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Relationship of cigarette smoking and snuff dipping to plasma fibrinogen, fibrinolytic variables and serum insulin. The Northern Sweden MONICA study

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Abstract

The influence of cigarette smoking and use of smokeless tobacco on plasma fibrinogen level, fibrinolytic variables, glucose tolerance and serum insulin was studied in a randomly selected population sample consisting of 604 men and 662 females between 25 and 64 years. Subjects were grouped according to tobacco habits as follows: regular smokers (>1 cig/day), ex-smokers, snuff dippers, and non-tobacco users. An oral glucose tolerance test was performed on 54% of the participants. Tissue plasminogen activator (tPA) activity and plasminogen activator inhibitor type 1 (PAI-1) activity were used to study fibrinolysis. Men who smoked had 0.34 g/l (95% CI 0.17 to 0.49) higher fibrinogen level than non-tobacco users and numbers of cigarettes smoked correlated with plasma fibrinogen levels ($r = 0.21$, $P = 0.006$). Female smokers had significantly higher fibrinogen levels than ex-smokers but the difference compared with non-smokers was not significant. Snuff dipping did not affect fibrinogen levels. We found no relationship between tPA activity, PAI-1 activity and tobacco use. Post-load plasma glucose was lower in women who smoked, otherwise no influence of tobacco use on glucose levels was seen. Lower post-load insulin levels (–8.8 mU/ml, 95% CI –2.4 to –16.3) than in non-smokers were also found in women who smoked. This was only partially explained by a lower body mass index in smokers. We conclude that cigarette smoking is associated with increased fibrinogen levels, unaltered fibrinolysis, normal glucose tolerance and insulin levels. The use of smokeless tobacco, as moist oral snuff, does not appear to affect these potential cardiovascular risk factors.

Keywords: Cigarette smoking; Smokeless tobacco; Fibrinogen; Tissue plasminogen activator activity; Plasminogen activator inhibitor type 1; Insulin; MONICA

1. Introduction

High plasma fibrinogen levels, low fibrinolytic activity and elevated endogenous serum insulin levels have all been suggested to be risk factors for

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cardiovascular disease. Fibrinogen is now an accepted risk factor [1], but there is as yet only limited evidence to support the claims for the others [2–5]. They may act as mediators for some of the atherothrombotic effects of traditional risk factors such as smoking, hypertension, obesity and hyperlipidaemia. There is evidence for cigarette smoking as a cardiovascular risk factor [6] and pathogenetic mechanisms have been reviewed [7]. Stopping smoking reduces this risk [6,7]. There is a stronger association between smoking and myocardial infarction and sudden death than between smoking and angina pectoris, and an effect on the thrombotic–fibrinolytic equilibrium is suspected [6,8]. Three population studies have all clearly shown the increase in fibrinogen levels due to cigarette smoking [9–11] and this increase is thought to explain much of the deleterious effect of smoking on coronary heart disease [12]. Whether or not smoking also induces hypofibrinolysis is not known, although an early population study indicated that this was the case [13]. Recently, cigarette smoking has been proposed to cause insulin resistance and hyperinsulinaemia [14].

In the USA and Sweden, smokeless tobacco in the form of moist oral snuff is becoming increasingly popular, especially in young men [15,16] but little is known about the health hazards of snuff use. A case–control study found no increased risk of myocardial infarction in snuff dippers [17]. Fibrinogen levels were normal in young men using snuff, whereas fasting serum insulin levels were raised in both snuff users and smokers [18].

The aim of this study was to assess the relationship between cigarette smoking and snuff use and fibrinogen levels, fibrinolytic variables and serum insulin in a randomly selected population sample and to assess whether there were any gender differences in these variables in smokers.

2. Materials and methods

This study was performed within the framework of the Northern Sweden MONICA Project which is a part of the WHO MONICA Project (Monitoring of Trends and Determinants in Cardiovascular Disease). In January–April 1990, a population sample was screened for cardiovascular risk factors. A total of 2000 subjects aged 25–64 years were invited. Within each age group (25–34, 35–44, 45–54, 55–64 years), 250 men and 250 women were randomly selected. Details of the selection and sampling procedures have been published [19]. In all, 1583 subjects participated in the study (79.2% of those invited). 754 subjects, chosen at random, fasted overnight (12 h) and underwent a 75 g oral glucose tolerance test (OGTT) according to WHO guidelines [20]. The study was approved by the Research Ethics Committee of Umeå University.

