Environmental and Experimental Biology (2011) 9: 23–28 *Original Paper*

The effect of chronic cigarette smoking on microvascular function, insulin resistance and inflammatory state

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Abstract

Cigarette smoking, a major risk factor for cardiovascular disease, can induce proinflammatory state and endothelial injury - the earliest manifestations of atherosclerotic changes. The aim of the study was to assess cutaneous vascular reactivity, insulin resistance and circulating levels of inflammatory cytokines in 20 healthy habitual smokers and 24 healthy non-smokers. The groups were matched for age. We used laser Doppler imaging with iontophoretic application of 1% acethylcholine solution and local heating 44 °C on the dorsum of the palm. Serum monocyte chemotactic protein-1, tumour necrosis factor-alpha and interleukin-6 were measured by xMAP technology. Insulin resdistance was assessed by HOMA-IR method. Local heating-induced neurally-mediated and endothelium-dependent vasodilatation was significantly decreased in elderly smokers vs. elderly non-smokers ($p < 0.05$). Young smokers showed significantly reduced endothelium-dependent vasodilatation vs. young non-smokers ($p < 0.05$). Ach-induced vasodilatation was significantly decreased in the elderly smokers and elderly non-smokers groups vs. young smokers and young non-smokers groups ($p < 0.05$). The level of tumour necrosis factor-alpha was significantly higher in both groups of smokers vs. non-smokers ($p < 0.05$). The level of monocyte chemotactic protein-1 was slightly higher in smokers. Only the elderly smokers group exhibited a tendency to higher values of HOMA-IR. Data showed that long-lasting cigarette smoking significantly impairs peripheral microvascular function due to increased inflammatory response.

Key words: cytokines, cigarette smoking, endothelium-dependent vasodilatation, insulin resistance, microvascular function.
Abbreviations: Ach, acethylcholine; BMI, body mass index; ES, elderly smokers; ENS, elderly non-smokers; HOMA-IR, values of insulin resistance; IL-6, interleukin-6; IR, insulin resistance; LDI, Laser Doppler imaging; LDI-Ach_{basal}, basal blood flow before Ach iontophoresis; LDI-Ach_{max}, acethylcholine iontophoresis-induced vasodilatation; LDI-$heating_{basal}$, basal blood flow before local heating; LDI-$heating_{1\ max}$, local heating-induced vasodilatation in early peak (known as neurally- mediated vasodilatation); LDI-$heating_{2\ max}$, local heating-induced vasodilatation in late phase (known as endothelium-dependent vasodilatation); MCP-1, monocyte chemotactic protein-1; NO, nitric oxide; PU, perfusion units; ROS, reactive oxygen species; TNF-α, tumour necrosis factor-alpha; YNS, young non-smokers; YS, young smokers.

Introduction

Assessment of microvascular function is of major importance in understanding the physiology of the vasculature and investigating the vascular effects of pathological conditions (Tesselaar, Sjöberg 2011). Impairment of vasodilatory function is one of the earliest manifestations of atherosclerotic changes in vessels (Ambrose, Barua 2004).

There are many studies in which the influence of several risk factors on vascular functions has been investigated. The most common health risk factor is cigarette and tobacco smoking. Numerous studies have demonstrated that acute and chronic exposure to tobacco smoke induces endothelial injury characterized by decreased vessel dilatation, prothrombotic and proinflammatory state. Recent studies on animals suggest that nicotine promotes the oxidative and inflammatory stress to the endothelium and induces pathological angiogenesis, leading to the progression of atherosclerotic lesions (Adamopoulos et al. 2008). The precise components and the potential mechanisms responsible for the deleterious effects of smoking have not yet been fully clarified (Adamopoulos et al. 2008). Some of the more often mentioned mechanisms include adverse effects on lipids, hemodynamic stress, oxidant injury, neutrophil activation, enhanced thrombosis, increased fibrinogen and blood viscosity (Benowitz, Gourlay 1997).

