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Oral snuff impairs endothelial function in healthy snuff users

M. ROHANI & S. AGEWALL
From the Department of Cardiology, Huddinge University Hospital, Karolinska Institute, Stockholm, Sweden

Abstract. Rohani M, Agewall S (Karolinska Institute, Stockholm, Sweden). Oral snuff impairs endothelial function in healthy snuff users. *J Intern Med* 2004; **255**: 379–383.

Aims. Oral snuff is a less dangerous drug and therefore a good substitute for cigarette smoking. The aim of this study was to determine whether oral moist snuff induces acute endothelial dysfunction. Previous studies have shown that endothelial dysfunction predicts cardiovascular morbidity.

Methods and results. Twenty healthy middle-aged snuff users underwent ultrasound assessment of endothelial-dependent flow-mediated dilatation (FMD) of the brachial artery. FMD measurements were performed in duplicate at baseline and then 20 and 35 min after the administration of 1 g portion-bag-packed moist snuff or placebo. Ten of the subjects were examined twice according to a randomized cross-over procedure, once with snuff and once with placebo. All images were arbitrarily analysed off-line by a single blinded observer. FMD values declined significantly from 3.4 ± 2.0% to 2.3 ± 1.3% ($P < 0.05$) 35 min after the administration of 1 g oral moist snuff. Heart rate, systolic and diastolic blood pressure increased significantly ($P < 0.05$) after snuff administration. All parameters remained unchanged after placebo.

Conclusions. Oral moist snuff significantly impaired FMD of the brachial artery. As endothelial dysfunction predicts cardiovascular morbidity, use of oral snuff should be discouraged.

Keywords: brachial artery, endothelial function, flow-mediated dilatation, nicotine, snuff, ultrasound.

Introduction

The use of oral moist snuff (ground and moistened dark tobacco, buffered to a pH of about 8.5 with sodium carbonate) is a widespread habit in Sweden. During the last two decades snuff sales have steadily increased and in 2001, 198 million cans were sold. Today, 10.4% of the population between 15 and 75 years of age in Sweden are snuff users. This increase has occurred in conjunction with a decrease in the consumption of cigarettes. In Sweden, which is the largest market in northern Europe, there are almost a million snuff consumers; about half of them are former smokers. Cardiovascular health hazards and endothelial damage associated with cigarette smoking are well known [1, 2] but conflicting results have been obtained regarding the role of smokeless tobacco as an important cardiovascular risk factor [3–5].

Previous data show that snuff use, in contrast to tobacco smoking, is not associated with an increase of the intima-media thickness (IMT) in the carotid and femoral arteries as assessed by the ultrasound method [6, 7]. However, endothelial dysfunction, especially reduction in the bioavailability of endothelium-derived nitric oxide is a key early event in atherogenesis, appearing long before the formation of structural atherosclerotic changes [8, 9]. The regular use of smokeless tobacco results in blood levels of nicotine similar to those observed in cigarette smokers [10] and animal studies have shown an impairment of endothelium-dependent dilation of arterioles after acute infusion of nicotine at a concentration similar to that observed in smokers [11]. The mechanism for altered responses of peripheral arterioles during exposure to nicotine appears to be related to the production of oxygen radicals as treatment with superoxide dismutase significantly restores impaired vasodilation [12]. However, the precise cellular pathway that accounts for the formation of the oxygen radicals remains unclear. Ultrasonographic assessment of flow-mediated dilatation (FMD) in the

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brachial artery is a noninvasive method that provides valuable insights into early atherogenesis.

Endothelial function assessed by this method correlates with an invasive testing of coronary endothelial function, as well as to the severity and extent of coronary atherosclerosis [13, 14]. The aim of this study was to determine whether intake of oral moist snuff causes acute endothelial dysfunction in the brachial artery of long-term snuff users.

Methods

Study population and design

Twenty middle-aged healthy snuff users were enrolled in the study. No participant had a history of cardiovascular disease or diabetes. None of the participants took any drug. The studies were always conducted early in the morning after 10-h fasting. FMD measurements were performed in duplicate at baseline, and then 20 and 35 min after the administration of 1 g portion-bag-packed moist snuff of the same brand or placebo. Ten of the subjects were studied twice according to a randomized cross-over procedure, once with snuff and once with placebo within a week of the first study. Amongst these 10 subjects, half of them were examined before and after placebo first and the other five subjects began with the snuff examination (Fig. 1). All ultrasound images were recorded on videotapes. The Ethics Committee of the Karolinska Institute, Huddinge University Hospital approved the study, and all subjects gave their informed consent to participate.

