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ORIGINAL ARTICLE

Prospective study of seasonal patterns in hemostatic factors in older men and their relation to excess winter coronary heart disease deaths

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Summary. *Background:* In England and Wales, approximately 20% extra deaths from coronary heart disease (CHD) occur between December and March, among older people. Circulating concentrations of tissue plasminogen activator (t-PA), von Willebrand factor (VWF) and fibrin D-dimer are associated with arterial disease, and tend to peak in winter. The potential contributions of these hemostatic activation measures to excess winter mortality are unknown. *Objectives:* To estimate contributions of hemostatic factors to excess winter mortality. *Methods:* Seasonal patterns in t-PA, VWF and D-dimer were investigated in 4088 men aged 60–79 years from 24 British towns. Data on established coronary risk factors were collected by questionnaire, physical examination and blood sampling. The adjusted mean increase in hemostatic markers during winter months, after adjustment for a range of coronary risk factors, was combined with associations of each marker with CHD mortality obtained from 9 years' follow-up of participants, to predict degree of excess CHD winter mortality. Associations of hemostatic markers with CHD incidence from large meta-analyses were also used. *Results:* All three markers showed peaks in winter; the adjusted mean increases during winter months were 0.21, 0.15 and 0.12 standard deviations for t-PA, VWF and log(D-dimer), respectively. Predicted excess hazard ratios for winter CHD mortality were 3.0%, 2.4% and 3.1%, respectively, in combination, representing an 8.6% excess. This increased to 14% when applying meta-analysis estimates. *Conclusions:* Seasonal patterns in three hemostatic markers predict at least 8.6% excess CHD mortality in winter in Great Britain, potentially accounting for over half the excess observed in recent years.

Keywords: coronary disease, epidemiology, observational studies, seasons, von Willebrand factor.

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Introduction

The tendency of most European countries to experience greater numbers of deaths in winter than over the rest of the year has been long recognized [1,2]. While the effect of cold weather may play a part, data comparisons across Europe suggest that the explanation is more complex [3]. Indeed some countries closer to the Mediterranean where winter temperatures are higher than in Scandinavian countries, sometimes appear to experience a sharper mortality difference according to season. The nature of building structures of non-Scandinavian homes has been blamed for failing to address the challenges of lower winter temperatures [4].

The United Kingdom has a substantial burden of excess winter deaths, estimated at over 36 000 for 2008–2009 in England and Wales; the highest numbers occurred in December and January [5]. The excesses occurred even among people under 65, but the burden rose steadily with age, being most marked in people aged 85 and over. It has long been established that diseases of the circulatory and respiratory system are the two leading causes of this excess [6]. This remained the case for the period 2005–2008, and while the excess winter mortality index, which estimates the *relative* increase in winter, is higher for respiratory disease, the index was still 15.8% for circulatory disease in men in 2007–2008, and was particularly marked in those aged over 85 years (20.6%) [7].

Seasonal fluctuations have been observed in a number of circulating hemostatic biomarkers, which are themselves linked

to coronary heart disease (CHD) and venous thromboembolism [8,9] These include: von Willebrand factor (VWF), a marker of endothelial disturbance that plays a key role in platelet adhesion and aggregation; tissue plasminogen activator (t-PA), another endothelial marker with a key role in fibrinolysis [10–12]; fibrin D-dimer, a marker of activated blood coagulation and fibrinolysis [11]; and fibrinogen, which is the precursor of fibrin, and C-reactive protein, a marker of the acute phase response [8,11–13].

Our first objective was to investigate patterns in these markers of hemostasis in men from the British Regional Heart Study who were examined over a 2-year period from 1998 to 2000, to ascertain whether higher levels were evident during winter months. Any hemostatic marker for which higher levels carry a short-term increased risk of CHD may help to account for the excess winter deaths reported in national statistics. Winter is defined in national statistics as including the months December, January, February and March. Therefore our second objective was to analyze relationships between the markers and 8–10 years' follow-up for CHD mortality. Our third objective was to calculate the difference in risk of CHD that would occur as a result of higher levels of the markers in winter, and thus the degree of the known excess in winter deaths that could be attributed to seasonal patterns of the hemostatic markers. As the strength of association of these markers with CHD *incidence* is also known from published meta-analyses of prospective studies [14–17], we applied also these more precise and reliable estimates.

