Seasonal Variations of Rheological and Hemostatic Parameters and Acute-Phase Reactants in Young, Healthy Subjects

M. Fröhlich, M. Sund, S. Russ, A. Hoffmeister, H.G. Fischer, V. Hombach, W. Koenig

Abstract The incidence of cardiovascular diseases is increased in winter months. Recent studies have shown seasonal changes in plasma viscosity, fibrinogen, and factor VII activity with elevated levels during winter. An increase in these factors generates a "hypercoagulable state," which may lead to a rise in cardiovascular morbidity and mortality. It has been suggested that an increase in upper respiratory infections might be the underlying cause for the raised acute-phase reactants, in particular fibrinogen, during the winter season. We investigated seasonal variations of 26 parameters, determining blood rheology and hemostasis in 16 healthy volunteers (8 men and 8 women) aged 20 to 41 years. They were seen at monthly intervals over a period of 1 year. Seasonal variation with peak fitted values in the winter months was found for plasma viscosity ($P<.001$ for the seasonal difference), red blood cell deformability ($P<.001$), whole blood viscosity ($P<.001$), hemoglobin ($P<.001$), hematocrit ($P<.001$), mean corpuscular volume ($P=.001$), platelet count ($P=.01$), α1-glycoprotein ($P<.001$), fibrinogen (measured by immunonephelometry; $P<.001$), plasminogen activator inhibitor-1 ($P=.002$), LDL cholesterol ($P=.003$), and triglyceride levels ($P<.001$). HDL cholesterol ($P<.001$) and cortisol ($P=.001$) showed inverse seasonal patterns, with a maximum during summertime. No statistically significant seasonal variations were seen for red blood cell aggregation, complement factor C4, total cholesterol, ceruloplasmin, haptoglobin, white blood cell count, and plasminogen. These data do not support the hypothesis that increased morbidity and mortality from cardiovascular diseases during winter may be mainly attributable to increased synthesis of acute-phase proteins due to infections. The cause for the seasonal variations in rheological and hemostatic parameters remains unclear and should be studied in more detail.
(Arterioscler Thromb Vasc Biol. **1997;17:2692-2697.)**

Key Words • seasonal variation • hemostatic parameters • blood rheology • acute-phase reactants • healthy young adults

There is evidence for an excess winter incidence in cardiovascular morbidity and mortality in elderly people. Data from England and Wales suggest a 30% increase in death from these causes during winter time.[1] Seasonal variations in blood pressure,[2,3] serum cholesterol, triglycerides,[4,5] and blood glucose[6] have been reported in several studies. However, variations in these factors are unlikely to explain the rise in acute cardiovascular events during winter time, since the pathophysiological hallmark of an acute myocardial infarct is an occlusive thrombus at the site of an atherosclerotic lesion.[7] There is evidence for a "thrombogenic state" in patients at increased risk for acute ischemic events.[8,9] Several studies have shown a strong and consistent association between increased levels of fibrinogen, factor VIIa, and PAI-1 and CHD events.[9-14] Fibrinogen could be shown to predict cardiovascular events independently of other risk factors.

A seasonal variability with peak concentrations during cold months was shown for fibrinogen.[15-17] Fibrinogen may contribute to atherothrombogenesis by several mechanisms: involvement in early atherosclerotic plaque formation (ie, providing an adsorptive surface for LDL accumulation), involvement in the response to endothelial damage, increased platelet aggregability by interaction with glycoprotein IIb/IIIa receptors on the platelet surface, increased RBC aggregation, and finally, contribution to PV (reviewed in Reference 18).

Several hypotheses have been proposed to explain the rise of plasma fibrinogen levels in winter. Some authors have suggested an increased incidence in winter respiratory infections, which might cause an acute-phase reaction and consecutively lead to an increase in fibrinogen.[15,19,20] Previous studies were carried out in cohorts of elderly people, and upper respiratory tract infections had not been excluded.[4,5,15,16]

We studied seasonal variations of a variety of hemorheological and hemostatic variables in young, healthy subjects and followed them for a 1-year period.

Methods

Sixteen healthy subjects, eight women and eight men, aged 20 to 41 years were asked to participate in the study. Exclusion criteria were chronic diseases, cardiovascular risk factors like arterial hypertension, hyperlipidemia, smoking, obesity, and diabetes mellitus. Subjects were asked not to change their lifestyle during the 1-year period. Each month, a questionnaire was administered and details were given of body weight, height, physical activity, nutrition, conditions of life, and profession. Inquiries were also made about health status, medications taken, acute illnesses, surgical interventions in the past, cardio-

Received 3/21/97; revision accepted 6/25/97.

From the Department of Internal Medicine II, Cardiology, University of Ulm Medical Center, and the GSF-National Research Center for Environment and Health, MEDIS Institute, Neuherberg (M.S.), Germany.

Correspondence to Dr Margit Fröhlich, Abteilung Innere Medizin II, Universität Ulm, Robert-Koch-Straße 8, D-89081 Ulm, Germany. E-mail margit.froehlich @ medizin.uni-ulm.de

Selected Abbreviations and Acronyms

AU = arbitrary units
BV = blood viscosity
CHD = coronary heart disease
CI = confidence interval
CRP = C-reactive protein
Hk = hematocrit
MCV = mean corpuscular volume
PAI-1 = plasminogen activator inhibitor-1
PV = plasma viscosity
RBC = red blood cell
WBC = white blood cell

vascular risk factors, allergies, and family history of cardiovascular diseases.