2.1. Blood sampling

Participants were instructed not to use tobacco during the hour preceding the examination, which took place between 07:00 and 14:00 h. Immediately before the OGTT (which started before 11:00 h), venous samples for determination of blood lipids, plasma glucose, serum insulin, plasma fibrinogen and fibrinolytic variables were obtained. A second sample for measurement of glucose and insulin levels was taken after 2 h. Glucose and insulin samples were frozen at −20°C within 3 h. Plasma was collected for nicotine and cotinine determinations from a subgroup. Sampling for measurement of plasma fibrinogen, tPA activity and PAI-1 activity was done in the sitting position with no special rest and with minimal occlusion. Blood was drawn into 5 ml vacuum tubes (Stabilyte, Biopool AB, Umeå, Sweden) pre-filled with 0.5 ml of 0.45 mol/l citrate buffer pH 4.3. This ensured stability of tPA activity without causing appreciable haemolysis. Tubes were centrifuged at room temperature at 2000 × g for 20 min, snap-frozen within 1 h and stored in liquid nitrogen.

2.2. Methods

Subjects were weighed wearing only light clothing without shoes on an electronic balance that was calibrated daily. Height was measured without shoes. Body mass index (BMI) was calculated as weight (kg)/[height (m)]^2. The circumference of the smallest part of the waist and the thickest part of the hip was measured in the standing position and the waist–hip ratio (WHR) was calculated. Blood pressure was measured with the subject in the sitting position after 5 min rest using the random zero method.

Participants were classified into five categories according to their pattern of tobacco use. Non-tobacco users did not use any kind of tobacco and had never been regular tobacco users ($n = 581$). Ex-smokers had previously been regular cigarette smokers but were not using any tobacco at the time of the study ($n = 238$). Smokers smoked at least one cigarette per day but did not use any other kind of tobacco ($n = 317$). Snuff dippers used moist snuff regularly but no other type of tobacco ($n = 104$, of whom 12 women were excluded from further analysis). Former smokers, who now used snuff, were included only if more than 1 year had passed since they stopped smoking. Snuff and cigarette users made regular use of both snuff and cigarettes but no other type of tobacco ($n = 42$, of whom 4 women were excluded from further analysis). Those who used both snuff and cigarettes were pooled with current smokers in the analysis as they resembled smokers in most respects. Nineteen pipe smokers and 19 cigar smokers were excluded. Insufficient data or overlap between groups also led to exclusions. Thus the study group finally consisted of 604 men (78% of total sample) and 662 women (82%). Current tobacco use per day was calculated as one cigarette = 1 g of tobacco and one can of moist snuff = 50 g of tobacco.

Blood for glucose analysis was drawn into sodium fluoride tubes and plasma glucose was analysed by the hexokinase method (Boehringer Mannheim, Germany) on a Hitachi 717. Serum insulin levels were determined by radio immunoassay with a double antibody solid phase technique (Phadeseph Insulin RIA, Pharmacia Diagnostics AB, Uppsala, Sweden). The detection limit was < 2.5 mU/l and the interassay coefficient of variation was 4.8% at the level of 52 mU/l. According to the manufacturer, this assay has a cross-reactivity with C-peptide of $< 0.1\%$ and with proinsulin of 40%. It is not known whether there is any cross-reactivity with proinsulin split products.

Plasma fibrinogen determinations were performed by a functional kinetic method (Fibrinogen Kinetic, Boehringer Mannheim) using a Hitachi 717 analyser. The assay was standardised with Standard Scandinorm (Diagnostica Stago, France). tPA activity and PAI-1 activity were determined by the coupled plasminogen/plasmin chromogenic assay, Spectrolyse/fibrin kit (#101101) and Spectrolyse/pl kit (# 101201), respectively (Biopool). Details regarding assay imprecision and analytical sensitivity have been published [21]. Serum total cholesterol, high density lipoprotein (HDL) cholesterol and serum triglyceride levels were determined by enzymatic methods (Boehringer Mannheim). Plasma nicotine and cotinine (the primary metabolite of nicotine which is linearly related to nicotine intake [22]) were determined by a single-step liquid–liquid extraction followed by capillary gas chromatography. Nicotine and cotinine were detected by means of a nitrogen-sensitive detector [23]. Cotinine values below 12 ng/ml were considered to confirm non-tobacco user status [24,25].

2.3. Statistics

Means and 95% confidence intervals (95% CI) are given. As the distributions for fibrinogen, tPA activity and PAI-1 activity as well as glucose, insulin and triglyceride levels were skewed we report means for these variables logarithmically transformed (i.e. geometric means). Pearson correlation coefficients were calculated with log transformed values for the dependent variables. We did not adjust tPA and PAI-1 activity values for time of sampling because the independent variables did not show any significant linear trend with time of sampling [21]. A one-way analysis of variance (ANOVA) procedure was used to test for differences in means between tobacco groups. Tukey's *b*-test was used to identify groups between which there were significant differences. Multiple linear regression was performed with a stepwise method. In this procedure age, BMI, WHR, height, cholesterol, HDL cholesterol, triglycerides and blood pressure were also included. Smoking was coded as current smoker (= 1) or non-smoker (= 0), as was snuff use. As many independent variables have a high correlation with one another (data not shown), tests for colinearity were performed. Variables were included into the final model only if tolerance values exceeded 0.75. Two-tailed significance tests were used and due to the multiple comparisons only P values below 0.01 were considered significant in all procedures except multiple regression analysis, in which P values below 0.05 were

