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Associations between cigarette smoking, pipe/cigar smoking, and smoking cessation, and haemostatic and inflammatory markers for cardiovascular disease

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Aims To examine the associations between cigarette smoking, pipe/cigar smoking, and years since quitting smoking, and inflammatory and haemostatic markers.
Methods and results A study in 2920 men aged 60–79 with no history of myocardial infarction, angina, stroke, or diabetes, and who were not on warfarin, from general practices in 24 British towns. After adjustment for other major cardiovascular risk factors, compared with never smokers, current cigarette smokers showed significantly higher levels of C-reactive protein (2.53 vs. 1.35 mg/L), white cell count (7.92 vs. 6.42 $\times$ 10^9/L), and fibrinogen (3.51 vs. 3.13 g/L). They also showed higher levels of haematocrit, blood and plasma viscosity, tissue plasminogen activator antigen, and fibrin D-dimer, and lower levels of albumin. Primary pipe/cigar smokers showed levels similar to never smokers. Ex-cigarette smokers and secondary pipe/cigar smokers showed intermediate levels although secondary pipe/cigar smokers showed higher odds of having elevated white cell count and fibrinogen than ex-cigarette smokers. Most inflammatory and haemostatic levels improved within 5 years of smoking cessation but took over 20 years to revert to levels of never smokers.
Conclusion These findings suggest that activation of inflammation and haemostasis may be potential mechanisms by which cigarette and pipe/cigar smoking increase cardiovascular risk.

Introduction

Cigarette smoking is a major risk factor for cardiovascular disease (CVD). Giving up smoking is associated with a substantial reduction in risk of coronary heart disease (CHD) and stroke, although the decrease in risk appears to be dependent on the duration of cessation.[1–3] There is increasing evidence that pipe/cigar smoking, particularly those who switch from cigarette smoking to pipe or cigar, carries an increased risk of CHD.[4,5] Proposed potential mechanisms by which smoking increases the risk of CVD include haemostatic disturbances,[6] lipid abnormalities,[7] and vascular endothelial dysfunction.[8,9] Inflammatory mechanisms play an important role in the development and progression of atherosclerosis.[10,11] Smoking is associated with a variety of markers of inflammation such as C-reactive protein, white cell count, fibrinogen, and albumin,[12–20] which have been shown to be independent risk factors for CHD.[11,21] Inflammation is another possible mechanism for the increased risk of CVD in smokers. Although most studies show haematological and inflammatory markers to be lowered after smoking cessation, few studies have examined the effects of duration of smoking cessation, and it appears that the effects of smoking on inflammatory markers may persist for many years.[12,17,20] Data on the effects of primary and secondary pipe or cigar smoking on haemostatic and inflammatory markers are limited. We have therefore, examined the effects of cigarette smoking and primary and secondary pipe/cigar smoking on inflammatory markers (C-reactive protein, white cell count, albumin, and fibrinogen) and several haemostatic factors shown to be significantly associated with risk of CHD,[11,22–25] including coagulation factors and markers (VIII, fibrin D-dimer), von Willebrand factor (vWF) and tissue plasminogen activator antigen (t-PA) (markers of endothelial dysfunction), and blood viscosity. We also examined the influence of smoking cessation on these inflammatory and haemostatic markers in relation to years since quitting and to the quantity of cigarettes smoked.

Methods

The British Regional Heart Study is a prospective study of CVD involving 7735 men aged 40–59, selected from the age–sex registers of

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one general practice in each of 24 British towns, who were screened between 1978 and 1980.[26] Postal questionnaires measuring medical history and lifestyle changes were sent to all survivors 5 years after screening (1983–85;Q5), again in 1992 (Q92) and in 1996 (Q96). In 1998–2000, all surviving men, now aged 60–79, were invited for a 20th year follow-up examination. All men completed a questionnaire (Q20) providing information on their medical history, smoking and drinking habits, physical activity, and occupation, had a physical examination, and provided a fasting blood sample. The men were asked to fast for a minimum of 6 h, during which they were instructed to drink only water and to attend for measurement at a specified time between 0800 and 1800 h. All men were asked to provide a blood sample, collected using the Sarstedt Monovette system. Of the 5565 surviving subjects, 4252 (77%) attended for examination and 4094 men (74%) had at least one measurement of the biological factors. We excluded 134 men currently on warfarin as well as men with a recall of a diagnosis of myocardial infarction (MI), angina, stroke, or diabetes ($n = 1036$), as these men have been shown to have elevated levels of haemostatic and inflammatory factors and because the diagnosis of CVD is associated with giving up smoking.[27]

Haemostatic and inflammatory variables

Blood was anticoagulated with K_2 EDTA (1.5 mg/mL) for measurement of haematocrit, white cell count, and platelet count in an automated cell counter; and plasma viscosity at 37°C in a semi-automated capillary viscometer (Coulter Electronics, Luton, UK). Blood viscosity was calculated from haematocrit and plasma viscosity.[28] Blood was also anticoagulated with 0.109 M trisodium citrate (9:1 v/v) for measurement of clottable fibrinogen (Clauss method) as well as coagulation factors VII, VIII, and IX in an MDA-180 coagulometer (Organon Teknika, Cambridge, UK). Plasma levels of t-PA antigen and D-dimer were measured with enzyme-linked immunosorbent assays (Biopool AB, Umea, Sweden) as was vWF antigen (DAKO, High Wycombe, UK). C-reactive protein was assayed by ultra-sensitive nephelometry (Dade Behring, Milton Keynes, UK).

