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Resonance-thrombography indices of the haemostatic process in relation to risk of incident coronary heart disease: 9 years follow-up in the Caerphilly Prospective Heart Disease Study

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Summary

Global assays, such as resonance-thrombography (RTG), which measure the interaction between platelets, coagulation and fibrinolysis have been used as summary measures of risk for over two decades but have not been evaluated in epidemiological studies. We examined whether RTG indices are risk indicators for incident coronary heart disease (CHD). RTG indices, related haematological variables and other risk factors were measured between 1984 and 1988 in a cohort of 2398 British men. Reaction time (*r*) and amplitude of fibrin leg (AF) were associated with lifestyle risk factors. During 9 years of follow-up, 282 (12%) men developed a major new CHD event, as classified by World Health Organization criteria. On adjustment for age, only *r* and AF measured at baseline were related to risk of incident CHD. On multivariate adjustment in a multiple logistic regression model that included age, diastolic blood pressure, body mass index, total and high-density lipoprotein cholesterol, lifestyle risk factors and use of prescribed medicine, these associations weakened but remained significant. Additional adjustment for fibrinogen, viscosity, white cell count and fibrin D-dimer either reduced these associations to non-significance (AF) or to borderline significance (*r*).

Keywords: coronary heart disease, resonance-thrombography, prospective study, fibrinogen, blood coagulation.

Coronary artery thrombosis plays a key role in the development of coronary heart disease (CHD), and arterial thrombosis has been described as 'haemostasis in the wrong place'. The haemostatic process involves the initial interaction of the injured vessel wall with circulating platelets followed by coagulation activation, resulting in the reduction of blood flow by a platelet-fibrin haemostatic plug (in injured micro-vessels) or a platelet-fibrin thrombus (in macro-vessels, for example after rupture of an arterial atherosclerotic plaque). The extent of fibrin formation is limited by activation of the endogenous fibrinolytic system, which lyses fibrin to form fibrin degradation products, such as fibrin D-dimer. These processes are all influenced by blood flow, which is in turn influenced by rheological and inflammatory variables including blood viscosity and white cell count.

Several studies have established that blood levels of several haematological variables related to haemostasis and thrombosis are associated with incident CHD. In the Caerphilly Prospective Heart Disease Study in middle-aged men, we have previously reported that incident CHD is associated with plasma fibrinogen, plasma viscosity and with cell count (Yarnell *et al*, 1991; Sweetnam *et al*, 1996), von Willebrand factor (VWF) (Rumley *et al*, 1999) which, like fibrinogen is an important mediator of platelet adhesion and aggregation, coagulation Factor VIII (Rumley *et al*, 1999) and fibrin D-dimer, a marker of activation of the coagulation and fibrinolytic systems (Lowe *et al*, 1998) Tissue plasminogen activator (t-PA) antigen and plasminogen activator inhibitor (PAI-1) activity were assayed as other markers of the fibrinolytic system (Lowe *et al*, 1998).

Measurement of these individual variables does not give an adequate measure of the global interaction between platelets, coagulation and fibrinolysis in the processes of haemostasis and thrombosis. Global assays of these interactions include thromboelastography (TEG), which was introduced by Hartert, who subsequently introduced flow stimulation into the clotting process in resonance-thrombography (RTG) (Hartert, 1981, 1982). The latter measurement has been advocated for the

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clinical laboratory screening of bleeding disorders (e.g. platelet dysfunction, dysfibrinogenaemias, disseminated intravascular coagulation and hyperfibrinolysis) (Hartert, 1981, 1982) but to our knowledge has not been evaluated as a risk marker for incident CHD in population-based prospective studies.

We therefore evaluated the association of four RTG indices with incident CHD, conventional CHD risk factors, and other haematological variables in the Caerphilly Prospective Heart Disease Study. The objective of this study was to establish whether or not these RTG indices were independently associated with incident CHD.