Table 1
Background data: men

	Non-tobacco users	Ex-smokers	Smokers	Snuff dippers	Snuff and cigarette users	P
n	220	130	124	92	38	
Age	45.3	49.9	46.7	42.0	44.9	**
	43.7–46.9	48.2–51.6	44.7–48.7	39.7–44.3	41.5–48.3	
BMI	25.5	26.4	25.7	25.3	25.9	
	25.1–25.9	25.9–26.9	25.1–26.3	24.6–25.9	24.6–27.3	
WHR	0.915	0.944	0.939	0.914	0.940	**
	0.908–0.922	0.936–0.953	0.928–0.949	0.902–0.926	0.916–0.963	
Diastolic BP	82.4	84.0	82.1	82.9	82.6	
(mmHg)	80.9–83.8	82.2–85.9	80.1–84.0	80.6–85.2	78.8–86.4	
Systolic BP (mmHg)	130	132	130	129	129	
	127–132	129–135	127–133	126–133	124–135	
Cholesterol (mmol/l)	6.2	6.6	6.2	6.3	6.7	
	6.0–6.4	6.4–6.8	6.1–6.4	6.0–6.6	6.2–7.3	
HDL cholesterol	1.28	1.29	1.24	1.36	1.21	
(mmol/l)	1.24–1.32	1.23–1.35	1.19–1.29	1.29–1.43	1.12–1.30	
Triglycerides	1.39	1.46	1.56	1.43	1.63	
(mmol/l)	1.28–1.51	1.31–1.63	1.36–1.78	1.22–1.67	1.38–1.93	

Means and 95% confidence intervals. HDL, high density lipoprotein; WHR, waist–hip ratio.
Significance testing with one-way ANOVA for differences across tobacco groups: $^{**}P < 0.001$.

Table 2
Background data: women

	Non-tobacco users	Ex-smokers	Smokers	P
n	361	108	193	
Age	46.7	45.3	43.6	*
	45.5–47.9	43.4–47.2	42.1–45.2	
BMI	25.4	24.9	24.4	
	24.9–25.9	24.2–25.6	23.8–25.0	
WHR	0.804	0.808	0.808	
	0.798–0.810	0.799–0.818	0.800–0.817	
Diastolic BP (mmHg)	80.6	77.5	76.7	**
	79.5–81.7	72.2–79.8	75.1–78.2	
Systolic BP (mmHg)	129	125	123	**
	127–131	121–128	120–125	
Cholesterol (mmol/l)	6.2	6.1	6.2	
	6.1–6.3	5.9–6.4	6.0–6.4	
HDL cholesterol (mmol/l)	1.58	1.58	1.49	*
	1.55–1.61	1.51–1.64	1.44–1.54	
Triglycerides (mmol/l)	1.19	1.12	1.29	
	1.11–1.28	1.02–1.22	1.19–1.39	

Means and 95% confidence intervals. HDL, high density lipoprotein; WHR, waist–hip ratio.
Significance testing with one-way ANOVA for differences across tobacco groups: $^{*}P < 0.01$, $^{**}P < 0.001$.

considered significant. The computer program SPSS for Windows, version 6.0, was used.

3. Results

Background variables for the different tobacco groups are given in Tables 1 and 2. Too few women ($n = 16$) used snuff to permit meaningful analysis and female snuff dippers were excluded. Male snuff dippers were significantly younger than smokers and ex-smokers, and non-smokers were younger than ex-smokers. Women who smoked were younger than non-smokers. BMI did not differ significantly between groups, although female smokers tended to have lower BMI than non-smokers ($P = 0.016$). Men who were current or previous smokers had greater WHR than non-tobacco users and snuff users. Both systolic and diastolic blood pressure were significantly lower in women who smoked than in non-smokers. HDL cholesterol levels were somewhat lower in women who smoked than in non-smokers. Adjusting for age reduced the difference in BMI between female smokers and non-smokers from 1.0 to 0.7 kg/m^2 ($P = 0.22$), the gap in diastolic blood pressure decreased from 3.9 to 3.1 mmHg ($P = 0.007$) and systolic blood pressure from 6.7 to 4.3 mmHg ($P = 0.044$). Otherwise the results were unchanged by age adjustment.

