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Relationship between smoking and cardiovascular risk factors in the development of peripheral arterial disease and coronary artery disease

Edinburgh Artery Study

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Aims The aim was to determine whether the effect of smoking on the development of peripheral or coronary artery disease might be mediated by other cardiovascular risk factors, including dietary antioxidant vitamin intake, serum low and high density lipoproteins, blood pressure, plasma fibrinogen, blood viscosity and markers of endothelial disturbance and fibrin turnover.

Methods and Results 1592 men and women aged 55–74 years were selected at random from 11 general practices in Edinburgh, Scotland and followed-up for 5 years. The incidences of peripheral arterial disease and coronary artery disease were 5·1% and 11·1%, respectively. Both conditions were more common in moderate and heavy smokers than in never smokers; cigarette smoking was a stronger risk factor for peripheral arterial disease than for coronary artery disease. Smoking was associated with reduced dietary antioxidant vitamin intake, serum high density lipoprotein cholesterol and diastolic blood pressure and with increased alcohol intake, serum triglycerides, blood viscosity, plasma fibrinogen, and markers of endothelial disturbance (tissue plasminogen activator and von Willebrand factor antigens). Simultaneous adjustment for these risk factors reduced the relative risk of peripheral arterial disease only slightly, from 3·94 (95% CI 2·04, 7·62) to 2·72 (95% CI 1·13, 6·53) in heavy smokers and from 1·87 (95% CI 0·91, 3·85) to 1·70 (95% CI 0·72, 3·99) in moderate smokers. Similar adjustment also had little effect on the risk of coronary artery disease associated with smoking.

Conclusion The combined effect of smoking on the cardiovascular risk factors studied may explain part of its influence on peripheral and coronary arterial disease, but the majority of the effect appears to be due to other mechanisms.
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Key Words: Peripheral arterial disease, coronary artery disease, smoking, risk factors.

Introduction

Cigarette smoking can result in a seven-fold increase in the risk of peripheral arterial disease[1,2], and at least a two-fold increase in the risk of coronary artery disease[3,4]. These two major forms of cardiovascular disease associated with smoking are the sequelae of atherothrombosis. However, the pathophysiological mechanisms by which smoking results in the development of atherothrombosis are unknown. Suggested mechanisms include endothelial disturbance, changes in fibrin formation and turnover, altered blood rheology, changes in lipids and lipoproteins and reduced availability of antioxidants. Thus, smokers have been shown in some studies to have increased plasma levels of von Willebrand factor (a marker of endothelial dysfunction)[5,6], raised plasma fibrinogen (the precursor of fibrin) and haematocrit[7,8], together with altered blood lipid and lipoprotein profiles[9] and reduced circulating antioxidants[10]. However, evidence that

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smoking-related changes in these risk factors explain the effect of smoking on disease is lacking.

In this report from the 5 year follow-up of the Edinburgh Artery Study, we aimed to determine the extent to which smoking-related changes in the levels of certain cardiovascular risk factors might explain the effect of lifetime cigarette smoking on the development of peripheral arterial disease and coronary artery disease in the general population. Risk factors studied included dietary antioxidant vitamin intake; high and low density lipoproteins; plasma fibrinogen; rheological factors (blood and plasma viscosity, haematocrit), tissue plasminogen activator and von Willebrand factor antigens (markers of endothelial dysfunction) and fibrin D-dimer (a marker of fibrin turnover).

Methods

Baseline study

The Edinburgh Artery Study began in 1988 as a cross-sectional survey of 809 men and 783 women aged 55–74 years. This population was selected at random, in 5-year age bands, from 11 general practices serving a range of socioeconomic and geographic areas throughout the city. The response rate was 65%, and follow-up of a sample of non-responders showed no substantial bias. Details of the study recruitment and examination process have been described[11]. Ethics committee approval was given for this study, and informed consent was obtained from each subject. The questionnaire included validated questions on social class[12], intermittent claudication[13], angina[13], frequency of food consumption[14] and alcohol intake[15]. The questionnaire also contained standard questions on cardiovascular history, including recall of a doctor's diagnosis of angina, myocardial infarction or intermittent claudication, and a detailed section on smoking habit.

During the clinical examination, 20 ml of fasting blood was taken, following which subjects consumed 75 g of glucose in the form of 335 ml Solripe Gluctoza Health Drink (Strathmore Mineral Water Co.). A second blood specimen was taken 2 h after the oral glucose load. A 12-lead electrocardiogram (ECG) was taken and coded independently by two observers using the Minnesota code[16]. Standing height (without shoes) was measured to the nearest 5 mm using a free standing metal ruler on a heavy base. Weight without shoes and outer clothing was measured to the nearest 100 g on digital scales (Soehnle) and supine brachial blood pressure was measured after a 10-min rest.

