Cigarette smoking is associated with an acute impairment of microvascular function in humans

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ABSTRACT

An effect on microvascular function has been proposed as a possible mechanism explaining the association of acute smoking with increased blood pressure and decreased insulin sensitivity. However, the effects of smoking on microvascular function have not been studied. We have investigated the acute effects of smoking on microvascular function in 12 healthy smokers. Before and after smoking a cigarette, we measured heart rate, blood pressure and capillary recruitment during peak reactive hyperaemia. We also measured endothelium-dependent and endothelium-independent vasodilatation of the skin microcirculation with iontophoresis of acetylcholine and sodium nitroprusside respectively combined with laser Doppler fluxmetry. To exclude non-specific changes, a control study with sham smoking was performed. The smoking and sham smoking studies were conducted in a randomized order. Compared with sham smoking, acute smoking caused increases in heart rate (smoking, 9.3 ± 4.1 beats/min; sham, 1.3 ± 3.0 beats/min; P < 0.001) and systolic blood pressure (smoking, 6.3 ± 8.8 mmHg; sham, 0.8 ± 4.4 mmHg; P < 0.05); decreases in absolute (smoking, −4.9 ± 6.9 per mm²; sham, 0.8 ± 2.1 per mm²; P = 0.01) and relative (smoking, −13.8 ± 21.4%; sham, 1.9 ± 6.9%; P = 0.02) capillary recruitment during peak reactive hyperaemia; and decreases in absolute (smoking, −62.4 ± 47.7 perfusion units (PU); sham, −30.8 ± 32.6 PU; P = 0.04) and relative (smoking, −147 ± 163%; sham, 32 ± 225%; P = 0.07) vasodilatation caused by acetylcholine. Absolute (smoking, −31.6 ± 58.5 PU; sham, −8.4 ± 44.0 PU; P = 0.3) and relative (smoking, −50.2 ± 219.0%; sham, −17.1 ± 139%; P = 0.7) vasodilatation caused by sodium nitroprusside were not affected. Thus acute smoking is associated with impaired capillary recruitment during peak reactive hyperaemia and impaired microvascular endothelium-dependent vasodilatation. These findings may explain the increased blood pressure and decreased insulin sensitivity that have been observed after acute smoking.

INTRODUCTION

One of the major risk factors for cardiovascular disease is cigarette smoking [1,2]. Cigarette smoking is associated with an acute increase in arterial wall stiffness [3,4] and immediate endothelial dysfunction of the large arteries [5,6], which are recognized to be important early phenomena in the pathogenesis of atherosclerosis. In addition, cigarette smoking is associated with an acute increase in blood pressure [7–10]. The mechanism behind this

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Abbreviations: PRH, post-occlusive reactive hyperaemia; PU, perfusion units.
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relationship is unclear. It has been proposed that the increased blood pressure is caused by impaired microvascular function, which may increase vascular resistance [11]. In addition, impaired microvascular function may also reduce insulin action and explain the decrease in insulin sensitivity that has been observed after smoking a cigarette [7,12]. In support of this concept, we have shown previously that microvascular function, measured as capillary recruitment during post-occlusive reactive hyperaemia (PRH) and acetylcholine-mediated vasodilatation in skin, is related to blood pressure and insulin sensitivity [13,14]. However, the effects of smoking on these microvascular functions have not been reported.

To determine whether smoking induces microvascular dysfunction, we assessed the acute effects of smoking a cigarette on capillary recruitment during PRH, as well as on skin microvascular reactivity to acetylcholine and sodium nitroprusside.

METHODS

Subjects

Twelve healthy smokers (average age 26 ± 6.2 years; range 19–37 years) were included in the study. All subjects were healthy, as judged by medical history, and non-diabetic according to ADA criteria [15]. The daily intake of cigarettes ranged from 1 to 23 (mean 12 ± 7). The subjects were normotensive, as determined by trilicate office blood pressure measurement, and did not use medication. Characteristics of the study subjects are given in Table 1. The study protocol was approved by the local Ethics Committee and conformed with the principles outlined in the Declaration of Helsinki. Informed consent was obtained from each subject.

