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Carotid and femoral atherosclerosis, cardiovascular risk factors and C-reactive protein in relation to smokeless tobacco use or smoking in 58-year-old men

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Abstract. Wallenfeldt K, Hulthe J, Bokemark L, Wikstrand J, Fagerberg B (Sahlgrenska University Hospital, Gothenburg University, Gothenburg, Sweden). Carotid and femoral atherosclerosis, cardiovascular risk factors and C-reactive protein in relation to smokeless tobacco use or smoking in 58-year-old men. *J Intern Med* 2001; **250:** 492–501.

Objectives. To examine the associations between smokeless tobacco use, smoking, cardiovascular risk factors, inflammation and ultrasound-assessed measures of atherosclerosis in the carotid and femoral arteries.

Subjects. The study was performed in a population-based sample of clinically healthy men ($n = 391$) all 58 years old. Exclusion criteria were cardiovascular or other clinically overt diseases or continuous medication with cardiovascular drugs.

Methods. The habits of smoking and oral moist snuff use were assessed by questionnaires. C-reactive protein (CRP) was assessed by high sensitive enzyme-linked immunosorbent assay (ELISA). Intima-media thickness (IMT) in the carotid bulb, the common carotid artery and the common femoral artery and plaque occurrence were measured by ultrasound.

Results. The use of oral moist snuff was associated with serum triglycerides and waist–hip ratio (WHR), but not with CRP or ultrasound-assessed measures of subclinical atherosclerosis. Smoking, on the other hand, was associated with CRP, the components in the metabolic syndrome and IMT as well as plaques in the carotid and femoral arteries. In comparison to never-smokers the current smokers had higher values of WHR, triglycerides, C-reactive protein and IMT in carotid bulb and femoral artery. Ex-smokers were in general more obese and had a femoral IMT that was in-between that of never-smokers and current smokers.

Conclusions. Tobacco smoking, but not oral moist snuff use, was associated with carotid and femoral artery IMT, and increased levels of CRP. Current smoking was also associated with abdominal obesity. Ex-smokers though, are generally more obese. Smoking was also associated with hyperinsulinaemia, dyslipidaemia and high blood pressure, i.e. the metabolic syndrome. The inhaled smoke from the combustion of tobacco seems to be an important aetiological factor in the atherosclerotic process.

Keywords: atherosclerosis, C-reactive protein, intima-media thickness, smokeless tobacco, smoking, ultrasound.

Introduction

Smoking is one of the major risk factors for cardiovascular disease and is also associated with an increased intima-media thickness (IMT) in the carotid and femoral arteries as assessed by the ultrasound method [1, 2]. Smoking is accompanied by physiological changes that promote or are associated with atherosclerotic disease, for example, low HDL cholesterol, hypertriglyceridemia, abdominal obesity and increased concentrations of circulating biochemical markers of inflammation [3, 4].

Much less is known about smokeless tobacco, although the regular oral use of snuff is associated

with blood levels of nicotine similar to those observed in cigarette smokers [5, 6]. Previously published data have indicated that oral use of moist snuff may be associated with an increased risk of cardiovascular death [7], whereas two other studies found no evidence that smokeless tobacco increased the risk for myocardial infarction [8, 9]. To our knowledge there is only one previous study that has examined the relationship between use of smokeless tobacco and IMT in the carotid artery, and no association was reported in this study encompassing 28 smokeless tobacco users and 40 never-users [1]. There are also only a few reports on the association between smokeless tobacco, risk factors for cardiovascular disease and markers of inflammation [10, 11].

The aim of the present study was to examine the associations between the use of oral moist snuff (henceforth referred to as snuff use), smoking, cardiovascular risk factors, C-reactive protein (CRP) and ultrasound-assessed measures of atherosclerosis in the carotid and femoral arteries in a population-based sample of 58-year-old men.