In the laboratory, tests for serum total cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides, thiocyanate, gamma-glutamyl transpeptidase, and plasma glucose were performed on a Cobas Bio analyser (Roche Products) using standard kits. Low density lipoprotein (LDL) cholesterol was calculated using the formula: LDL cholesterol = total cholesterol − HDL cholesterol − triglycerides/5[17]. Fibrinogen was measured in citrated plasma by a thrombin-clotting turbidometric method[18]. Tissue plasminogen activator antigen was measured using an ELISA (Biopool, Umea, Sweden); von Willebrand factor antigen was measured by an ELISA (DAKO, Copenhagen, Denmark) as was fibrin D-dimer (AGEN, Parsippeny, New Jersey, U.S.A.). Blood and plasma viscosities were measured from a blood sample anticoagulated with dry dipotassium edetate ($1{\cdot}5\ \mathrm{mg\ .\ ml^{-1}}$) at high shear rates (over $300\ \mathrm{s^{-1}}$) in a Coulter-Harkness viscometer at 37 °C[18]. Haematocrit was measured using a Hawksley microcentrifuge and reader[18]. Quality control was measured by means of blind duplicate samples taken intermittently throughout the study.

Five year follow-up

Details of the follow-up procedure have been described[19]. Participants were followed-up over a 5-year period for cardiovascular events and death. To identify all deaths, participant's records were flagged at the U.K. National Health Service Central Registry. To obtain details of non-fatal events, information was sought from general practitioners, hospitals, the Information Services Division of the Scottish Office Home and Health Department and by annual questionnaire to the subjects themselves. All cardiovascular events and deaths were further investigated using hospital or general practitioner records. Subjects were invited to a 5-year follow-up examination, where they completed a self-administered questionnaire which included questions on smoking, cardiovascular events and the World Health Organisation (WHO) angina and intermittent claudication questionnaires[13]. A 12-lead ECG was taken and coded as at baseline.

Definitions of cardiovascular events

Criteria to define cardiovascular events were adapted from those agreed by the American Heart Association[20] and have been described previously[19]. Myocardial infarction was coded if two of the following were present: (i) cardiac pain lasting at least 20 min, (ii) diagnostic or equivocal ECG codes, (iii) elevated or equivocal cardiac enzyme levels. Diagnostic ECG codes in the absence of elevated enzyme levels or cardiac pain were also included (silent myocardial infarction). Fatal myocardial infarction was recorded if there was post-mortem evidence of acute myocardial infarction, definite criteria for myocardial infarction were present within 4 weeks prior to death or ICD-9 codes for cause of death were 410–414. A diagnosis of angina pectoris required either: (i) positive WHO angina questionnaire[13] and recall of a doctor's diagnosis of angina, or (ii) positive WHO angina questionnaire plus ECG ischaemia, or (iii) clinical diagnosis of angina made following investigation by

the general practitioner or in hospital. Intermittent claudication was diagnosed using the WHO questionnaire[13]. Multiple events of the same type occurring in the same subject were reported only once.

Disease categories

Three categories of disease were identified: (i) coronary artery disease (fatal or non-fatal myocardial infarction or angina), or (ii) peripheral arterial disease (intermittent claudication) and (iii) 'healthy' (no coronary artery disease or intermittent claudication). Subjects with angina, myocardial infarction or intermittent claudication at baseline were excluded from the analysis, as were a small number of subjects (n=18) who developed both coronary artery disease and intermittent claudication during follow-up (to allow separate analysis of the two disease states).

Data analysis

Data were analysed on the Edinburgh University mainframe computer using the SPSS-X and SAS software packages. Cigarette smoking was calculated in packyears (years of cigarette smoking multiplied by the average number of packs smoked per day) with the value zero entered for lifelong non-smokers. The number of units of alcohol consumed in the previous week was used to indicate current drinking levels. The current smoking and alcohol histories were considered sufficiently valid, since stated consumption correlated with mean thiocyanate and gamma-glutamyl transpeptide levels respectively. Nutrient intakes for individuals were estimated by multiplying the nutrient content of a typical portion size of the specified food item in the questionnaire by the frequency of consumption and summing over all food items. Nutrient intakes were adjusted for total energy intake, as described previously[21].

Mean levels of risk factors were compared between subjects with peripheral arterial disease or coronary artery disease and the 'healthy group', and the significance of differences were assessed by Student's t-test for continuous risk factors and chi-square test for categorical risk factors. Distributions of packyears, α-tocopherol, β-carotene, alcohol consumption, triglycerides, plasma glucose, tissue plasminogen activator antigen, von Willebrand factor antigen and fibrin D-dimer were positively skewed, so logarithmic or square root transformations were used in all analyses. Current smokers, recent ex-smokers (those ex-smokers who had stopped smoking less than 5 years previously), distant ex-smokers and never smokers were defined according to baseline self-reported cigarette smoking habits.