Anti-hypertensive agents were used by 12.2% of men who smoked, by 4.5% of snuff dippers and non-tobacco users. Of women who smoked, 7.3% used anti-hypertensive medication, as did 11.9% of non-smokers. Oral contraceptives were used by

Table 3
Exposure data for current tobacco users

	Sex	Smokers	Snuff dippers	Snuff and cigarette use	P
Lifetime duration (years)	M	29.4 27.4–31.5	17.0 15.1–19.0	27.5 24.7–30.4 16.4[a] 13.0–19.8	
	F	25.7 24.4–27.0			
Cigarettes/day	M	16.5 15.3–17.8		10.1 8.0–12.2	**
	F	12.7 12.0–13.4			
Cans[b] of snuff/week	M		3.2 2.9–3.5	2.5 2.2–2.9	*
Tobacco use/day (g)	M	16.5 15.3–17.8	22.9 20.7–25.0	28.2 25.0–31.4	
	F	12.7 12.0–13.4			
Plasma cotinine[c] (ng/ml)	M	242 209–275	351 277–425	308 242–373	*
	F	257 222–292			
Plasma nicotine[c] (ng/ml)	M	9.8 7.6–12.1	15.5 9.6–21.4	9.5 5.0–14.1	
	F	13.0 10.4–15.7			

Means and 95% confidence intervals.
[a]Duration of snuff use among those who use both snuff and cigarettes.
[b]A can contains 50 g snuff.
[c]Plasma cotinine and nicotine from 149 men and 172 women.
Significance testing with one-way ANOVA for differences across tobacco groups: *$P < 0.01$, **$P < 0.001$.

7.1% of the women and 5.0% used oestrogen replacement therapy. The proportion of hormone users did not differ between tobacco groups. These data are not standardized for age.

Data on tobacco exposure are given in Table 3. The mean age when starting to smoke was 17 years in both sexes. Smokers had a longer duration of tobacco use than snuff users. Ex-smokers had smoked for a total of 18.6 and 17.0 years, in men and women respectively. They ceased to smoke 14 and 13 years ago (means). Women who smoked had the lowest current tobacco exposure, but similar cotinine levels to male smokers, while male snuff dippers and those who used both snuff and cigarettes had the highest consumption. This was validated by the higher plasma cotinine found in snuff users than in smokers. Higher nicotine levels in snuff dippers indicated more recent or greater tobacco exposure (i.e. higher steady state levels).

Cotinine and nicotine samples were obtained from 321 subjects (25.4% of the whole study group). In non-tobacco users and male ex-smokers, all cotinine values were below 12.0 ng/ml. One out of 24 sampled women self-reported to be ex-smokers had a high plasma cotinine, 173 ng/ml, implying recent tobacco use. Another two female ex-smokers had threshold cotinine values, 27 and 32 ng/ml. All tobacco users except two (1.8%) had cotinine levels above 55 ng/ml. Only one non-tobacco user (1.0%) had a plasma nicotine above 4.0 ng/ml. Cotinine levels correlated with number of cigarettes per day in men, $r = 0.38$ ($P = 0.013$, $n = 41$), and in women, $r = 0.28$, although not significantly ($P = 0.06$, $n = 52$). Cotinine concentrations were correlated to the number of snuff cans dipped per week, $r = 0.60$ ($P = 0.003$, $n = 22$).

In men, plasma fibrinogen levels were significantly higher in current smokers than in non-tobacco users and snuff dippers ($P < 0.001$) (Table 4). Higher fibrinogen levels were found in ex-smokers than in non-users ($P = 0.01$) and snuff users ($P = 0.005$). In women, fibrinogen levels were significantly higher in current smokers than in ex-smokers ($P = 0.006$) (Table 4). As there is an interaction between age and tobacco use on fibrinogen levels, fibrinogen values were plotted against age groups and tobacco habits (Fig. 1). In men, fibrinogen levels were higher in smokers than in non-tobacco users and snuff users in all four age groups. The difference was not so striking in women, where the low levels in ex-smokers were prominent. Univariate correlation analysis in current smokers showed duration of smoking to be a

Table 4
Plasma fibrinogen and fibrinolytic variables according to tobacco habits

	Sex	Non-tobacco users	Ex-smokers	Current smokers	Snuff users	P
n	M	216	129	162	90	
	F	252	107	194		
Fibrinogen (g/l)	M	3.24	3.45	3.58	3.16	**
		3.14–3.33	3.32–3.58	3.45–3.71	3.01–3.31	
	F	3.59	3.38	3.66		*
		3.49–3.68	3.22–3.54	3.54–3.78		
tPA activity (IU/ml)	M	0.81	0.76	0.78	0.90	
		0.76–0.88	0.68–0.84	0.71–0.85	0.80–1.01	
	F	0.92	0.94	0.85		
		0.87–0.97	0.85–1.04	0.79–0.91		
PAI-1 activity (U/ml)	M	5.5	6.7	6.4	5.4	
		5.0–6.1	5.8–7.8	5.5–7.3	4.5–6.5	
	F	5.7	5.0	5.4		
		5.3–6.2	4.3–5.7	4.8–6.0		

Geometric means, 95% confidence intervals.
Significance testing with one-way ANOVA for differences across tobacco groups: *$P < 0.01$. **$P < 0.001$.

