Some long term effects of smoking on the haemostatic system: a report from the Caerphilly and Speedwell Collaborative Surveys

J W G YARNELL, P M SWEETNAM, S ROGERS, P C ELWOOD, D BAINTON,*
I A BAKER,* R EASTHAM,† J R O'BRIEN,‡ M D ETHERINGTON‡

*From the Medical Research Council Epidemiology Unit, South Wales; the *Bristol and Weston Health Authority; the †Pathology Department, Frenchay Hospital, Bristol; and the ‡Central Laboratory, St Mary's Hospital, Portsmouth*

SUMMARY   Data from two community studies on men from South Wales and the west of England suggest that the effects of smoking on the haemostatic system remain for many years after giving up. Long term correlations between several variables, including plasma fibrinogen and white cell count, and the length of time after giving up were seen in ex-smokers. Dose response relations were apparent in current smokers in terms of the white cell count and two haematological variables, the packed and mean cell volumes. These long term correlations probably reflect the toxicity of other agents in tobacco smoke besides nicotine and carbon monoxide, which act only in the short term. Identification of these agents may further our understanding of the mechanism by which cigarette smoking is associated with atherosclerotic disease.

Evidence is accumulating that haemostatic factors have a pathogenetic role in ischaemic heart disease[1][2] and stroke.[3] Smoking habit is known to affect substantially several haemostatic factors[4][5] and is also a major risk factor for ischaemic heart disease.[6][7] This paper examines the activity of several haemostatic factors in non-smokers, smokers, and ex-smokers in two community studies on men from South Wales and the west of England.

Material and methods

STUDY POPULATIONS

All men aged between 45 and 59 years resident in the town of Caerphilly and five outlying villages (total population 41 000) were included in the study. Subjects were selected principally from electoral registers and a private census carried out by letter and house-to-house survey. Age and sex registers and practice records were also used as an additional check on the eligible population. Twenty one general practitioners served the area covered by the survey working in seven independent surgeries.

In Bristol computerised age and sex registers of patients were available for all 16 general practitioners who worked from two health centres serving Speedwell, a largely residential district of east Bristol (total population 32 000). All male subjects aged 45 to 59 years of age on September 1 1979 were selected and invited by letter from their general practitioner to participate in the survey.

SURVEY METHODS

In Caerphilly a total of 2818 men were included and invited to attend one of seven local clinics for a medical examination. A total of 2512 (89%) subjects were examined. A questionnaire was filled in by all subjects for details of medical history, occupation, and smoking habit, and other data.

In the Bristol population 2550 men were included in the study, and 2348 (92%) subjects were seen at the Speedwell clinic. Questionnaires used in this survey were identical in essential respects with those used in Caerphilly.

LABORATORY METHODS

All subjects were seen at a morning haematology clinic, and a venous blood sample was obtained with

minimal haemostatis after an overnight fast. Fibrinogen concentration was estimated by two methods; a nephelometric determination after heat precipitation in buffered saline (fibrinogen N)[8] and a clotting assay (fibrinogen C).[9] Viscosity was estimated on plasma in edetic acid by the method of Harkness.[10] The heparin neutralising activity of platelet poor plasma was measured using the heparin-thrombin clotting time.[11] The relevance of this test has been discussed previously,[12] but may be summarised as a non-specific measure of the heparin neutralising activity of several plasma proteins (with platelets removed), which have associations with ischaemic heart disease.

A full blood count, which included a white cell count, haematocrit, and mean corpuscular volume, was done using a Coulter model S-plus cell counter in whole blood anticoagulated with edetic acid. Cell counts were carried out within four to 10 (mean six) hours after venepuncture, and the nephelometric fibrinogen and plasma viscosity, usually within 12 hours after collection. These measurements were carried out in Frenchay Hospital, Bristol for both studies. Delay or time from venepuncture to time of assay tended to be slightly shorter for samples collected in Speedwell.

The assays on platelet poor plasma (the heparin-thrombin clotting time and clottable fibrinogen) were carried out after a standard delay overnight of 28 to 32 hours. In hot or warm weather conditions a frozen cooler pack was placed inside the container. These latter assays were carried out for both studies at St Mary's Hospital, Portsmouth. Samples from the Speedwell study were subject to about 28 hours' delay from time of collection; samples from Caerphilly were subject to about 32 hours' delay. For technical reasons, fewer of these assays on platelet poor plasma were carried out than was the case for the hae-

matological and other tests carried out at Frenchay Hospital in Bristol. This was particularly true of samples from Caerphilly.

