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Effect of Smoking on Endothelial Function and Wall Thickness of Brachial Artery

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Background Impaired flow mediated dilatation (FMD) and increased wall thickness (WT) of the brachial artery have been associated with atherosclerosis and its risk factors. In this study we sought to determine brachial artery wall thickness in chronic smokers and the instantaneous effect of smoking on brachial artery endothelium dependent vasodilator function in smokers and non-smokers.

Method and Results Using a high-resolution ultrasound, WT of posterior brachial artery wall, the diameter of brachial artery at rest and during reactive hyperemia (FMD %), as well as after sublingual administration of nitroglycerine (nitroglycerine mediated dilatation (NMD) %) was measured in 20 smokers and 20 non-smokers. Wall thickness (WT) of the posterior brachial artery wall and the wall index (WI) were greater in smokers than non-smokers. The baseline brachial artery diameter was comparable in smokers and non-smokers. Flow mediated dilation (FMD) was found to be less in smokers than non-smokers. The NMD in smokers also did not differ significantly from that in non-smokers. Flow mediated dilation significantly reduced after smoking compared to baseline in both groups. However, NMD remained unchanged after smoking in both groups.

Conclusions Increased WT and impaired endothelium-dependent dilatation of brachial artery suggests that cigarette smoking disrupts vessel wall morphology long before atherosclerosis is manifest. (*Circ J* 2004; **68:** 1123 – 1126)

Key Words: Acute smoking; Brachial artery wall thickness; Endothelial function

Cigarette smoking is a well-known cardiovascular risk factor, and it affects both the coronary and peripheral circulation[1–4] Because cigarette smoke contains a large number of oxidants, it has been hypothesized that the adverse effect of smoking could result in oxidative damage to vascular endothelium[5] Indeed, endothelial dysfunction in brachial and coronary arteries has been demonstrated in long-term smokers and even in passive smokers[6–8] However, it has also been found that acute cigarette smoking causes vasoconstriction of the epicardial coronary artery and increases the coronary resistance vessel tone[9,10] Acute cigarette smoking has also been shown to cause a transient increase in pulse rate and blood pressure[11] Although previous studies have apparently found that chronic cigarette smoking is associated with endothelial dysfunction, data regarding the dose-dependent effects of smoking on endothelial dependent vasodilatation are limited and inconclusive[6–8, 12,13]

Flow mediated dilatation in systemic as well as coronary arteries is mediated by the endothelium through the release of dilator substances that act on the underlying smooth muscle; these endothelium-derived relaxing factors have been identified as nitric oxide[14] Anderson et al[15] found that coronary artery endothelium dependent vasomotor responses to acetylcholine and flow-mediated vasodilatation in the brachial artery were similar. Thus, endothelial function in peripheral vessels such as the brachial artery can be measured noninvasively and inferentially correlated to responses within the coronary vasculature. In addition, Weideinger et al[16] recently showed that brachial artery wall thickness (BA-WT) is independently correlated with the presence of coronary artery disease (CAD) and that BA-WT can provide a novel noninvasive marker of atherosclerosis.

Therefore, the purpose of this study was to determine chronic as well as instantaneous effects of smoking on brachial artery endothelial function in long-term smokers and non-smokers, and the effect of chronic smoking on BA-WT.

Methods

Subjects

Twenty healthy long-term heavy smokers (15 males, 5 females, mean age 27±9 years, smoking average of 25 cigarettes/day) and age-matched 20 healthy non-smoking hospital staff (14 males, 6 females, mean age 25±7 years) were studied. A complete physical and echocardiographic examination was performed prior to the study. The participants were free from the other risk factors for CAD and none were taking any any medication during the study (Table 1). All participants gave their informed consent and the institutional review board approved the study protocol.

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Table 1 Study Subjects Baseline Characteristics

Variable	*Smokers*	*Nonsmokers*	*p values*
No. (M/F)	*15/5*	*14/6*	*NS*
Age (years)	*27±9*	*25±7*	*NS*
Total cholesterol (mg/dl)	*177±27*	*171±25*	*NS*
HDL cholesterol (mg/dl)	*43±6*	*42±6*	*NS*
Triglycerides (mg/dl)	*135±24*	*129±21*	*NS*

NS, Statistically not significant; HDL, high density lipoprotein.