Clinical and experimental studies indicate that either active or passive exposure to cigarette smoke promotes vasomotor dysfunction, atherogenesis and thrombosis in multiple vascular beds (Ambrose, Barua 2004). Cigarette smoke is a complex mixture of chemicals that includes nicotine as well as toxic substances such as carbon monoxide, hydrogen cyanide, nitrogen oxides, aldehydes, N-nitrosamines, ketones, quinine and polyaromatic

hydrocarbons (Benowitz, Gourlay 1997; Roemer et al. 2004; Fujioka, Shibamoto 2006). Cigarette smoking is associated with elevated oxidative stress, which may in turn alter endothelial function through reduced nitric oxide (NO) bioavailability (Jefferis et al. 2010). Oxygen-free radicals in cigarette smoke inactivate NO and directly damage endothelial cells. It has been shown that cigarette smoke exposure in humans impairs endothelium-dependent vasodilation in macrovascular and microvascular beds (Ambrose, Barua 2004; Ijzerman et al. 2003).

Some studies have shown smoking to be associated with decreased insulin sensitivity (Unverdorben et al. 2009), and smokers tend to be relatively insulin-resistant, with evidence of endothelial dysfunction compared with non-smokers (Reaven, Tsao 2003). Cross-sectional studies indicate that waist circumference is higher in smokers than in non-smokers and is positively associated with the number of pack-years of smoking (Chiolero et al. 2008).

The aim of this study was to evaluate alterations in cutaneous vasomotor reactivity – neuronal regulation and endothelium-dependent vasodilatation, serum cytokines and insulin sensivity in healthy chronic cigarette smokers and non-smokers.

Materials and methods

Subjects

20 healthy habitual smokers and 24 healthy non-smokers, only male participants, were included in the study. They were matched by age and body mass index (BMI): 10 young smokers (YS), 12 young non-smokers (YNS), 10 elderly smokers (ES), and 12 elderly non-smokers (ENS). We did not include participants with acute inflammatory condition or chronic inflammatory state, hypertension and metabolic syndrome. All subjects had not been using any medication for some months before the examination. Characteristics of the study subjects are shown in Table 1.

All subjects gave their informed consent to the protocol, which was approved by the local Medical Ethics Committee of the University of Latvia for Biomedical Research.

Biochemical measurements

Blood samples for cytokines and other blood tests were taken after a 12-h fast. Blood samples were collected without anticoagulant and were allowed to coagulate for 20 to 30 min at room temperature. Sera were separated by centrifugation for 20 min at 1600 × g. All specimens were immediately aliquoted, frozen, and stored at –80 0C. xMAP multiplex immunobead assay technology was used to test for MCP-1, TNF-α, IL-6 by Luminex200 analyzer (Luminex Corp., Austin, TX). For quantification of insulin resistance, we used homeostasis model assessment (HOMA-IR = fasting glucose × fasting insulin / 22.5). The HOMA-IR values have been shown to correlate well with values obtained using the "gold standard" clamp technique (Bonora et al. 2000). Fasting concentrations of insulin and glucose were analysed by standard methods (Gallois et al. 1996).

Blood flow measurements

For evaluating cutaneous endothelium-dependent and neurally regulated microvascular dilatation we used laser Doppler imaging technique (MoorLDI2, Moor Instruments Ltd., UK) in conjunction with local heating 44 °C (LDI-heating) and iontophoretic application of 1% acethylcholine solution (LDI-Ach) on the dorsum of the palm. The iontophoretic stimulation produces a stimuli-response curve which indicates the endothelium-dependent vasodilatation.