Disease incidence was calculated according to lifetime smoking exposure by dividing subjects into three groups by packyear values. Subjects with a packyear of 0 were classified as never smokers and the remaining subjects were divided into two approximately equal groups; those with a packyear ≤ 25 (moderate smokers) and those with a packyear >25 (heavy smokers). Mean risk factor levels were calculated in the never smokers, moderate smokers and heavy smokers, and a test for linear trend across the three smoking groups done.

To determine whether the influence of smoking on disease was affected by any of the risk factors, log-linear models were used to estimate relative risks (95% confidence intervals) of disease for moderate and heavy smokers, using never smokers as the base category, in both the peripheral arterial disease and coronary artery disease groups. The relative risks were adjusted for age and sex, and each risk factor was then entered separately into the model to identify its effect on the risk of disease. Finally, a model in which all risk factors were used as covariates simultaneously was performed for each of the two disease groups.

Results

Of the original 1592 subjects entered into the Edinburgh Artery Study, 1267 were free of angina, intermittent claudication and previous myocardial infarction at baseline. After 5 years of follow-up, 64 (5·1%) of these had developed peripheral arterial disease but not coronary artery disease (angina or myocardial infarction) and 141 (11·1%) had developed coronary artery disease but not peripheral arterial disease (only 18 subjects developed both conditions). One thousand and forty-four (1044) subjects who were free of disease at baseline remained free of both conditions by follow-up ('healthy' group).

Table 1 shows the baseline characteristics of subjects developing peripheral arterial disease and coronary artery disease and of those in the healthy group. Compared with subjects remaining healthy, both disease groups had significantly higher lifetime cigarette smoking, systolic blood pressure, serum LDL cholesterol, serum triglycerides, plasma fibrinogen, tissue plasminogen activator antigen, blood viscosity, plasma viscosity and haematocrit ($P<0·05$). Subjects developing coronary artery disease also had lower mean HDL cholesterol levels and higher fasting plasma glucose ($P<0·01$). Comparison of the two disease groups indicated that lifetime cigarette smoking was greater in the peripheral arterial disease group than in the coronary artery disease group (mean $\sqrt{}$packyears 4·46 and 3·41 respectively, $P<0·05$). Subjects with peripheral arterial disease were also more likely to be current or recent ex smokers than those developing coronary artery disease ($P<0·001$).

Smoking habit did not alter greatly during follow-up in subjects developing disease. Seven (10·9%) subjects who developed peripheral arterial disease stopped smoking during follow-up and one (1·6%) subject who was an ex-smoker at baseline resumed smoking. Similarly, seven (5·0%) subjects who developed coronary artery disease stopped smoking and one (0·7%) subject who was an ex-smoker at baseline resumed smoking.

Table 1 Baseline characteristics of subjects with PAD and CAD and those in the 'healthy' group

	Means or percentages (SE)			Significant differences between PAD and CAD
	PAD (n=64)	CAD (n=141)	Healthy (n=1044)	
Age (years)	66·1 (0·7)	65·1 (0·5)	64·2 (0·2)	ns
Sex (% male)	53·7	57·9	48·1	ns
Smoking				
Current/recent ex (%)†	53·1***	29·4	30·7	0·001
Packyears (√)	4·46 (0·35)***	3·41 (0·24)***	2·69 (0·09)	0·01
Vitamin C (mg)	53·3 (3·3)	59·2 (2·3)	59·8 (0·8)	ns
α-tocopherol (ln mg)	2·20 (0·06)	2·36 (0·04)	2·30 (0·01)	0·02
β-carotene (ln mg)	7·90 (0·12)	8·05 (0·08)	8·04 (0·03)	ns
Alcohol ($\sqrt{}$units . week^{-1})	2·5 (0·3)	2·2 (0·2)	2·2 (0·1)	ns
Systolic BP (mmHg)	148·0 (2·8)*	148·7 (2·1)***	141·2 (0·6)	ns
Diastolic BP (mmHg)	80·7 (1·6)	82·5 (1·0)	80·8 (0·4)	ns
BMI (kg . m^{-1})	25·2 (0·5)	25·8 (0·3)	25·3 (0·1)	ns
LDL cholesterol (mmol . l^{-1})	5·68 (0·15)***	5·39 (0·09)**	5·14 (0·04)	ns
HDL cholesterol (mmol . l^{-1})	1·43 (0·05)	1·35 (0·03)***	1·49 (0·01)	ns
Triglycerides (ln mmol . l^{-1})	0·45 (0·05)***	0·41 (0·04)**	0·29 (0·01)	ns
Fasting glucose (ln mmol . l^{-1})	1·75 (0·03)	1·78 (0·02)**	1·73 (0·01)	ns
2-hour glucose (ln mmol . l^{-1})	1·72 (0·05)	1·77 (0·03)	1·72 (0·01)	ns
Fibrinogen (g . l^{-1})	2·92 (0·07)***	2·75 (0·06)*	2·62 (0·02)	ns
t-PA ($\sqrt{}$ng . ml^{-1})	2·86 (0·07)**	2·83 (0·05)***	2·63 (0·02)	ns
Fibrin D-dimer (ln ng . ml^{-1})	4·49 (0·09)	4·46 (0·06)	4·38 (0·19)	ns
vWF ($\sqrt{}$IU . dl^{-1})	10·80 (0·28)	10·37 (0·19)	10·34 (0·07)	ns
Blood viscosity (mPa . s^{-1})	3·68 (0·07)*	3·70 (0·06)**	3·52 (0·02)	ns
Plasma viscosity (mPa . s^{-1})	1·344 (0·009)*	1·343 (0·008)**	1·322 (0·003)	ns
Haematocrit (%)	47·1 (0·5)**	46·4 (0·4)*	45·6 (0·1)	ns