A 5% sample of duplicate specimens was presented to each laboratory during the course of the studies. These were labelled and coded prior to desptach and presented "blind" to the laboratory staff with the usual samples. The coefficients of variation for the duplicate pairs (n = 130–155) for fibrinogen N, fibrinogen C, viscosity, heparin-thrombin clotting time, white cell count, haematocrit, and mean corpuscular volume were 7, 11, 2, 29, 3, 2 and 1%, respectively (log transformed values for heparin-thrombin clotting time and fibrinogen C were 7 and 4%, respectively). A second blood sample was taken from a small group of men (n = 8–12). Coefficients of variation for these pairs of results for fibrinogen N, fibrinogen C, viscosity, heparin-thrombin clotting time, white cell count, and haematocrit were 10, 18, 2, 21, 12 and 6%, respectively. (These data are from Caerphilly subjects but the quality control results from Speedwell subjects are very similar.) Preliminary reports[12 13] from both studies summarise the laboratory and survey methods.

Analyses of variance, together with linear regression analysis, were used to assess differences between and trends among groups. Most of the haemostatic variables were lognormally distributed. All results, however, were presented in natural units. Analysis of logarithmically transformed data produced virtually identical results.

Results

Blood samples were obtained for 2486 subjects from the Caerphilly sample, and 2273 subjects from the Bristol clinic and hospital.

Table 1 *Mean (SD) concentrations of plasma fibrinogen and viscosity in men from Caerphilly and Speedwell according to smoking habit*

	No of subjects		Fibrinogen N (g/l)†		Fibrinogen C (s)		Viscosity (cP)	
Smoking habit	Caerphilly	Speedwell	Caerphilly	Speedwell	Caerphilly	Speedwell	Caerphilly	Speedwell
Never	390	335	3·49 (0·8)	3·34 (0·7)	14·0 (2·4)	14·6 (2·7)	1·69 (0·08)	1·66 (0·09)
Ex-smokers (years since giving up):								
10 or more	349	500	3·55 (0·8)	3·44 (0·8)	13·7 (2·5)	14·1 (2·6)	1·70 (0·09)	1·67 (0·09)
5–9	158	163	3·60 (0·8)	3·52 (0·9)	13·8 (2·3)	14·3 (3·3)	1·70 (0·10)	1·66 (0·10)
1–4	128	129	3·78 (0·9)	3·59 (0·9)	13·3 (2·5)	13·6 (3·1)	1·72 (0·09)	1·67 (0·11)
< 1 year	76	30	3·72 (0·8)	3·76 (0·9)	13·2 (2·2)	13·1 (2·9)	1·71 (0·09)	1·68 (0·11)
Pipe or cigar	257	229	3·85 (0·9)	3·52 (0·8)	12·8 (2·4)	13·7 (3·1)	1·71 (0·10)	1·65 (0·09)
Cigarettes (per day):								
1–14	352	269	3·94 (0·9)	3·84 (0·8)	12·4 (2·2)	12·7 (2·6)	1·72 (0·11)	1·68 (0·09)
15–24	441	347	3·99 (0·8)	3·76 (0·8)	12·5 (2·4)	12·7 (2·5)	1·72 (0·10)	1·68 (0·09)
25 or more	310	217	4·05 (0·8)	3·83 (0·8)	12·4 (2·2)	12·4 (2·5)	1·73 (0·10)	1·69 (0·10)
All men*	2472	2260	3·79 (0·8)	3·58 (0·8)	13·1 (2·4)	13·6 (2·8)	1·71 (0·10)	1·67 (0·09)

*Row totals do not addup as smoking habit not known for some subjects.
†No of subjects as in table 2.

Table 2 *Mean (SD) of white cell count and heparin-thrombin clotting time in men from Caerphilly and Speedwell according to smoking habit*