Methods

The British Regional Heart Study (BRHS) is a prospective study of cardiovascular disease (CVD) involving 7735 men aged 40–59 years when screened between 1978 and 1980, drawn from a single general practise in each of 24 British towns [18]. The population studied was socio-economically representative of British men and represented all major British regions, in particular representing the geographical variation in mortality due to CVD [18]. In 1998–2000, all surviving men, by then aged 60–79 years (mean age 68.7 years), were invited for a 20th year follow-up examination, which 4252 men (77% of survivors) attended. They were requested to fast for a minimum of 6 h, during which time they were instructed to drink only water, and to attend for measurement at a prespecified time between 08.00 and 18.00 h. All men were asked to provide a blood sample, collected using the Sarstedt-Monovette system. Four thousand and eighty-eight men had at least one hemostatic marker measured; participants with no biomarkers present were excluded.

Cardiovascular risk factors

Anthropometric measurements including body weight, height and waist circumference (WC) were carried out. The men completed a questionnaire, which included questions on their medical history and lifestyle behaviour. Details of measurements and classification methods for smoking status, physical activity, body mass index, social class, blood pressure, total cholesterol, high-density lipoprotein (HDL)-cholesterol, triglycerides and glucose have been described [19].

Geographical region

Men were classed as living in the south of England, Midlands/Wales, north England or Scotland according to a previous classification based on the town of residence when recruited to the study [20]. For this analysis, men who had moved to a different region were excluded.

Mean outdoor temperature on the day of examination in the town of examination was obtained from the UK Meteorological Office. Participants examined on the same day in the same town were therefore assigned the same outdoor temperature; however, the indoor temperature was recorded at the time of examination by nurses for each participant.

Hemostatic and inflammatory biomarkers

At the 20-year examination, levels of t-PA antigen, D-dimer, VWF antigen, CRP and fibrinogen were measured in citrated plasma, as described elsewhere [21]. t-PA and D-dimer were measured with enzyme-linked immunosorbent assays (ELISAs) (Biopool AB, Umea, Sweden), as was VWF antigen (Dako, High Wycombe, UK). Plasma fibrinogen was assayed by the Clauss method in an MDA-180 coagulometer (Organon Teknika, Cambridge, UK). CRP was assayed by ultra-sensitive nephelometry (Dade Behring, Milton Keynes, UK). Intra- and inter-assay CVs were, respectively: 4.1% and 6.6% for t-PA; 3.2% and 4.2% for vWF; and 4.7% and 5.2% for D-dimer [21].

Follow-up

All men have been followed-up from initial examination (1978–1980) to June 2008 for all cause mortality and cardiovascular morbidity and follow-up has been achieved for 99% of the cohort. In the present analyses, mortality for CHD is based on follow-up from rescreening in 1998–2000 at mean age 60–79 years over a mean follow-up period of 9 years (range 8–10 years). Information on death was collected through the established tagging procedures provided by the National Health Service registers. Fatal CHD events were defined as death with CHD (ICD9 codes 410–414) as the underlying code.

Statistical methods

Distributions of tPA, VWF, fibrin D-dimer, CRP and fibrinogen were examined according to month of year as well as age group (under 70 years vs. 70+) and region of residence. Possible influences of age, smoking status, physical activity, alcohol intake, height, body mass index, indoor temperature, average outdoor temperature and time of blood measurement were incorporated as covariates in multivariable regression

analysis (see below). As the distribution of D-dimer and CRP were positively skewed, analysis was carried out on their log-transformed values throughout.