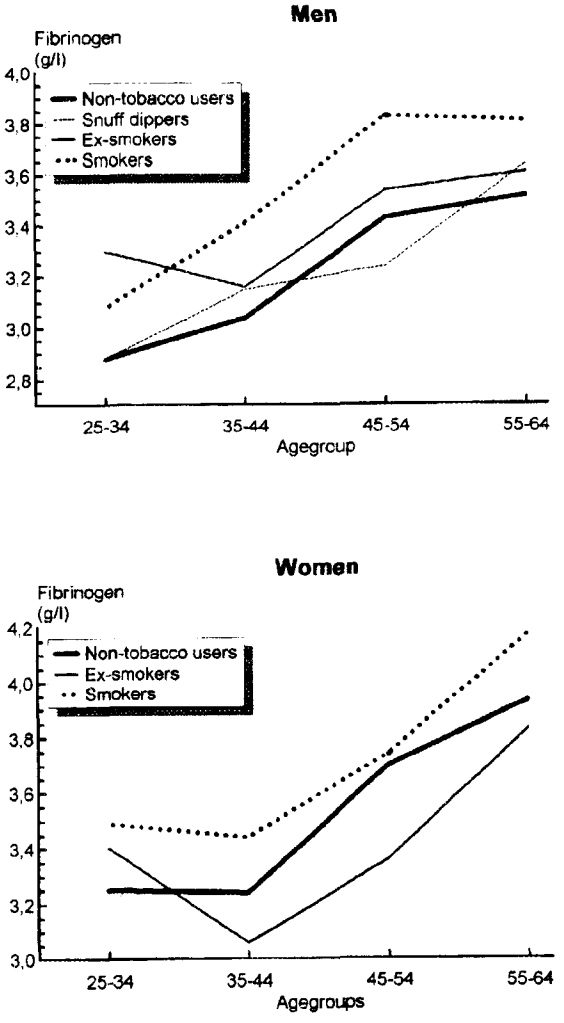


Fig. 1. Plasma fibrinogen according to age group and tobacco habits, in men (upper) and women (lower).

strong predictor of fibrinogen levels, but when age was controlled for this association disappeared. In men, the number of cigarettes smoked daily remained correlated to higher plasma fibrinogen levels ($r = 0.21$, $P = 0.006$) after age adjustment. As the relation between number of cigarettes and fibrinogen levels may not be linear, age-stratified scatterplots were studied (Fig. 2). An increase in fibrinogen levels may be discerned already below 10 cigarettes per day. Above this the increase seems more modest. Therefore, the correlation coefficient may underestimate this more curvilinear relation. No gender differences can be seen. Plasma nicotine and cotinine levels in current smokers showed insignificant positive relationships to fibrinogen levels in men. After age adjustment, no significant correlations were found between fibrinogen and measures of use such as duration, number of cigarettes, cotinine or nicotine levels in women. In multiple linear regression analysis, current smoking (yes or no), but no other measure of exposure, was a significant predictor of higher fibrinogen levels in both men and women (standardised regression coefficient 0.09, $P = 0.005$ and $P = 0.003$ in men and women, respectively). In female ex-smokers, the number of years since stopping smoking (controlling for age) showed a low inverse relationship to fibrinogen levels, $r = -0.21$ ($P = 0.029$). No measurement of snuff use was related to fibrinogen levels in univariate or multivariate analysis. In snuff dippers, neither plasma nicotine nor cotinine was correlated to fibrinogen.

No significant differences in tPA activity were noted between the tobacco groups (Table 4), either before or after age adjustment. An interaction was implied between age and tobacco use on tPA activity levels in men. By age stratification, a pattern could be discerned with lower tPA activity in smokers below 45 years. This was significant ($P < 0.001$) compared with snuff dippers between 35 and 44 years old, but not compared with non-users. Above this age, the activity was essentially the same in all groups. In women, age stratified tPA activity was similar between tobacco groups (data not shown). There were no relationships between cigarette smoking and tPA activity in univariate correlation or in multiple linear regression analysis. tPA activity was not related to snuff use. Tobacco habits showed no relationship to PAI-1 activity (Table 4) and this was not altered by age adjustment. Neither in univariate nor multivariate analysis were there any significant correlations with the different tobacco exposure variables.

Fasting glucose levels did not differ between the groups (data not shown). Post-load plasma glu-