Methods

The Caerphilly Collaborative Heart Disease Study began in 1979 with the overall objective of examining the determinants and predictive ability of new and classical risk factors for incident CHD. During the initial recruitment phase (1979–83) 2512 men aged 45–59 years were examined, representing 90% of the population of men in this age group from the town of Caerphilly, South Wales, UK, and its surrounding villages (total population 40 000). Since then they have been examined at 5-year intervals. At the first re-examination between 1984 and 1988, when the men were aged 49–64 years, men of the same age who had moved into the defined geographical area were also deemed to be eligible. A total of 2398 men were recruited into the reconstructed cohort and they form the baseline population for the current study.

The general design and methods of the Caerphilly Study have been described elsewhere (Bainton *et al*, 1992; Sweetnam *et al*, 1996). Briefly, at the survey between 1984 and 1988 the men were invited to attend an afternoon/evening clinic where a detailed medical and lifestyle history were obtained, the London School of Hygiene and Tropical Medicine (LSHTM) chest pain questionnaire (Rose *et al*, 1982) was administered, a full 12-lead electrocardiogram (ECG) was recorded, and height, weight and blood pressure were measured. Alcohol consumption was obtained by the use of a self-administered questionnaire checked at the clinic and converted from standard units of volume to millilitres of pure alcohol per week (Yarnell *et al*, 1983). Prescribed medication has been shown previously to influence levels of haemostatic and inflammatory markers (Yarnell *et al*, 2000, 2001) and was included as a confounding variable. Only two subjects included in this analysis were taking anticoagulants during the baseline phase of this study (1984–88), and were classed among those taking prescribed medication. The men were then invited to return to an early morning clinic where a blood sample was taken and a fasting blood sample was obtained from 2225 men (93%).

Follow-up procedure and definition of incident CHD

The incidence of CHD was measured up to the third re-examination of the men. In Caerphilly this took place between 1993 and 1997 with a nearly constant interval of 105 (SD = 6) months after the baseline, first re-examination. All men had been flagged with the National Health Service Central Registry and all death certificates were coded to the 9th Revision of the International Classification of Diseases (ICD). Fatal CHD was defined as any death coded to ICD 410-414 inclusive. At each re-examination, the LSHTM chest pain questionnaire was re-administered and another ECG was recorded. The LSHTM chest pain questionnaire was extended to include questions about admission to hospital with severe chest pain. These, together with lists from Hospital Activity Analysis of all men admitted to local hospitals with a diagnosis of ICD 410-414, were used as a basis for a search of hospital notes for events satisfying World Health Organisation criteria 'for definite non-fatal' myocardial infarction (MI). Finally, the appearance on any follow-up ECG of major or selected moderate Q waves (Minnesota codes 1-1-any, 1-2-1 to 1-2-5 or 1-2-7) when there were no Q waves (1-1-any, 1-2-any or 1-3 any) on either the recruitment ECG or the baseline, first re-examination ECG was taken as evidence that an MI had occurred during the follow-up period.

Blood collection, storage and analysis

Blood was taken between 7.00 and 10.00 AM for 91% of the men, before 7.00 AM for 7% and between 10.00 and 11.00 AM for 2%. The blood was collected without venous stasis into evacuated containers using a 19-gauge butterfly needle and Sarstedt monovette adapters (Leicester, UK). Various aliquots were used for measurement of different haemostatic variables.

Citrated plasma

Measurements were made during 1994, when the plasma had been stored at −70° for 6–10 years. Enzyme-linked immunosorbent assays (ELISAs) were used for measurement of VWF, D-dimer and t-PA as previously described (Lowe *et al*, 1998; Rumley *et al*, 1999). PAI-1 activity was measured using a chromogenic assay (Chromogenix, Stockholm, Sweden) (Lowe *et al*, 1998). Clottable fibrinogen was later measured by an automated Clauss assay in a Coag-A-Mate X 2 coagulometer (Organon Teknika, Cambridge, UK) with the use of a separate stored plasma sample (Sweetnam *et al*, 1998). One-third of these samples had previously been used for another purpose, so that a total of only 1378 samples was available.