Smoking habit	No of subjects		Heparin-thrombin clotting time (s)		White cell count (10^9/l)†	
	Caerphilly	Speedwell	Caerphilly	Speedwell	Caerphilly	Speedwell
Never	356	322	35·6 (13·3)	37·8 (14·4)	5·89 (1·2)	6·01 (1·3)
Ex-smokers (years since giving up):						
10 or more	325	486	33·8 (12·6)	35·0 (13·4)	6·16 (1·8)	6·14 (1·4)
5–9	143	158	35·0 (12·0)	36·0 (16·7)	6·35 (1·6)	6·40 (2·0)
1–4	115	127	32·4 (12·4)	34·3 (15·9)	6·55 (1·5)	6·73 (1·7)
< 1 year	73	29	31·6 (10·5)	31·0 (15·8)	6·81 (1·8)	7·32 (2·0)
Pipe or cigar	240	224	29·8 (11·1)	34·2 (13·9)	7·13 (2·0)	6·95 (1·7)
Cigarettes (per day):						
1–14	313	256	28·1 (11·3)	29·9 (14·2)	7·68 (2·0)	8·02 (2·1)
15–24	397	336	28·6 (11·8)	29·4 (12·4)	8·08 (2·0)	8·20 (2·2)
25 or more	284	214	27·3 (10·4)	27·5 (12·1)	8·25 (2·2)	8·52 (2·0)
All men*	2253	2192	31·1 (12·2)	33·2 (14·2)	7·10 (2·0)	7·04 (2·0)

*Row totals do not add up as smoking habit not known for some subjects.
†No of subjects as in table 1.

Table 1 shows the mean values of fibrinogen N, fibrinogen C, and plasma viscosity by smoking habit for the Caerphilly and Speedwell men. Clotting time of fibrinogen C was measured in seconds; hence a shorter clotting time indicates greater clotting activity. The correlations were similar in both areas. Mean values of tests differed significantly ($p < 0.001$) between current smokers, ex-smokers, and those who had never smoked. Current cigarette smokers had the highest concentrations. Both ex-smokers and cigar and pipe smokers had values that were intermediate between those of the current smokers and those of the men who had never smoked, who had the lowest values. There was no clear evidence of a dose response in the current cigarette smokers, and any such trends were not significant.

Among the ex-smokers there was, in both areas, a significant ($p < 0.01$) trend for fibrinogen N to decline with the increase in the number of years after giving up. Thus the men who gave up in the past year had concentrations that were nearly as high as those of the current smokers. The men who gave up smoking 10 years ago or more had levels that had nearly, but not quite, returned to those of the men who had never smoked. A similar trend was seen for plasma viscosity, but this was less pronounced and was not significant.

Data for fibrinogen C (clottable fibrinogen) showed a similar correlation between smoking habit and fibrinogen N. Again there was no dose response correlation with current cigarette smoking, but there was a noticeable trend that correlated with the length of time an ex-smoker had given up.

Table 2 shows the results for the heparin-thrombin clotting time and white cell count for both the Caerphilly and Speedwell subjects. A shorter clotting time indicated greater clotting activity: the correlations were similar in the two areas. Mean times for both tests differed significantly ($p < 0.001$) between current smokers, ex-smokers, and those who had never smoked, with ex-smokers giving intermediate times. In smokers there was a significant ($p < 0.01$) dose response relation with amount smoked and the white cell count, the heaviest smokers having the highest counts. Among ex-smokers ($p < 0.001$) white cell count decreased significantly ($p < 0.001$) as the length of time after giving up smoking increased. Those who had given up in the past year had white cell counts that were about 10% lower than even the light cigarette smokers. The count declined further as length of time without smoking increased. The men who gave up 10 or more years ago, however, still had counts a little higher than those of the men who had never smoked.

In smokers the heparin-thrombin clotting time tended to decrease with the amount smoked; this trend was significant ($p < 0.05$) for the Speedwell men but not for the Caerphilly men. Among ex-smokers heparin-thrombin clotting time tended to increase with duration not smoking (up to nine years) and the clotting time was the longest in those who had never smoked. These correlations, however, did not reach significance.

Table 3 shows the relation between two red cell variables and smoking habit. Those who had never smoked and the ex-smokers had similar haematocrit values. Haematocrit was much higher ($p < 0.001$) in current smokers, and there was a strong dose response ($p < 0.01$) with the amount of cigarettes smoked. Mean corpuscular volume was also highest in current smokers, and there was a significant ($p < 0.05$) dose response correlation with amount

Table 3   Mean (SD) values of haematocrit and mean corpuscular volume in men from Caerphilly and Speedwell according to smoking habit