The response of skin blood flow to a step increase in local temperature is biphasic, with an early peak occurring within minutes, followed by a nadir, and then a late phase with a progressive rise to a plateau in 20 to 30 min. (Golay et al. 2004). A slower late phase is endothelium-dependent vasodilatation that relies on local production of NO, because it is suppressed by inhibitors of NO synthase and insensitive to local anesthesia (Minson et al. 2001). The early peak appears to be predominantly mediated by an axon reflex mechanism and it has been found to be diminished by local anesthesia (Minson et al. 2001). A temperature of 44 °C can induce nociceptive response and thus modify the mechanisms of vasodilation, possibly through the release of neuropeptides (Golay et al. 2004).

Laser Doppler flux was expressed in arbitrary perfusion units (PU).

Cutaneous vasomotor function, insulin sensivity and levels of inflammatory cytokines were compared between the groups – YS vs. YNS and ES vs. ENS.

Table 1. Characteristics of the study subjects. YS, young smokers; YNS, young non-smokers; ES, elderly smokers; ENS, elderly non-smokers. *, pack-years was calculated by formula: number of pack years = (number of cigarettes smoked per day × number of years smoked) / 20

Characteristics	YS (n = 10)	YNS (n = 12)	ES (n = 10)	ENS (n = 12)
Age (years)	26 ± 6	26 ± 8	47 ± 7.4	52 ± 8.4
BMI (kg m^{-2})	24 ± 4	23 ± 4	26.7 ± 3.5	23.7 ± 2.4
Waist circumference (cm)	88.6 ± 12.1	79 ± 9	94.7 ± 11.3	84.8 ± 12.4
Cigarettes per day	14 ± 5	-	17 ± 4	-
Pack-years*	8.4 ± 4	-	16.7 ± 7	-

Table 2. Levels of blood biomarkers in young smokers (YS), young non-smokers (YNS), elderly smokers (ES), and elderly non-smokers (ENS)

Biomarker	YS (mean ± SD)	YNS (mean ± SD)	p value	ES (mean ± SD)	ENS (mean ± SD)	p value
TNF-α (pg mL^{-1})	6.5 ± 1.4	4.8 ± 1.5	< 0.05	6.9 ± 1.3	4.29 ± 1	< 0.05
MCP-1 (pg mL^{-1})	480.5 ± 167.5	391.3 ± 131	ns	458.6 ± 173.5	402.5 ± 201.4	ns
IL-6 (pg mL^{-1})	3.6 ± 1.6	5.6 ± 4.7	ns	4.5 ± 2.5	5.4 ± 3.8	ns
HOMA-IR	1.5 ± 1.4	1.8 ± 1.2	ns	2.25 ± 0.9	1.7 ± 1	ns

Study design

Measurements were made in a quiet, temperature-controlled room (22 ± 0.5 °C), with the subjects in the supine position. All subjects were asked to avoid from caffeine- and alcohol-containing drinks on the examination day, and to refrain from smoking for at least 3 h before the examination. Microvascular measurements were obtained after 15 min of acclimatization and were performed with the investigated hand at heart level. The basal blood flow was studied for 3 min and the microcirculatory measurement of local heating 44 °C was performed for 25 min. Iontophoretic application of 1% acethylcholine solution was performed for 13 min on the palm of the other hand, and the basal blood flow was also calculated the first 3 min.

Statistical analysis

Data were analysed by STATISTICA 7.0 software (StatSoft Inc, USA). After testing normality, data were expressed as mean ± SD and significant differences between groups were determined using the Mann-Whitney U test (despite a normal distribution of data, a nonparametric statistical method was used due to a low number of participants). A two-tailed value of $p < 0.05$ was considered to be significant.

Results

Cytokines and HOMA-IR

Circulating level of TNF-α was significantly higher in young and elderly smokers than in both groups of non-smokers (Table 2). The level of MCP-1 was higher in both groups of smokers, but did not reach a significant difference. The level of IL-6 did not significantly differ between the groups. The HOMA-IR values was slightly higher in elderly smokers vs. other study groups (Table 2).