Cosinor analysis

Cosinor analysis [22] is a natural way of modelling cyclic behaviour that provides parameters of interest, in particular the amplitude of the cycle. It has been applied to another British cohort [11]. A simple cosinor model is given by

$$Y_i(t) = M + B\sin(\Psi t) + C\cos(\Psi t) + \varepsilon_i$$

where Y_i = level of hemostatic marker for participant i, M represents the overall mean, t = calendar month of measurement, and $\Psi = 2\pi/n$ where $n = 12$ for the number of months in the year. The model is simply a linear regression model with sin (Ψt) and cos (Ψt) as the regressors; ε_i is the residual term for the deviation of individual levels of Y from that predicted.

As levels of some established risk factors had previously been shown to vary between the towns that had formed the part of the BRHS's multistage sampling procedure, adjustment for the town effect was necessary [23]. This was achieved by the use of multilevel models where participants formed the first level and towns formed the second.

Therefore, the multilevel sinusoidal model including the covariates was expressed as:

$$Y_{ij}(t) = (M + U_{0j}) + B * \sin\frac{2\pi t}{12} + C * \cos\frac{2\pi t}{12} + \sum(\beta_k * Z_{ijk}) + \varepsilon_{ijk}$$

where Y_{ij} = level of hemostatic marker for participant i in town j, Z_{ijk} = level of covariate k for participant i in town j, and β_k = effect of covariate Z_k. U_{oj} is the 'second level' residual term representing the deviation of the town mean from the overall mean. t is the calendar time of the year in months.

From this model, the predicted means for each month of the year were estimated, and the difference in mean predicted level between 'winter months' as defined by UK national statistics, namely December, January, February and March, and non-winter months, defined as April to November, was calculated. Details of calculation of the confidence interval are given as Data S1.

Association with CHD mortality

Cox proportional hazards models were used to calculate the hazard ratio for each of the hemostatic markers, converted into standard deviation (SD) scores. Thus the increased hazard of mortality from CHD associated with an SD increase in each hemostatic marker, h, was estimated. Therefore, to predict hazard ratios for winter compared with non-winter months we calculated h^D, where D represented the estimated difference in SD units between winter and non-winter months for a particular marker. The hazard ratios were mutually adjusted for one another (and age) and thus could be multiplied together to estimate the combined hazard ratio for differences between winter and non-winter months for all three. A similar calculation was carried out for major CHD incident events. Hazard ratios were not adjusted for season of measurement because we sought a global view of the overall association between each hemostatic marker and CHD endpoints.

The proportional hazard assumption was tested using the Schoenfeld test. Also, log(cumulative hazard) plots were produced to assess whether hazards of CHD mortality were stronger in the very short, as opposed to longer, follow-up times.

Use of odds ratios from published meta-analysis

Results were presented in 2010 of odds ratios per 2 SD increase in baseline levels of t-PA, VWF and D-dimer for CHD *incidence*, drawing from 12 prospective studies with more than 4000 CHD events [16]. Odds ratio equivalents for differences observed between winter and non-winter months were obtained for each marker and multiplied together.

Results

Seasonal variations of hemostatic markers

Table 1 shows the unadjusted distributions of hemostatic and inflammatory markers by month of year: t-PA, VWF and D-dimer showed peaks in winter and troughs in summer, while fibrinogen and CRP showed no clear winter to summer variation, so were not considered further. The peaks for the three hemostatic markers all occurred in December or January and the troughs occurred in June or July. The magnitude of each marker's seasonal variation was plotted in terms of Z scores (deviation of each month's mean from the overall mean, divided by the pooled within-month standard deviation). Negative values are shown for summer months and positive values for winter. Figure 1 shows the variation for t-PA, VWF and D-dimer.

Distributions of established cardiovascular risk factors were examined for participants measured in winter and non-winter months (Table 2). Mean blood pressure (especially diastolic) was higher among those measured in winter, mean HDL cholesterol was higher and current smoking was less prevalent. Fieldwork in winter more commonly involved towns in the south of England while the opposite was true for towns in the north of England. Indoor temperature was only slightly lower on average when fieldwork was conducted in winter than at other times (mean 22.5 °C vs. 23.4 °C), though mean outdoor temperature was clearly lower (mean 5.8 °C vs. 12.2 °C).