Study design

Measurements were conducted in a quiet, temperature-controlled room (23.4 ± 0.4 °C) at 08.00 h, after a 10 h fast, with the subjects in the sitting position. All subjects abstained from caffeine- and alcohol-containing drinks overnight, and had to refrain from smoking for at least 6 h before examination. All microcirculatory measurements were performed with the investigated hand at heart level. Measurements were obtained after allowing 20 min of rest and acclimatization. The acute effects of smoking were compared with the effects of sham smoking. The study design was randomized, with two sessions. These were performed on separate days within 2 weeks. After 20 min of rest, haemodynamic and microvascular measurements were obtained. The participants were then asked to smoke a cigarette of a filter type (containing ~ 1.0 mg of nicotine and ~ 15 mg of tar) or to simulate smoking with a drinking straw with a filter (sham smoking). Subjects were instructed to take a puff of 5 s duration every 15 s, and the whole cigarette had to be smoked within 5 min. After smoking or sham smoking, microvascular measurements were repeated. In addition, blood pressure and heart rate were determined before and after smoking and sham smoking.

Haemodynamic measurements

Systolic blood pressure, diastolic blood pressure and heart rate were determined with an automatic device (Colin Press-Mate BP-8800). Measurements were performed before and 20–30 min after smoking or sham smoking. The average of three measurements during each period were calculated. The interval between the three consecutive measurements was 5 min.

Assessment of capillary recruitment

The capillaroscopy studies were conducted before and 30 min after smoking or sham smoking. Nailfold capillaries in the dorsal skin of the third finger of the left hand were visualized by an epi-illuminated microscope as described previously [13,14,16]. Capillaries were visualized in a standardized manner, making it possible to visualize the same field in the smoking and sham smoking studies. Capillaries were visualized approx. 1.5 mm proximal to the terminal row of capillaries. One visual field of 1 mm² was recorded before and after 4 min of arterial occlusion, and the images were stored on videotape. The number of capillaries was counted off-line by an experienced investigator (R.G.I.J.) from a freeze-framed reproduction of the videotape, and from the running videotape when it was uncertain whether a capillary was present or not. The investigator counting the capillaries was unaware of whether the videotapes were from the smoking or the sham smoking experiment. Capillary density was defined as the number of erythrocyte-perfused capillaries per mm² of nailfold skin. PRH after 4 min of arterial occlusion with a digital cuff was used to assess functional recruitment of capillaries [13,14,16]. The number of capillaries in the resting state was counted during a 15 s period, with only continuously perfused capillaries being counted, as described previously [17]. Directly after release of the cuff, the number of perfused capillaries was counted. Percentage capillary

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Characteristics of the subjects</th>
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<tbody>
<tr>
<td>Parameter</td>
<td>Value</td>
</tr>
<tr>
<td>n (male/female)</td>
<td>12 (9/3)</td>
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<tr>
<td>Age (years)</td>
<td>26 ± 6.2</td>
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<tr>
<td>Waist-to-hip ratio</td>
<td>0.83 ± 0.06</td>
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<tr>
<td>Body mass index (kg/m²)</td>
<td>22.6 ± 2.9</td>
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<tr>
<td>Fasting plasma glucose (mmol/l)</td>
<td>4.9 ± 0.8</td>
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<td>Fasting serum total cholesterol (mmol/l)</td>
<td>4.6 ± 0.8</td>
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<tr>
<td>Fasting HDL-cholesterol (mmol/l)</td>
<td>1.5 ± 0.4</td>
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recruitment during PRH was assessed by dividing the increase in capillary density during PRH by the capillary density in the resting state. The intra-subject coefficient of variation was $17.2 \pm 12.1\%$ (measured on two occasions in seven subjects).