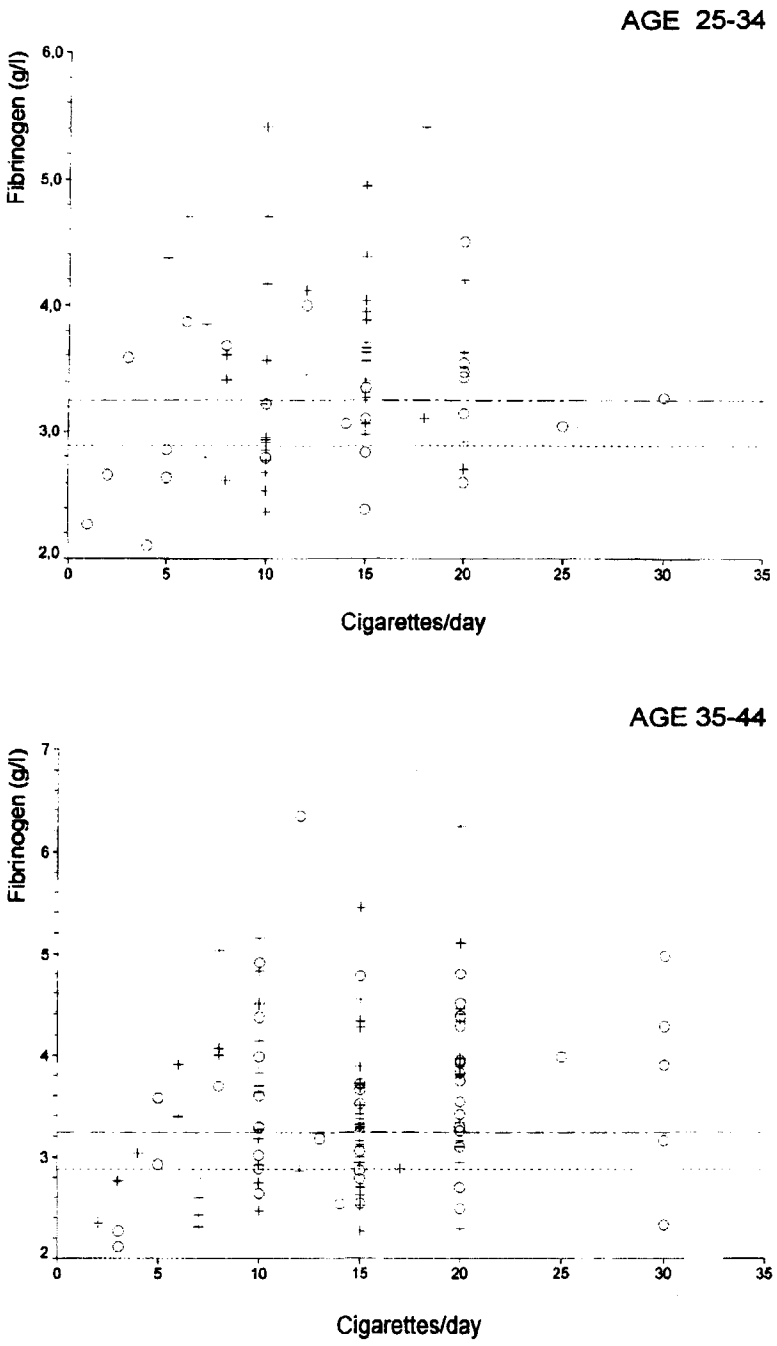
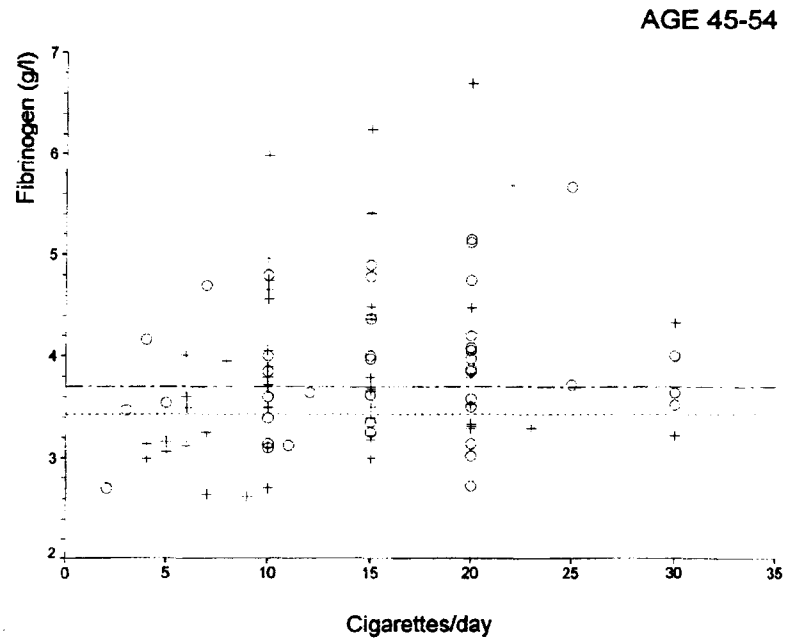


Fig. 2. Age-stratified scatterplots of plasma fibrinogen levels and number of cigarettes per day. Men (+), women (O). Geometric means for non-tobacco users are shown as (– – –) for men and (–·–·–) for women.