Materials and methods

Study subjects

The inclusion criteria were age 58 years, male sex and Swedish ancestry. Exclusion criteria were cardiovascular or other clinically overt disease, treatment with cardiovascular drugs for ischaemic heart disease, heart failure, hypertension, diabetes mellitus and hyperlipidemia or unwillingness to participate. The present report is a substudy to a previously published study that has been described in the detail elsewhere [12, 13]. Briefly, the subjects were 58-year-old men, randomly selected from the general population; 1728 men were invited to a screening examination by mail. Of these, 83% sent a reply and 1188 men were willing to participate. Out of these, 818 men were found to be eligible.

These men were at screening preliminary divided into quintiles of estimated insulin sensitivity by using a body mass index (BMI)/blood glucose algorithm that had been shown to be a good estimate of insulin sensitivity. Every man in quintile 1 (indicating low insulin sensitivity) and quintile 5 (indicating high insulin sensitivity) and every fifth man in quintiles 2–4 (indicating intermediate sensitivity) was invited to further examinations ($n = 391$).

The subjects received both written and oral information before they gave their consent to participate. The study was approved by the Ethics Committee at Sahlgrenska University Hospital.

Measurements

All measurements were performed in the morning. Venous blood samples were drawn after a mean fasting period of 12 h, serum was separated and frozen within 4 h in −70°C. Body weight, height, waist and hip circumference were measured and BMI and WHR were calculated.

Information on general health and tobacco habits were obtained by a self-administered questionnaire. Present use of snuff was defined as at least one snuff-dipping per day. Current smoking was defined as every-day use of at least one cigarette per day. Ex-smoking and previous use of smokeless tobacco were defined as no use during the last 3 months. 'Snuff-years' were defined as the amount of moist snuff in grams taken per day multiplied by the total number of years taking snuff. The total number of years of smoking was multiplied by the number of cigarettes smoked daily. The product was called 'cigarette-years'. Information on smoking and snuff use habits was available in all 391 study subjects; however, 'cigarette-years' and 'snuff-years' could only be calculated in 384 and 385 men, respectively.

Blood pressure was measured twice when the subject had been resting in the supine position for 5 min with appropriate cuff-size in relation to arm size as previously described [14]. The diastolic blood pressure was determined as Korotkoff phase V.

Ultrasonography

Intima-media thickness. Examination was performed with an ultrasound scanner (Acuson 128; Acuson, Siemens, Mountain view, CA, USA) with a 7-MHz linear transducer aperture of 38 mm. The electrocardiographic signal (lead II) was simultaneously recorded to synchronize the image capture of the top of the R-wave to minimize variability during the cardiac cycle. Both the left and right carotid arteries were scanned at the level of the bifurcation and images for IMT measurements were recorded from

the far wall in the common carotid artery and the carotid artery bulb, and from the right femoral artery. The software program gives the average thickness of the IMT. Measurements in the common femoral artery were made in a similar way as for the carotid artery but along a 15-mm-long section proximal to the bifurcation [15]. IMT was defined as the distance from the leading edge of the lumen–intima interface to the leading edge of the media–adventitia interface of the far wall. At the position of the thickest part of the wall (visually judged), a frozen longitudinal image was captured and recorded on videotape. The procedure was repeated four times to achieve four separate images for analysis. A short sequence of real-time images was also recorded on videotape to assist in the interpretation of the frozen images. The images were measured in an automated analysing system [16], based on automatic detection of the echo structures in the ultrasound image but with the option to make manual corrections by the operator. The interobserver variation for IMT has been considered satisfying as the coefficient of variation in the method for measurement in the common carotid artery was 5.3% in double-sided, automated reading. The corresponding figures for examinations of the carotid artery bulb and the common femoral artery were 6.0 and 16.9%, respectively. The method has been described in detail elsewhere [17]. Measurements of carotid and femoral artery IMT were available in 379 and 373 patients, respectively.