Assessment of endothelium-dependent and endothelium-independent vasodilatation
Endothelium-dependent and endothelium-independent vasodilatation of the skin microcirculation were evaluated by iontophoresis of acetylcholine and sodium nitroprusside respectively in combination with laser Doppler fluxmetry [13,14], before and 15 and 35 min after smoking or sham smoking. A protocol of multiple fixed doses (current intensity x delivery time) was employed, resulting in an incremental dose–response curve. Skin temperature was monitored. Acetylcholine (1%; Miochol; Bournonville Pharma) was delivered using an anodal current; seven doses (each 0.1 mA for 20 s) were delivered, with a 60 s interval between doses. Sodium nitroprusside (0.1%; Nipride; Roche) was delivered using a cathodal current; nine doses (each 0.2 mA for 20 s) were delivered, with a 90 s interval between doses. Acetylcholine-dependent laser Doppler flux was measured before and after smoking or sham smoking on the right hand on dorsal skin of the middle phalanx of the third and second fingers respectively. Nitroprusside-dependent laser Doppler flux was measured before and after smoking or sham smoking on the left hand on the middle phalanx of the fourth and second fingers respectively. Approx. 5 min was allowed to elapse between the acetylcholine and nitroprusside measurements. During the smoking and sham smoking studies, the same fingers were used for each measurement. Intra-subject coefficients of variation for the percentage increase from baseline to the plateau phase (final two iontophoretic deliveries) was 13.5$\pm$7.7% for acetylcholine and 18.7$\pm$23.4% for sodium nitroprusside (measured on two occasions in seven subjects).

Statistical analysis
Data are expressed as means $\pm$ S.D., unless stated otherwise. Comparisons of haemodynamic variables and microvascular measurements before and after smoking or sham smoking were performed using ANOVA for repeated measures. To investigate whether the influence of smoking was significantly different from the influence of sham smoking, the interaction between the factors smoking and time was used in ANOVA for repeated measures. Pearson’s correlation was used to investigate the association between changes in microvascular function and the number of cigarettes smoked per day. A two-tailed $P$ value of $< 0.05$ was considered significant. All analyses were performed using the statistical software package SPSS version 9.0.

RESULTS

Haemodynamic variables
Table 2 shows haemodynamic variables before and after smoking and sham smoking. Heart rate was significantly increased after smoking ($P < 0.001$); this increase was significantly different from the change in the sham smoking study (smoking, 9.3$\pm$4.1 beats/min; sham, $-1.3\pm3.0$ beats/min; $P < 0.001$). Systolic blood pressure was also significantly increased after smoking ($P < 0.05$); this increase was significantly different from the change in the sham smoking study (smoking, 6.3$\pm$8.8 mmHg; sham, 0.8$\pm$4.4 mmHg; $P < 0.05$). Diastolic blood pressure was not influenced by either smoking or sham smoking.

Capillary recruitment
Capillaroscopy data are shown in Table 3 and Figure 1. Capillary density in the resting state was not influenced by either smoking or sham smoking. However, absolute and relative capillary recruitment were decreased after smoking ($P < 0.05$ compared with baseline). During the sham smoking study, no significant changes were observed. The observed decreases in absolute and relative capillary recruitment during the smoking study were significantly different from the changes observed during the sham smoking study [absolute: smoking, $-4.9\pm6.9$ per mm$^2$; sham, $0.8\pm2.1$ per mm$^2$ ($P = 0.01$); relative: smoking, $-13.8\pm21.4\%$; sham, $1.9\pm6.9\%$ ($P = 0.02$)]. The effect of smoking a cigarette on capillary

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before</th>
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<tr>
<td>Heart rate (beats/min)</td>
<td>66.6 $\pm$ 7.0</td>
<td>75.9 $\pm$ 7.5$^{+}$</td>
<td>65.2 $\pm$ 7.5</td>
<td>63.9 $\pm$ 6.7</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>114.7 $\pm$ 10.1</td>
<td>120.9 $\pm$ 12.4$^{+}$</td>
<td>115.6 $\pm$ 7.4</td>
<td>114.8 $\pm$ 6.0</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>61.8 $\pm$ 7.2</td>
<td>64.8 $\pm$ 7.2</td>
<td>63.1 $\pm$ 6.8</td>
<td>62.7 $\pm$ 4.6</td>
</tr>
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</table>
Table 3  Microvascular function before and after smoking and sham smoking
Values are expressed as means ± S.D. ACh, acetylcholine; SNP, sodium nitroprusside. Significance of differences: * P < 0.05, ** P < 0.01 for before compared with after smoking or sham smoking; † P < 0.05 for change during smoking study compared with change during sham smoking study.