Smoking

The men were asked about current cigarette smoking status and pipe/cigar smoking at both initial examination (Q1) and re-examination (Q20), and cigarette smoking status was assessed at all follow-up questionnaires during the 20 year period. The men were classified into five groups according to their smoking status at re-examination: (i) never smokers ($n = 873$)—those who had never smoked cigarettes and did not currently smoke pipe/cigar; (ii) ex-cigarette smokers ($n = 1503$)—those who previously smoked cigarettes and did not currently smoke pipe or cigars; (iii) primary pipe/cigar smokers ($n = 44$)—those who had never smoked cigarettes and currently smoked pipe or cigars; (iv) secondary pipe/cigar smokers ($n = 109$)—former cigarette smokers who currently smoked pipe or cigars; and (v) current cigarette smokers ($n = 391$)—irrespective of whether they have ever smoked pipe or cigars. This classification has been used in previous studies in this cohort.[3] At initial examination, the age at which they had started smoking was obtained and data were available on years since a man had last regularly smoked cigarettes prior to screening, as well as the number of cigarettes smoked previously. During follow-up in 1992 and 1996 and at re-examination, the men were also asked to provide the age at which they gave up smoking. From this information, the approximate number of years since a man had last smoked regularly was calculated for smokers at initial examination who gave up during the follow-up period. The number of cigarettes reported at screening (Q1) was used to assess the number of cigarettes previously smoked in these ex-smokers. From the combined information at screening (Q1; 1978–80) and follow-up questionnaires, the ex-smokers at re-examination were classified into subgroups according to approximate years since quitting (<5, 5–9, 10–19, and ≥ 20 years) and previous quantity of cigarettes smoked (<20 and ≥ 20 per day). In 94% of the men who had given up smoking since initial examination, the age at which they reported giving up smoking was consistent with giving up smoking since initial examination. In the remaining 6% of men who reported giving up ≥ 20 years ago but were smokers at initial examination, time since quitting was taken as the approximate number of years since they last reported smoking during follow-up. Smoking data were not available in four men, leaving 2920 men for analyses.

Reported current smoking status was validated using carboxyhaemoglobin (COHb) measurements.[29] Current cigarette smoking status was strongly associated with COHb. Mean COHb level in current cigarette smokers was 2.95% compared with 0.61%, in those who had given up <5 years, and 0.41% in never smokers. Both pipe and cigar smokers showed higher levels than ex-cigarette smokers (1.55 and 1.57% for primary and secondary pipe/cigar smokers).

Cardiovascular risk factors

Details of classification methods and measurements of the cardiovascular risk factors (including social class and physical activity) have been described.[26,30] Body mass index (BMI; weight/height^2 in kg/m^2) was calculated for each man at re-examination. The men were asked to report the total number of alcoholic drinks per week and were classified into five groups on the basis of their total daily intake: none, <1 per day, 1–2 per day, 3–4 per day, and ≥ 5 units [one drink per unit (UK) = 10 g alcohol]. A physical activity score was derived for each man at Q20 and the men were grouped into six broad categories: inactive, occasional, light, moderate, moderately-vigourous, and vigourous.[30] The longest-held occupation of each man was recorded and coded in accordance with the Registrar General's occupational classification into six social class groups: I, II, III non-manual (non-manual) or III manual, IV, and V (manual). The Armed Forces formed a separate group. Systolic blood pressure at Q20 (Dinamap reading) was adjusted to accord with the Hawksley random zero sphygmanometer readings at baseline[31] by subtracting 8 mmHg from the reading. Blood pressure was adjusted for observer variation.[32] Total cholesterol and HDL-cholesterol were measured on a Hitachi 747 automated analyser using the methods of Siedel[33] and Sugichi,[34] respectively.

Statistical analysis

The distributions of white cell count, C-reactive protein, and fibrin D-dimer were highly skewed and log transformation was used. Analysis of covariance was used to obtain adjusted mean levels for the smoking groups fitting smoking as a categorical variable. To take into account potential confounders, we adjusted for factors which have been shown to be associated with haemostatic and inflammatory markers, for example, age, BMI, systolic blood pressure, HDL-cholesterol, physical activity, social class, and alcohol intake.[15,16] In the adjustment, age, BMI, systolic blood pressure, and HDL-cholesterol were fitted as continuous variables, because many of the haemostatic/inflammatory factors have shown to increase with increasing age, BMI, systolic blood pressure, or HDL-cholestrol.[15,16] Physical activity (five levels), social class (three levels), and alcohol intake (five levels) were fitted as categorical variables. In the adjustment for categorical variables, dummy coding was fitted for each level. Thus, for example, four dummy variables were used for alcohol (five levels). Mean values were adjusted to the overall mean of the covariate. The *F*-test was used to assess the overall difference in mean among the five smoking groups. As one of the primary aims of the study was to determine whether current cigarette smoking is associated with increased levels, we also compared current smokers to never smokers. Two-tailed tests were used and $P < 0.05$ was considered statistically significant. We had an *a priori* hypothesis that if cigarette smoking had an influence on haemostatic and inflammatory