Vascular reactivity

Results on vascular reactivity are shown in Table 3. Cutaneous basal blood flow did not significantly differ between study groups. Cutaneous vasodilatory response to local heating was significantly decreased in elderly smokers compared with elderly non-smokers, which was shown in early peak – LDI-heating$_{1\ max}$ ($p < 0.05$) (Fig. 1) and late phase – LDI-heating$_{2\ max}$ ($p < 0.05$) (Fig. 2).

he YS group had lower neurally regulated vasodilatation, indicated by LDI-heating$_{1\ max}$ values (Fig. 2) and significantly reduced endothelium-dependent vasodilatation in LDI-heating$_{2\ max}$ ($p < 0.05$) (Fig. 1), in comparison with those in the YNS group.

LDI-Ach$_{max}$ values were significantly lower in both elderly groups (ES and ENS) than in YS and YNS ($p < 0.05$) (Fig. 3). There was no significant difference between YS and YNS, and between ES and ENS in LDI-Ach$_{max}$ values.

Discussion

The vascular alterations involve functional and structural changes of the arteries, and increasing vascular stiffness occurs with age (Marín 1995). Impaired microcirculatory vasodilatation and altered endothelial cell function has been shown to occur at advanced age and obesity (Abularrage et al. 2005; Marín 1995). Dysfunction of microcirculation occurs in a similar fashion in multiple tissue beds long before the onset of atherosclerotic symptoms (Abularrage et al. 2005).

It has been demonstrated in some studies that the early peak and the late phase in skin blood flow during local heating are diminished with advanced age. Minson and colleagues observed that axon reflex- and NO-dependent vasodilation are reduced in the skin of healthy older

Table 3. Values of microvascular measurements in young smokers (YS), young non-smokers (YNS), elderly smokers (ES), and elderly non-smokers (ENS)

Measurements	YS PU (mean ± SD)	YNS PU (mean ± SD)	p value	ES PU (mean ± SD)	ENS PU (mean ± SD)	p value
LDI-heating$_{basal}$	81.4 ± 23.9	80 ± 25.5	ns	88.3 ± 39.9	88.7 ± 39.9	ns
LDI-heating$_{1\ max}$	440.5 ± 78.7	505.5 ± 123	ns	358.5 ± 98.3	530.3 ± 133	< 0.05
LDI-heating$_{2\ max}$	446.5 ± 114	557.5 ± 111	< 0.05	393.1 ± 98.8	572.4 ± 138	< 0.05
LDI-Ach$_{basal}$	68 ± 27.5	61.2 ± 23.5	ns	71.2 ± 31.5	65.8 ± 21.7	ns
LDI-Ach$_{max}$	403.4 ± 102	380 ± 105.4	ns	263.6 ± 62 *	299.8 ± 70 *	ns

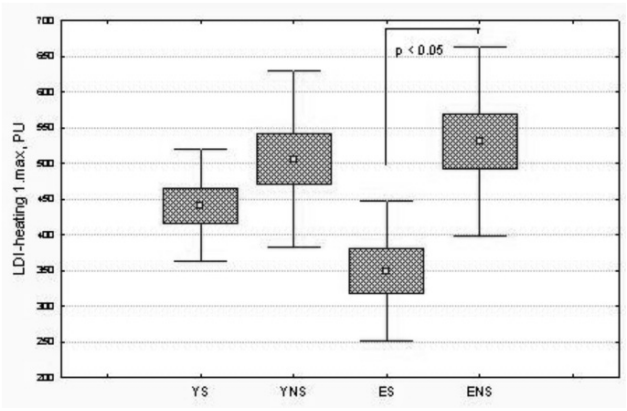

Fig. 1. Local heating-induced (44 °C) neurally regulated vasodilatation (LDI-heating$_{1\ max}$) in young smokers (YS), young nonsmokers (YNS), elderly smokers (ES), and elderly non-smokers (ENS). Data are expressed as mean ± SD.