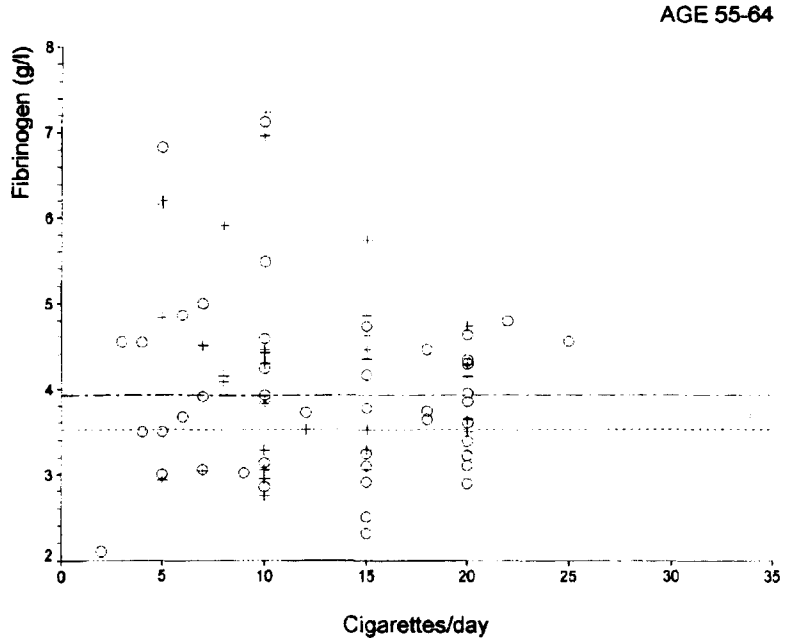


Fig. 2 (continued).

Table 5
Fasting and post-load serum insulin values according to tobacco habits

	Sex	Non-tobacco users	Ex-smokers	Smokers	Snuff users	P
n	M	125	73	80	42	
	F	196	60	105		
Fasting insulin (mU/l)	M	6.2	6.5	6.1	5.8	
		5.6–6.7	5.5–7.5	5.2–7.0	4.9–7.0	
	F	6.4	5.2	5.5		
		5.9–6.9	4.7–5.9	5.1–6.0		
Post-load insulin (mU/l)	M	25.0	24.9	20.3	20.6	
		21.5–29.0	20.0–31.0	16.8–24.6	15.9–26.8	
	F	35.1	25.3	26.3		*
		31.0–39.6	20.8–30.8	22.8–30.4		

Geometric means, 95% confidence intervals. n = numbers with complete dataset for fasting insulin and for post-load insulin. Measurements are missing from some subjects.
Significance testing with one-way ANOVA for differences across groups: $^*P < 0.01$.

cose in men was not related to tobacco habits, but in women post-load glucose levels were lower in both smokers and ex-smokers than in non-tobacco users ($P < 0.001$). The difference was 0.7 mmol/l (95% CI 0.4 to 1.0) between smokers and non-users. In a multiple linear regression analysis smoking was not a predictor of low post-load glucose levels. Fasting serum insulin levels were independent of tobacco habits, although there was a trend towards lower levels in women who smoked (Table 5). There were no univariate relationships between measurements of cigarette exposure and fasting insulin levels. In men, post-load insulin levels did not vary with tobacco use. Women who smoked had 8.8 mU/ml lower post-load insulin (95% CI 2.4 to 16.3, $P < 0.01$) than non-users. This finding persisted after age adjustment, but only BMI was a positive predictor of post-load insulin levels in multiple linear regression analysis. Being a regular smoker showed an inverse relation of borderline significance with post-load insulin (standardised regression coefficient −0.10, $P = 0.058$).

4. Discussion

During the past 20 years the consumption of moist snuff has tripled in the USA [15] and doubled in Sweden [16]. The prevalence of snuff dipping is highest among young men, and use of both snuff and cigarettes is common [15,16]. As many current young smokers report being former users of smokeless tobacco [15,16], snuff use may well be a first step towards taking up smoking. There is no evidence of a parallel decrease in smoking among young Swedish men, which led to the conclusion that an increase in the use of snuff is no prerequisite — and also no guarantee — of a decrease of smoking [16]. Data comparing the relation between smokeless tobacco and cigarette smoking and putative cardiovascular risk factors such as elevated plasma fibrinogen levels, decreased fibrinolysis and hyperinsulinaemia are lacking.

Non-tobacco users, smokers and snuff dippers differ not only in tobacco use but also in age and a variety of anthropometric and metabolic variables which are possibly related to the tobacco use per se. By controlling for variations in these variables, any integrated effects of tobacco use on the organism would be lost. Therefore, we chose only to adjust for differences in age, or age stratification, and to address the issue of direct tobacco effects in multiple regression analysis with possible confounders included. However, level of education and other life-style variables not reported here (such as physical activity and alcohol use) could be related to the type of tobacco used and could substantially influence results.

As has been previously shown [9–12], men who smoked had higher plasma fibrinogen levels, with

a dose–response relationship [26–28]. There were non-significant differences in this study between women non-smokers and smokers, most of which showed low or non-existent correlations between the number of cigarettes and fibrinogen levels, as earlier noted [11,26,27]. This may be due to the fact that women smoked substantially fewer cigarettes per day than men. Only in two studies have cotinine levels been used to validate self-reported smoking habits [12,28].