EDTA plasma

Fresh dipotassium edentate-anticoagulated samples were used to measure nephelometric fibrinogen, plasma viscosity and white cell count as described previously (Yarnell *et al*, 1987, 1991). α_2-Macroglobulin and α_1-antitrypsin were measured nephelometrically with Beckman antisera kits (High Wycombe, UK). Lipid measures were made by using enzymatic assays as described previously (Bainton *et al*, 1992).

Resonance-thrombography

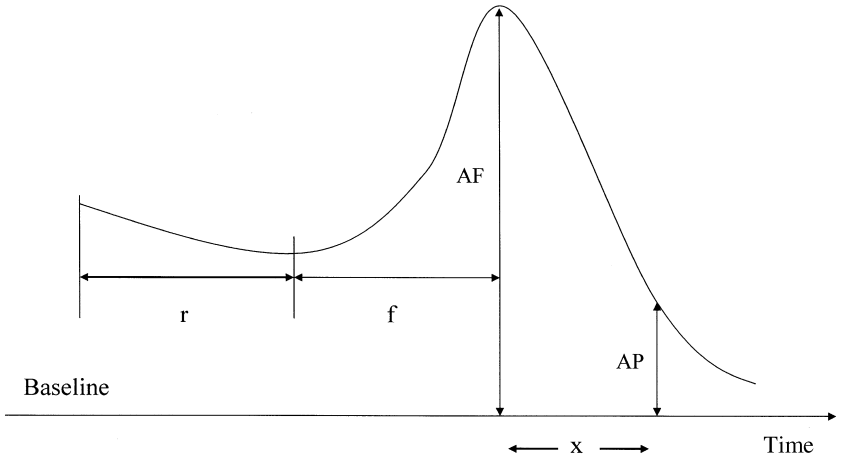
Resonance-thrombography is a successor to the technique of thrombo-elastography (Hartert, 1981, 1982). RTG is a method for measuring the start and progression of clot formation in flowing blood, involving formation of elastic fibrin molecules and their condensation by platelets. The resonance-thrombograph consists of a cylinder suspended as a pendulum on an elastic bar. The cylinder dips into a cylindrical container heated to 37°C. The 1·3 mm wide gap between them is filled with 0·3–0·4 ml of citrated whole blood up to the container's rim. All measurements were made in the Department of Haematology, Cardiff Royal Infirmary, by a dedicated technician within 7 h of collection of the samples. The action of this measuring device consists of an orbital movement of the cylinder within the container. The frequency of this electromagnetically forced movement is constant. The cylinder, by its movement, encircles a tiny orbit. In the measuring procedure the clot is subject to permanent strain by the orbital movement. Its oscillation causes a stretching of fibrin molecules and gives an assay of clot elasticity by recording a differentiating resonance effect. The instrument used was described in greater detail elsewhere (Hartert, 1982). The RTG delivers distinguishable and characteristic types of graphs for qualitative and quantitative differences in fibrin production and similarly separate changes in platelet activity are recorded. RTG parameters are shown in Fig 1. Reaction time (r) measures the pre-phase of coagulation, f is the duration of the ascending leg of the waveform mainly determined by the growing fibrin structure – the fibrin time, AF is its amplitude. Both f and r reflect clot formation, AF reflects the final clot stiffness. The amplitude AP, representing the angle of the platelet (P) leg, is mainly determined by platelet activity. In the present study, r (min), f (min), AF (mm) and AP (mm) were measured. To simplify its measurement, the amplitude of the platelet leg (AP) was measured 300 s after maximum amplitude (AF). Due to the shape and completeness of the trace it was not always possible to measure this latter parameter, and 550 measurements could not be made for this reason.

Statistical methods

The distributions of RTG indices had a positive skew and all were transformed to logarithms. For each, this transformation produced a more nearly symmetrical Gaussian distribution and stabilized variances. Comparisons of mean values between men who developed major CHD and those who did not were made by analysis of covariance (Nie *et al*, 1996).