Study Design

After a light breakfast following overnight fasting, the participants were taken to a dimly lit room with an average temperature of 22–24°C. The participants were asked to refrain from alcohol and beverages containing caffeine and strenuous exercise for 24 h prior to the study. Smokers were asked to refrain from smoking at least 8 h before the study. The studies were performed between 09.00 h and 12.00 h. The participants rested on beds in supine position for at least 15 min. Using a 11.0-MHz linear array transducer of a combined sonography system (Toshiba, Nemio 20, Tokyo, Japan), the BA-WT was measured. Flow mediated dilation, as well as NMD were also measured at supine position. After completion of baseline records, all participants were asked to smoke a cigarette (1.1 mg nicotine, 15 mg tar); inhalation of smoke was discouraged. Measurements of FMD and NMD of BA were repeated 30 min after smoking in both groups.

Assesment of BA-WT

Brachial artery wall thickness was assessed in 2 ways on the peak of the vessel arch. First, the intima and media distance between lumen-intima and media adventitia border using electronic calipers. Measurements were obtained at 2 sites per image in 4 different images per patient. The 2 sites per image were defined as being at, or close to, 1 mm of the "peak" of the vessel arch. Usually, this peak is the location that gives the clearest image. Care was taken to limit the distance between the 2 measurements sites to 3 and 5 mm.The mean of the 8 measurements was defined as BA-WT. Second, diameters were measured as the distance between the anterior to the posterior media-adventitia border (diameter media) and anterior to posterior lumen-intima border (diameter intima) to obtain calculated BA-WT (diameter media-diameter intima/2). Finally, a WI was derived to correct WT for vessel diameter: WI=([diameter media-diameter intima]/diameter media)×100.

Assesment of FMD

The right BA, proximal to the antecubital fossa, was imaged longitudinally using the linear-array transducer. Flow-mediated endothelium-dependent vasodilatation was assessed by measuring the BA diameter at baseline and during reactive hyperemia as previously described![7] Reactive hyperemia was induced by deflating a cuff previously inflated to 300 mmHg for 4.5 min in the forearm. Flow mediated dilation was assesed at 50–60 s after deflating the cuff. Arterial flow velocity was measured at baseline and during reactive hyperemia using pulsed-wave Doppler. Blood flow was calculated by multiplying the velocity time integral of the Doppler flow signal by the heart rate and the vessel cross-sectional area ($3.14\times D^2/4$). After 10 min, the endothelium-independent response was assessed by the change in artery diameter at 3–4 min after a 400 μg of sublingual nitroglycerine (NTG). The parameters were measured for 3 consecutive cardiac cycles, and the average was taken.

The percentages of diameter and flow changes during reactive hyperemia and after sublingual NTG were calculated. All measurements were performed by 2 independent obervers who were uninformed of the clinical and study details. The interobserver and intraobserver variability for FMD and BA-WT were 0.04±0.03 mm, 0.03±0.02 mm, and 0.06±0.02 mm, 0.05±0.03 mm, respectively.

Statistical Analysis

Statistical analysis was performed with SPSS for Windows version 11.0 (SPSS Inc, Chicago, Illinois). Data are presented as mean±SD. For continuous variables The Mann–Whitney U test was used for continuous variables and the chi-square test was used for categorical changes. Differences in the variables before and after smoking within each group were determined using the Wilcoxon signed rank test. Pearson correlation analysis was conducted to investigate the correlation between the directly measured BA-WT and calculated BA-WT. A p value <0.05 was considered to indicate statistical significance.