Fibrinogen levels declined with increasing time since smoking cessation in women, as recently noted by Lee et al. [28], which strengthens the causative role of smoking in increasing fibrinogen levels. As previously noted [28,29], women who formerly smoked had lower fibrinogen levels than non-smokers. A possible explanation for this is that in our study women who were former smokers maintained lower BMI than non-smokers. This fits well with the finding of intermediate fibrinogen values for male ex-smokers who had higher BMI than non-tobacco users. Thus a potentially beneficial effect of smoking cessation on fibrinogen levels might partly be offset by a concomitant weight gain.

A novel finding was the lack of influence of smokeless tobacco use on fibrinogen levels. Although snuff dippers had a shorter duration of tobacco use, daily tobacco exposure and plasma cotinine were higher than in smokers. A recent cross-over study [30] showed that transdermal nicotine in contrast to smoking does not influence fibrinogen levels. This suggests that the effect of smoking on fibrinogen levels is not mediated by circulating nicotine absorbed through the dermis, buccal or intestinal mucosa. Potential causative mechanisms could be local reactions in the lung, inhaled carbon monoxide or polycyclic aromatic hydrocarbons.

If we accept that the higher fibrinogen level is a major mediator of the atherothrombotic effects of smoking, this implies that the use of smokeless tobacco carries less risk for cardiovascular events. This is supported by a recent case–control study in men under 65 years of age [17], in which smokers had a doubled risk of myocardial infarction while no increased risk was found in snuff users. On the other hand, hypertension and disability retirement in people with cardiovascular disease have been reported to be more common in Swedish construction workers using smokeless tobacco [31], an observation that suggests that further exploration of the relationship between smokeless tobacco and circulatory disorders is necessary.

Impaired fibrinolysis has often been found in patients with existing cardiovascular disease, but a causal role has been difficult to establish [4]. Only recently, the first report was published in which low fibrinolytic activity (measured by a global assay — fibrin plate lysis time) predicted myocardial infarction in younger men [5]. There have still not been any population studies using specific assays for tPA activity. Only two population studies report on fibrinolysis and smoking. In the Northwick Park Heart Study, male smokers had impaired fibrinolytic activity by global assays [5,13], but Lowe et al. found no effect of smoking on fibrinolysis as measured by a plasmin-released fibrinogen peptide [32]. Experimental studies on the acute effects of smoking show either increased fibrinolytic activity measured as tPA activity [33,34] or unchanged fibrinolysis measured by PAI-1 activity [35]. The chronic effects of smoking were studied in small and highly selected samples and regular smokers were found to have increased PAI-1 activity [35] and lower tPA activity [36]. No changes were seen in fibrinolytic variables in young male smokers and snuff dippers compared with non-tobacco users [18].

We found no consistent differences regarding tPA activity and PAI-1 activity between the groups, and we conclude that being a regular smoker or snuff dipper does not have detrimental effects on fibrinolytic activity, as assayed by tPA activity or PAI-1 activity. A word of caution may be in order because of the relatively low cigarette consumption among the smokers in our study. Only 10% of the men and 2% of the women smoked more than 20 cigarettes per day. This does not preclude the possibility that acute effects of tobacco use on fibrinolysis exist. We studied chronic effects; judged by plasma nicotine [25] the great majority of subjects had adhered to the instructions not to smoke or use snuff for at least 1 h before the examination.

We found no evidence of glucose intolerance associated with tobacco use. On the contrary, lower post-load glucose and serum insulin levels

were found in women who smoked. The lower serum insulin, but not the lower glucose, was only partly explained by the lower BMI in women who smoked. In other population-based studies, lower post-load glucose and insulin levels in male smokers were noted during OGTT [3,37] which persisted after adjustment for BMI [2]. If fasting plasma insulin levels are taken as a crude measure of insulin resistance in epidemiological studies [38], then our results do not support the hypothesis that habitual smokers are insulin resistant when not smoking [14] or that snuff use would be associated with higher fasting insulin levels [18]. Intravenous GTT failed to disclose any signs of insulin resistance or hyperinsulinaemia in female smokers [39], but men who smoked had impaired glucose elimination [37]. Acute smoking, but not snuff dipping, was found to impair insulin action in an experimental setting [40]. Endogenous fasting insulin levels are the strongest predictor of low tPA activity [41]. If smoking did lead to persistent hyperinsulinaemia, smokers would be expected to have lower tPA activity, but we could not verify this. Thus, results from population samples (using serum insulin levels) and experimental studies (using euglycaemic clamps) disagree. But we are probably looking at different facets of what is now known as the insulin resistance syndrome.

We conclude that cigarette smoking is associated with increased fibrinogen levels, unaltered fibrinolysis and intact glucose tolerance without hyperinsulinaemia. The use of smokeless tobacco, as moist oral snuff, does not appear to affect these cardiovascular risk factors.

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