When the multilevel sinusoidal model specified above was applied to each marker, predicted means and their confidence intervals were calculated for the 12 calendar months (see Fig. 2). Even after adjustment, the mean levels were highest in December or January and lowest between June and August. From the sinusoidal curves fitted in this model, mean levels were estimated for each calendar month, and averages taken for winter months (December to March) vs. non-winter

Table 1 Mean (SD) of biochemical markers by calendar month

Month	t-PA (ng mL^{-1})	VWF (IU dL^{-1})	Log_e (D-dimer [ng mL^{-1}])	Fibrinogen (g L^{-1})	Log_e (CRP [mg L^{-1}])
Jan	12.27 (5.23)	155.2 (52.3)	4.52 (0.86)	3.26 (0.66)	0.66 (1.03)
	n = 259	n = 259	n = 259	n = 259	n = 257
Feb	11.48 (4.57)	136.4 (46.5)	4.45 (0.76)	3.25 (0.76)	0.54 (1.09)
	n = 530	n = 530	n = 530	n = 529	n = 526
Mar	11.38 (4.18)	138.2 (45.9)	4.36 (0.85)	3.26 (0.82)	0.43 (1.18)
	n = 405	n = 405	n = 405	n = 405	n = 405
Apr	11.69 (4.55)	135.2 (41.9)	4.46 (0.85)	3.22 (0.71)	0.54 (1.08)
	n = 419	n = 419	n = 419	n = 419	n = 419
May	11.12 (4.43)	137.1 (45.8)	4.37 (0.85)	3.10 (0.60)	0.60 (0.96)
	n = 194	n = 194	n = 193	n = 194	n = 192
Jun	10.58 (4.19)	129.5 (42.4)	4.36 (0.84)	3.18 (0.71)	0.47 (1.17)
	n = 448	n = 448	n = 446	n = 447	n = 444
Jul	9.95 (4.29)	145.2 (46.7)	4.41 (0.97)	3.30 (0.72)	0.63 (1.14)
	n = 356	n = 356	n = 356	n = 356	n = 353
Aug	11.33 (4.30)	144.9 (42.7)	4.41 (0.72)	3.39 (0.70)	0.55 (1.09)
	n = 151	n = 151	n = 151	n = 151	n = 149
Sep	10.00 (4.11)	142.7 (47.9)	4.45 (0.89)	3.33 (0.68)	0.42 (1.06)
	n = 394	n = 394	n = 393	n = 394	n = 391
Oct	11.48 (4.51)	135.7 (41.8)	4.48 (0.80)	3.35 (0.83)	0.69 (1.14)
	n = 327	n = 327	n = 327	n = 327	n = 325
Nov	10.90 (4.14)	145.7 (49.6)	4.52 (0.88)	3.30 (0.69)	0.63 (1.10)
	n = 399	n = 399	n = 399	n = 398	n = 396
Dec	11.28 (4.48)	146.8 (44.1)	4.41 (0.71)	3.37 (0.85)	0.66 (1.12)
	n = 201	n = 201	n = 201	n = 201	n = 199
Mean	11.12	141.03	4.43	3.28	0.57
Pooled SD	4.40	45.8	0.84	0.73	1.11
Total N	4083	4083	4079	4080	4056