Assessment of plaque occurrence. The carotid and femoral arteries were scanned both longitudinally and transversely to assess the occurrence of plaques [14]. A plaque was defined as a distinct area with an IMT more than 50% thicker as compared with neighbouring sites (visually judged). A semiquantitative subjective scale was used to grade the size of plaques into: grade 1, one or more small plaques ($<\sim 10$ mm^2); grade 2, moderate to large plaques (the differentiation between grades 1 and 2 was made subjectively in most cases, and quantitative measurements were made in the computerized system [18] only when the correct classification was not obvious to the observer); grade 3, plaques giving flow disturbances [15]. In the present study no plaque of grade 3 was found in the femoral artery and three subjects had plaques of grade 3 in the carotid artery. Therefore, plaques of grades 2 and 3 were merged into one group of moderate to large plaques. This analysis included plaques in the near wall as well as the far wall of the vessel. Analyses of plaques were performed in both the right and left carotid artery. The largest plaque in either artery was used in the present analysis. In a re-reading reproducibility study ($n = 45$) of plaque size there were high correlation coefficients for the right and left carotid arteries ($r_s = 0.96$ and $r_s = 0.96$, respectively); and also for the right femoral artery ($r_s = 0.86$). Measurements of plaques in the carotid and femoral artery were available in 367 and 389 patients, respectively.

Biochemical analysis

Cholesterol and triglyceride levels were determined by fully enzymatic techniques [19, 20]. HDL was determined after precipitation of apolipoprotein (apo) B-containing lipoproteins with manganese chloride and dextran sulphate. LDL was calculated as described by Friedewald *et al.* [21]. Blood glucose was measured with the glucose oxidase technique. Plasma insulin was determined in all subjects with a radioimmunoassay (Pharmacia Insulin RIA, Pharmacia Diagnostics, Uppsala, Sweden). All lipid analyses were performed at the Wallenberg Laboratory. CRP was measured by a commercially available ELISA kit (Medix Biochemica, Kauniainen, Finland). Frozen serum samples from 36 healthy men obtained at the same occasion were evaluated twice on different days and the correlation coefficient was 0.99 (no systematic difference).

Statistics

All statistics were analysed using SPSS for Windows 10.0 (SPSS Inc., Chicago, IL, USA). Skewed variables such as CRP, triglycerides, fasting blood glucose and plasma insulin were log-transformed before statistical testing. The characteristics of the subjects are described as means and standard deviations if nothing else is indicated. Intergroup comparisons were made by using ANOVA with Dunnett's *T*-test for *post-hoc* analysis. Nonparametric Spearman's rank correlation test was used in the correlation analysis. Multiple regression was used in the multivariate analyses and dummy technique was used to categorize subjects into

smokers, ex-smokers and those who had never smoked. A similar definition of groups was used for snuff-using habits. $P < 0.05$ (two-sided) was regarded as statistically significant.

Results

As presented in Tables 1 and 2, 96 men (25% of all) were current smokers and 48 men (12% of all) were current snuff takers, whereas 139 men (36% of all) had never smoked or used snuff regularly. Eighty-one men were current ($n = 48$, 12% of all) or previous snuff-takers ($n = 33$, 8% of all) and of these only four subjects had never smoked.

Amongst the 427 men who were not included in the study, 22% were current smokers, 14% were current snuff-users and 37% were never-smokers. The mean values for cigarette-years and snuff-years were also very similar in the two groups; 334 cigarette-years in the study group versus 293 in the excluded group ($P = 0.15$), and 100 snuff-years versus 89 ($P = 0.33$), respectively.