| Variable | Smoking | | | | Sham smoking | | | |
|----------|---------|---------|---------|---------|------------|---------|---------|---------|---------|
|          | Before  | After   | Before  | After   |            | Before  | After   |            |         |
| Capillary recruitment |         |         |         |         |            |         |         |            |         |
| Baseline density (per mm²) | 41.1 ± 11.4 | 41.7 ± 10.4 | 40.9 ± 8.8 | 41.0 ± 9.9 |            |         |         |            |         |
| Peak density (per mm²) | 52.8 ± 13.8 | 48.5 ± 12.0 † | 53.5 ± 14.2 | 54.4 ± 15.0 |            |         |         |            |         |
| Absolute increase (per mm²) | 11.8 ± 7.7 | 6.8 ± 3.7 † | 12.6 ± 7.6 | 13.4 ± 6.7 |            |         |         |            |         |
| Recruitment (%) | 30.3 ± 21.8 | 16.6 ± 8.1 † | 30.2 ± 16.1 | 32.1 ± 11.7 |            |         |         |            |         |
| ACh-mediated vasodilatation |         |         |         |         |            |         |         |            |         |
| Skin temperature (°C) | 31.0 ± 0.8 | 29.5 ± 1.1 ** | 30.8 ± 0.7 | 30.2 ± 1.8 |            |         |         |            |         |
| Baseline perfusion (PU) | 31.5 ± 12.6 | 21.4 ± 11.7 * | 33.8 ± 13.7 | 24.4 ± 11.7 ** |            |         |         |            |         |
| Plateau perfusion (PU) | 140.9 ± 55.3 | 68.2 ± 59.8 ** | 162.2 ± 57.9 | 121.9 ± 63.7 ** |            |         |         |            |         |
| Absolute increase (PU) | 109.4 ± 50.0 | 47.0 ± 52.9 † † | 128.4 ± 55.8 | 97.6 ± 60.6 ** |            |         |         |            |         |
| ACh-induced vasodilatation (%) | 368 ± 157 | 221 ± 203 ** | 419 ± 145 | 450 ± 275 |            |         |         |            |         |
| SNP-mediated vasodilatation |         |         |         |         |            |         |         |            |         |
| Skin temperature (°C) | 31.4 ± 1.6 | 30.0 ± 1.5 † † | 31.3 ± 1.1 | 30.8 ± 1.7 * |            |         |         |            |         |
| Baseline perfusion (PU) | 29.6 ± 7.7 | 21.5 ± 9.6 † † | 23.3 ± 5.3 | 23.9 ± 7.6 |            |         |         |            |         |
| Plateau perfusion (PU) | 120.2 ± 52.9 | 80.4 ± 64.1 ** | 88.4 ± 40.8 | 80.4 ± 42.7 |            |         |         |            |         |
| Absolute increase (PU) | 90.6 ± 50.2 | 59.0 ± 56.9 ** | 64.9 ± 39.1 | 56.5 ± 41.4 |            |         |         |            |         |
| SNP-induced vasodilatation (%) | 313 ± 181 | 263 ± 162 | 277 ± 156 | 260 ± 201 |            |         |         |            |         |

Figure 1  Capillary recruitment during peak reactive hyperaemia before and after smoking and sham smoking
The results are expressed as means ± S.E.M.; * P < 0.05 for change during smoking study compared with change during sham smoking study.

Endothelium-dependent and -independent vasodilatation of the skin microcirculation
Results are shown in Table 3 and Figure 2. Smoking significantly diminished the absolute and relative vasodilatation induced by acetylcholine [absolute: before smoking, 109.4 ± 50.0 perfusion units (PU); after, 47.0 ± 52.9 PU (P < 0.01); relative: before, 368 ± 157%; after, 221 ± 203% (P < 0.01)]. The significant attenuation of the absolute and relative increases after iontophoresis of acetylcholine during the smoking study were different from the changes observed during the sham smoking study [absolute: smoking, −62.4 ± 47.7 PU; sham,
−30.8 ± 32.6 PU (P = 0.04); relative: smoking, −147 ± 163%; sham, 32 ± 225% (P = 0.07). The effect of smoking a cigarette on the absolute and relative increases after iontophoresis of acetylcholine was not related to the average number of cigarettes smoked per day (r = −0.01, P = 1.0 and r = −0.16, P = 0.6 respectively).

Smoking did not impair the vasodilatation induced by sodium nitroprusside. The changes in the absolute and the relative increases after iontophoresis of sodium nitroprusside during the smoking study were not significantly different from the changes observed during the sham smoking study [absolute: smoking, −31.6 ± 58.5 PU; sham, −8.4 ± 44.0 PU (P = 0.3); relative: smoking, −50.2 ± 219.0%; sham, −17.1 ± 139% (P = 0.7)].

