion pressure. In its strict sense this behavior of the vascular bed cannot be investigated in humans, because isolated changes of perfusion pressure cannot be induced. However, in the human choroid, nonlinear pressure-flow relationships have been found during experimental changes in perfusion pressure.8–13 There is evidence that chronic smoking markedly perturbs cerebrovascular autoregulation.14 Effects of smoking on the vascular integrity of the choroid, however, are unknown so far.

In the present study, we compared choroidal blood flow regulation between smokers and nonsmokers. During an experimental increase in perfusion pressure as provoked by isometric exercise, net choroidal vasoconstriction is necessary to keep blood flow constant. Numerous mediators may be involved in this vasoconstrictor response, because blood flow in the choroid is controlled by a number of hormonal, neural, and paracrine regulatory systems.15 We hypothesized that this vasoconstrictor response is altered in chronic smokers compared with age-matched nonsmoking control subjects.

METHODS

Subjects

The present study was performed in compliance with the Declaration of Helsinki and the Good Clinical Practice guidelines. After approval of the study protocol by the Ethics Committee of the Vienna University School of Medicine and after written informed consent was obtained, 24 healthy male volunteers were enrolled in the study (age range, 19–35 years; smokers: mean, 24.7 ± 3.1 [SD]; nonsmokers: mean, 24.9 ± 3.2). Twelve participants had been smoking for at least 2 years and regularly smoked between 15 and 25 cigarettes per day. The other 12 volunteers had no history of smoking.

All volunteers were drug free for at least 3 weeks before inclusion and passed a prestudy screening during the 4 weeks before the first study day that included physical examination and medical history, 12-lead electrocardiogram; complete blood cell count; activated partial thromboplastin time and thrombin time; clinical chemistry (sodium, potassium, creatinine, uric acid, glucose, cholesterol, triglycerides, alanine aminotransferase, aspartate transcarbamylase, γ-glutamyltransferase, alkaline phosphatase, total bilirubin, total protein); hepatitis A, B, and C and HIV-serology; urinalysis; random urine drug screen; and ophthalmic examination. Subjects were excluded if any clinically relevant abnormality was found as part of the pretreatment screening. In addition, subjects with ametropia of more than 3 D, amsiometria more than 1 D, or any evidence of eye disease that might interfere with the purpose of the present trial were excluded.

To distinguish objectively between smokers and nonsmokers, it was necessary to determine the level of cotinine in the urine. This investigation was performed with a homogenous immunnoassay (EMIT technique; Diagnostic Reagents, Inc., Los Angeles, CA).16 In addition,
the subjects were asked to complete the Fagerstrom Tolerance Questionnaire.17

**Experimental Design**

This study was an observer-masked design performed in two cohorts. Subjects were asked to refrain from alcohol and caffeine for at least 12 hours before trial days. In addition, they were advised to abstain from smoking for at least 2 hours before the beginning of measurements to exclude any acute effects of cigarette smoking. Dilation of one pupil was obtained with tropicamide (mydriaticum, Agepha-Augentropfen; Agepha GmbH, Vienna, Austria).

After a 20-minute resting period, baseline measurements with confocal laser Doppler flowmetry were obtained with subjects in a sitting position. The measurements were continued without cessation with subjects squatting for 6 minutes. This exercise was performed in a position in which the upper and the lower leg are as close as possible to a right angle. For the subjects’ security a nurse was standing behind each of them during the squatting period.

**Systemic Hemodynamics**

Systolic, diastolic, and mean arterial blood pressure (SBP, DBP, MAP, respectively) were measured on the upper arm with an automated oscillometric device. Pulse rate (PR) was automatically recorded from a finger-pulse oximeter (HP-CMS patient monitor; Hewlett-Packard, Palo Alto, CA). Systemic hemodynamics were measured in 1-minute intervals during the squatting period and in 10-minute intervals during resting periods. Pulse rate and a real-time electrocardiogram were monitored continuously.