Duplicate samples were assayed blindly by each laboratory throughout the recruitment period. Coefficients of variation were calculated for the RTG indices as follows: $r = 8{\cdot}3\%$; $f = 15{\cdot}7\%$; AF = 19·2% (all $n = 22$); AP = 22·7% ($n = 13$).

Evidence of ischaemia at baseline was assessed from the chest pain questionnaire and the ECG. Three categories, namely angina, history of at least one episode of prolonged, severe chest pain and ECG ischaemia were defined as reported previously (Bainton *et al*, 1988). Among the cohort of 2398 men, 736 (31%) had some evidence of ischaemia at baseline. This was similar to the prevalence found by the British Regional Heart Study (Shaper *et al*, 1984) for men of a similar age. Exclusion of such a large group, among whom almost half of the incident events occurred, did not seem satisfactory. Neither did the usual practice of excluding just a very small percentage (usually <5%) of men for whom there is good clinical evidence of a previous MI. Instead, we have chosen to include all men and to adjust for the presence of ischaemia at baseline by including this as a confounder in the logistic regression analyses with the occurrence, or not, of a major incident CHD event as the binary dependent variable (Yu *et al*, 2003). The distributions of RTG indices were divided into



Indices: r, lag time (s); f, fibrin time (s); AF, maximum amplitude (mm); AP, amplitude of platelet leg (mm), measured x=300 seconds after maximum amplitude.

Fig 1. Resonance-thrombography: derivation of the four indices.

equal 'thirds' and the results were presented as the relative odds of major incident CHD in each 'third', relative to a baseline 'third', which was always taken as the 33% of men with the lowest levels, with adjustment for age and other risk factors. These were referred to throughout as relative odds and where appropriate they were shown together with 95% confidence intervals (CI) estimated from the logistic regression models. Standardized relative odds were calculated as summary measures (the proportionate change in relative odds associated with standard deviation change in the RTG index).

Results

Of the total cohort of 2398 British men, 282 (11·8% of all men) developed major new CHD events over the follow-up period of nearly 9 years. Of these 282, 132 (46·8%) were fatal and 150 were non-fatal (53·2%). Fasting blood samples were available from 2225 (93%) men; of these, 261 (11·7%) developed major new CHD. Results for RTG indices were available for up to 1491 (67%) of fasting men, of whom 176 (11·8%) developed new major CHD. Common results were available for 1470 men for *r*, *f* and AF and 920 for AP; which was unavailable for a larger number of subjects as a delay of five additional minutes was required for its measurement.

Table I shows the geometric mean of RTG indices according to smoking status, social class, history of diabetes, work activity, prescribed medicine, family history of CHD and pre-existing CHD at baseline. The mean level of *r* was significantly different between manual and non-manual social class ($P = 0{\cdot}002$). For AF, there were also significant differences between non-smokers, ex-smokers and current smokers ($P = 0{\cdot}018$), between manual and non-manual social class ($P = 0{\cdot}001$) and between subjects with and without prescribed medicine ($P = 0{\cdot}003$).

Table II shows the Pearson correlation coefficients between RTG indices and some indicators of CHD risk. Even low correlations achieved statistical significance because of the large number of subjects in the study but only correlations in excess of 0·1 would be likely to have any biological relevance;

Table I. Geometric mean of RTG indices according to smoking status, social class, history of diabetes, work activity, prescribed medicine, family history of CHD and pre-existent CHD at baseline between 1984 and 1988 in men from Caerphilly, South Wales, UK.