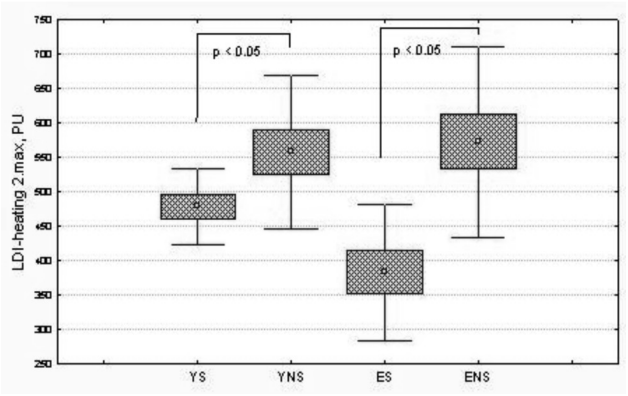

Fig. 2. Local heating-induced (44 °C) endothelium-dependent vasodilatation (LDI-heating$_{2\ max}$) in young smokers (YS), young nonsmokers (YNS), elderly smokers (ES), and elderly non-smokers (ENS). Data are expressed as mean ± SD.

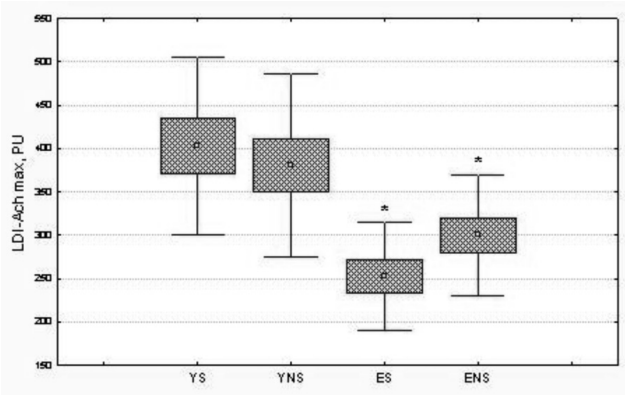

Fig. 3. Acetylcholine-induced endothelium-dependent vasodilatation (LDI-Ach$_{max}$) in young smokers (YS), young nonsmokers (YNS), elderly smokers (ES), and elderly non-smokers (ENS). Data are expressed as mean ± SD. *, $p < 0.05$ vs. younger adults.

individuals with mean age 77 ± 5 years; both functional and structural changes occur in the skin vasculature with aging that may account for the reduced skin blood flow to this stimulus (Minson et al. 2002). Andersson et al. (2003) reported that reactivity in the cutaneous microvessels is attenuated with advancing age. They found that vasorelaxation following heat was greater in young adults than in elderly subjects, whose mean age was 74 ± 2 years in the study. In our study there was no difference in cutaneous vasodilatory response to local heating in young and elderly (mean age 52 ± 8 years) non-smokers (Table 3). This disparity in age in our and the above studies may be the reason why our results differed the others.

Nevertheless, Edvinsson et al. (2008) observed significantly reduced vasodilatory response to local heating (44 °C) in smokers compared with non-smokers. The mean age in both groups was 64 ± 2 years (Edvinsson et al. 2008). We also observed significantly attenuated cutaneous vasodilatory response to local heating in elderly smokers compared with elderly non-smokers (Fig. 1 and 2). Also, young smokers had significantly decreased endothelium-dependent vasodilatation in late phase (Fig. 2) and an obvious trend to decreased neurally regulated vascular reactivity in early peak (Fig. 1). This finding indicates that smoking is a relevant risk factor which can result in vascular alterations and damage to endothelium-dependent vasodilation in any age.

