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Smokeless tobacco use and atherosclerosis: an ultrasonographic investigation of carotid intima media thickness in healthy middle-aged men

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Abstract

There is well-documented evidence of accelerated atherosclerosis in smokers but the mechanisms still remain unclear. The relationship to the use of smokeless tobacco, involving high exposure to nicotine, have not been evaluated before. The possible role of nicotine was investigated in a clinical study of the intima media thickness in the carotid artery of 143 healthy, middle-aged men (35–60 years old) with different tobacco consumption habits. B-mode ultrasonography was performed and biochemical risk factors for cardiovascular disease (serum lipids, serum lipoproteins and plasma fibrinogen) were determined. Long term smokeless tobacco users ($n = 28$) did not differ significantly from never-users ($n = 40$) regarding bulb intima media thickness (0.80 ± 0.13 versus 0.78 ± 0.12 mm) or common carotid intima media thickness (0.67 ± 0.11 versus 0.68 ± 0.11 mm), whereas smokers ($n = 29$) had significantly increased wall measurements (bulb 0.87 ± 0.19, $P = 0.002$ common carotid 0.74 ± 0.13, $P = 0.03$) compared to never-users. Only in smokers were biochemical risk factors significantly altered towards an elevated risk. Significant effects of interaction of smoking and increased s-cholesterol levels on carotid intima media thickness were also found. Smokeless tobacco users showed similar tendencies, but without definite statistical significance. On the basis of these data, it appears most likely that the increased occurrence of atherosclerosis in smokers is caused by other components of tobacco smoke than nicotine. © 1997 Elsevier Science Ireland Ltd.

Keywords: Atherosclerosis; Smokeless tobacco; Nicotine; Ultrasonography; Risk factors

1. Introduction

Sweden has the most widespread use of 'snuff dipping' in the European Union, with 21% of all males using wet snuff (ground and moistened dark tobacco, buffered to a pH of about 8.5 with sodium carbonate). A 'pinch' of about 2–3 g tobacco is placed in contact with the oral mucosa behind the upper lip. The average usage time is about 14 h/day [1–3]. The regular use of smokeless tobacco results in blood levels of nicotine similar to those observed in cigarette smokers [4,5]. Cigarette smoking is associated with the development of severe atherosclerosis [6] possibly via mechanisms involving increased oxidative stress and nitric oxide (NO) inactivation in the vascular endothelium [7–9]. Whether or not nicotine, as a major component of tobacco, might promote the atherosclerotic process has not been determined, but increased mortality from cardiovascular disease has been reported among smokeless tobacco users [10,11].

The ultrasonographic assessment of the intima media thickness and the carotid artery lumen diameter is a non-invasive method of measuring the presence and/or

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progression of vascular atherosclerosis [12,13]. Several studies have demonstrated a consistent association between intima media thickness and cardiovascular risk factors, including atherosclerosis of the coronary arteries [14–16]. The method allows the detection of minimal atherosclerotic lesions, thereby facilitating studies of the early, silent phases of atherosclerotic disease before clinical events due to coronary atherosclerosis or other atherosclerotic manifestations have occurred. All ultrasonographic studies of carotid atherosclerosis involving smoking habits demonstrate the consistent role of the latter as a major risk factor [15,17–20].

The aim of the present study was to investigate the possible influence of long-term exposure to smokeless tobacco on the atherosclerotic process by ultrasonographic determination of intima media thickness, lumen diameter and the occurrence of atherosclerotic plaques in the carotid artery in middle-aged men. In addition to tobacco consumption habits, the influence of other cardiovascular risk factors, such as serum lipoproteins, insulin, blood pressure, body stature and a family history of cardiovascular disease, were considered.