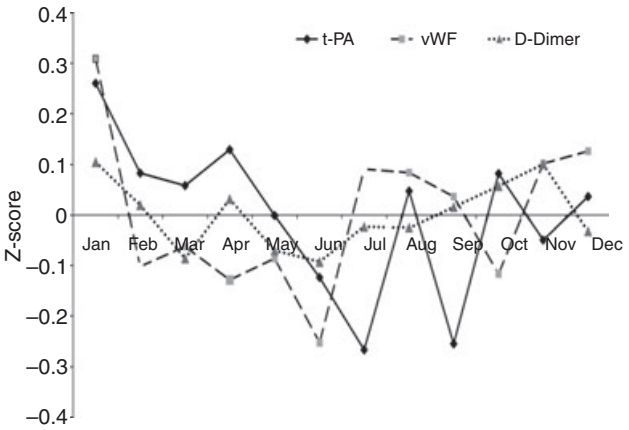

Fig. 1. Monthly variation in three hemostatic markers, expressed as standard deviation (Z) scores.

months (April to November). The mean difference was then expressed as the number of standard deviations, and were 0.21 (95% CI 0.04–0.37), 0.15 (0.0–0.30) and 0.12 (0.03–0.22) standard deviations for t-PA, VWF and D-dimer, respectively, equivalent to differences of 0.92 ng mL^{-1} in t-PA, 6.82 IU dL^{-1} in VWF and 0.103 in log_{10}(D-dimer). For the median D-dimer in our sample of 76 ng mL^{-1}, this last difference would be equivalent to approximately 20 ng mL^{-1}. No significant seasonal variations were observed either for systolic or diastolic blood pressure, or for total or HDL serum cholesterol.

Hemostatic markers and CHD mortality

Among 4088 participants, 1123 deaths occurred between examination in 1998–2000 and June 2008. Of these, 276 were due to CHD, occurring at a rate of 8.3/1000 person years. The winter months accounted for 109 of the CHD deaths (27 per month), while non-winter months accounted for 167 (21 per month). The age-adjusted hazard ratios associated with standard deviation increases in t-PA, VWF and log(D-dimer) were 1.20, 1.28 and 1.35, respectively. When mutually adjusted for each other as well as age, these were 1.14 (95% CI, 1.02–1.28), 1.18 (1.05–1.33) and 1.28 (1.15–1.44). Applying these to the number of standard deviations' increase in hemostatic markers over winter months would predict hazard ratios of 1.028, 1.025 and 1.031, giving 1.086 when multiplied together. Thus the increases in the three hemostatic markers in winter would predict an 8.6% excess CHD mortality. Additional adjustment for cholesterol, blood pressure and BMI made very little difference (results not shown).

Over the same period of follow-up, 378 major CHD events occurred (including the 276 fatal events) with a rate of 1.05/1000 person years. Hazard ratios per SD increase for the hemostatic markers, mutually adjusted and adjusted for age, were 1.11, 1.12 and 1.15. Hence hazard ratios associated with CHD mortality were stronger than for CHD incidence. Factors representing the relative strength of association, obtained as log(hazard ratio for mortality)/log(hazard ratio for incidence), were 1.32, 1.42 and 1.83, respectively. Model assumptions were checked using the Schoenfeld residual test; this showed that the

Table 2 Distributions of demographic and cardiovascular risk variables for men measured in winter months, compared with men measured in non-winter months

	Winter (n = 1395)	Non-winter (n = 2684)
Age (years)	68.8 (5.5)	68.6 (5.5)
Body mass index (kg m^{-2})	27.0 (3.6)	26.8 (3.7)
Systolic blood pressure (mm Hg)	149.9 (24.1)	148.6 (24.2)
Diastolic blood pressure (mm Hg)	86.0 (11.1)	84.8 (11.2)
Total cholesterol (mM)	6.02 (1.07)	5.99 (1.08)
HDL cholesterol (mM)	1.35 (0.36)	1.31 (0.33)
Current smoker, n (%)	157 (11)	366 (14)
Physically inactive, n (%)	140 (10)	296 (11)
Heavy drinking, n (%)	40 (3)	81 (3)
Residence in south England, n (%)	633 (45)	708 (26)
Residence in Midlands/Wales, n (%)	195 (14)	439 (16)
Residence in north England, n (%)	392 (28)	1233 (46)
Residence in Scotland, n (%)	129 (9)	295 (11)
Indoor temperature (°C)	22.5 (2.0)	23.4 (2.2)
Outdoor temperature (mean on day, °C)	5.8 (3.1)	12.2 (3.7)

effect of each marker did not change over follow-up time to a statistically significant extent (all $P > 0.15$).