	r (min)+	*f* (min)+	AF (mm)+	AP(mm)+
Smoking status				
Nonsmoker	6·69	2·41	18·17	2·94
Ex-smoker	6·75	2·38	16·93	2·91
Current smoker	6·82	2·37	16·63	3·00
Test of difference++	0·474	0·771	0·018*	0·653
Social class				
Manual	6·86	2·39	16·53	2·94
Non-manual	6·60	2·36	17·94	2·99
Test of difference++	0·002**	0·450	0·001***	0·587
History of diabetes				
Yes	7·08	2·33	15·51	3·20
No	6·76	2·38	17·04	2·95
Test of difference++	0·178	0·628	0·155	0·320
Work activity				
High	6·81	2·39	16·81	2·92
Low	6·72	2·36	17·28	3·00
Test of difference++	0·266	0·356	0·235	0·381
Prescribed medicine				
Yes	6·83	2·37	16·40	2·94
No	6·71	2·39	17·53	2·96
Test of difference++	0·124	0·647	0·003**	0·811
Family history of CHD				
Yes	6·74	2·37	17·48	2·92
No	6·77	2·38	16·92	2·96
Test of difference++	0·802	0·819	0·347	0·728
Evidence of CHD at baseline				
Yes	6·79	2·42	16·93	2·93
No	6·76	2·36	17·02	2·97
Test of difference++	0·789	0·219	0·830	0·657

RTG, resonance-thrombography; CHD, coronary heart disease; *r*, reaction time; *f*, fibrin formation time; AF, amplitude of fibrin leg; AP, amplitude of presenting the angle of platelet leg.
+Values are geometric mean.
++Values are *P*-values by one-way analysis of variance. All RTG indices are logarithmically transformed.
Significantly different at *0·05, **0·01 and ***0·001.

Table II. Pearson correlation coefficients between RTG indices and indicators of CHD risk.

Variables	*r* (min)	*f* (min)	AF (mm)	AP (mm)
Age (years)	0·057*	−0·048	−0·146**	−0·040
Body mass index (kg/m^2)	−0·022	−0·011	−0·031	−0·040
Diastolic blood pressure (mmHg)	0·003	0·002	0·003	−0·013
Systolic blood pressure (mmHg)	0·015	−0·053*	−0·053*	−0·038
Alcohol consumption+ (ml/week of pure alcohol)	−0·026	0·075**	0·060*	0·024
Total cholesterol (mmol/l)	0·046	−0·015	−0·104**	−0·082*
HDL cholesterol (mmol/l)	−0·049	−0·035	0·094**	−0·016
Triglycerides (mmol/l)+	−0·007	0·009	−0·041	−0·017
D-dimer (ng/ml)+	0·077**	−0·175**	−0·258**	−0·024
von Willebrand factor (IU/dl)+	−0·032	−0·117**	−0·073**	0·043
Tissue plasminogen activator (ng/ml)+	−0·012	0·024	−0·080**	−0·055
Plasminogen activator inhibitor (% pool)+	−0·069**	0·003	−0·034	−0·020
Nephelometric fibrinogen (g/L)+	0·202**	−0·153**	−0·488**	−0·021
Plasma viscosity (mPa s)+	0·145**	−0·179**	−0·406**	−0·048
White cell count (10^9/l)+	0·092**	−0·066*	−0·220**	−0·080*
Clottable fibrinogen (g/l)+	0·183**	−0·281**	−0·498**	−0·114**
Serum viscosity (mPa s)+	0·087**	−0·083**	−0·202**	−0·022
Haemoglobin (g/dl)+	0·031	0·314**	0·094**	0·165**
Gamma glutamyl transferase (IU/l)+	−0·053*	0·001	−0·070**	0·008
α_2-macroglobulin (mg/dl)+	0·056*	−0·030	−0·099**	−0·004
α_1-antitrypsin (mg/dl)+	0·024	−0·096**	−0·164**	−0·060

HDL, high-density lipoprotein.
*$P < 0·05$, **$P < 0·01$.
+Values of the variable are logarithmically transformed.
Abbreviations as in Table I.

r was positively associated with nephelometric and clottable fibrinogen and plasma viscosity; *f* was positively associated with haemoglobin, and was negatively associated with D-dimer, VWF antigen, nephelometric and clottable fibrinogen and plasma viscosity. AF was negatively associated with age, total cholesterol, D-dimer, nephelometric and clottable fibrinogen, plasma and serum viscosity, white cell count, and α1-antitrypsin. AP was negatively associated with clottable fibrinogen and haemoglobin. The strongest correlations were between AF and inflammatory and rheological markers, such as the two measurements of fibrinogen and plasma viscosity.