2. Methods

2.1. Subjects

143 healthy firemen, 35–60 years of age, were recruited to participate in an ultrasonographic examination of the carotid artery in connection with their annual compulsory fitness test. Tobacco consumption habits and family histories of cardiovascular disease and/or parental death before the age of 60 were recorded by means of a questionnaire in connection with blood sample collections and body stature measurements, all performed by the same specially trained nurse. Tobacco consumption habits were categorized according to the subgroups shown in Table 1, where duration of the tobacco consumption habit is also presented. For intergroup comparisons concerning exposure to tobacco, three major groups were selected: never-users of tobacco ($n = 40$), smokeless tobacco users ($n = 28$) and smokers ($n = 29$). Subjects who were ex-smokers, ex-smokeless tobacco users or who had changed their tobacco consumption habits were only included in the analysis of the whole study group. Five subjects who were daily smokers and occasionally also used smokeless tobacco were regarded as regular smokers. The informed consent of all subjects was obtained before the study and the study protocol were approved by the Ethics Committee of the Karolinska Hospital.

2.2. Carotid ultrasonography

The examination was performed with an ultrasound scanner (Acuson 128 Mountain View, CA) equipped with a linear 5 and 7 MHz probe. In the majority of cases we used the 7 MHz probe, but in a few cases the 5 MHz probe was used due to deeply positioned vessels making it difficult to properly identify the vessel wall. The patient was placed in the supine position with his head turned to the left. The ultrasound probe was placed over the right carotid artery and a complete examination of the vessel was made including the morphology and flow conditions using 2-D mode and Doppler (colour and spectral).

Simultaneous ECG recordings were made and at the point of best visibility of the wall structures, the image was frozen on the R-wave (end diastole) over the bulb area visualizing the distal part of the common carotid artery and the proximal part of the bulb. Altogether, 6 images were frozen (3 of the distal common carotid artery and 3 of the proximal bulb) and recorded on videotape.

2.3. Measurements of intima media thickness and lumen diameter

The frozen images from the videotapes were analyzed by a computerized analyzing system consisting of a PC-controlled frame-grabber (Imaging Technology FG-

Table 1
Classification of the study population into tobacco habit groups and duration of tobacco habits

Tobacco habit	n	Years of tobacco use (median (25th, 75th percentiles))
Never-users of tobacco	40	0
Ex-users for ≥ 5 years[a]	15	13 (10–20)
Ex-users for ≤ 5 years[b]	10	24 (20–28)
Smokeless tobacco users[c]	28	25 (19–27)
Ex-smokers, now smokeless tobacco users[d]	19	24 (18–31)
Smokers[e]	24	30 (25–30)
Smokers + smokeless tobacco user[f]	5	30 (20–31)
Ex-smokeless, now smoking[g]	2	33 (30–35)
Total	143	

[a] Stopped smoking or using smokeless tobacco more than five years prior to examination.
[b] Stopped smoking or using smokeless tobacco less than five years prior to examination.
[c] Daily smokeless tobacco use for more than 6 months.
[d] Stopped smoking more than six months ago, daily smokeless tobacco users for more than six months.
[e] Daily smoking for more than six months.
[f] Daily smoking plus daily or occasional smokeless tobacco use.
[g] Stopped using smokeless tobacco more than six months ago, daily smokers for more than six months.

100) PC with an extra monitor and a digitizer (Summagraphics MM-1201). The intima media thickness was defined as the distance between the leading edge of the intima lumen interface and the leading edge of the media adventitia interface of the far wall. The lumen diameter was defined as the distance between the leading edges of the intima lumen interface of the near wall and the lumen intima interface of the far wall. The lumen diameter was measured in the distal part of the common carotid artery. The intima media thickness was measured along a 10 mm long section of the distal part of the common carotid artery and in the proximal part of the bulb. The minimal, maximal and mean values for intima media thickness and lumen diameter from each of the 3 frozen images, from the 2 measured sections, were calculated. Plaque formations were registered regardless of the location in the vessel and were defined as a 100% increase in intima media thickness compared to adjacent normal-sized intima media thickness. Both the sonographic recordings and the analysis of measurements were done by the same examiner in a blinded fashion without access to information on the subjects' tobacco consumption habits.