Characteristics of the study subjects by snuff-use status

The analysis of the characteristics of the subjects by history of snuff-taking showed only that never-snuff users had lower serum triglyceride concentrations than previous or current snuff-takers and that a previous habit of using snuff was associated with the highest number of cigarette-years (Table 1). Amongst the men who were current snuff-takers 32 of 48 (67%) were previous smokers compared

Table 1 Characteristics of the subjects by habits of snuff intake

	Never snuff user ($n = 310$)	Ex-snuff user ($n = 33$)	Current snuff user ($n = 48$)	*P*-value
Current smokers, *n* (%)[a]	70 (23)	12 (36)	14 (29)	
Ex-smokers, *n* (%)[a]	101 (33)	19 (58)	32 (67)	
Never smoked, *n* (%)[a]	139 (45)	2 (6)	2 (4)	
Cigarette-years	293 ± 397	627 ± 554	400 ± 309	1–2**
Body mass index (kg m^{-2})	26.3 ± 4.4	27.3 ± 4.4	26.7 ± 4.4	ns
Waist–hip ratio	0.94 ± 0.07	0.96 ± 0.06	0.95 ± 0.07	ns
Waist circumference (cm)	95.9 ± 12.2	98.5 ± 11.3	96.9 ± 12.2	ns
Cholesterol (mmol L^{-1})				
Total	5.98 ± 1.11	6.08 ± 1.15	6.18 ± 1.06	ns
HDL	1.28 ± 0.37	1.17 ± 0.33	1.28 ± 0.38	ns
LDL	4.04 ± 0.96	4.12 ± 1.02	4.09 ± 0.97	ns
Triglycerides (mmol L^{-1})	1.47 ± 0.94	2.11 ± 1.76	1.78 ± 0.93	†
Apolipoprotein A1 (g L^{-1})	1.43 ± 0.23	1.37 ± 0.22	1.43 ± 0.24	ns
Apolipoprotein B (g L^{-1})	1.21 ± 0.27	1.24 ± 0.27	1.25 ± 0.29	ns
Fasting blood glucose (mmol L^{-1})	4.88 ± 1.08	4.70 ± 0.47	5.22 ± 1.96	ns
Plasma insulin (μU mL^{-1})	9.70 ± 6.13	11.10 ± 6.68	11.09 ± 6.93	ns
C-reactive protein (mg L^{-1})	2.47 ± 4.52	2.27 ± 2.34	2.64 ± 3.27	ns
Intima-media thickness (IMT) (mm)				
Common carotid artery	0.80 ± 0.13	0.80 ± 0.14	0.82 ± 0.12	ns
Carotid bulb	0.99 ± 0.26	0.96 ± 0.22	1.04 ± 0.26	ns
Femoral artery	1.05 ± 0.49	1.20 ± 0.49	1.12 ± 0.43	ns
Carotid plaques (%)				ns
None	59	48	50	
Small	17	27	24	
Large	24	24	26	
Femoral plaques (%)				ns
None	63	56	54	
Small	13	6	10	
Large	24	38	35	

Values are means ± SD; ns = not significant.
**$P < 0.01$, †ANOVA $P = 0.001$.
[a]Percentage of the subjects in each column.