factors, these levels would be highest in current smokers and would regress to those of never smokers with increasing years after cessation. We therefore tested for linear trends with decreasing years of smoking by assigning quantitive values (1–6) to the six smoking groups and fitting smoking as a continuous variable (*Figure 1*). Elevated levels of the haemostatic and inflammatory markers were defined as the top tertile of the distribution, as many studies or meta-analyses present data by top tertile and have shown this to be associated with significantly increased risk of CHD.[11,22–24] Multiple logistic regression was used to obtain the adjusted odds ratio (relative risk) of having elevated levels of the inflammatory and haemostatic factors, with the exception of albumin where the lowest tertile was used to assess the odds of low albumin for the smoking categories, using never smokers as the reference group. This also allows us to compare the magnitude of relationships between current smoking and haemostatic/inflammatory markers. There were very few missing values for the individual factors. The majority of variables (fibrinogen, fibrin D-dimer, t-PA, vWF, factors VII, VIII, and IX) had less than 10 men with missing values. Data on CRP was missing in 31 men. The maximum missing value for any factor was 47 for plasma viscosity (1.6%).

Results

Table 1 shows the personal characteristics and cardiovascular risk factors at Q20 by the five smoking categories. There were significant differences in age, BMI, physical activity, alcohol, social class, and systolic blood pressure levels among the groups. There were no significant differences overall in HDL-cholesterol levels among the smoking categories, but current cigarette smokers showed significantly lower levels than never smokers ($P < 0.01$). There was no significant difference in serum total cholesterol levels among the groups.

Smoking status and haemostatic/inflammatory markers

Table 2 shows the adjusted mean levels of haemostatic and inflammatory markers for the smoking categories adjusted for age, BMI, physical activity, alcohol, social class, systolic blood pressure, and HDL-cholestrol. Cigarette smoking was strongly and positively associated with inflammatory markers (C-reactive protein, white cell count, fibrinogen) and inversely associated with albumin. Significant positive associations were also seen with blood and plasma viscosity, haematocrit, t-PA antigen, and fibrin D-dimer. Cigarette smokers showed significantly lower levels of factor VIII than never smokers, but no association was seen with vWF. Ex-cigarette smokers and secondary pipe/cigar smokers had intermediate levels between never and current smokers. Secondary pipe/cigar smokers tended to have levels similar to ex-cigarette smokers, with the exception of white cell count and haematocrit ($P = 0.0002$ and $P < 0.05$, respectively) which were elevated and factor VIII which was reduced ($P = 0.01$), even though the majority of secondary pipe/cigar smokers reported giving up cigarette smoking >20 years previously (72 compared with 66% in ex-smokers). Levels of factor VIII in secondary pipe/cigar smokers were similar to current smokers. Primary pipe/cigar smokers tended to have levels of all factors similar to never smokers.

When current cigarette smokers were divided into light (1–19 per day; $n = 274$)) and heavier (≥20 per day; $n = 117$) cigarette smokers, heavier smokers showed higher levels of white cell count, fibrinogen, blood and plasma viscosity, and haematocrit than lighter smokers, but this was not seen for C-reactive protein, albumin, t-PA, or fibrin D-dimer. Among current smokers in whom the majority (98%) had smoked for >30 years [mean smoking years 50 (SD 7.7) years], there was no relationship between duration of smoking and mean levels of inflammatory and haemostatic variables, after adjustment for number of cigarettes smoked.

Table 3 shows the adjusted relative odds of having elevated levels of inflammatory and haemostatic markers (top tertile) for the smoking categories relative to never smokers. The number of primary pipe/cigar smokers was very small and data are not presented. Cigarette smoking was most strongly associated with elevated markers of inflammation, in particular C-reactive protein, white cell count, and fibrinogen; and also with the endothelial marker, t-PA. Both secondary pipe/cigar smokers and ex-cigarette smokers showed significantly increased odds of raised levels of inflammatory markers (C-reactive protein, white cell count, and fibrinogen) and elevated t-PA compared with never smokers, but the increase was greater in secondary pipe/cigar smokers than in ex-cigarette smokers. Secondary pipe/cigar smokers showed significantly higher odds of having elevated white cell count ($P = 0.03$) and fibrinogen ($P = 0.05$) than ex-cigarette smokers. Only current cigarette smoking was associated with significant increased odds of elevated blood and plasma viscosity and haematocrit levels.

Smoking cessation and haemostatic and inflammatory markers

Levels of inflammatory and haemostatic markers tended to regress to those of never smokers with increasing duration of cessation and for most variables reverted to those of never smokers after 20 years cessation (*Figure 1*). Haematocrit levels reverted to that of never smokers within 5 years of cessation. However, compared with never smokers, C-reactive protein, fibrinogen, white cell count, plasma and blood viscosity, fibrin D-dimer, and t-PA levels remained significantly raised and albumin remained significant lowered among ex-smokers even after 10–19 years of cessation (all $P < 0.05$). *Table 4* compares the absolute difference in mean levels between smokers and ex-smokers according to duration since quitting. Overall, about half of the reduction had taken place within 5–9 years of cessation.