2.4. Blood pressure

In connection with the ultrasound examination, the systolic and diastolic blood pressures were recorded from the right upper arm as the mean of 2 measurements after 5 min of rest in the supine position. The examination was performed with a tonometer with cuff size adjusted to the circumference of the arm. The blood pressure was recorded to the nearest 5 mmHg.

2.5. Blood chemical analysis

One day before the ultrasonographic examination, a venous blood sample was drawn, without a tourniquet, from an antecubital vein after overnight fasting and 8 h abstention from the use of tobacco. Serum levels of cholesterol, triglycerides and subfractions of lipoproteins (low-density lipoprotein (LDL), high-density lipoprotein (HDL), apolipoprotein (apo) A1, apo B and plasma fibrinogen were analyzed by routine laboratory methods. Plasma levels of nicotine and cotinine were also analyzed. Cotinine, the main metabolite of nicotine, but with a much longer half-life, is linearly and directly related to nicotine intake [21].

A maximal exercise test on a MedGraphics™ computerised test bicycle, with continuous recording of heart rate, systolic blood pressure and ECG was also performed after the ultrasonographic investigation, and has been described in detail elsewhere [22].

2.6. Statistics

Means and S.D. were calculated for all the anthropometric, chemical and ultrasonographic measurements for the whole study population and for the three tobacco habit groups separately. Intergroup comparisons were made for never-users of tobacco with smokeless tobacco users and smokers respectively, using analysis of variance (ANOVA) and Fisher's PLSD (protected least significant difference) test for post hoc significance tests. For skewed variables non-parametric tests were used for comparisons between the groups (Mann-Whitney when comparing two groups, Kruskall-Wallis when comparing three groups). Interaction effects were analyzed by two-way analysis of variance. Odds ratios (ORs) and 95% confidence intervals (C.I.) were calculated for questionnaire answers in the three tobacco habit groups with the never-users as a reference group. Covariates that might influence cardiovascular morbidity were entered in a multivariate regression model to check for their possible association with ultrasonographic measurements. Multiple linear regression was also used to adjust intima media measurements and chemical analyses for age. Univariate linear regression was used to analyze the nature of relationships between single ultrasonographic measurements, anthropometric data and established cardiovascular risk factors.

3. Results

3.1. Characteristics of the study groups

As shown in Table 1 all present tobacco users had been exposed to tobacco for more than 20 years. The group of smokeless tobacco users did not differ significantly from the never-users regarding age, body mass index or waist hip ratio, whereas the group of smokers was slightly older (on average, 5 years, $P < 0.001$) and although no significant difference in body mass index was observed, smokers showed a tendency to central obesity with a significantly higher waist-hip ratio, as shown in Table 2. After overnight abstinence the mean blood cotinine levels were significantly ($P < 0.001$), and on average 37% higher among smokeless tobacco users compared to smokers, indicating a higher total exposure to nicotine in smokeless tobacco users.

Blood pressure values at rest were similar in the 3 groups. In the questionnaire the responses of smokeless tobacco users and never-users were similar regarding a family history of cardiovascular disease but smokers had higher odds ratios than both smokeless tobacco users and never-users. Smokers also had significantly higher coffee and alcohol intake (see Table 3).

Table 2
Basic characteristics of the study population

	All subjects[a] ($n = 143$)	Never-users of tobacco ($n = 40$)	Smokeless tobacco users ($n = 28$)	Smokers ($n = 29$)	P-value*
Anthropometric data					
Age (years)	45.1 ± 6.6	43.1 ± 6.2	44.4 ± 6.3	48.0 ± 5.4	<0.001
BMI (kg/m^2)	25.5 ± 2.2	25.1 ± 2.2	25.5 ± 2.3	25.4 ± 2.1	
Waist/hip ratio (cm/cm)	0.90 ± 0.05	0.87 ± 0.04	0.87 ± 0.05	0.93 ± 0.06	<0.001
Blood pressure, HR					
SBP (mmHg)	122 ± 12	121 ± 12	122 ± 11	122 ± 15	
DBP (mmHg)	77 ± 7	76 ± 7	77 ± 8	78 ± 8	
Heart rate (beats/min)	59 ± 10	57 ± 11	58 ± 9	62 ± 10	0.01
Tobacco use					
Number of cigarettes/day		0	0	18 ± 11	
Grams of smokeless tobacco/day		0	32 ± 17	0	
Plasma nicotine[b]		0.2 ± 0.4	3.7 ± 2.5	5.6 ± 4.1	
Plasma cotinine[b]		3.8 ± 2.5	338 ± 176	248 ± 144	