In our study, the vasodilatory response to 1% Ach iontophoresis was significantly decreased in both elderly groups – smokers and non-smokers compared with young smokers and non-smokers (Table 3). There was no difference between young adults – YS and YNS, and between elderly subjects – ES and ENS. These findings are in agreement with several other reports. In the study of Andersson and colleagues the vasodilatory response to Ach was significantly higher in young adults than in healthy elderly subjects (Andersson et al. 2003). Pellaton et al. (2002) observed no significant difference in Ach-induced increase of skin blood flow between younger smokers and younger non-smokers. However, in older smokers Ach-induced skin blood flow increases were significantly attenuated in comparison with non-smokers (Pellaton et al. 2002). Also, Al-Shaer et al. (2006) demonstrated that vasodilatation to Ach in healthy elderly was significantly decreased when compared to young controls. Studies with iontophoretic administration of Ach to human skin vessels have demonstrated that vasodilatation to Ach in the forearm skin microcirculation is mediated largely by a prostanoid-dependent mechanism (Khan et al. 1997; Noon et al. 1998). Also, a reduction in prostanoid-dependent vasodilatation with healthy ageing in response to exogenous Ach has been demonstrated (Minson et al. 2002). Holowatz et al. (2005) found that NO did not directly contribute to Ach-mediated vasodilatation in either age group. They suggested that older subjects exhibit alterations in cyclooxygenase vasoactive products, favouring vasoconstriction and attenuated

vasodilator prostanoid contribution to Ach-mediated vasodilatation.

There was no difference between the groups in basal blood flow in our study. These findings are in agreement with several other reports. Edvinsson et al. (2008) did not observe a difference in basal flow between elderly smokers and non-smokers. Also, Al-Shaer et al. (2006) reported that there was no difference between young and elderly healthy non-smoking volunteers.

The higher levels of inflammatory cytokines observed in elderly smokers in this study, might indicate an inflammatory response to functional and structural changes in the microvasculature. Oxidative stress may mediate this adverse effect, since cigarette smoke contains large amounts of free radicals such as superoxide anion and hydroxyl radicals that degrade NO released from the endothelium (Neunteufl et al. 2002). ROS formation in the peripheral vasculature can be induced by cytokines such as IL-6 and TNFα, and thus the inflammatory response can induce endothelial dysfunction, possibly by enhancing the production of ROS, which can scavenge NO (Andersson et al. 2003; Ijzerman et al. 2003). Cigarette smoking is associated with an increased level of multiple inflammatory markers, including IL-6 and TNFα, in smokers (Ambrose, Barua 2004; Unverdorben et al. 2009).

Some studies have shown smoking to be associated with decreased insulin sensitivity and increased visceral fat accumulation. It has been reported that insulin resistance was higher in smokers compared with non-smokers (Unverdorben et al. 2009) and smokers tended to be relatively insulin resistant with evidence of endothelial dysfunction compared with non-smokers (Reaven, Tsao 2003). Dzien et al. (2004) reported that smoking was associated with a metabolic profile, indicating a higher degree of insulin resistance in men with and without clinically manifested cardiovascular disease. In our study we observed that elderly smokers exhibited a tendency to higher values of insulin resistance, body mass index and waist circumference vs. elderly non-smokers. Cross-sectional studies have indicated that waist circumference is higher in smokers than in non-smokers (Chiolero et al. 2008). Waist circumference is an indicator of the amount of visceral adipose tissue and is positively associated with the number of pack-years of smoking (Chiolero et al. 2008). Our study did not find any correlations between pack-years and IR, waist circumference and BMI.

In this study elderly smokers demonstrated significantly decreased vasomotor reactivity of the skin microvasculature. Also, the levels of inflammatory cytokines were higher in cigarette smokers than in non-smokers. However, elderly smokers showed higher values of insulin resistance, and there was no direct association with smoking status. Our findings suggest that long-lasting cigarette smoking significantly impairs vascular function and inflammatory state in middle age smokers.

Acknowledgements

This study was supported by grant No. 2010.10-4/VPP-4/5 of the framework of the Latvian National Program and by the European Social Fund within the project «Support for Doctoral Studies at University of Latvia».

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Received 9 February 2011; received in revised form 12 March 2011; accepted 16 March 2011