The decrease in skin temperature during the smoking study was greater than that observed during the sham smoking study [−1.4 ± 1.4 °C and −0.6 ± 1.5 °C respectively (P = 0.08) for the baseline measurements preceding the acetylcholine procedure; −1.4 ± 0.9 °C and −0.6 ± 0.8 °C respectively (P = 0.02) for the baseline measurements preceding the sodium nitroprusside procedure].

**DISCUSSION**

We have examined the acute effects of smoking on skin microcirculatory function, the only site available in humans in which to directly and non-invasively assess microcirculatory dynamics. Acute smoking was associated with impaired recruitment of capillaries and impaired microvascular endothelium-dependent vasodilatation, whereas endothelium-independent vasodilatation was not affected. This is in accordance with experimental studies that have demonstrated detrimental effects of cigarette smoke on the microcirculation [18,19]. Our findings are also in accordance with a study by Tur et al. [20], who demonstrated a lower peak and a slower recovery of skin laser Doppler blood flow during peak reactive hyperaemia after smoking in humans. However, capillary recruitment and microvascular endothelium-dependent vasodilatation were not measured in that study, and a control experiment with sham smoking was not performed.

One could argue that the impaired microvascular function after smoking was due to the observed decrease in skin temperature. However, we find this unlikely, because the microvascular dilatation after the iontophoresis of sodium nitroprusside was not affected by smoking. Therefore the decreased skin temperature may be the consequence, and not the cause, of impaired microvascular function.

The present study does not clarify the mechanism responsible for the acute smoking-induced decrease in microvascular vasodilatation, but a role for nicotine or oxygen free radicals has been suggested. It has been demonstrated that administration of nicotine is associated with an acute impairment of vascular function in experimental studies [21,22] and in human veins [23]. Nicotine may cause sympathetic activation and, in addition, inhibit the activity of nitric oxide synthase [22], which may explain our finding of impaired endothelium-dependent vasodilatation. Alternatively, clinical [24] and experimental [25] studies have suggested a role for oxygen-derived free radicals. Cigarette smoke contains large amounts of free radicals [26,27], which may injure the endothelium. This hypothesis is supported by studies demonstrating that the antioxidants vitamin C and vitamin E can attenuate the acute impairment of endothelium-dependent vasodilatation in the brachial artery [6,28]. Whether these findings can be extrapolated to microvascular function remains to be established.

In our present study, acute smoking caused an increase in heart rate. The size of this effect was in accordance with other studies reporting an effect of acute smoking on heart rate [5,6,29–31]. The observed increase in systolic blood pressure after acute smoking is also in accordance with other studies [7–10]. We did not measure insulin sensitivity in the present study. However, previous studies have demonstrated that acute cigarette smoking is associated with a decrease in insulin sensitivity [7,12]. This decreased insulin sensitivity after smoking may be caused, in part, by the effect of smoking on microvascular function [11]. Experimental [32,33] and human studies [34–36] suggest that impaired microvascular function contributes to an increase in vascular resistance and antedates an increase in blood pressure. In addition, impaired microvascular function has been suggested to reduce insulin sensitivity by decreasing the delivery of insulin and glucose [11,13,37]. Our finding of an acute effect of smoking on microvascular function may be relevant to understanding how smoking can increase blood pressure and decrease insulin sensitivity. However, it should be emphasized that other mechanisms, such as sympathetic activation [30] and increased stiffness of large vessels [3,4], may also be involved in the observed increase in blood pressure after smoking.

We did not measure microvascular function in an age- and sex-matched group of non-smokers. However, skin capillary recruitment, as well as endothelium-dependent and endothelium-independent vasodilatation, were diminished in the smokers compared with non-smokers that have been investigated in our previous studies. The finding of impaired skin endothelium-dependent and endothelium-independent vasodilatation in smokers is in accordance with a recent study of chronic smokers [38].

In summary, our present data provide the first direct evidence for a smoking-induced acute impairment of capillary recruitment in humans. In addition, our data demonstrate that smoking resulted in impaired microvascular endothelium-dependent vasodilatation, without an influence on microvascular endothelium-independent
vasodilation. These findings offer a potential explanation for the association of acute smoking with increased blood pressure and decreased insulin sensitivity.

REFERENCES


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