Values are means ± S.D.
No significant differences were found on comparing smokeless tobacco users with never-users.
BMI, body mass index; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure.
[a] Also includes subjects with mixed tobacco use and former users of tobacco.
[b] After overnight abstension.
* Comparisons between smokers and never-users with analysis of variance (ANOVA), significance level $P < 0.05$.

3.2. Chemical analyses

Smokeless tobacco users did not show any significant differences in the measured blood components compared to never-users, as shown in Table 4, in which all values are adjusted for age differences. However, in smokers all biochemical cardiovascular risk factors, such as serum lipids, lipoproteins and plasma-fibrinogen, were significantly altered compared to never-users.

3.3. Intima media thickness and plaque occurrence

Smokeless tobacco users did not differ significantly from never-users regarding any of the intima media wall measurements or lumen diameters of the common carotid or the bulb area. Smokers, however, showed a 5–20% greater maximal intima media thickness than never-users of tobacco, after adjusting for age differences. The mean intima media thickness in smokers was significantly greater for both the common carotid and the bulb wall, whereas no significant differences were found in the vessel lumen diameter. The results for the different tobacco consumption habit groups are presented in Table 5. A comparison of the distribution of the different values of the common carotid and bulb wall measurements in the tobacco groups are illustrated in Fig. 1.

Plaques were diagnosed in two smokeless tobacco users (7.1%) and in 11 smokers (37.9%), whereas no plaque was found among the never-users. In age-adjusted, intergroup comparisons, the difference was significant for smokers ($P < 0.001$), but not for smokeless tobacco users ($P = 0.32$), compared to never-users. No signs of stenosis of the vascular lumen were diagnosed and flow conditions were similar in all groups. The presence of carotid plaques had a significant positive correlation to systolic blood pressure (Table 6).

3.4. Correlation between anthropometric measurements, tobacco consumption habits, cardiovascular risk factors and carotid wall measurements

Age was the factor with the most pronounced correlation with both common carotid and bulb area intima media thickness ($r = 0.34$, $r = 0.37$ $P < 0.001$) as shown in Table 6. An increase of 0.01 mm/year was found for the mean bulb intima media thickness, but on comparing the different tobacco habit groups, the mean increase was 0.005 mm/year in never-users, 0.006 mm/year in smokeless tobacco users and 0.02 mm/year in smokers. This indicates a 4-fold rate of increase for smokers compared to never-users. In a multivariate model, taking biochemical risk factors, age, body mass index, tobacco use and blood pressure into account, no single factor but age correlated significantly with intima media thickness. In a univariate regression analysis, apo B, the apo B/apo A-I ratio, total cholesterol, triglycerides and LDL-cholesterol levels correlated highly significantly with increased intima media thickness as well as with the occurrence of plaque (Table 6).

Smoking was significantly correlated with both intima media thickness and plaque occurrence. The corre-

Table 3
Questionnaire based data on the study population

	Never-users of tobacco ($n = 40$)	Smokles tobacco users ($n = 28$)			Smokers ($n = 29$)		
	%	%	OR	C.I.	%	OR	C.I.
Coffee intake high[a]	13	21	1.9	0.5–7.0	24	2.2	0.6–7.9
Alcohol intake medium/high[b]	70	75	1.3	0.4– 0.8	97	12.0	1.5–98
Family history of MI	28	18	0.6	0.2– 1.9	41	1.9	0.7 – 5.1
Parent died ≤60 years of age	23	14	0.6	0.2– 2.1	34	1.8	0.6 – 5.3