Table 2 Characteristics of the subjects by smoking status

	Never smokers ($n = 143$)	Ex-smokers ($n = 152$)	Current smokers ($n = 96$)	*P*-value
Current snuff users, *n* (%)[a]	2 (1%)	32 (21%)	14 (15%)	
Snuff-years	23 ± 181	178 ± 419	87 ± 232	1–2***
Cigarette-years	0 ± 0	479 ± 405	617 ± 398	2–3*
Body mass index (kg m^{-2})	25.8 ± 4.1	27.6 ± 4.5	25.6 ± 4.4	1–2**, 2–3**
Waist–hip ratio	0.93 ± 0.07	0.96 ± 0.07	0.95 ± 0.06	1–2***, 1–3*
Waist circumference (cm)	93.8 ± 11.2	99.1 ± 12.7	95.4 ± 11.9	1–2**
Cholesterol (mmol L^{-1})				
Total	5.98 ± 1.14	6.11 ± 1.06	5.90 ± 1.12	ns
HDL	1.31 ± 0.36	1.28 ± 0.38	1.21 ± 0.36	ns
LDL	4.08 ± 1.02	4.09 ± 0.91	3.95 ± 0.96	ns
Triglycerides (mmol L^{-1})	1.32 ± 0.64	1.73 ± 1.16	1.69 ± 1.28	1–2***, 1–3**
Apolipoprotein A1 (g L^{-1})	1.43 ± 0.22	1.44 ± 0.23	1.38 ± 0.23	ns
Apolipoprotein B (g L^{-1})	1.18 ± 0.29	1.24 ± 0.26	1.22 ± 0.27	ns
Fasting blood glucose (mmol L^{-1})	4.84 ± 1.08	4.98 ± 1.25	4.88 ± 1.26	ns
Plasma insulin (μU mL^{-1})	9.04 ± 4.39	11.29 ± 8.00	9.37 ± 5.73	1–2**, 2–3*
C-reactive protein (mg L^{-1})	1.43 ± 1.82	2.45 ± 3.55	4.05 ± 6.68	1–2**, 1–3***
Intima-media thickness (IMT) (mm)				
Common carotid artery	0.78 ± 0.12	0.81 ± 0.13	0.81 ± 0.13	ns
Carotid bulb	0.95 ± 0.22	1.00 ± 0.22	1.05 ± 0.35	1–3*
Femoral artery	0.87 ± 0.28	1.10 ± 0.47	1.31 ± 0.62	1–2***, 1–3***, 2–3*
Carotid plaques (%)				ns
None	62	55	53	
Small	15	23	16	
Large	23	22	31	
Femoral plaques (%)				***
None	80	54	46	
Small	10	13	14	
Large	10	33	41	

Values are mean ± SD; ns = not significant.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.
[a]Percentage of the subjects in each column.

with 101 of 310 (33%) amongst those who never used snuff. There were no associations between snuff use and ultrasound-assessed measures of atherosclerosis. Neither in the carotid artery nor in the femoral artery did the occurrence of plaques associate with snuff-years (Fig. 1). Fourteen of the current snuff-users were also current smokers. Recalculation of data after removal of these 14 men did not change the results in Table 1 (data not shown).

Characteristics of the study subjects by smoking status

Table 2 shows that in comparison with never-smokers current smokers were characterized by higher values of WHR, serum triglyceride concentration, CRP, IMT carotid bulb and femoral artery IMT. In comparison to ex-smokers, current smokers had more cigarette-years, lower plasma insulin concentration and higher femoral artery IMT.

In comparison with never-smokers the ex-smokers had more snuff-years, higher values of BMI, WHR, waist circumference, serum triglycerides, plasma insulin, CRP and femoral artery IMT. The ex-smokers also had higher BMI than current smokers.

The occurrence of large atherosclerotic plaques in the femoral artery increased in parallel with a history of previous or current smoking. There was no statistically significant difference in the prevalence of plaques in the carotid artery. However, both in the carotid artery and in the femoral artery the occurrence of plaques was associated with increasing numbers of cigarette-years (Fig. 1).