P-value for difference in mean between diseased and healthy group: ***$P \leq 0{\cdot}001$, **$P \leq 0{\cdot}01$, *$P \leq 0{\cdot}05$.
ns=not significant; PAD=peripheral arterial disease; CAD=coronary artery disease; BMI=body mass index; LDL/HDL=low/high density lipoprotein cholesterol; t-PA=tissue plasminogen activator antigen; vWF=von Willebrand factor antigen. †Current smokers or those who had stopped smoking less than 5 years previously.

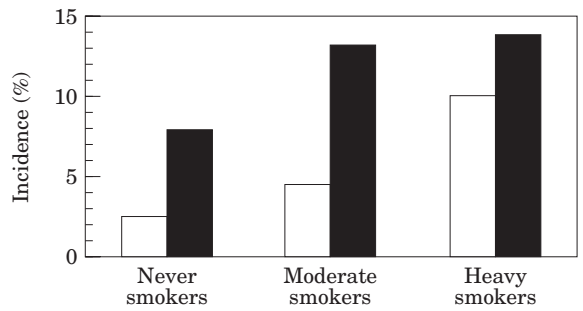

Figure 1 **Incidence (%) of peripheral arterial disease (□) and coronary artery disease (■) in never smokers (packyears=0), moderate smokers (0 <packyears ≤25) and heavy smokers (packyears >25).**

To assess risk of disease according to lifetime smoking, subsequent analysis was performed on subjects grouped according to their packyear values. Figure 1 illustrates that the incidence of peripheral arterial disease rose from 2·6% in never smokers (packyears=0) to 4·5% in moderate smokers (0 <packyears ≤25) and 9·8% in heavy smokers (packyears >25). The incidence of coronary artery disease rose from 7·4% in never smokers, to 13·1% in moderate smokers and 13·7% in heavy smokers.

Table 2 compares mean risk factor levels in never smokers, moderate smokers and heavy smokers. Heavy smokers were slightly older than never and moderate smokers, and were more likely to be male ($P<0{\cdot}05$ for linear trend). Heavy smokers also had considerably lower intakes of dietary antioxidant vitamins and higher weekly alcohol consumption ($P<0{\cdot}001$ for linear trend). Serum HDL cholesterol levels were higher and serum triglyceride levels were lower in never smokers than in moderate and heavy smokers ($P<0{\cdot}001$), but there was no significant difference in LDL cholesterol ($P>0{\cdot}05$). Plasma glucose levels 2 h after a standard glucose tolerance test were also higher in never smokers. Plasma fibrinogen, tissue plasminogen activator antigen, von Willebrand factor antigen, blood viscosity, plasma viscosity and haematocrit levels all increased across smoking groups ($P<0{\cdot}001$).

Tables 3 and 4 show the relative risks of peripheral arterial disease and coronary artery disease, respectively, associated with moderate and heavy smoking, before and after adjustment for the other cardiovascular risk factors. The age- and sex-adjusted relative risks of peripheral arterial disease associated with smoking were 1·87 (95% CI 0·91, 3·85) for moderate smokers and 3·94 (95% CI 2·04, 7·62) for heavy smokers. Similarly adjusted relative risks of coronary artery disease were