Smoking habit	Haematocrit (%)			Mean corpuscular volume (fl)	
	Caerphilly	Speedwell		Caerphilly	Speedwell
Never	46·3 (3·1)	45·5 (3·0)		89·4 (3·9)	89·4 (3·9)
Ex-smokers (years since giving up):					
10 or more	45·7 (3·2)	45·6 (2·9)		90·0 (4·1)	89·7 (4·3)
5–9	45·8 (3·7)	45·9 (3·4)		91·0 (5·2)	90·8 (4·3)
1–4	46·2 (3·2)	45·6 (3·4)		90·1 (4·1)	90·6 (4·3)
< 1	46·0 (3·6)	44·9 (3·1)		90·8 (4·3)	90·1 (5·3)
Pipe or cigar	46·4 (3·2)	46·1 (2·9)		91·1 (4·4)	91·5 (4·1)
Cigarettes (per day):					
1–14	46·7 (3·8)	46·5 (3·1)		91·8 (4·6)	91·7 (4·8)
15–24	47·2 (3·7)	46·7 (3·3)		92·3 (4·5)	92·3 (4·8)
25 or more	48·2 (3·4)	47·3 (3·0)		92·7 (4·3)	92·7 (4·4)
All men*	46·6 (3·5)	46·1 (3·2)		91·1 (4·5)	90·9 (4·6)

*Row totals do not add up as smoking habit unknown for some subjects.

smoked. Ex-smokers had hematocrit values intermediate between those of current smokers and the men who had never smoked. There was, however, no consistent correlation with length of time after having given up among the ex-smokers.

Discussion

Previous studies[4] [13] [14] have shown that age is an important determinant of plasma fibrinogen and viscosity. The age ranges of the present studies were narrow—45–59 years (Caerphilly) and 45–63 years (Speedwell)—and age effects were independent of the findings. The range of mean ages in different smoking categories was only two years for both Caerphilly and Speedwell, and the pattern within the smoking categories differed completely from that found in the haemostatic variables; non-smokers and the heaviest smokers had similar ages and were the youngest; ex-smokers and the lightest smokers were similar, and on average were two years older than the other groups.

The correlation between the haemostatic factors and ischaemic heart disease and the intercorrelations between the haemostatic factors, have been reported previously[4] [12] among subsamples of the population studied here. Our data show, however, in agreement with those from Yarnell et al,[13] that the relation between smoking habit and haemostatic factors is quite independent of the relation between smoking habit and prevalence of ischaemic heart disease. In the Speedwell pilot study[12] inconsistent relations between fibrinogen, smoking habit, and ischaemic heart disease were found, but this was probably due to the small numbers studied. Intercorrelations between the haemostatic factors were found, but were not sufficiently large to render any of the tests in the

present report redundant. Correlation coefficients based on a large number of comparisons were similar in both populations and were similar to those reported previously[12] [13]: fibrinogen N v fibrinogen C, r = −0·6; fibrinogen C v heparin-thrombin clotting time, r = −0·7; white cell count v fibrinogen N, r = 0·4.

Smoking habit was assessed by means of a questionnaire and no independent validation was obtained in the present data. One possibility to account for the findings is that some men who claimed to be ex-smokers were still smoking and that there was a higher number of these among those who claimed to have given up more recently. This would give trends among ex-smokers for all variables related to current smoking habit such as haematocrit and mean cell volume, but this is not the case. Furthermore, recent data from the same population indicates that ex-smokers gave an accurate account of their smoking habit (unpublished observations). Urinary cotinine was measured and the cotinine:creatinine ratios among ex-smokers were similar to those of subjects who had never smoked, and there was no suggestion of a trend related to the length of time after giving up. The cotinine:creatinine ratios were at least 10 times greater among current smokers. Light smokers had lower values than heavy smokers.

Plasma concentrations of nicotine[15] and carboxyhaemoglobin[16] fall rapidly on giving up smoking (within hours and a few days, respectively). Whether these acute effects of smoking provide an adequate explanation for the association between smoking habit and increased risk of ischaemic heart disease remains controversial,[17–19] and the present findings suggest that the biological consequences of smoking extend for many years after giving up.

Raised plasma fibrinogen concentrations have been shown to be caused by increased fibrinogen turnover in patients with ischaemic heart disease,[20] and increased counts of white cells imply a chronic inflammatory response. The mechanism for these effects is uncertain, but toxic materials in tobacco smoke include tar products and cadmium.[21]

By contrast, the results of the red cell variables (haematocrit and mean corpuscular volume) indicate that this biological response may be related only to the acute effects of smoking (a response to minor anoxia due to carboxyhaemoglobin). A detailed review of the pathological responses to tobacco smoke can be found elsewhere.[22] Whatever the mechanism to account for the present findings, these suggest that the chronic effects of smoking retain a carry over effect for many years among ex-smokers. In terms of public health it may be worth noting that subjects who never smoked retain the lowest levels of activity of these haemostatic factors.