To assess whether the effects of giving up smoking are also dependent on the number of cigarettes smoked, we divided ex-cigarette smokers according to number of cigarettes smoked in the past. With the exception of C-reactive protein, there was little difference in mean levels of inflammatory and haemostatic markers between ex-light (<20 per day) and ex-heavier cigarette smokers (≥20 per day) after adjusting for years since quitting smoking and confounders. For C-reactive protein, ex-light smokers showed significantly lower mean levels than ex-heavier smokers after adjustment for duration of quitting and confounders (adjusted mean 1.52 vs. 1.79 mg/L; $P = 0.003$). *Figure 2* shows mean levels of C-reactive protein according to years since quitting and number of cigarettes smoked adjusting for age and lifestyle factors. Although current light and heavy cigarette smokers

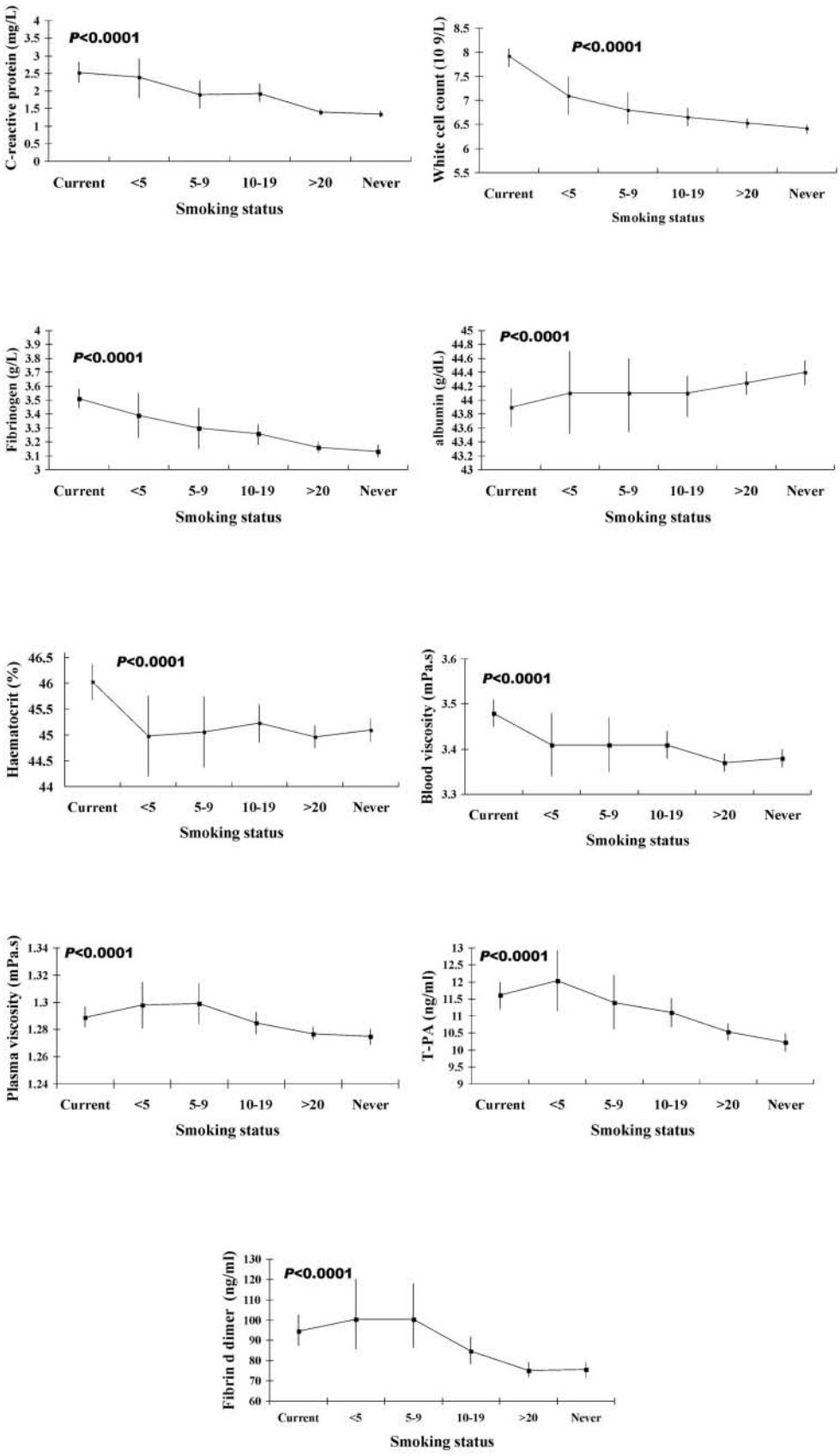

Figure 1 Adjusted mean levels of inflammatory and haemostatic markers by cigarette smoking status and years since quitting. Adjusted for age, social class, physical activity, alcohol intake, BMI, systolic blood pressure, and HDL-cholesterol.