Applying estimates from published meta-analysis

In estimating the effect of hemostatic markers on CHD mortality, we used follow-up from the BRHS for CHD mortality. Only 276 events occurred and thus our estimates were imprecise. Estimates of the relationship between the three hemostatic markers and CHD *incidence* (fatal and non-fatal) have recently been more precisely quantified in a meta-analysis of published literature [16]: odds ratios seen were equivalent to 1.20, 1.16 and 1.25 per SD increase in t-PA, VWF and D-dimer, respectively. Applying these effects would yield an expected excess mortality of 9.1%.

Relative strengths of association for effects of the markers on CHD mortality as compared with effects on CHD incidence were applied to the meta-analysis odds ratios, which were reported only for CHD incidence. The relative risks of CHD mortality associated with increased levels of hemostatic markers seen in winter months would then become $1.028^{1.32} \times 1.025^{1.42} \times 1.031^{1.83} = 1.14$, representing a predicted 14% excess CHD mortality.

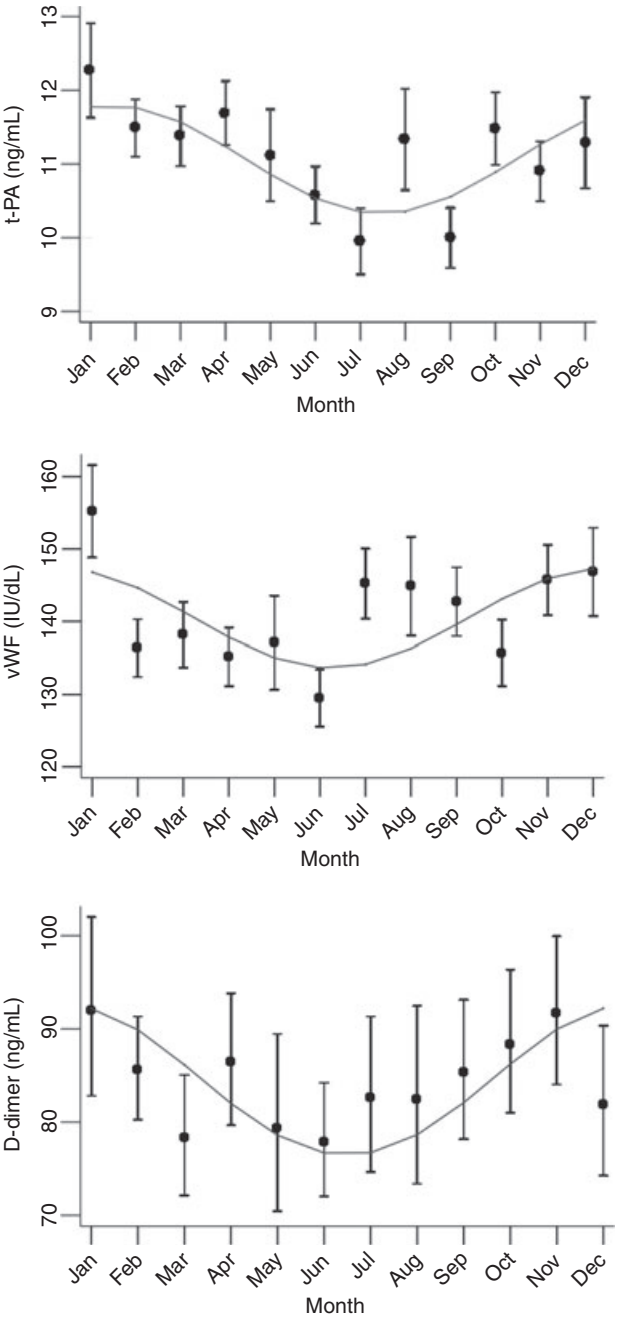

Fig. 2. Seasonal variation of biomarkers. The monthly-observed mean is shown with 95% confidence interval; the smooth line represents the fitted seasonal curve from a model that was adjusted for age, smoking status, physical activity, alcohol intake, height, body mass index, indoor temperature, average outdoor temperature and time of blood measurement.

