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Impact of long-term exposure to cigarette smoking on skin microvascular function

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ABSTRACT

In order to evaluate the impact of cigarettes smoking and smokers' clinical characteristics on skin microvascular function, we measured the skin forearm blood flux, basally and during post-occlusive reactive hyperaemia, in 100 current smokers (mean age 51 ± 11 years; range: 18 to 86 years) and in 66 healthy never-smokers matched for age and sex, by using laser Doppler fluximetry (LDF). Basal and post-ischemic LDF tracings were analyzed in the frequency domain within 0.009–0.02 Hz, 0.021–0.06 Hz and 0.061–0.2 Hz ranges, related to endothelial-dependent, sympathetic-dependent and myogenic-dependent vasomotion, respectively, using an adapted version of the Fourier analysis. The post-ischemic percentage change from baseline of the area under the LDF curve (AUC%) was significantly lower in smokers than in never-smokers [162.5% (139.3–183.0) vs 190.1% (156.3–216.8); $p = 0.00016$]. Compared to controls, smokers also showed a reduced basal power spectral density (PSD) in the myogenic-dependent vasomotion ($p = 0.0034$) and a reduced post-ischemic percentage increase in PSD of the endothelial-dependent vasomotion ($p = 0.0010$) and sympathetic-dependent vasomotion ($p = 0.0016$). An inverse relationship was observed in smokers between AUC% and smoking exposure duration ($r = 0.23$, $p = 0.018$), pack-years ($r = 0.33$, $p = 0.0007$), age ($r = 0.26$, $p = 0.008$) and body mass index ($r = 0.21$, $p = 0.037$). In the multiple linear regression model, pack-years was the only variable independently associated with AUC% ($r = 0.21$, $p = 0.03$). This study confirms that smoking is associated with cutaneous microvascular dysfunction and shows that the severity of this impairment is independently related to the duration and intensity of the exposure to smoking.

Introduction

Cigarette smoking represents one of the main preventable causes of death worldwide. Besides the damage on the respiratory system, a large amount of data demonstrates that long-term cigarette smoking is associated with a generalized impairment of the vascular function, at the level of both conduit (McGill, 1988; Whisnant et al., 1990; Witteman et al., 1993; Willeit et al., 2000) and resistance arteries (Rangemark and Wennmalm, 1992; Jacobs et al., 1993; Heitzer et al., 1996; Pellaton et al., 2002; Dalla Vecchia et al., 2004; Rossi et al., 2007a; Avery et al., 2009). Impairment of vascular function considerably increases the risk of the development of cardiovascular diseases, such as myocardial infarction, stroke and peripheral artery disease in current smokers (Murray and Lopez, 1996; Powell, 1998). In particular, the impairment of microvascular function in smokers has been demonstrated in studies based on laser-Doppler fluximetry (LDF) at the level of the cutaneous microcirculation. In these studies smokers exhibited a reduction in the cutaneous vasodilatory response to different stimuli, such as ischemia (Pellaton et al., 2002), thermal stimulus (Avery et al., 2009), and acethylcoline iontophoresis (Rangemark and Wennmalm, 1992; Pellaton et al., 2002), accounting for both a myogenic and an endothelial dysfunction of the skin microvasculature. A further finding, suggesting a microvascular dysfunction in current smokers, comes from studies that examined the blood flow oscillations, the so called blood flowmotion, detected at the level of cutaneous microcirculation by means of the spectral analysis of LDF signal (Rangemark and Wennmalm, 1992; Pellaton et al., 2002). In studies based on this method, smokers exhibited a reduced power spectral density (PSD) increase of some cutaneous blood flow oscillations within the frequency range of 0.009 and 1.6 Hz, either in response to ischemia (Rossi et al., 2007a), or in response to a thermal stimulus (Avery et al., 2009). Some of these blood flow oscillations are suggested to be related to rhythmical variations in cutaneous microvessel diameter, the so called vasomotion (Colantuoni et al., 1994). PSD reduction in smokers of the blood flow oscillations related to vasomotion accounted for the impairment of this particular attitude of the microvascular wall. Since vasomotion has been suggested to play an important role in optimizing the tissue blood flow distribution (Parthimos et al., 1996; Sakurai and Terui, 2006), reduction of cutaneous vasomotion in smokers may further aggravate the functional impairment of the microcirculation. If, as recently

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suggested, skin microcirculation mirrors the state of microcirculation in other vascular beds, including cardiac muscle (Tur et al., 1991; Jung et al., 2001; Shamim-Uizzaman et al., 2002; Holowatz et al., 2008), the impairment of the skin microvascular function demonstrated in smokers has a relevance that goes beyond the involvement of the cutaneous tissue.

One might expect that the negative effects of long-term cigarette smoking on microvascular function would become more pronounced as the number of years and the intensity of smoking increase. However, the small number of smokers who were examined in previous studies aimed to clarify this aspect (Pellaton et al., 2002; Avery et al., 2009), did not provide conclusive data on the possible influences of smoking habit variables on microvascular function, or on the interaction between chronic smoking habit and other clinical variables on the same function.

The aim of this study was to identify smoking habit characteristics and/or clinical variables that independently affect microvascular function in current smokers. With this purpose, the skin post-occlusive reactive hyperemia (PORH) and skin vasomotion were assessed in a large group of current smokers and in a control group of never smokers by means of LDF coupled with spectral analysis of LDF signal.

Materials and methods

Subjects

This study included current cigarettes smokers who consecutively attended a smoking cessation program at the Smoking Cessation Centre of the University-Hospital of Pisa from April 2009 to February 2011.