Table 2 Mean risk factor levels at baseline in the three packyear groups

	Means or percentages (SE)			Significant linear trend
	Never smokers (n=492)	Moderate smokers (n=398)	Heavy smokers (n=336)	
Age (years)	64·2 (0·3)	63·9 (0·3)	65·1 (0·3)	$P \leq 0{\cdot}05$
Sex (% male)	36·5	53·6	65·3	$P \leq 0{\cdot}001$
Vitamin C (mg)	64·1 (1·2)	61·0 (1·3)	51·3 (1·3)	$P \leq 0{\cdot}001$
α-tocopherol (ln mg)	2·34 (0·02)	2·34 (0·02)	2·19 (0·02)	$P \leq 0{\cdot}001$
β-carotene (ln mg)	8·13 (0·03)	8·07 (0·04)	7·86 (0·05)	$P \leq 0{\cdot}001$
Alcohol ($\sqrt{}$units . week^{-1})	1·6 (0·1)	2·4 (0·1)	2·9 (0·1)	$P \leq 0{\cdot}001$
Systolic BP (mmHg)	142·8 (1·0)	142·3 (1·1)	141·9 (1·3)	ns
Diastolic BP (mmHg)	81·9 (0·5)	80·4 (0·6)	80·0 (0·7)	$P \leq 0{\cdot}05$
BMI (kg . m^{-1})	25·4 (0·2)	25·4 (0·2)	25·2 (0·2)	ns
LDL cholesterol (mmol . l^{-1})	5·23 (0·05)	5·25 (0·06)	5·07 (0·06)	ns
HDL cholesterol (mmol . l^{-1})	1·52 (0·02)	1·46 (0·07)	1·41 (0·02)	$P \leq 0{\cdot}001$
Triglycerides (ln mmol . l^{-1})	0·26 (0·02)	0·30 (0·02)	0·38 (0·03)	$P \leq 0{\cdot}001$
Fasting glucose (ln mmol . l^{-1})	1·73 (0·01)	1·73 (0·01)	1·74 (0·01)	ns
2-hour glucose (ln mmol . l^{-1})	1·79 (0·02)	1·69 (0·02)	1·68 (0·02)	$P \leq 0{\cdot}001$
Fibrinogen (g . l^{-1})	2·55 (0·03)	2·67 (0·03)	2·77 (0·04)	$P \leq 0{\cdot}001$
t-PA ($\sqrt{}$ng . ml^{-1})	2·56 (0·03)	2·67 (0·35)	2·83 (0·03)	$P \leq 0{\cdot}001$
Fibrin D-dimer (ln ng . ml^{-1})	4·40 (0·03)	4·35 (0·03)	4·45 (0·03)	ns
vWF ($\sqrt{}$IU . dl^{-1})	10·22 (0·10)	10·17 (0·11)	10·81 (0·12)	$P \leq 0{\cdot}001$
Blood viscosity (mPa . s^{-1})	3·44 (0·03)	3·52 (0·03)	3·72 (0·04)	$P \leq 0{\cdot}001$
Plasma viscosity (mPa . s^{-1})	1·316 (0·004)	1·323 (0·004)	1·342 (0·005)	$P \leq 0{\cdot}001$
Haematocrit (%)	44·9 (0·2)	45·7 (0·2)	46·9 (0·2)	$P \leq 0{\cdot}001$

ns=not significant; BMI=body mass index; LDL/HDL=low/high density lipoprotein cholesterol; t-PA=tissue plasminogen activator antigen; vWF=von Willebrand factor antigen.

1·59 (95% CI 1·04, 2·42) and 1·66 (95% CI 1·07, 2·58), respectively. On adjusting these relative risks for each risk factor individually, the risk of peripheral arterial disease did not alter greatly and remained highly significant for heavy smokers. The relative risk of coronary artery disease was also little affected, and the 95% confidence limits of all relative risks before and after adjustment overlapped considerably. The most consistent change, present in both disease groups, was a marginal reduction in relative risk after adjustment for plasma fibrinogen. Simultaneous (multiple) adjustment for all the risk factors studied reduced the relative risks of both diseases slightly, especially for heavy smokers developing peripheral arterial disease; from 3·94 (95% CI 2·04, 7·62) to 2·72 (95% CI 1·13, 6·53). However, the 95% confidence limits of the relative risks before and after multiple adjustment still overlapped considerably.

Discussion

Our prospective results confirm the importance of smoking as a risk factor for the development of peripheral arterial disease as well as for coronary artery disease in the general population. In addition, a higher mean packyear value and a higher percentage of current and recent ex-smokers in subjects developing peripheral arterial disease compared with those developing coronary artery disease support previous findings that cigarette smoking is a stronger risk factor for peripheral arterial disease than for coronary artery disease[21,22]. Although subjects developing both symptomatic peripheral arterial disease and coronary artery disease were excluded from the present analysis, it is likely that there was some overlap between the two groups with respect to subclinical disease, making this difference in smoking effect even more remarkable. Explanations for the greater effect of cigarette smoking on the development of peripheral arterial disease have been discussed previously[23,24] and may reflect different anatomical structures and/or haemodynamics of the peripheral and coronary arteries.