MI, myocardial infarction in either of the parents; OR, odds ratio comparisons made with never-users as reference group; C.I. = 95% confidence interval.
[a] Self reported low, medium or high.
[b] Self reported none, low, medium or high.

lation between smoking and intima media thickness was more evident for the carotid bulb wall than for the common carotid wall but most pronounced for plaque (Table 6). The amount of cigarettes consumed per day and the number of years smoking were significantly correlated with the occurrence of plaque ($r = 0.29$, $P = 0.03$ and $r = 0.43$, $P < 0.001$ respectively), but not with intima media thickness.

The effects of the interaction of increased serum cholesterol levels and tobacco consumption habits on carotid intima media thickness are illustrated in Fig. 2. Significantly higher values of bulb intima media thickness were noted for smokers with high serum cholesterol levels ($P < 0.001$) than for those with low ones. A similar pattern was also noted for smokeless tobacco users, but without statistical significance on comparing subjects with high and low cholesterol levels. In never-users, higher serum cholesterol values did involve a small, but nonsignificant, increase in mean intima media thickness.

There was a positive correlation between the values for the common carotid and the bulb area intima media thickness ($r = 0.6$, $P < 0.001$). This correlation was strong for never-users and smokeless tobacco users ($r = 0.7$ $P < 0.001$) whereas smokers showed no significant correlation between these measurements ($r = 0.3$, $P = 0.08$). A weak, but significant, correlation between systolic blood pressure and increased intima media thickness in both parts of the carotid wall was found in all three groups (Table 6).

3.5. Correlation between electrocardiographic findings and plaque occurrence

During the maximal exercise test, the electrocardiographic (ECG) recordings showed horizontal ST segment depressions ≥1 mm, without clinical signs of ischemia in three ex-smokers and slight horizontal ST-depressions <1 mm were found in 19 subjects (3 never-users, 3 smokeless tobacco users, 7 smokers, 6 ex-smokers). There was a significant correlation ($r = 0.32$; $P < 0.001$) between the presence of carotid plaques and electrocardiographic recordings of slight ST-T changes during exercise. The odds ratio to show signs of discrete and subclinical coronary ischemia on ECG was 6.7 (C.I. 2.1–21.8) in individuals diagnosed with plaques, compared to those without plaques. Subjects with a bulb intima media thickness over the median value (0.83 mm) had an odds ratio of 3.0 (C.I. 1.1–8.1) to show slight ST-T changes compared to those with values below the median.

3.6. Correlation between intima media measurements and family history of diabetes and myocardial infarction

None of the investigated subjects had reported a personal history of cardiovascular diseases or diabetes. Regarding family history the group of smokers reported the occurrence of myocardial infarction in either of their parents more frequently (41%, $n = 12$) than smokeless tobacco users (18%, $n = 5$) and never-users (28%, $n = 11$). The odds ratios are presented in Table 3 along with the occurrence of parental death before the age of 60 years, which was also most common among smokers. The bulb intima media wall thickness was significantly increased (mean difference 0.06 mm, $P = 0.02$) in subjects with a family history of myocardial infarction. Subjects with a family history of diabetes also had a significantly increased mean bulb intima media thickness (mean difference 0.12 mm, $P = 0.001$) compared to subjects without a family history of diabetes. There was no difference in age distribution between subjects with and without family history of either myocardial infarction or diabetes.