The criteria for selection of smokers were to be free from heart failure, liver failure, renal failure, severe arterial hypertension or dermatological disease. Information on smoking exposure and presence of other cardio-vascular risk factors were collected in the enrolled smokers by standardised procedures. Expired carbon monoxide was always measured at enrolment. Life-time exposure to cigarette smoking was assessed as pack-years, a parameter which was obtained by multiplying the number of cigarettes smoked per day by the number of years the person has smoked, and dividing by 20 (the number of cigarette in a pack) (Prignot, 1987).

Apparently healthy normotensive volunteers who had never smoked, age and sex-matched with smokers, were recruited among visitors to the Clinical and Experimental Medicine Department of University-Hospital of Pisa, as controls. Exclusion criteria for control subjects were a family history of diabetes, to practice with regularity an intense sport activity, and passive smoking exposure. Smokers exposed to passive smoking were defined those who answered “yes” to the standard question asked at recruitment “Are you usually exposed to smoking of other persons at home, at work or in other places?”. The same question was used for selecting the control subjects.

Smoker and control subjects underwent skin PORH test using LDF within 1 week of their enrollment in the study.

The protocol of this study was approved by the local Ethics Committee and was in accordance and with the Helsinki Declaration of 1975, as revised in 2000. Each enrolled subject gave his written consent to take part to the study.

Test of skin post-occlusive reactive hyperaemia

Cutaneous PORH test was performed in smokers and control subjects using a method previously reported (Rossi et al., 2007a). The test was carried out in the morning, over a period of about 60 min in each case, in a quiet room with air conditioning, whose temperature was systematically measured and ranged from 21 °C to 23.5 °C.

Subjects were asked to abstain from food, drugs, alcohol, coffee and tea 8 h prior to the LDF measurement. Each subject had 20 min of acclimatisation in the supine position before the test was initiated. After acclimatisation, basal skin blood flux in his/her right forearm was recorded by means of an LDF apparatus (Periflux PF4, Perimed, Järfälla, Sweden), equipped with a not heated probe (PF408). This apparatus allowed skin blood flux to be detected in a cutaneous tissue volume of about 1 mm, and measured in perfusion units (PU) (1 PU = 10 mV). The LDF probe was fixed to the medial surface of right forearm, the precise measurement site being selected so as to avoid proximity to any of the larger blood vessels, hairs and blemishes. The laser characteristics were: 780 nm wavelength, 10 Hz–19 KHz bandwidth, 0.1 s time constant, 32 Hz sampling frequency. Probe calibration was performed before each test session, by a specific device (Perimed, Järfälla, Sweden) containing colloidal latex particles whose Brownian motion provides the standard values. The LDF signal was recorded continuously by an interfaced computer (Compaq, Hewlett Packard, Netherlands) equipped with a dedicated software (Perisoft, Perimed, Järfälla, Sweden).

After the basal skin blood perfusion had been recorded for 20 min, right forearm ischemia was induced by inflating a pneumatic cuff (which was positioned on the right arm prior to the basal blood flux measurement) to 30 mm Hg above the systolic blood pressure of the subject, for 3 min. After this time, the pneumatic cuff was instantaneously deflated and forearm skin blood flux was then recorded for 10 min. The choice of 3 min for the occlusion was based on a previous study (Tee et al., 2004) showing that 3 min ischemia caused a greater increase in skin perfusion compared to 1 or 2 min, whereas prolonging ischemia for >3 min produced patient discomfort.

Basal skin blood flux was taken as the mean value in PU during the 3 min interval before the occlusion. Maximal post-ischemic skin blood flux (peak-flux) was taken as the highest blood flux value recorded after ischemia. The skin PORH was expressed as the percentage change in the post-ischemic area under the LDF curve above baseline (AUC%). In particular, AUC% was obtained as percentage change of the post-ischemic area under the LDF curve (PU per s) registered during the first 3 min after pneumatic cuff deflation, above the baseline area under the LDF curve (PU per s) registered during the last 3 min before pneumatic cuff inflation. The time taken to reach the maximum post-occlusive blood flux from pneumatic cuff release (peak-time) was also measured in smokers and controls.

Assessment of skin vasomotion

Skin vasomotion was assessed by means of the spectral analysis of skin LDF signal, using a dedicated software (Perisoft, Perimed, Järfälla, Sweden) which was already used in previous studies (Rossi et al., 2007a, 2007b, 2008; Schmiedel et al., 2007). This software measures in PU^2/Hz the PSD of LDF signal oscillations, using the basic fast Fourier transform algorithm. In the fast Fourier transform algorithm, we used, the beginning and the end of the signal were attenuated by using a windowing “Parzen” function, to avoid the well known “leakage phenomenon” (frequency components in the spectra “leaking” into other frequencies). In the windowing “Parzen” function, a short-time Fourier transform, with a different window length for each frequency interval, was used. According to the same previous studies (Rossi et al., 2007a, 2007b, 2008; Schmiedel et al., 2007), the frequency spectrum from 0.009 to 1.6 Hz was divided into the following five sub-intervals: 0.009–0.02 Hz, 0.021–0.06 Hz, 0.061–0.2 Hz, 0.21–0.6 Hz. and 0.61–1.6 Hz. Each of these LDF signal frequency intervals is related to cutaneous endothelial-dependent vasomotion, sympathetic-dependent vasomotion, myogenic-dependent vasomotion, respiratory activity and heart activity, respectively (Kvernmo et al., 1999; Stefanovska et al., 1999).

The absolute PSD of each of the five sub-intervals considered was measured during 5 min before ischemia, as well during 5 min after the peak of skin PORH. Data analysis, performed using the Perisoft dedicated software, allowed to identify the highest PSD within each of the five frequency intervals considered. This value was considered the absolute PSD value of that given frequency interval. The PSD value of the total spectrum was obtained by the sum of the PSD value of each frequency interval considered. Post-ischemic per cent change in the PSD value of