Although one concern in the present study is false reporting of smoking history, the prevalence of smoking deception in Scotland is probably low[25]. Furthermore, smoking histories were consistent with serum thiocyanate levels measured in the laboratory[21]. Since all subjects were free of clinical disease when smoking histories were obtained, false reporting of smoking habit associated with pre-existing disease should have been minimal. Lifetime smoking status was calculated at baseline and may have changed in the period prior to the development of symptomatic disease. However, this is unlikely to have had a major impact on the results since only 11% of subjects developing peripheral arterial disease and 5% of those developing coronary artery disease stopped smoking during follow-up, and these subjects were ex-smokers for 5 years or less prior to developing disease.

Table 3 Logistic regression on subjects with peripheral arterial disease by lifetime smoking status, adjusting for cardiovascular risk factors

Adjusted for	Relative risk (95% CI)	
	Moderate smokers (n=18)	Heavy smokers (n=33)
Age and sex	1·87 (0·91, 3·85)	3·94 (2·04, 7·62)
Vitamin C	1·88 (0·91, 3·86)	3·78 (1·95, 7·35)
α-tocopherol	1·90 (0·92, 3·89)	3·72 (1·92, 7·24)
β-carotene	1·88 (0·91, 3·86)	3·89 (2·00, 7·54)
Alcohol	1·71 (0·83, 3·58)	3·55 (1·80, 7·02)
Systolic pressure	1·88 (0·91, 3·85)	3·96 (2·05, 7·68)
Diastolic pressure	1·88 (0·92, 3·87)	3·88 (2·00, 7·54)
BMI	1·87 (0·91, 3·85)	3·95 (2·04, 7·64)
LDL cholesterol	1·81 (0·88, 3·72)	4·07 (2·11, 7·84)
HDL cholesterol	1·85 (0·90, 3·81)	3·97 (2·06, 7·67)
Triglycerides	1·83 (0·89, 3·75)	3·70 (1·91, 7·16)
Fasting glucose	1·85 (0·90, 3·80)	3·95 (2·05, 7·62)
2-hour glucose	1·92 (0·91, 4·06)	4·51 (2·27, 8·96)
Fibrinogen	1·75 (0·85, 3·59)	3·60 (1·86, 6·97)
t-PA	1·98 (0·95, 4·14)	3·75 (1·89, 7·44)
Fibrin D-dimer	1·89 (0·92, 3·87)	3·80 (1·96, 7·37)
vWF	1·90 (0·92, 3·89)	3·73 (1·92, 7·72)
Blood viscosity	2·10 (0·99, 4·47)	3·66 (1·78, 7·51)
Plasma viscosity	2·01 (0·96, 4·20)	3·92 (1·98, 7·81)
Haematocrit	1·94 (0·93, 4·06)	3·58 (1·80, 7·12)
Multiple adjustment	1·70 (0·72, 3·99)	2·72 (1·13, 6·53)

BMI=body mass index; LDL/HDL=low/high density lipoprotein cholesterol; t-PA=tissue plasminogen activator antigen; vWF=von Willebrand factor antigen.

The pathophysiological mechanisms responsible for the effect of smoking on the development of atherosclerotic disease are unknown. However, we found that smoking was associated with changes in the levels of several cardiovascular risk factors which may help to explain at least some of this effect. It should be noted that, despite the large size of the overall cohort studied, the number of subjects developing disease was relatively small (64 peripheral arterial disease and 141 coronary artery disease); this may have resulted in some biologically important results failing to reach statistical significance, especially where the two groups were compared directly.

LDL and HDL cholesterol and triglycerides

Adverse alterations in the lipid profile of smokers were consistent with previous studies in which the levels of triglycerides and LDL cholesterol averaged 9·1% and 1·7% higher, respectively, in smokers than non-smokers, and levels of HDL cholesterol were approximately 5·7% lower[9]. Although these differences are small, they might be expected to affect atherogenesis to a significant degree if maintained throughout adulthood. However, we found that adjustment for LDL cholesterol, HDL cholesterol and triglycerides had very little effect on the smoking-related risk of either peripheral arterial disease or coronary artery disease, suggesting that they do not have a major mediatory role in the impact of smoking on either disease state. Accordingly, the effect of smoking on cardiovascular disease mortality in the Lipid Research Clinics Follow-up Study was independent of both HDL and LDL cholesterol[26]. The lack of an effect of HDL cholesterol may be related to the fact that of the two major subcomponents of HDL cholesterol, HDL2 and HDL3, only the former may be protective[27,28], whereas cigarette smoking has been reported to correlate inversely with HDL3, but not HDL2 levels[29].