Discussion

This study has shown that circulating levels of three hemostatic markers (VWF, t-PA and fibrin D-dimer), each of which has been demonstrated to have an independent relationship with CHD incidence in the BRHS and other studies [14–17], tend to have higher values in winter months. The seasonal changes in hemostatic factors in older men across the UK are consistent with findings from the National Child Development Study, a UK-wide cohort of men and women studied when aged 45 years [11]. Graphs presented for that study suggest the magnitude of difference was roughly similar for t-PA, larger for VWF and smaller for D-dimer. As in that and other previous studies, we observed weaker variations in fibrinogen and CRP; hence the seasonal variation in VWF, t-PA and D-dimer does not appear to be due to a systemic inflammatory response, for example owing to winter infections [8,24]. As deaths are more common in winter, and deaths from circulatory disease are jointly most responsible as a cause (together with respiratory disease), it is

plausible that an increased thrombotic tendency is substantially responsible for this excess. The differences seen in the current study would predict an 8.6% excess in CHD mortality. Official statistics suggest that the relative excess death rate from circulatory diseases among men in the same winter months (December to March) for this age group is 15–20% [7]. The excess in the current study was of similar magnitude (27 per month in winter vs. 21 per month outside winter).

We have also estimated the effect of hemostatic markers on CHD mortality with data from 12 studies with over 4000 CHD events [16]. The odds ratios seen in this meta-analysis were stronger than in BRHS for all three markers. Using corrected relative risks for CHD mortality associated with increased levels of hemostatic markers then led to a predicted 14% excess CHD mortality.

The BRHS comprises a socially and geographically representative sample of British men born between 1918 and 1939, who were aged 60–79 when the measures reported here were taken. In the initial recruitment in 1978–1980, the order in which towns were surveyed was deliberately chosen to avoid the confounding of regional patterns in CHD mortality with seasonal patterns. When the men were remeasured in 1998–2000, the same ordering of town measurement was intended but could not entirely be followed for logistical reasons. The result was that towns in the south of England (where CHD mortality is lower) were more likely to be measured in winter than other towns. However, town effects were allowed for using multilevel modelling.

If the increases in circulating levels of hemostatic markers during winter months were responsible for excess mortality due to circulatory causes, we would need to assume that the markers' levels had a very short-term impact. We have used data from 8 to 10 years' follow-up to make estimates of their impact. However, plots of cumulative hazard functions did not suggest that the impact in early follow-up (up to 1 month) differed from later follow-up.

The potential causality of circulating VWF levels in CVD is supported by the association of its major genetic determinant (non-O blood groups) with risk of CHD, stroke, peripheral arterial disease and venous thromboembolism [25]. Circulating D-dimer levels are associated with several prothrombotic genetic mutations, including the factor V Leiden mutation [26], which has been associated with risk of CHD, stroke and venous thromboembolism [27,28]. As yet, genetic determinants of t-PA have not been associated with CVD risk.

It should be noted that circulating levels of VWF, t-PA and D-dimer have been associated in prospective studies not only with incident CHD, but also incident stroke, peripheral arterial disease and venous thromboembolism [9]. Seasonal variations in these markers among older women need also to be explored.

These results support a potential role for increased thrombotic tendency in increased cardiovascular morbidity and mortality in winter months. Older people whose risk of CVD is already high might potentially be protected by antithrombotic therapies such as aspirin, especially during the winter season. However, the balance of risks and benefits of aspirin in CVD prevention should be considered [29].

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Disclosure of Conflict of Interests

The authors state that they have no conflict of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Method for deriving the confidence interval for the difference in mean predicted levels.

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