**Applanation Tonometry and Ocular Perfusion Pressure**

The intraocular pressure (IOP) was measured with a Perkins applanation tonometer (Clement Clarke, Edinburgh, UK). Oxybuprocaine hydrochloride was used to anesthetize the cornea. Ocular perfusion pressure (OPP) was calculated as OPP = \( \frac{1}{3} \times MAP - IOP \). This formula is based on the evidence that the pressure in choroidal veins almost equals the IOP.19,20 During the isometric squatting exercise, we observed only small changes in IOP over baseline after 6 minutes. Hence, we used a linear regression model to extrapolate the IOPs at the other time points of squatting.

**Table 1. Baseline Values of Ocular and Systemic Hemodynamic Parameters**

<table>
<thead>
<tr>
<th></th>
<th>Smokers</th>
<th>Nonsmokers</th>
<th>P (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>83.8 ± 5.3</td>
<td>78.8 ± 2.3</td>
<td>0.225</td>
</tr>
<tr>
<td>PR (bpm)</td>
<td>77.4 ± 2.7</td>
<td>69.7 ± 3.6</td>
<td>0.098</td>
</tr>
<tr>
<td>OPP (mm Hg)</td>
<td>40.8 ± 2.2</td>
<td>37.5 ± 1.5</td>
<td>0.215</td>
</tr>
<tr>
<td>IOP (mm Hg)</td>
<td>15.0 ± 1.0</td>
<td>15.0 ± 1.0</td>
<td>0.894</td>
</tr>
<tr>
<td>CBF (AU)</td>
<td>21.9 ± 10.8</td>
<td>22.9 ± 8.9</td>
<td>0.577</td>
</tr>
</tbody>
</table>

Data are means ± SEM (n = 12, each). AU, arbitrary units.

**Table 2. Effects on Systemic and Ocular Hemodynamic Parameters of 6 Minutes of Squatting**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Smokers</th>
<th>Nonsmokers</th>
<th>P (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>84 ± 3</td>
<td>79 ± 2</td>
<td>0.562</td>
</tr>
<tr>
<td>IOP (mm Hg)</td>
<td>15 ± 1</td>
<td>15 ± 1</td>
<td>0.631</td>
</tr>
<tr>
<td>1</td>
<td>99 ± 4</td>
<td>100 ± 5</td>
<td>0.995</td>
</tr>
<tr>
<td>2</td>
<td>113 ± 5</td>
<td>108 ± 4</td>
<td>0.657</td>
</tr>
<tr>
<td>3</td>
<td>113 ± 5</td>
<td>112 ± 3</td>
<td>0.311</td>
</tr>
<tr>
<td>4</td>
<td>115 ± 4</td>
<td>117 ± 3</td>
<td>0.211</td>
</tr>
<tr>
<td>5</td>
<td>116 ± 3</td>
<td>119 ± 3</td>
<td>0.191</td>
</tr>
<tr>
<td>6</td>
<td>121 ± 3</td>
<td>120 ± 4</td>
<td>0.143</td>
</tr>
</tbody>
</table>

Data are means ± SEM (n = 12, each). ND, not done.
DISCUSSION

Based on the results of the present study, blood flow regulation in the choroid appears to be less effective in chronic smokers than in nonsmokers. Although CBF values showed a satisfactory reproducibility within subjects during isometric exercise, there was an interindividual variability as shown in the large error bars in Figures 1 and 4. This can be explained by the physical fitness of the subjects, which influences the exercise-induced increase in MAP, as well as the fact that the choroidal autoregulatory range showed a high variability among subjects. In the present study, the baseline MAP and OPP tended to be higher in smokers than in nonsmokers, but the differences in the increase of both parameters during isometric exercise was less distinctive. This is in contrast to a significantly more pronounced increase of CBF when compared with the nonsmoking group. The PR of the smoking group tended to be higher during the whole study day, which could be attributed to a reduced physical capacity among chronic smokers.