Dietary antioxidant vitamins and alcohol

It has also been suggested that antioxidant vitamin deficiencies and increased alcohol intake may contribute to increased atherosclerotic disease in smokers. The relationship between alcohol intake and risk of cardiovascular disease has been investigated widely in recent years with most evidence suggesting that whilst heavy drinkers have a higher risk of disease than moderate smokers, complete abstainers are also at increased risk[30]. Although an association between smoking and increased alcohol intake has been reported previously[31–34], we found that adjustment for such differences in weekly alcohol intake in our study had little effect on the risk of disease associated with

Table 4 Logistic regression on subjects with coronary artery disease by lifetime smoking status, adjusting for cardiovascular risk factors

Adjusted for	Relative risk (95% CI)	
	Moderate smokers (n=52)	Heavy smokers (n=46)
Age and sex	1·59 (1·04, 2·42)	1·66 (1·07, 2·58)
Vitamin C	1·59 (1·05, 2·42)	1·65 (1·06, 2·58)
α-tocopherol	1·60 (1·05, 2·44)	1·72 (1·10, 2·68)
β-carotene	1·60 (1·05, 2·44)	1·64 (1·05, 2·56)
Alcohol	1·58 (1·03, 2·43)	1·72 (1·09, 2·70)
Systolic pressure	1·59 (1·04, 2·42)	1·69 (1·09, 2·62)
Diastolic pressure	1·58 (1·03, 2·42)	1·65 (1·06, 2·57)
BMI	1·59 (1·04, 2·42)	1·67 (1·07, 2·59)
LDL cholesterol	1·56 (1·02, 2·37)	1·72 (1·11, 2·67)
HDL cholesterol	1·59 (1·04, 2·41)	1·68 (1·08, 2·60)
Triglycerides	1·57 (1·03, 2·39)	1·62 (1·04, 2·51)
Fasting glucose	1·62 (1·06, 2·47)	1·69 (1·09, 2·62)
2-hour glucose	1·65 (1·07, 2·55)	1·81 (1·15, 2·85)
Fibrinogen	1·50 (0·98, 2·29)	1·52 (0·97, 2·38)
t-PA	1·44 (0·92, 2·25)	1·57 (0·99, 2·48)
Fibrin D-dimer	1·49 (0·97, 2·31)	1·64 (1·05, 2·57)
vWF	1·49 (0·96, 2·30)	1·66 (1·06, 2·60)
Blood viscosity	1·48 (0·93, 2·36)	1·60 (0·99, 2·59)
Plasma viscosity	1·64 (1·07, 2·53)	1·66 (1·05, 2·61)
Haematocrit	1·62 (1·05, 2·48)	1·69 (1·08, 2·65)
Multiple adjustment	1·22 (0·72, 2·07)	1·61 (0·91, 2·85)

BMI=body mass index; LDL/HDL=low/high density lipoprotein cholesterol; t-PA=tissue plasminogen activator antigen; vWF=von Willebrand factor antigen.

smoking, except for a marginal reduction in the risk of peripheral arterial disease. We also confirmed the inverse relationship between smoking and dietary intake of antioxidant vitamins reported previously[32,35], with particularly low levels in the heaviest smokers. This could potentially result in the oxidation and increased atherogenicity of LDL cholesterol in smokers[36]. However, adjustment for dietary antioxidant vitamins had very little effect on the relationship between smoking and either peripheral arterial disease or coronary artery disease. Despite this finding, it is still possible that reductions in serum antioxidant levels due to smoking might be important in the aetiology of atherosclerosis. In addition to the well-established limitations in the accuracy of nutrient intake evaluation using dietary recall questionnaires, there is evidence that serum levels of antioxidants are lower in smokers than in non-smokers for the same level of dietary intake[37], possibly due to excessive consumption to counteract the damaging oxidative effects of cigarette smoke.

Plasma fibrinogen

Plasma fibrinogen levels are known to be raised in smokers[7,8]. We found that adjustment for fibrinogen consistently reduced the risk of both coronary artery disease and peripheral arterial disease associated with moderate and heavy smoking very slightly, although the significance level of the relationship between smoking and disease did not alter greatly. Previous studies have also indicated that changes in plasma fibrinogen might contribute to the effect of smoking on cardiovascular disease. Thus, when fibrinogen was added to the multivariate model for prediction of cardiovascular disease in the Framingham Study, the coefficient for smoking became much reduced and was no longer statistically significant, although each independently contributed to risk in cross-sectional analysis[38]. Also, on adjustment for smoking, the relationship of fibrinogen with peripheral arterial disease and coronary artery disease decreased very slightly (but remained significant) in cross-sectional and case control analyses from the Edinburgh Artery Study[6,21]. These results suggest that part of the effect of smoking on disease may be mediated by changes in plasma fibrinogen levels, but that the majority of the effect is caused by other mechanisms.