Results

There was no difference in body mass index, heart rate, systolic and diastolic blood pressure between smokers and non-smokers (25±5 vs 26±5 kg/m^2, 66±7 vs 64±9 beats/min, 120±9 vs 118±8 mmHg, 69±7 vs 68±8 mmHg, respectively). Mean duration of smoking was 10±2 years in smokers. Directly measured BA-WT and calculated BA-WT were greater in smokers than non-smokers (0.39±0.05 vs 0.33±0.03 mm, p=0.0001 and 0.37±0.05 vs 0.32±0.03 mm, p=0.0001, respectively) and the 2 methods showed a close correlation (r=0.93, p<0.0001). The WI was also greater in smokers than non-smokers (WI: WT/vessel diameter×100; 18.6±1.8 vs 14.7±1.2, p=0.0001). Baseline BA diameter was comparable in smokers and non-smokers (4.4±1.7 mm vs 4.5±1.5 mm, p=0.8). Flow mediated dilation was found to be significantly lower in smokers than non-smokers (4.7±1.6% vs 9.2±4.6%, p=0.0001). Blood flow during reactive hyperemia was similar in both groups (527±157% vs 511±151%, p=0.7). The NMD in smokers also did not differ significantly from that in non-smokers (14.9±2.6% vs 14.4±1.8%, p=0.4). Blood flow during reactive hyperemia did not alter significantly after smoking in either group (527±157% vs 544±168%, p=0.2, and 511±151% vs 520±149%, p=0.4, respectively). Flow mediated dilation reduced significantly after smoking compared to baseline, but NMD remained unchanged after smoking both in smokers and non-smokers (Figs 1 and 2, respectively).

Discussion

In the present study we have noted that: (1) WT and WI of the posterior BA wall are greater in smokers than non-smokers, (2) FMD of BA was significantly impaired in smokers compared to non-smokers, and (3) short-term smoking reduces FMD in brachial artery both in smokers and non-smokers.

Endothelial dysfunction is evident at an early stage in development of coronary atherosclerosis. Brachial artery wall thickness measurements with high-resolution ultrasound appeared to be a feasible, reproducible, and had low

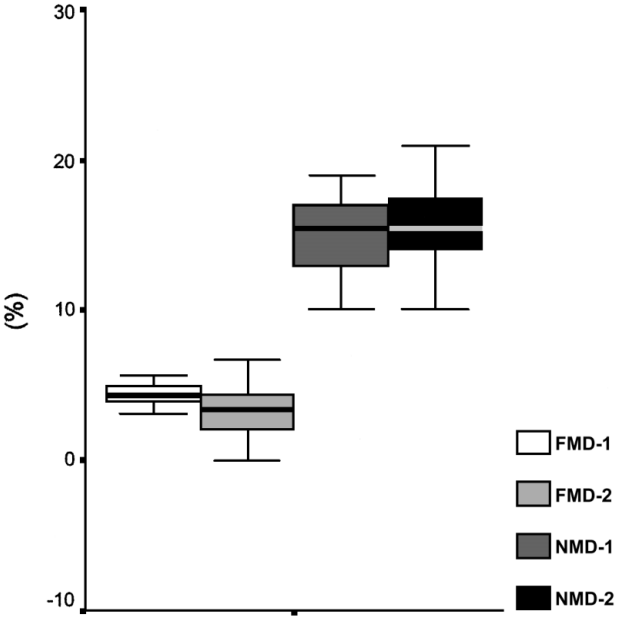

Fig 1. Comparison of flow-mediated dilatation (FMD) and nitroglycerine-mediated dilatation (NMD) of brachial artery before (1) and after smoking a cigarette (2) in smokers (Statistical significance level; FMD1 vs FMD2; 4.7±1.6% vs 3.1±2.3%, p=0.017, NMD1 vs NMD2; 14.9±2.6% vs 15.5±2.9%, p=0.16).