In its strict sense, autoregulation can be investigated only in an isolated organ where perfusion pressure can be varied experimentally, but in humans such experiments are obviously not possible. In particular, the neural input to the choroidal vessels has also to be considered, because isometric exercise induces sympathetic and parasympathetic stimulation. There is evidence from various animal studies that ocular sympathetic vasoconstriction elicited by an increase in OPP. In the human choroid, blood flow regulation is neither influenced by the muscarinic receptor antagonist atropine nor by the nonse-

The pressure-flow relationship during the squatting periods is presented in Figure 4. In smokers, CBF increased at changes in OPP of more than 49% over baseline, whereas CBF in nonsmokers remained stable until an increase in OPP of 74% above baseline. This difference between the two groups was significant (P < 0.001). At an almost 100% increase in OPP, CBF increased by 19.7% in the control group, but as much as 32.2% in the group of smokers.

FIGURE 1. Relative change of CBF during squatting compared with the pre-exercise value. Data are presented as means ± SEM (n = 12, each). *Significant difference versus baseline (one-way ANOVA, post hoc testing); #significant difference between smokers and nonsmokers (ANOVA, interaction between time and group).

FIGURE 2. The effect of squatting on OPP. Data are presented as means ± SEM (n = 12, each). *Significant difference versus baseline (one-way ANOVA, post hoc testing).

FIGURE 3. The effect of isometric exercise on PR. Data are presented as means ± SEM (n = 12, each). *Significant difference versus baseline (one-way ANOVA, post hoc testing).

RESULTS

No adverse events were observed during the study. The results of the Fagerstrom Tolerance Questionnaire were 0 ± 0 points for the nonsmoking group and 4.0 ± 0.6 points for the smoking group, indicating nicotine-dependence among the smokers. Cotinine levels in the urine were 45.1 ± 11.5 ng/mL in nonsmokers and 2025.7 ± 336.1 ng/mL in smokers. This again clearly indicates that the smokers were accurately selected, because the urine cotinine concentration in nonsmokers is normally below 500 ng/mL.24

There were no significant differences between the baseline values of the two groups (Table 1). As expected, 6 minutes of isometric exercise induced a significant increase in MAP (Table 2), CBF (Fig. 1), OPP (Fig. 2), and PR (Fig. 3) in smokers and nonsmokers (P < 0.001, each). The increase in CBF was significantly higher in the smoking group (P < 0.001), whereas MAP (P = 0.18), PR (P = 0.18) and OPP (P = 0.43) increased comparably in smokers and nonsmokers. IOP remained unchanged during isometric exercise in both groups (Table 2).

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selective β-adrenoceptor antagonist propranolol,10 which, however, does not exclude a role of neural input in this regulatory process. The vasoconstrictor angiotensin II also does not contribute to choroidal blood flow regulation during changes in perfusion pressure in animals or humans.12,28 By contrast, NO appears to be involved into human choroidal blood flow regulation during isometric exercise.11 This could partially explain the results of the present study, because recent animal and human studies29,30 have shown that cigarette smoking is associated with reduced endothelium-dependent vasodilation, NO generation, and endothelial nitric oxide synthase (eNOS) activity. The expression of eNOS protein is increased in human smokers in the presence of reduced eNOS activity.30 Moreover, the endothelin system is involved in choroidal blood flow regulation in animals28,31 and in humans12 during isometric exercise. Smokers have higher endothelin (ET)-1 plasma levels after cigarette smoking,32 whereas basal ET-1 levels are slightly reduced in nonsmokers (p < 0.01).36 A limitation of the present study is that only subfoveal CBF was assessed with the LDF technique. Little is known about potential regional differences in CBF regulation, and our results cannot necessarily be extrapolated to the more peripheral choroid. In addition, care must be taken to compare baseline CBF data between the two groups, because absolute data, as obtained with this device, strongly depend on the light-scattering properties of the tissue.

In conclusion, smoking alters the mechanisms involved in choroidal blood flow regulation. Further studies are needed to identify which pathways are favored targets of smoking-induced vascular damage.

References