Fibrin turnover and endothelial disturbance

In addition to possible increased deposition of arterial fibrin due to elevated circulating fibrinogen in smokers, alterations in fibrin turnover and/or endothelial disturbance might be important in mediating the effect of cigarette smoking on disease. Tissue plasminogen

activator (t-PA) antigen levels reflect inactive t-PA/plasminogen activator inhibitor complexes rather than free active t-PA[39,40]. Thus, raised levels potentially indicate elevated plasminogen activator inhibitor activity and impaired fibrinolytic activity, as well as increased release of tPA secondary to endothelial disturbance. Our results are consistent with several studies reporting raised t-PA antigen levels in coronary artery disease[41,42], although recent prospective studies suggest that tPA is not an independent risk factor for incident ischaemic heart disease in the healthy population[43,44]. Fibrin D-dimer, an index of cross-linked fibrin turnover has been shown to be an independent predictor of ischaemic heart disease events[45,46], as has von Willebrand factor (a marker of endothelial disturbance)[46,47]. Despite associations between smoking and elevated t-PA and von Willebrand factor antigen levels, adjustment for these factors had little impact on the effect of smoking on either peripheral arterial disease or coronary artery disease. Nor did adjustment for fibrin D-dimer levels have any major impact. Previous reports on the importance of fibrin turnover and endothelial disturbance on the relationship between smoking and atherosclerotic disease have been conflicting. A recent case control study found that controlling for the effects of smoking significantly reduced the relationships between peripheral arterial disease and both von Willebrand factor and fibrin D-dimer[6]. However, our results are consistent with a larger study in which smoking adjustment had a negligible effect on the associations of von Willebrand factor and fibrin D-dimer with the risk of intermittent claudication[48]. Thus, present evidence does not suggest that these markers of endothelial disturbance and fibrin turnover mediate a significant part of the relationship between smoking and disease.

Blood rheology

Smokers have also been shown previously to have reversible increases in blood viscosity compared to non-smokers, due to increased haematocrit and plasma viscosity[49,50]. Increases in plasma viscosity are largely due to elevated plasma fibrinogen levels, together with other 'acute phase reactant' proteins such as alpha$_2$ macroglobulin[49]. Adjustment for changes in blood viscosity, plasma viscosity and haematocrit with smoking had no consistent effect on the risks of peripheral arterial disease and coronary artery disease associated with smoking in the present study. Similarly, in a recent case control study, the risk of intermittent claudication for a given rise in plasma viscosity was reduced only marginally after adjustment for lifetime smoking[48].

Body mass index, blood pressure and glucose

Of the many physiological responses to cigarette smoking, alterations in blood pressure and body mass index are perhaps the least likely to contribute to its atherogenic effect, since smokers have lower body mass indices than nonsmokers[32,51–53] and probably lower long-term blood pressures[54,55]. In addition, the only significant relationship between plasma glucose levels, disease and smoking in this study was a slightly higher mean fasting plasma glucose level in subjects developing coronary artery disease and slightly lower mean levels 2 h after a standard glucose tolerance test in moderate and heavy smokers compared with never smokers; the latter may be related to the higher insulin levels reported in smokers than in non-smokers[56]. It is unsurprising therefore that adjustment for plasma glucose, systolic blood pressure, diastolic blood pressure and body mass index had little impact on the effect of smoking on disease.

Simultaneous adjustment for the whole range of risk factors studied resulted in a slight reduction in the risk of disease associated with smoking. This was most marked for peripheral arterial disease, where adjustment for multiple risk factors explained approximately one quarter of the risk of disease associated with heavy smoking (relative risk reduced from 3·94 to 2·72). However, this effect was much less marked for coronary artery disease, and in both disease states smokers continued to have between a 1·22 and 2·72 times greater risk of disease than non-smokers after taking into account the other risk factors. It should be noted that the log-linear models used to evaluate the mechanisms whereby smoking causes peripheral arterial disease and coronary artery disease are likely to underestimate the role of the variables included in the model. Also, since the cohort studied is relatively old and the levels of several of the factors studied tend to alter throughout life, it might be that any mediatory role occurs during the development of atherosclerosis earlier in life. However, the conclusion most consistent with our results is that whilst the combined effect of smoking on the risk factors studied might explain *some* of the influence of smoking on disease, the majority of its effect appears to be mediated by other mechanisms, at least in older people. Such mechanisms could include a direct toxic effect of whole smoke, nicotine and/or carbon monoxide on endothelial cells[56], increased platelet reactivity and aggregability[58], and/or a detrimental effect of the elevated white blood cell count found consistently in smokers[59,60].

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