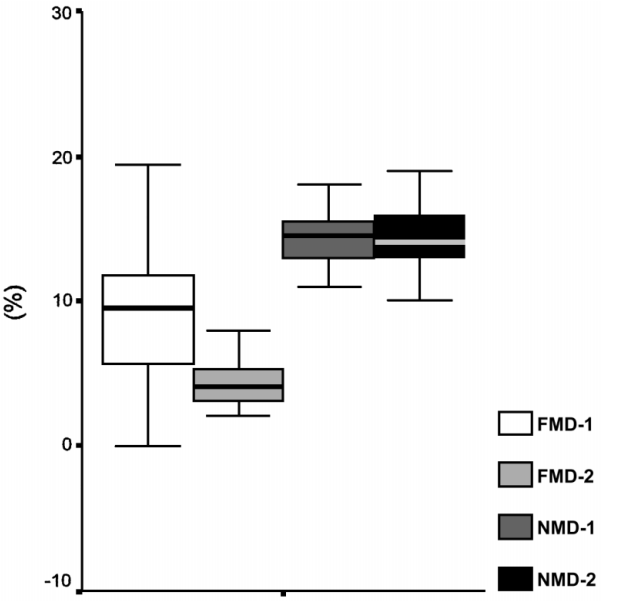

Fig 2. Comparison of flow-mediated dilatation (FMD) and nitroglycerine-mediated dilatation (NMD) of brachial artery before (1) and after smoking a cigarette (2) in non-smokers (Statistical significance level; FMD1 vs FMD2; 9.2±4.6% vs 4.3±2.4%, p=0.002, NMD1 vs NMD2; 14.4±1.8% vs 14.6±2.3%, p=0.4).

interobserver variability values that are similar to published data obtianed in other vascular beds such as the carotid artery[18] In the present study, we found that BA-WT and WI were higher in smokers than non-smokers. Accordingly, Sorensen et al[19] showed that atherosclerosis in the brachial artery is a frequent finding and that correlations between BA and coronary and carotid lesions are at least as strong as between the latter 2 arterial beds. Thus, our results are in accordance with the data from a previous study that found a close correlation between the smoking and carotid artery intima-media thickness[20] Cryer et al[21] found prompt increases in systolic blood pressure and heart rate 2.5 min after the commencement of smoking, with a maximal hemodynamic response to smoking within 5 min. In this study, serum cathecholamine levels increase and return to baseline levels 30 min after the commencement of smoking. Previous studies have found that within the first 10 min of active smoking, there is a rise in plasma or serum endothelin-1 level, which is followed by a decline over time[22,23] Therefore, we have repeated the evaluation of the brachial artery endothelial function, 30 min after smoking. Our results suggest that acute, as well as chronic cigarette smoking, impair endothelial function.

The mechanism of smoking-induced endothelial dysfunction is unclear and complex. Several mechanisms might account for the smoking-induced alterations in coronary endothelial function. Smoking is associated with a direct toxic effect on human endothelial cells, reduces endothelial prostacyclin production and increases leukocyte adhesion to endothelial cells[24–27] Cigarette smoke contains a large number of oxidants; the role of oxygen-derived free radicals in mediating endothelial dysfunction can be modulated by the potent antioxidant vitamin C[28–30] Alternatively, smoking increases endothelial angiotensin II production, which reduces nitric oxide activity, and might contribute to endothelial damage in smokers[31] Increased platelet and serum fibrinogen, as well as decreased serum plasminogen levels known to occur in smokers, might also impair endothelial function in smokers[32] It has been suggested that FMD is mediated by the endothelium-derived relaxing factor, which is known as NO[33] It has been shown that inhaling a single cigarette decreases exhaled NO in smokers. This can inhibit the enzyme NO synthase, whereas inhalation of NO itself and carbonmonoxide, both constituent of tobacco smoke, have no effect on exhaled NO in nonsmoking controls[34]

It is well known that cigarettes are a source of nicotine. Endothelial-dependent dilatation of arterioles in the hamster was modestly impaired by infusion of low concentrations of nicotine and acute infusion of nicotine[35] The concept that nicotine causes endothelial dysfunction via an increase in oxidative stress has been supported by animal studies demonstrating that chronic exposure to nicotine and acute infusion of nicotine cause an impairment of endothelium-dependent arteriolar dilatation that can be restored by superperfusion with superoxide dismutase[36] Overall, smoking-induced endothelial dysfunction is most likely associated with increased oxidative stress and its toxic products.

It is also well known that endothelium-dependent vasodilatation varies during the menstrual cycle. We included 11 young females in our study thus, this could have partly affected our results. However, the majority of females were not menstruating during the study period. There was also no difference in the number of females in both groups. Thus, we believe that the effects of the menstrual cycle on our results are probably minimal.

In conclusion, chronic as well as acute cigarette smoking disrupts vessel wall morphology. Increased BA-WT can be an early indicator of subclinical atherosclerosis in long-term smokers. However, whether acute cigarette smoking-induced endothelial dysfunction can lower the threshold of

cardiovascular events needs to be determined by further large-scale studies.

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