Assessment of FMD and blood flow

After having taken a medical history and having measured supine resting pulse and blood pressure the ultrasound procedures for assessing endothelium-dependent FMD were performed as described in the guidelines by Corretti *et al.* [15].

Briefly, using a high-resolution ultrasound scanner (GE Vingmed System Five, Horten, Norway) with a 10.0-MHz linear array transducer, the left brachial artery was measured twice at rest in the recumbent position in a temperature-controlled room (23 °C) after a 10-min equilibration period by an ultrasonographer. Scans of the brachial artery were taken proximal to the antecubital fossa at baseline and 2.5 min after release of the blood pressure cuff (12.5 cm wide) placed on the forearm at arterial occlusion. The procedures were repeated 20 and 35 min after placing 1 g of a portion-bag-packed snuff in the upper gingivolabial sulcus. Pulse and blood pressure were measured before each ultrasound assessment in the right arm. The velocity of arterial flow was measured with a pulsed Doppler at a 60° angle to the vessel at baseline rest image and immediately after cuff release.

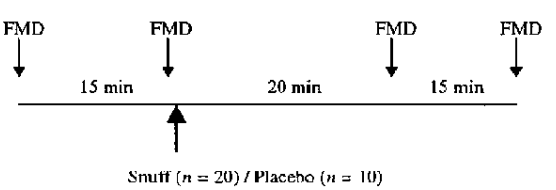

Fig. 1 Flow-mediated dilation at baseline calculated as a mean value of the two measurements. Ten subjects were studied once with snuff and the other 10 subjects were randomized to placebo or snuff and studied twice by a cross-over procedure within a week of the first study. Snuff (n = 20)/placebo (n = 10).

Data analysis

Images were digitally acquired from the videotape and measured in random order by a single observer blinded to the conditions under which the ultrasonic images were obtained. Measurement of the brachial artery diameter was defined as the distance from the leading edge of the near wall to the leading edge of the far wall of the artery along a line perpendicular to the artery's long axis [16]. Brachial artery diameter was calculated in diastolic frames taken coincidentally with the R wave on the electrocardiogram twice at rest and then 45, 60 and 75 s after cuff deflation. A mean of the diameter after 45, 60 and 75 s was calculated. Diameter changes were expressed as the percentage change relative to the mean baseline value (100%). Volume flow was calculated by multiplying the velocity time integral of the Doppler flow signal for a single pulse wave by the heart rate and the cross-sectional area of the vessel. Volume flow was measured during rest and the peak response at the maximum flow in a single cardiac cycle 10–20 s after cuff release.

Statistical methods

Results are presented as mean and standard deviations. Paired t-test was used to compare continuous

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variables. All tests were two-sided and $P < 0.05$ was regarded as statistically significant.

Results

Figure 1 shows the flow chart of the study. Twenty subjects participated in the study (18 males and two females), mean age 34 years. Mean blood pressure was 109/74 and heart rate was 55 beats per minute (Table 1). FMD values declined significantly from baseline 3.4 ± 2.0% to 3.1 ± 2.4% (NS) and 2.3 ± 1.3% ($P = 0.004$), at 20 and 35 min, respectively, after the administration of snuff (Table 2), but remained unchanged in the placebo group. Basal vessel size (at baseline, 20 and 35 min after snuff administration: 3.80 ± 0.34, 3.78 ± 0.35 and 3.81 ± 0.30 mm) remained unchanged (Table 2). Heart rate, systolic and diastolic blood pressure increased significantly after snuff administration (Fig. 2), but remained unchanged in the placebo group. The percentage increase in blood flow during reactive hyperaemia increased slightly but not significantly 20 min after snuff administration (at baseline, 20 and 35 min after snuff administration: 338 ± 138, 365 ± 125 and 319 ± 105%) and remained unchanged after placebo (baseline 431 ± 140, 446 ± 144 and 441 ± 117%).

Table 1 Baseline characteristics ($n = 20$)

Age (years)	34 ± 6
SBP (mmHg)	109 ± 10
DBP (mmHg)	74 ± 5
HR (bpm)	55 ± 9

DBP, diastolic blood pressure; SBP, systolic blood pressure; HR, heart rate.

Table 2 Flow-mediated dilatation characteristics at baseline, 20 and 35 min after the administration of moist snuff ($n = 20$)

	Baseline	20 min	35 min
Baseline brachial artery diameter (mm)	3.80 ± 0.34	3.78 ± 0.35	3.81 ± 0.30
Peak hyperaemic blood flow	438 ± 140	465 ± 125	419 ± 105
Blood flow increase (%)	338 ± 138	365 ± 125	319 ± 105
FMD (%)	3.4 ± 2.0	3.1 ± 2.4	2.3 ± 1.3*

Values are mean ± SD. *$P < 0.05$.