Table 4
Blood analysis of the study population

	Never-users of tobacco ($n = 40$)	Smokeless tobacco users ($n = 28$)	Smokers ($n = 29$)	Significance) P-value*
Serum cholesterol (mmol/l)	5.2 ± 0.9	5.3 ± 1.0	5.8 ± 1.1	0.005
HDL cholesterol (mmol/l)	1.4 ± 0.3	1.3 ± 0.3	1.0 ± 0.3	<0.001
LDL cholesterol (mmol/l)	3.4 ± 0.8	3.5 ± 0.9	3.8 ± 1.0	0.02
Serum triglycerides[a] (mmol/l)	1.0 ± 0.5	1.2 ± 0.5	2.6 ± 2.2	<0.001
Apo A-I (g/l)	1.52 ± 0.22	1.41 ± 0.22	1.35 ± 0.19	0.003
Apo B (g/l)	1.24 ± 0.28	1.34 ± 0.38	1.60 ± 0.45	<0.001
Apo B/apo A-I	0.83 ± 0.23	0.96 ± 0.33	1.23 ± 0.38	<0.001
Fibrinogen[a] (g/l)	2.61 ± 0.59	2.73 ± 0.48	3.20 ± 0.67	<0.001

Age-adjusted means ± S.D.
No significant differences were found on comparing smokeless tobacco users with never-users.
[a] Skewed variables, non-parametric test of Kruskal-Wallis.
* Comparisons between smokers and never-users with analysis of variance (ANOVA), significance level $P<0.05$.

4. Discussion

Up to now the influence of tobacco consumption on the progression of atherosclerosis has been investigated mostly in smokers. All studies of smoking and intima media thickness have established a strong correlation between smoking and an accelerated progression of carotid atherosclerosis [15,17–20]. The role of nicotine in this process has not been defined but if the nicotine content of tobacco smoke plays an important role smokeless tobacco users should be expected to demonstrate similar findings to those in smokers since they are exposed to the same or even higher quantities of nicotine (Table 2). The higher levels of cotinine found in smokeless tobacco users compared to smokers, although levels of blood nicotine are about the same, are in agreement with other studies [4,23]. This might be due to a higher absorption of nicotine through the gastrointestinal mucosa caused by swallowing followed by a first pass liver metabolism of nicotine to pharmacologically inactive cotinine [23,24].

The mean values found for the common carotid and bulb area intima media thickness are in agreement with those of previous investigators [14,19,25,26] as was the age-related rate of increase in intima media thickness [27]. The possible influence of different tobacco consumption habits examined in the present study does not, however, indicate that the influence of smokeless tobacco use should be associated with an accelerated atherosclerosis similar to that in smokers. The significantly larger intima media thickness of 5–20% found in the carotid artery of smokers, as compared to never-users, is in line with the findings of other investigators [19,27].

The levels of established metabolic risk factors for cardiovascular disease were similar to those found in other studies [14,25] and significant alterations involving an elevated cardiovascular risk were observed only in smokers. All metabolic parameters in smokeless tobacco users showed, however, a slight tendency towards elevated cardiovascular risk levels rather than equal or lower values compared to never-users.

In agreement with several other studies [19,26,28,29], blood levels of biochemical cardiovascular risk factors were significantly correlated with increased intima media thickness. There was also an apparent effect of interaction between increased serum cholesterol and smoking on the carotid intima media thickness (Fig. 2). Smoking subjects with high cholesterol levels showed a clear increase in carotid bulb intima media thickness, although none of the subjects exhibited clinical signs of cardiovascular disease. A similar, but nonsignificant, pattern of interaction was observed in smokeless tobacco users but the differences were too small to claim that smokeless tobacco use can aggravate atherogenesis in individuals with raised levels of cardiovascular risk factors in a way similar to that in smokers.

Development of atherosclerotic lesions in the carotid artery occurs at a relatively late stage in life compared to other arteries such as the coronary arteries. Ten percent of 40-year-old men and 80% of 60-year-old men had signs of atherosclerosis of the carotid artery in a study by Salonen et al. [30]. Although smokers exhibit a more rapid progression of atherosclerosis in the femoral arteries than in the carotid artery the ultrasound measurements were performed only on the far wall of the carotid vessel as this method seems to be the most adequate for an accurate assessment of intima media thickness at present [12,13,31]. The bulb was measured despite its curved interfaces, which makes the ultrasonographic reading less sharp, but the bulb still represents an area where very early signs of atherosclerosis are first observed [19] as indicated also in this study.