Table 1 Personal characteristics and CV risk factors by smoking status

	Never ($n = 873$)	Ex ($n = 1503$)	Primary pipe/ cigar smokers ($n = 44$)	Secondary pipe/ cigar smokers ($n = 109$)	Current smokers ($n = 391$)	*P*-value difference between groups
Age (years)	66.8 ± 5.15	68.5 ± 5.60	68.3 ± 5.07	67.8 ± 5.53	67.3 ± 5.29	<0.0001
BMI (kg/m^2)	26.4 ± 3.44	27.0 ± 3.39	26.3 ± 2.90	26.6 ± 3.42	25.7 ± 3.60	<0.0001
Inactive (%)	26.7	33.2	18.2	33.0	38.3	<0.0001
Manual (%)	40.8	55.5	38.6	60.6	68.6	<0.0001
Alcohol ≥3 drinks/day (%)	8.0	14.0	13.2	18.3	16.7	<0.0001
SBP (mmHg)	148.3 ± 22.8	151.6 ± 23.7	150.5 ± 27.1	145.3 ± 22.0	148.1 ± 26.4	<0.0001
HDL-C (mmol/L)	1.36 ± 0.34	1.35 ± 0.34	1.39 ± 0.45	1.37 ± 0.36	1.31 ± 0.36	0.28
Cholesterol (mmol/L)	6.08 ± 1.03	6.11 ± 1.06	6.12 ± 0.92	6.07 ± 1.06	6.07 ± 1.11	0.90

Mean expressed as mean ± standard deviation. SBP, systolic blood pressure; HDL-C, HDL-cholesterol.

showed similar levels of C-reactive protein, the level of reduction of C-reactive protein in ex-smokers appeared to be dependent on quantity smoked. Among ex-light smokers, C-reactive protein levels decreased within 5 years of cessation, whereas for heavier smokers, reduction was not apparent until after 5 years.

Discussion

In this study of older men, current cigarette smoking was significantly associated with increased levels of inflammatory markers (C-reactive protein, white cell count, fibrinogen), reduced albumin level, increased coagulation activation (fibrin D-dimer), increased levels of the endothelial marker, t-PA antigen, and increased plasma and blood viscosity, and haematocrit. Each of these factors has been previously shown to be associated with risk of CVD.[10,11,21–25] The strongest associations were seen with inflammatory factors (C-reactive protein, white cell count, and fibrinogen). Our findings confirm those of other studies[6,9,12–20,22,23] and extend previous reports by examining the effects of smoking cessation on these factors in relation to time since quitting and number of cigarettes smoked and the effects of switching to pipe/cigar smoking. No association was seen between current cigarette smoking and vWF; previous studies, however, have reported conflicting findings.[35–37] However, smokers, showed significantly lower levels of factor VIII than never smokers, as previously reported.[35]

Potential biases and limitations

The study population is not strictly a random population sample, being influenced by survival and response, both of which will tend to lead to under-representation of smokers in the study sample. Although the prevalence of smoking may be underestimated, there is no reason to believe that under-representation *per se* should bias the associations investigated between smoking categories (and levels of smoking in subgroup analyses) and biological markers. For survival and response to cause selection bias, these factors would have to have been strongly related to smoking category (or smoking level for subgroup analyses) and to levels of the inflammatory and haemostatic markers assessed within each particular smoking category. If levels of biological markers are linked to survival/response, then survivors/responders may have tended to have lower levels of markers than the non-survivors would have had (if it had been possible to include them). This would have resulted in the strength of the associations being underestimated. There were very few missing values for the individual factors, and it is therefore unlikely that missing values would bias the results.

Although we have observed strong relationships between smoking and many of the inflammatory and haemostatic factors shown to be associated with CHD, our cross-sectional study cannot provide direct evidence as to whether these factors do indeed mediate the relationship between smoking and CHD. However, the associations between smoking and several factors, which are themselves strongly associated with risk of CHD (particularly C-reactive protein, fibrinogen, and t-PA), are strong and reversible after smoking cessation. It is, therefore, likely that these factors mediate at least part of the increased CHD risk in smokers.

Cigarette smoking and haemostatic and inflammatory markers

It has been established that fibrinogen levels are higher in smokers than in non-smokers, and it has been estimated that up to 50% of the increase in risk of CHD in smokers may be associated with fibrinogen levels.[6,38] In recent years, several studies have also reported strong associations between current smoking and C-reactive protein, which is similarly predictive of CHD events to fibrinogen (odds ratio, top third to bottom third, about 1.5).[11,21] The effects of smoking on fibrinogen synthesis may be a part of a generalized inflammatory reaction, as smoking is strongly related to other measures of inflammation, in particular C-reactive protein and white cell count, and (inversely related) albumin.[39] Raised levels of these inflammatory markers may partly reflect elevations of inflammatory cytokines such as interleukin-6 and TNFα, which are major regulators of the reactant plasma protein component of the inflammatory response;[40,41] interleukin-6 levels are increased in smokers.[42] While these blood changes in smokers may simply be markers of smoking-induced tissue damage, it is also possible that high fibrinogen levels may