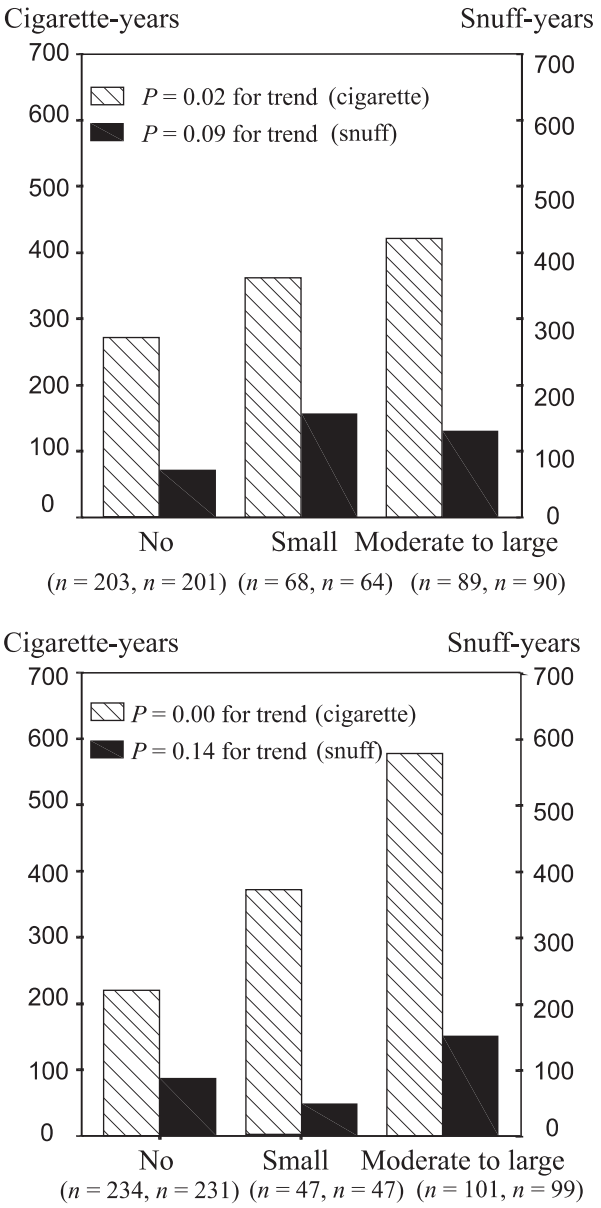

Fig. 1 Upper panel: bar graphs showing the relationship between plaque occurrence in the carotid artery and mean cigarette- and snuff-years, respectively. Lower panel: bar graphs showing the relationship between plaque occurrence in the femoral artery and mean cigarette- and snuff-years, respectively.

Uni- and multivariate associations

Cigarette-years showed statistically significant positive associations with BMI, waist circumference, WHR, systolic blood pressure and serum concentrations of triglycerides, plasma insulin, CRP, and a negative correlation with HDL cholesterol (Table 3). Snuff-years were only related to serum triglycerides and to WHR. Cigarette-years correlated with snuff-years ($r = 0.25$; $P < 0.01$).

Cigarette-, but not snuff-years, was statistically significant associated with IMT in the common carotid artery, the carotid bulb and the femoral artery. The *r*-value was highest for the association with femoral artery IMT ($r = 0.42$).

Eighty-one men were previous or current snuff takers, whereas 248 men were smokers or ex-smokers. In order to obtain comparable statistical power in the analyses of correlates to cigarette- and snuff-years, respectively, all never smokers and a random selection of 81 smokers or ex-smokers were included in an additional correlation analysis in accordance with Table 3. The results showed that IMT in the femoral artery, carotid bulb, common carotid artery, CRP, triglycerides, WHR and snuff-years remained significantly associated with cigarette-years (data not shown). Similar results were found when the 14 smokers in the current snuff-user group were excluded from the analyses (data not shown).

In a multiple regression analysis with femoral artery IMT as the dependent variable and independent dummy variables for smoking and snuff-taking habits (1 = never-user, 2 = ex-user, 3 = current-user) showed that only smoking was associated with femoral artery IMT (β-coefficient 0.35; $P < 0.001$). The introduction of the product of smoking habits and snuff-taking habits as an independent variable as a measure of a possible interaction between smoking and snuff-taking did not change the outcome of the analysis, indicating that snuff-years did not contribute to the variation in femoral artery IMT. Similar statistically significant findings were made for the carotid bulb IMT as dependent variable and smoking and snuff-use as independent variables (data not shown).