References

1 Davies MJ, Thomas A. Thrombosis and acute coronary-artery lesions in sudden ischaemic death. *N Engl J Med* 1984;**310**:1137–40.

2 Meade TM, Chakrabarti R, Haines AP, North WRS, Stirling Y, Thompson SG. Haemostatic function and cardiovascular death: early results of prospective study. *Lancet* 1980;i:1050–4.

3 Wilhelmsen L, Svardsudd K, Korstan-Bengsten K, Larsson B, Welin L, Tibblin G. Fibrinogen as a risk factor for stroke and myocardial infarction. *N Engl J Med* 1984;**311**:501–5.

4 Yarnell JWG, Sweetnam PM, Elwood PC, *et al.* Haemostatic factors and ischaemic heart disease: the Caerphilly study. *Br Heart J* 1985;**53**:483–7.

5 Meade TW, Chakrabarti R, Haines AP, North WRS, Stirling Y. Characteristics affecting fibrinolytic activity and plasma fibrinogen concentrations. *Br Med J* 1979;i:153–6.

6 Reid DD, Hamilton PJS, McCartney P, Rose G, Jarrett RJ, Keen H. Smoking and other risk factors for coronary heart-disease in British civil servants. *Lancet* 1976;ii:979–84.

7 Kannel WB. Update on the role of cigarette smoking in coronary heart disease. *Am Heart J* 1981;**101**:319–28.

8 Thorp JM, Horsfall GB, Stone MC. A new red-sensitive micro-nephelometer. *Medical and Biological Engineering* 1967;**5**:51–6.

9 Clauss A. Gerrinnungephysiologische Schnellmethode sur Bestimmung des Fibrinogens. *Acta Haematol* 1957;**17**:237–46.

10 Harkness J. The viscosity of human blood plasma; its measurement in health and disease. *Biorheology* 1971;**8**:171–93.

11 O'Brien JR, Etherington M, Jamieson S, Lawford P, Sussex J, Lincoln SV. Heparin neutralizing activity test in the diagnosis of acute myocardial infarction. *J Clin Pathol* 1975;**28**:975–9.

12 Baker IA, Eastham R, Elwood PC, Etherington M, O'Brien JR, Sweetnam PM. Haemostatic factors associated with ischaemic heart disease in men aged 45 to 64 years: The Speedwell Study. *Br Heart J* 1982;**47**:490–4.

13 Yarnell JWG, Fehily AM, Milbank J, Kubicki AJ, Eastham R, Hayes TH. Determinants of plasma lipoproteins and coagulation factors in men from Caerphilly. *J Epidemiol Community Health* 1982;**37**:137–40.

14 Lowe GDO, Drummond MM, Forbes CD, Barbanel JC. The effects of age and cigarette-smoking on blood and plasma viscosity in men. *Scott Med J* 1980;**25**:13–7.

15 Hill P, Haley NJ, Wynder EL. Cigarette smoking: carboxyhemoglobin, plasma nicotine, cotinine and thiocyanate vs self-reported smoking data and cardiovascular disease. *J Chron Dis* 1983;**36**:439–49.

16 Galea G, Davidson RJL. Haematological and haemorheological changes associated with cigarette smoking. *J Clin Pathol* 1985;**38**:978–84.

17 Kaufman DW, Helmrich SP, Rosenberg L, Miettinen OS, Shapiro S. Nicotine and carbon monoxide content of cigarette smoke and the risk of myocardial infarction in young men. *N Engl J Med* 1983;**308**:409–13.

18 McGill HC. Potential mechanisms for the augmentation of atherosclerosis and atherosclerotic disease by cigarette smoking. *Prev Med* 1979;**8**:390–403.

19 Hartz AJ, Anderson AJ, Brooks HL, Manley JC, Parent GT, Barboriak JJ. The association of smoking with cardiomyopathy. *N Engl J Med* 1984;**311**:1201–6.

20 Pilgeram LO. The turnover rate of autologous plasma fibrinogen ^{14}C in subjects with coronary thrombosis. *Thrombosis et Diathesis Haemorrhagica* 1968;**20**:31–43.

21 Lewis GP, Jusko WJ, Coughlin LL, Hartz S. Cadmium accumulation in man: Influence of smoking, occupation, alcohol habit and disease. *Journal of Chronic Diseases* 1972;**25**:717–26.

22 Anthony HM. Reactive changes in the blood of smokers and the development of arteriosclerosis and chronic obstructive pulmonary disease; a review. *Reviews on Environmental Health* (In press).