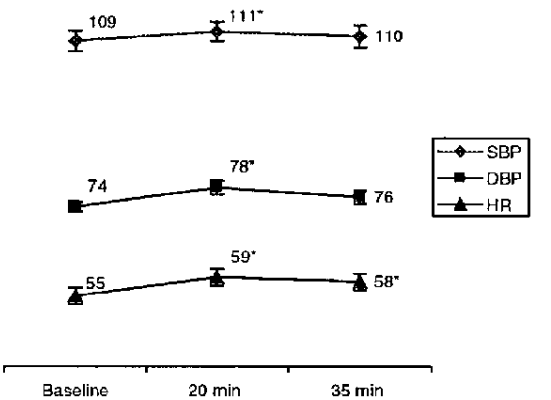

Fig. 2 Effect of oral snuff on heart rate (HR), systolic (SBP) and diastolic (DBP) blood pressure. Oral snuff significantly increased HR 20 and 35 min after snuff administration, SBP and DBP 20 min after snuff administration (*$P < 0.05$).

Discussion

The present study shows that the administration of 1 g oral moist snuff causes acute endothelial dysfunction in the brachial artery of chronic snuff users. However, the precise mechanisms by which oral snuff leads to this endothelial dysfunction are unknown.

Application of nasal nicotine spray causes endothelial dysfunction [17]. Endothelial dysfunction is present in chronic smokers as well as after acute cigarette smoking [18–20]. Thus, it is most likely that the acute impairment of endothelial function after the use of cigarettes, nicotine spray and snuff are induced by nicotine. Furthermore, we found the same haemodynamic responses to oral snuff as previously demonstrated by other investigators [1, 21]. The predominant haemodynamic effects of nicotine result from activation of the sympathetic nervous system mediated by release of noradrenaline and adrenaline [22]. Sympathetic stimulation evoked by baroreceptor unloading markedly reduces FMD, possibly by decreasing NO availability [23].

In addition to studies of the sympathetic effect on FMD, many investigators have begun to examine the role of oxygen radicals and tetrahydrobiopterin (BH4) in impaired FMD both in humans and animals [11, 24, 25]. Qin *et al.* have shown that application of superoxide dismutase or BH4 during infusion of nicotine could prevent impaired NOS-dependent vasodilation in an animal model, suggesting that the formation of oxygen radicals, possibly via an

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alternation in the utilization of BH4, contributes to this impairment [25].

In line with previous studies the present study indicates that oral moist snuff is associated with higher ambulatory blood pressure, increased heart rate, an increased risk of type 2 diabetes and endothelial dysfunction [21, 26]. Thus, these factors might contribute to an increased cardiovascular risk in snuff users.

Flow-mediated dilation is a surrogate variable, which predicts cardiovascular events [27, 28]. Based on the results of the present study and the known haemodynamic effects of snuff we suggest oral snuff should be considered as a public health risk. However, further investigations are needed to find out whether long-term snuff consumption is associated with higher risk of cardiovascular events. Otherwise, the health care system will most likely face a new, growing, harmful habit in the near future. Smokeless tobacco may not be as harmful as smoking cigarettes but it involves a large number of the population, as there are almost one million snuff users in Sweden alone. A proportionately small increase in cardiovascular morbidity or developing insulin resistance due to this habit has obvious effects on the public health level.

Many of the snuff consumers were recruited from smokers who intended to quit smoking. Use of snuff results in blood levels of nicotine similar to those observed in cigarette smokers [10] and therefore many subjects find it very difficult to quit the use of snuff later on. There are numerous studies regarding smoking cessation whereas we lack snuff use cessation studies [29]. Taken together, we do believe there is a significant health risk if snuff is marketed as a harmless substitute for smoking.

In conclusion oral moist snuff significantly impairs endothelial function and has negative haemodynamic effects. As endothelial dysfunction predicts cardiovascular morbidity, use of oral snuff should be discouraged.

Conflict of interest statement

No conflict of interest was declared.

Acknowledgements

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Correspondence: Stefan Agewall MD PhD FESC, Department of Cardiology, Huddinge University Hospital, Karolinska Institute, S 141 86 Stockholm, Sweden (fax: +46 8 585 867 10; e-mail: stefan.agewall@hs.se).

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