C-reactive protein

C-reactive protein was associated with femoral IMT ($r = 0.16$; $P = 0.003$) and cigarette-years ($r = 0.32$; $P < 0.001$), but not with snuff-years ($r = 0.06$; $P = 0.21$). A regression analysis was performed with log CRP as dependent variable and cigarette- and snuff-years as independent variables. The results showed that only smoking status turned out to be an independent covariate to log CRP

Table 3 Univariate associations between cigarette- and snuff-years versus risk factors for cardiovascular disease and intima-media thickness (IMT) in the carotid and femoral arteries in 58-year-old men ($n = 391$)

	Spearman's *r*-value	
Risk factor	Cigarette-years	Snuff-years
Snuff-years	0.25**	
Body mass index (kg m^{-2})	0.14**	0.09
Waist circumference (cm)	0.21***	0.07
Waist–hip ratio (cm)	0.29***	0.11*
HDL cholesterol (mmol L^{-1})	−0.14**	−0.04
Triglycerides (mmol L^{-1})	0.25***	0.16**
Plasma insulin (μU mL^{-1})	0.12*	0.04
Systolic blood pressure (mmHg)	0.10*	0.08
C-reactive protein (mg L^{-1})	0.32***	0.01
IMT (mm)		
Common carotid artery	0.17**	0.06
Carotid bulb	0.13*	0.09
Femoral artery	0.42***	0.03

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

(β-coefficient 0.30; $P < 0.001$). The introduction of the product of cigarette- and snuff-years as an independent variable did not change the outcome of the analysis (data not shown). Excluding the subjects with serum CRP concentration above 10 mg L^{-1} did not change the results (data not shown).

Discussion

The results from this population-based study of 58-year-old men showed that 12% of the subjects were currently using smokeless tobacco taken as snuff and 8% were previous snuff-users. In contrast to tobacco smoking the use of snuff was not associated with ultrasound-assessed measures of atherosclerosis in the carotid and femoral artery. Thus, neither a current or previous use of snuff nor the total consumption calculated as snuff-years were associated with the thickness of the intima-media complex or with the occurrence of atherosclerotic plaques in the carotid or femoral arteries.

Current smoking, on the other hand, was associated with an increase of the IMT in the carotid bulb and the femoral artery. Cigarette-years were associated with IMT in the carotid bulb, in the common carotid artery and in the femoral artery. In addition, the occurrence of atherosclerotic plaques both in the carotid and femoral arteries were associated with the total number of cigarette-years.

Before these findings are further discussed some methodological issues have to be addressed. Subjects with clinical manifestations of cardiovascular disease were excluded. This might have reduced the possibility to detect an impact of tobacco use on the risk for development of atherosclerosis. Hence, in the present study no large plaque was found in the femoral artery and only three subjects had large plaques in the carotid artery. However, by using this approach we avoided the confounding effect of concomitant treatment, which would have affected several of the studied variables. Some of the results regarding effects of smoking confirmed previously known data, indicating a sufficient study power. There were only 81 men who were current or previous snuff users, to be compared with 248 current or previous smokers, implying a greater statistical power to examine covariates to smoking than to snuff-taking. Therefore, an alternative correlation analysis was done after random selection of a number of smokers corresponding to the same numbers as snuff-takers. This analysis did not change the conclusions from the original analysis. In addition, a *post-hoc* calculation based on available data indicated that the study had a power of 80% to detect a 12% difference in common carotid artery IMT ($P < 0.05$) between not-smoking snuff-takers and those not using snuff and not smoking.

There was a close relation between the habits of smoking and snuff-taking and it might have been difficult to separate the effects of either habit on the development of atherosclerosis. However, this close association between snuff use and smoking, would, if anything, have supported associations between snuff use, risk factors and atherosclerosis. Still, no such observations were made. Multiple regression techniques were also applied to examine whether there was any interaction effects between smoking habits and the use of snuff on IMT. The results consistently showed that only tobacco smoking was associated with atherosclerosis. It may also be argued that carotid artery IMT is not a valid measure of the atherosclerotic process in relevant vascular beds such as the coronary arteries. Today, there is a great body of evidence from cross-sectional and prospective studies demonstrating that carotid artery IMT is a fair measure of the atherosclerotic disease resulting in manifest coronary artery disease

and stroke [22, 23]. In addition, we actually measured the occurrence of atherosclerotic plaques.