Table 2 Smoking status and adjusted mean levels (95% CI) of haemostatic and inflammatory markers

	Never ($n = 873$)	Ex-smokers ($n = 1503$)	Primary pipe cigar smokers ($n = 44$)	Secondary pipe cigar smokers ($n = 109$)	Current smokers ($n = 391$)	*P*-value[a]	*P*-value[b]
CRP (mg/L)[c]	1.35 (1.26,1.46)	1.58 (1.49,1.66)	1.22 (0.91,1.68)	1.72 (1.42,2.10)	2.53 (2.27,2.80)	<0.0001	<0.0001
White cell count ($\times 10^9$/L)[c]	6.42 (6.30,6.49)	6.62 (6.48,6.69)	6.48 (5.99,7.03)	7.24 (6.96,7.61)	7.92 (7.69,8.08)	<0.0001	<0.0001
Fibrinogen (g/L)	3.13 (3.08,3.18)	3.20 (3.17,3.24)	3.16 (2.96,3.37)	3.26 (3.13,3.39)	3.51 (3.44,3.54)	<0.0001	<0.0001
Albumin (g/L)	44.4 (44.2,44.6)	44.2 (44.0,44.3)	44.0 (43.2,44.8)	44.0 (43.5,44.5)	43.9 (43.6,44.2)	0.002	0.02
HCT (%)	45.1 (44.9,45.3)	45.0 (44.9,45.2)	45.2 (44.2,46.2)	45.7 (45.1,46.3)	46.0 (45.7,46.4)	<0.0001	<0.0001
Blood viscosity (mPa s)	3.39 (3.36,3.40)	3.38 (3.37,3.40)	3.36 (3.28,3.45)	3.43 (3.38,3.49)	3.48 (3.45,3.51)	<0.0001	<0.0001
Plasma viscosity (mPa s)	1.275 (1.270,1.280)	1.281 (1.278,1.286)	1.263 (1.241,1.285)	1.277 (1.263,1.292)	1.289 (1.282,1.297)	0.003	0.02
Factor VIII (iu/dL)	129.9 (127.7,131.9)	132.1 (130.5,133.7)	131.2 (122.0,140.5)	124.5 (118.7,130.3)	124.5 (121.3,127.7)	0.007	0.0003
VWF (iu/dL)	134.9 (131.6,137.7)	136.3 (134.1,138.6)	140.3 (137.1,153.4)	132.8 (123.5,140.3)	138.8 (134.3,143.4)	0.14	0.45
t-PA (ng/mL)	10.2 (9.9,10.5)	10.8 (10.6,11.0)	10.7 (9.5,11.9)	10.8 (10.3,11.6)	11.6 (11.2,12.0)	<0.0001	<0.0001
D-dimer (ng/mL)	75.6 (71.5,79.4)	79.8 (76.7,83.1)	78.3 (62.2,98.5)	81.1 (70.1,93.7)	94.4 (88.2,102.5)	<0.0001	0.0002

Adjusted for age, social class, physical activity, alcohol intake, BMI, systolic blood pressure, and HDL-cholesterol.
CRP, C-reactive protein; HCT, haematocrit.
[a]Comparisons between current smokers and never smokers.
[b]Differences between groups.
[c]Geometric mean used.

promote CVD through arterial wall infiltration and effects on blood viscosity,[24] platelet aggregation, and fibrin formation.[38,39] Smoking also increases viscosity by increasing haematocrit.[43] High C-reactive protein levels may also play a role in development of atherosclerosis. As reported previously, smoking is positively associated with fibrin D-dimer, a measure of ongoing fibrin formation and degradation that is related to CHD risk.[22] The increased D-dimer in smokers probably reflects increased coagulation activation.[37] However, further analyses revealed that this relationship was largely because of the effects of smoking on fibrinogen, as adjustment for fibrinogen attenuated the relationship between smoking and fibrin-D-dimer.

Current smoking was associated with a significant increase in t-PA antigen, which has been reported in previous studies.[16,43] T-PA is synthesized by endothelial cells, and *in vivo* studies have demonstrated major impairment of t-PA release from the vascular endothelium of smokers.[44] Hence, the elevated levels of t-PA antigen in smokers are not likely to be due to increased endothelial release of t-PA; they may be measures of increased circulating complexes of t-PA with its inhibitor, plasminogen activator inhibitor type 1 (PAI-1), which was not assayed in the present study.[23] Increased levels of t-PA antigen have been associated with increased risk of CHD.[23] (odds ratio, top third to bottom third, about 1.5), ischaemic stroke,[45] and peripheral vascular disease,[46] conditions strongly associated with smoking. Thus, disturbed fibrinolysis (or endothelial dysfunction) may be another possible mechanism by which smoking can lead to arterial thrombosis and MI and stroke. Although smoking is known to be associated with endothelial dysfunction, we observed no association between current smoking and vWF factor (another marker of endothelial dysfunction), which is consistent with other reports.[47,48] A recent meta-analysis has shown that vWF is only weakly associated with risk of CHD.[11]