Table III shows the mean, median and geometric mean for RTG indices in men who developed CHD, and men who did not. The mean difference was statistically significant between subjects with and without incident CHD only for AF by analysis of covariance after adjustment for age ($P = 0·003$); but also approached statistical significance for *r* ($P = 0·057$).

Logistic regression models were used to analyse the relation of RTG indices to the risk of incident CHD. Table IV shows the results of relative odds and 95% CI for major incident CHD events. Also shown are the standardized relative odds (SRO) – the proportionate change in relative odds associated with an increase of 1 standard deviation in the RTG indices. After adjustment for age, *r* was a predictor of risk of incident CHD; relative odds with increasing tertile categories, when compared with the lowest third category, were 2·22 (95% CI, 1·41–3·50) and 1·87 (95% CI, 1·19–2·93), respectively. Participants in the second and third tertile group showed a 122% and

Table III. Mean, median and geometric mean for RTG indices in men who developed CHD, and men who did not during 9 years follow-up.

	Incident CHD				Free of incident CHD					
	No.	Mean (SD)	Median	GM	No.	Mean (SD)	Median	GM	Mean difference+	*P*-value
r (min)	172	7·10 (1·20)	7·00	6·99	1298	6·91 (1·52)	7·00	6·74	0·035 (0·018)	0·057
f (min)	172	2·48 (0·67)	2·50	2·38	1298	2·51 (0·91)	2·50	2·38	0·001 (0·026)	0·956
AF (mm)	172	16·83 (6·90)	16·00	15·35	1298	18·71 (7·33)	18·00	17·22	−0·106 (0·035)	0·003
AP (mm)	113	3·29 (1·60)	3·00	2·98	807	3·28 (1·88)	3·00	2·95	0·014 (0·044)	0·746

SD, standard deviation; GM, geometric mean.
+Values are adjusted-age mean difference (logarithmic scale) and standard error.
++The significant test between subjects with and without incident CHD by analysis of covariance; the tests were adjusted for age.
Abbreviations as in Table I.

Table IV. Age and multivariate-adjusted relative odds (95% CI) of incident CHD by thirds of the level of each RTG index during 9 years follow-up.

	Tertile group			
Indices	1	2	3	SRO++
r (min)+	1·00	2·22 (1·41, 3·50)***	1·87 (1·19, 2·93)**	1·19 (91·00, 1·41)*
	1·00	2·17 (1·35, 3·47)***	1·72 (1·07, 2·76)*	1·16 (0·97, 1·39)
	1·00	2·12 (1·26, 3·55)**	1·77 (1·05, 2·98)*	1·15 (0·93, 1·41)
f (min)+	1·00	1·00 (0·67, 1·48)	1·04 (0·71, 1·52)	1·01 (0·86, 1·18)
	1·00	1·13 (0·75, 1·71)	1·16 (0·78, 1·74)	1·06 (0·90, 1·26)
	1·00	1·32 (0·84, 2·08)	1·49 (0·94, 2·35)	1·19 (0·98, 1·44)
AF (mm)+	1·00	0·80 (0·55, 1·15)	0·56 (0·37, 0·84)**	0·80 (0·69, 0·93)**
	1·00	0·86 (0·59, 1·27)	0·64 (0·41, 0·98)*	0·88 (0·75, 1·04)
	1·00	0·93 (0·60, 1·45)	0·80 (0·48, 1·33)	0·98 (0·80, 1·20)
AP (mm)+	1·00	0·82 (0·50, 1·35)	1·04 (0·66, 1·66)	1·04 (0·85, 1·26)
	1·00	0·82 (0·49, 1·37)	1·13 (0·70, 1·83)	1·08 (0·88, 1·33)
	1·00	0·77 (0·43, 1·36)	1·19 (0·71, 2·01)	1·14 (0·90, 1·45)

CI, confidence interval; SRO, standardized relative odds.
+First row indicates age-adjusted relative odds and 95% CI; second row indicates multivariate-adjusted relative odds and 95% CI; third row indicates additional adjustment for D-dimer antigen, plasma viscosity, nephelometric fibrinogen and white cell count.
Adjusted multiple variables are age, diastolic blood pressure, body mass index, smoking habit, alcohol consumption, social class, prescribed medicine, total cholesterol and HDL cholesterol.
++Standardized relative odds are the proportionate change in relative odds associated with an increase of 1 standard deviation in the RTG index level.
Significantly different at *0·05, **0·01 and ***0·001.
Abbreviations as in Table I.