An interesting observation was the significant correlation between the presence of carotid plaques or increased carotid bulb intima media thickness and electrocardiographic recordings of slight ST-T changes

Table 5
Results of the ultrasonographic examination of the right carotid artery

	Never-users of tobacco ($n = 40$)	Smokeless tobacco users ($n = 28$)	Smokers ($n = 29$)	Significance (P-value*)
Wall thickness (mm)				
Comm. carotid (mean)	0.68 ± 0.11	0.67 ± 0.11	0.74 ± 0.13	0.03
Comm. carotid (max)	0.79 ± 0.15	0.81 ± 0.13	0.83 ± 0.15	0.08
Carotid bulb (mean)	0.78 ± 0.12	0.80 ± 0.13	0.87 ± 0.19	0.002
Carotid bulb (max)	0.95 ± 0.15	1.01 ± 0.18	1.14 ± 0.34	<0.001
Lumen diameter (mm)				
Lumen (mean)	5.79 ± 0.67	5.83 ± 0.61	5.73 ± 0.47	0.81
Lumen (min)	5.63 ± 0.65	5.63 ± 0.60	5.55 ± 0.46	0.78
Plaque[a] (%)	0	7.1	37.9	<0.001

Age-adjusted means ± S.D.
No significant differences were found on comparing smokeless tobacco users with never-users.
Comm, common; Mean, mean values of three measurments; Max, largest measurment value; Lumen diameter measured in the proximal part of the common carotid artery.
[a] 100% increase in intima media thickness compared to adjacent normal-sized wall thickness.
* Comparisons between smokers and never-users with analysis of variance, significance level $P < 0.05$.

during exercise. This supports earlier findings demonstrating the association of carotid intima media thickness and coronary atherosclerosis [28,32–35].

The investigated subjects represented a selection of healthy and physically well trained middle-aged men, in whom fewer signs of cardiovascular disease could be suspected than in a random sample of the population [36]. However, this study population has a high prevalence of smokeless tobacco users (30%) and a homogenous socio-economic makeup that eliminates other confounders that might influence the interpretation of the results.

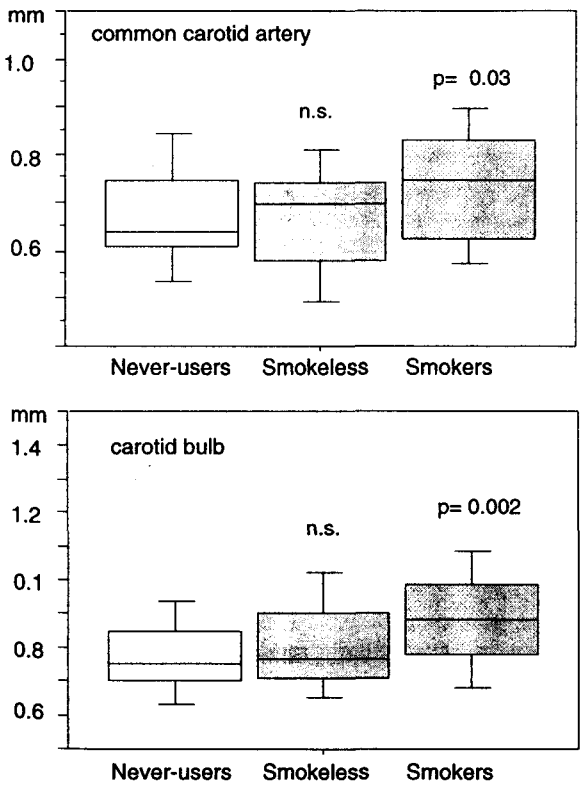

Fig. 1. Intima media wall thickness of the common carotid artery and the carotid bulb in the different tobacco habit groups. Box plot showing the 10th, 25th, 50th, 75th and 90th percentiles of the mean values. ANOVA and Fisher's tests for comparisons with never-users.