Smoking cessation and cardiovascular risk

Our findings indicate that cessation from smoking results in a rapid reduction in haemostatic and inflammatory markers, but levels remained significantly raised after 10–19 years and did not revert to that of never smokers until after 20 years; for C-reactive protein, the reduction was dependent on the number of cigarettes smoked. Similar results regarding the persistent effect of smoking on inflammation and haemostasis have been reported previously.[14,19,22] In the Speedwell Study[22] and in the MONICA Augsberg Study,[19] C-reactive protein was still significantly raised 10 years after smoking cessation, and in the EPIC-Norfolk Study,[20] white blood cell count did not revert to that of never smokers until after 20 years. Levels of t-PA antigen levels were still significantly raised overall in ex-smokers, and reduction was only evident after 5 years cessation, reverting to that of never smokers after 20 years. Hence, the increases in inflammatory markers and t-PA antigen persist for many years, perhaps because of persisting, slowly reversible changes in smoking-damaged tissues such as the arteries and the lungs. The rapid reduction in haematocrit within 5 years of smoking cessation to levels seen in never smokers suggest that this biological response may be more closely related to the acute effects of smoking, such as hypoxia.[49] However, blood and plasma viscosity remained elevated

Table 3 Adjusted relative odds (95% CI) of being in the top tertile of the distribution of haemostatic and inflammatory variables compared with never smokers

	Never	Ex-smokers	Secondary pipe/cigar smokers	Current smokers
CRP $\geq$ 2.37 mg/L	1.00	1.50 (1.22,1.83)	1.55 (0.99,2.44)	3.38 (2.58,4.43)
White cell count $\geq 7.5 \times 10^9$/L	1.00	1.29 (1.06,1.58)	2.01 (1.32,3.09)	3.77 (2.86,4.92)
Fibrinogen $\geq$ 3.41 g/L	1.00	1.25 (1.02,1.52)	1.90 (1.23,2.94)	2.93 (2.24,3.83)
Albumin $\leq$ 4.3 g/L[a]	1.00	1.11 (0.93,1.33)	1.28 (0.84,1.94)	1.75 (1.36,2.27)
HCT $\geq$ 47%	1.00	1.01 (0.83,1.21)	1.41 (0.92,2.16)	1.71 (1.32,2.22)
Blood viscosity $\geq$ 3.62 mPa s	1.00	0.96 (0.75,1.18)	1.13 (0.68,1.89)	1.92 (1.43,2.57)
Plasma viscosity $\geq$ 1.31 mPa s	1.00	1.22 (1.00,1.49)	1.26 (0.81,1.99)	1.61 (1.23,2.11)
Factor VIII $\geq$ 141 iu/dL	1.00	1.21 (1.00,1.47)	0.78 (0.49,1.24)	0.74 (0.56,0.98)
VWF $\geq$ 153 iu/dL	1.00	1.05 (0.86,1.28)	1.02 (0.64,1.59)	1.15 (0.88,1.51)
t-PA $\geq$ 12 ng/mL	1.00	1.31 (1.07,1.60)	1.55 (0.99,2.43)	2.17 (1.64,2.87)
D-dimer $\geq$ 98.5 ng/mL	1.00	1.15 (0.94,1.41)	1.32 (0.84,2.08)	1.52 (1.15,2.00)

Adjusted for age, social class, physical activity, alcohol intake, BMI, systolic blood pressure, and HDL-C; data on primary/cigar smokers ($n = 44$) are not presented; CRP, C-reactive protein; HCT, haematocrit.
[a]Except for albumin—bottom tertile.

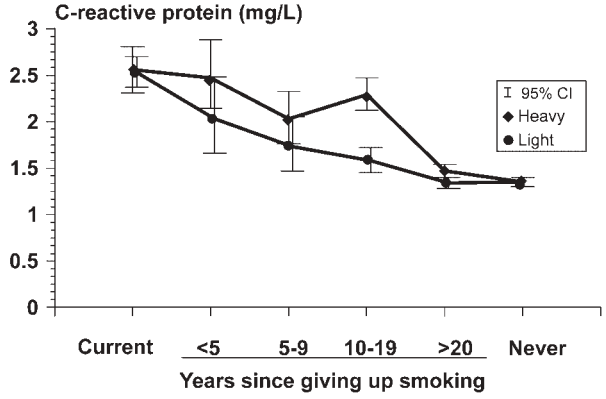

Figure 2 Adjusted mean C-reactive protein levels in relation to number of cigarettes smoked and years since quitting. Adjusted for age, social class, physical activity, alcohol intake, BMI, systolic blood pressure, and HDL-cholestrol.

after 10 years, probably because of persistent increases in inflammatory proteins such as fibrinogen.[49]

Smoking cessation is associated with a significant reduction in risk of MI and stroke, and benefit is seen relatively soon after cessation.[1,3] Although some studies suggest that risk of MI reverts to that of never smoking within 5 years,[50] other studies suggest that the risk, albeit reduced, is still higher in former smokers after 10 years.[51,52] About 50% of the absolute reduction in blood levels of inflammatory and haemostatic markers appears to occur within the first 10 years. If these blood changes are related to MI and stroke, their reductions are consistent with the benefit in risk of CHD seen within 10 years of smoking cessation.[52] In the recent Reykjavik Study, it was estimated that the relative risk of CHD in the top tertile of C-reactive protein relative to the bottom tertile (a mean difference of 1.2 mg/L in C-reactive protein) was about 1.45.[11] When compared with current smokers, we observed a reduction of 0.53 mg/L in C-reactive protein levels within 10 years of smoking cessation and a mean reduction of $\sim$1.1 mg/L after 20 years. This would correspond to about an 18% reduction in risk of CHD after 10 years and a 30% reduction in risk after 20 years.