87% increase in risk in 9-year incidence of CHD, respectively. After adjusting the data additionally for age and other risk factors listed in Table IV, the relative odds were 2·17 (95% CI, 1·35–3·47) and 1·72 (95% CI, 1·07–2·76) respectively; men in the second and third tertile group showed a 117% and 72% increase in risk. After additional adjustment for D-dimer antigen, plasma viscosity, nephelometric fibrinogen and white cell count, men in the second and third tertile group still showed increases in risk. But the tests for trend that were summarized by SROs were not significant, either after multivariate adjustment, or after adjustment for additional haematological variables.

A similar analysis was carried out for AF. After adjustment for age, relative odds with increasing tertile groups, when compared with the lowest tertile group, were 0·80 (95% CI, 0·55–1·5) and 0·56 (95% CI, 0·37–0·84); men in the lowest tertile group showed a 44% reduction in risk. After adjusting the data additionally for age and other risk factors, the relative odds were 0·86 (95% CI, 0·59–1·27) and 0·64 (95% CI, 0·41–0·98), respectively; men in the lowest tertile group showed a 36% reduction in risk. But the tests for trend were not significant, either after multivariate adjustment or after adjustment for additional haematological variables.

Discussion

We have shown for the first time in a prospective study that two of the four RTG indices were associated with incident CHD. Men with incident CHD had a longer initial reaction time (*r* index; $P = 0{\cdot}057$ on univariate analysis). The amplitude of fibrin formation (AF) was also significantly shorter in men who subsequently developed incident CHD than in men who did not ($P = 0{\cdot}003$ on univariate analysis). The two other parameters studied did not show a significant association with incident CHD.

In general, classical CHD risk factors showed weak associations with RTG indices. Smoking, manual social class and use of prescribed medicine were associated with shorter AF index (Table I), as were age, systolic blood pressure and cholesterol (Table II). In contrast, haematological risk predictors including fibrinogen, plasma and serum viscosity, and fibrin D-dimer were strongly associated with shorter AF index, and longer reaction time (*r* index; Table II). These findings are consistent with previous reports for fibrinogen and its degradation products (Hartert, 1981, 1982; Zuckerman *et al*, 1981). Increasing fibrinogen levels delay the onset of clot formation by inhibiting the rate of fibrin polymerization (Blomback *et al*, 1989) and produce denser fibrin clots (Lim *et al*, 2003).

Adjustment for classical CHD risk factors reduced the associations of RTG indices with incident CHD to borderline statistical significance (Table IV), while the additional adjustment for haematological risk predictors, including fibrinogen and white cell count (Danesh *et al*, 1998), plasma viscosity (Danesh *et al*, 2000) and fibrin D-dimer (Danesh *et al*, 2001) reduced these associations further, especially for AF index (Table IV). We therefore conclude that RTG indices did not add significantly to the multivariate prediction of CHD risk in the present study. Further, larger studies are required to

determine with confidence the size of their associations with CHD risk and their use (if any) in CHD risk prediction.

Our results suggest that the association of some classical CHD risk factors (e.g. smoking) (Yarnell *et al*, 1987) as well as some haematological variables, with CHD risk (Danesh *et al*, 1998, 2000, 2001) may be partly attributable to their effects on the clotting of flowing blood *in vitro*, and hence potentially on coronary thrombus formation *in vivo*. Further experimental work is required to test these hypotheses – for example the effect of smoking reduction or cessation on RTG indices.

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