In this study, convincing differences concerning metabolic risk factors for cardiovascular disease were found in smokers vis-à-vis never-users, as previously confirmed in the majority of studies [6,37,38]. Although no significant differences were noted in the comparisons of smokeless tobacco users with never-users, all metabolic data (Table 4) showed a similar tendency towards raised levels of cardiovascular risk, which might indicate a smaller, but still noticeable, metabolic influence of nicotine compared to smoking. The sympathomimetic effect of nicotine is known to facilitate lipolysis but the mechanisms for altering lipid metabolism and regulating homeostasis in smokers are still largely unknown. The noted differences between smokeless tobacco users and never-users did not reach statistical significance, however.

It is concluded that the possible deleterious effects of nicotine do not seem to include a significant influence on the progression of atherosclerosis in the carotid artery. Smokeless tobacco users did not show a significant increase in intima media thickness as compared to never-users in spite of exposure to smokeless tobacco for more than 20 years. The pathogenesis of the accelerated atherosclerosis in smokers seems to be causally related to other components of tobacco smoke other than nicotine. The findings of this study do not exclude a negative influence of smokeless tobacco use on the cardiovascular system, as nicotine has a wide range of both central and peripheral effects on the nervous system, as well as on metabolic homeostasis.

Table 6
Univariate correlation analysis of mean values of carotid wall measurements or plaque occurence and age, systolic blood pressure, tobacco habits and blood levels of metabolic cardio-vascular risk factors in the study population

	Common carotid		Carotid bulb		Plaque occurence	
	r	*P*-value	*r*	*P*-value	*r*	*P*-value
Age	0.34	<0.001	0.37	<0.001	0.19	0.02
Syst. BP	0.18	0.03	0.26	0.002	0.32	<0.001
Smoking	0.21	0.03	0.33	0.001	0.44	<0.001
Smokeless tobacco	0.06	0.52	0.07	0.49	0.08	0.40
Apo B/apo A-I	0.29	<0.001	0.29	<0.001	0.28	<0.001
apo B	0.32	<0.001	0.31	<0.001	0.29	0.004
Total cholesterol	0.31	<0.001	0.33	<0.001	0.23	0.006
Triglycerides	0.31	<0.001	0.45	<0.001	0.36	<0.001
LDL cholesterol	0.24	0.003	0.28	0.001	0.21	0.01
HDL cholesterol	−0.11	0.16	−0.18	0.03	−0.21	0.01
Fibrinogen	0.21	0.01	0.14	0.09	0.39	<0.001

Significance level $P<0.05$.

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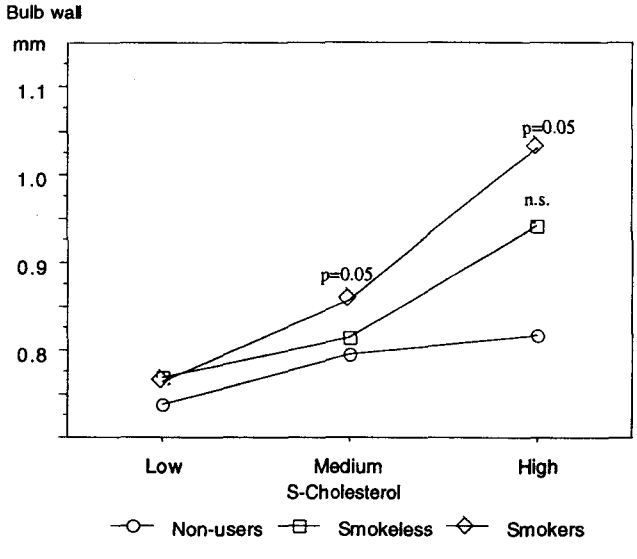

Fig. 2. Interaction line for carotid bulb intima media wall thickness in different tobacco habit groups. Subjects divided according to three cholesterol levels: low 3.20–4.70, medium 4.71–6.80 and high 6.81–7.90 mmol/l. The differences between smokers and never-users are significant both at medium and high cholesterol levels, indicating a multiplicative effect of smoking and increased cholesterol levels. The differences between smokeless tobacco users and never-users were not significant.

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