Pipe and cigar smoking

Many studies have shown that those who switch to pipe or cigar smoking retain elevated risk of MI and stroke.[4,5] We observed that primary pipe/cigar smokers showed similar levels of inflammatory and haemostatic markers to never smokers but ex-cigarette smokers who switched to pipe/cigar showed significantly elevated levels of inflammatory and haemostatic markers, in particular fibrinogen and white cell count, and to a lesser degree C-reactive protein and t-PA, even though the majority had given up >20 years previously. COHb levels were similar in primary and secondary pipe/cigar smokers in this study, thus the higher inflammatory and haemostatic marker levels in secondary pipe/cigar smokers are unlikely to be due to the fact that secondary pipe/cigar smokers inhale more than primary cigar smokers do, as has been reported.[53] Few studies have examined the relationship between pipe and cigar and inflammatory and haemostatic markers, and most do not distinguish between primary or secondary pipe/cigar smokers. In one study that has done so, secondary pipe/cigar smokers showed higher levels of fibrinogen than primary pipe/cigar smokers, as observed in this present study.[6] In the other studies, pipe/cigar smokers have been shown to have significantly elevated levels of inflammatory markers (C-reactive protein, white cell count, and fibrinogen) when compared with never smokers, with levels tending to approach those of current smokers.[14,16] The higher levels of inflammatory markers and t-PA antigen in secondary pipe/cigar smokers may be of relevance to the high risk retained in those who switch from cigarettes to pipe/cigar smoking.

Conclusion

The findings in this study confirm a reversible association of smoking with inflammatory and haemostatic markers, which is time dependent and which may take up to 20 years to revert to levels seen in never smokers. Those who switch to pipe/cigar smoking retain significantly elevated levels of inflammatory markers and t-PA antigen. The present findings, together with previous reports, suggest that the

Table 4 Absolute difference in adjusted means (95% CI compared with current smokers) according to duration of cessation of smoking

	Duration of cessation				Trend among ex-smokers
	<5 years	5–9 years	10–19 years	≥20 years	
CRP (mg/L)[a]	75	96	341	991	
	−0.21 (−0.35, 1.07)	−0.63 (−1.02, −0.12)[b]	−0.60 (−0.86, −0.26)[b]	−1.13 (−1.30, −0.95)[b]	<0.0001
White cell count, (10^9/L)[a]	−0.83 (−1.23, −0.31)[b]	−1.12 (−1.50, −0.68)[b]	−1.27 (−1.50, −0.96)[b]	−1.43 (−1.68, −1.70)[b]	0.006
Albumin (g/L)	0.23 (−0.42, 0.88)	+0.18 (−0.41, 0.78)	0.16 (−0.23, 0.55)	0.35 (0.03, 0.67)[b]	0.30
Fibrinogen (g/L)	−0.12 (−0.29, 0.05)	−0.12 (−0.37, −0.06)[b]	−0.25 (−0.36, −0.15)[b]	−0.35 (−0.44, −0.27)[b]	0.0006
HCT (%)	−1.05 (−1.90, −0.20)[b]	−0.97 (−1.74, −0.20)[b]	−0.81 (−1.31, −0.30)[b]	−1.07 (−1.48, −0.66)[b]	0.38
Blood viscosity, (mPa s)	−0.07 (−0.14, 0.00)	−0.07 (−0.13, 0.00)	−0.07 (−0.11, −0.03)[b]	−0.11 (−0.14, −0.07)[b]	0.01
Plasma viscosity (mPa s)	0.009 (−0.010, 0.027)	+0.01 (−0.007, 0.027)	−0.004 (−0.016, 0.007)	−0.012 (−0.021, −0.003)[b]	0.003
t-PA (ng/mL)	0.42 (−0.05, 1.40)	−0.21 (−1.11, 0.69)	−0.51 (−1.10, 0.08)	−1.08 (−1.56, −0.60)[b]	<0.0001
D-dimer (ng/mL)[a]	6.9 (−10.6, 27.8)	+6.5 (−9.8, 25.5)	−9.8 (−18.5, 0.46)	−19.4 (−25.7, −11.43)[b]	<0.0001

CRP, C-reactive protein; HCT, haematocrit.
[a]Geometric mean.
[b]Significantly different from current smokers.

associations of cigarette smoking and pipe/cigar smoking with CVD may in part be associated with increased activation of inflammation and coagulation.

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