Taken together, our data indicate that tobacco smoking, but not snuff use, was associated with atherosclerosis. This finding raises the question of possible underlying mechanisms. The present results show that the current smoking status and the number of cigarette-years were related to a number of factors associated with atherosclerotic disease, i.e. increases in WHR, plasma insulin, serum triglyceride concentration, CRP and a low serum HDL cholesterol concentration [3, 4, 24]. Hence, it may be speculated that tobacco smoking may cause atherosclerosis through a number of proatherosclerotic mechanisms. In contrast, snuff use was only associated with weak increases in the serum triglyceride concentration and WHR. Amongst the few previously published studies, one study observed no difference between users and nonusers of snuff in abdominal obesity, circulating blood lipids or blood pressure [9], whereas another study reported on higher ambulatory blood pressure in snuff-users and smokers compared with nonusers of tobacco [25].

It is well known that tobacco smoking is associated with the development of subclinical atherosclerosis and clinical atherosclerotic diseases such as myocardial infarction or stroke [22, 23]. As regards the use of snuff, there is one previous, small study of Swedish firemen showing no association between snuff taking and carotid artery IMT [1]. Moreover, previous case–control studies have demonstrated that, contrary to the habit of tobacco smoking, the use of smokeless tobacco was not associated with an increased risk of future myocardial infarction [8, 9]. In one of the previous studies of 25–64-year-old men, the odds ratio for myocardial infarction amongst snuff users was even reduced after adjustments for cardiovascular risk factors [9]. However, in a large prospective study of Swedish construction workers the age-adjusted risk of dying from cardiovascular disease was 1.4 for smokeless tobacco users and 1.9 for smokers in comparison with those who did not use tobacco [7].

To sum up, the results from the previous and present studies indicate that smokeless tobacco is associated with much less or no risk for atherosclerotic disease than tobacco smoking. It is a well-known fact that snuff-users have the same blood nicotine concentrations as smokers and even higher concentrations of cotinine, a major metabolite to nicotine [5, 6, 26, 27]. Taken together, these data clearly indicate that nicotine in itself may not be the most important aetiological factor in the atherosclerotic process but rather the inhaled smoke from the combustion of tobacco, which is known to result in a great number of products with known or potential adverse effects [28].

We observed that CRP as a sensitive circulating measure of inflammation was only associated with smoking and not with the use of snuff. Different biochemical markers of inflammation are known to indicate a risk of cardiovascular disease, although the underlying mechanisms have not been clarified [29–31]. One important component of the atherosclerotic process is inflammation and it has been postulated that this may be mirrored by circulating biochemical markers such as CRP [30, 31]. We are only aware of two studies that have examined if biochemical markers of inflammation are increased in snuff-takers [10, 11]. The results from these studies showed that snuff use, in contrast to tobacco smoking, was not associated with any increase in plasma fibrinogen as a measure of inflammation. In the present study, the results showed that smoking, but not snuff use, was associated with an increase in CRP. It is notable that previous smokers had a mean femoral IMT and plaque occurrence in-between the findings made in never-smokers and current smokers, respectively. The median time elapsed since smoking cessation was 6 years. These results indicate that the effect of smoking on the femoral artery wall is remaining for a considerable time in ex-smokers.

The limitations of the present study were that only men were studied and that it was a cross-sectional study. Consequently, only associations were studied and no conclusions can be drawn regarding causality. The results can only be inferred to 58-year-old men with varying degrees of insulin sensitivity [12, 13]. However, this is a cohort of the general population that is characterized by a high risk of atherosclerotic disease, as showed by the fact that about half of the studied men had atherosclerotic plaques in their carotid arteries [12].

The conclusions are that the oral use of moist snuff was not associated with any signs of ultrasound-assessed atherosclerosis in the carotid or femoral arteries or with elevated CRP concentrations. In contrast, tobacco smoking was associated both with an increase of the IMT and the occurrence

of atherosclerotic plaques in the carotid and femoral arteries. Smoking was also accompanied by abdominal obesity, dyslipidemia, hyperinsulinemia and inflammation, i.e. factors that are associated with development of atherosclerotic disease.

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