Fasting venous blood samples were obtained in 4-week intervals during identical conditions (in the morning, at room temperature) for a given individual. EDTA blood was immediately centrifuged, and obtained aliquots were stored at −70°C. Blood pressures were measured in the sitting position.

RBC count, WBC count, hemoglobin, and Hk were measured in a Coulter counter from EDTA blood. Whole BV was determined in EDTA blood by a controlled shear stress rheometer at different shear stresses (100, 200, 500, and 2000 Pa) and 37°C, first at native Hk and second at 45% Hk (Carrimed); PV was measured by a falling ball viscometer at 37°C (Haake) that had been calibrated against a Coulter-Harkness capillary viscometer (Coulter Electronics). RBC aggregation was measured by a photooptic method (MA 1 aggregometer, Myrenne) and RBC deformability by the St George Filtrometer (Carrimed), both in EDTA blood. Nuclepore filters of the same batch (Nuclepore) were taken throughout. All viscosity measurements were done within 2 hours after venipuncture. CRP, ceruloplasmin, and α1-glycoprotein were measured by nephelometry (BNA nephelometer). Total and HDL cholesterol and triglycerides were determined by routine enzymatic tests. LDL cholesterol was calculated by the method of Friedewald et al.[21] Fibrinogen was determined by two different methods: immunonephelometry (Behringwerke) and by the clotting method of Clauss.[22] In addition, plasminogen, α2-macroglobulin, PAI-1 (Kabi Vitrum/Pharmacia) were measured. Haptoglobin was determined by immunodiffusion (Behringwerke AG) and complement factor C4 by immunonephelometry (Behringwerke). Assays were done for interleukin-1α and -1β, tumor necrosis factor α (ELISA, R&D Diagnostics), and cortisol (RIA, Dianovo Immunotech GmbH). RBC sedimentation rate was determined according to the method of Westergren.

Routine laboratory tests, including RBC count, WBC count, hemoglobin, Hk, total cholesterol, HDL cholesterol, triglycerides, fibrinogen (Clauss method[22]), cortisol, and viscosity measurements were subject to continuous quality control. The remaining parameters were determined in batches. Coefficients of variation for repeated measurements were 3.3% and 2.1% for BV 2000 and BV 500, 0.7% for PV, 10.3% for RBC aggregation, 5.9% for RBC deformability, 7% for fibrinogen (measured by immunonephelometry), 3.8% for plasminogen, 7.6% for PAI-1, 3.4% for complement factor C4, 4.0% for ceruloplasmin, and 10% for α1-glycoprotein.

Statistical Methods

Seasonal variation was assumed to follow a sinusoidal curve with a period of 1 year. This curve can be written as

$$f(t)=a+b\cdot\sin(2\pi t/365)+c\cdot\cos(2\pi t/365)$$

where t is the examination day number counted from January 1, 1993. In this model, a represents the annual mean. No seasonal variation occurs when b and c are both zero or, equivalently, when the seasonal difference, ie, the difference between the curve's maximum and minimum, is zero. The seasonal difference is

$$\{\text{abs}\}\ f[\tan(b/c)\cdot 365/2\pi]-f[\tan(b/c)\cdot 365/2\pi+365/2].$$

For each variable investigated, this model was fitted to the data as a multiple-regression model with $\sin(2\pi t/365)$ as one independent variable and $\cos(2\pi t/365)$ as the second independent variable. Random errors were assumed to be normally distributed. The Shapiro test and other sample statistics were used to assess departures from normality. However, for no variable were the departures too serious to necessitate a transformation or any other action. Since the data of each patient represented repeated measurements, a correlated error structure had to be considered. After trying several candidates, the compound symmetry structure appeared to be satisfactory for all variables. The regression coefficients a, b, and c were estimated by restricted maximum likelihood.

The statistical test for the seasonal difference was performed at a nominal 5% level. All computations were carried out using SAS software, version 6.11 for Windows 3.1, in particular, its procedure MIXED.[23]

Results

Rheological Parameters

Table 1 summarizes the results for a variety of hemorheological parameters. The Table presents the annual mean derived from the fitted sinusoidal curve, the month in which the curve's maximum occurred, the value of the

TABLE 1. Seasonal Variations of Hemorheological Parameters

Variable	Annual Mean	Month of Maximum	Seasonal Difference		
			Value	95% CI	*P*
BV 100, mPa · s	7.49	March	0.54	0-1.08	.05
BV 200, mPa · s	5.93	February	0.25	−0.05-0.56	.103
BV 500, mPa · s	4.60	February	0.26	0.12-0.35	.001
BV 2000, mPa · s	3.75	February	0.22	0.12-0.31	<.005
RBC aggregability, AU	8.68	January	0.36	−0.03-0.75	.07
Hemoglobin, g/dL	14.16	December	0.54	0.28-0.81	<.001
Hk, %	42.16	February	2.34	1.63-3.05	<.001
MCV, fL	86.57	March	0.22	0.42-1.5	.001
PV, mPa · s	1.197	January	0.073	0.051-0.049	<.001
Platelet count, 10^9/L	242.72	November	11.27	2.73-19.82	.01
RBC def, AU, inversely	13.84	November	1.49	0.96-2.02	<.001

RBC def indicates RBC deformability. Whole BV was measured at different shear stresses (100, 200, 500, and 2000 mPa).

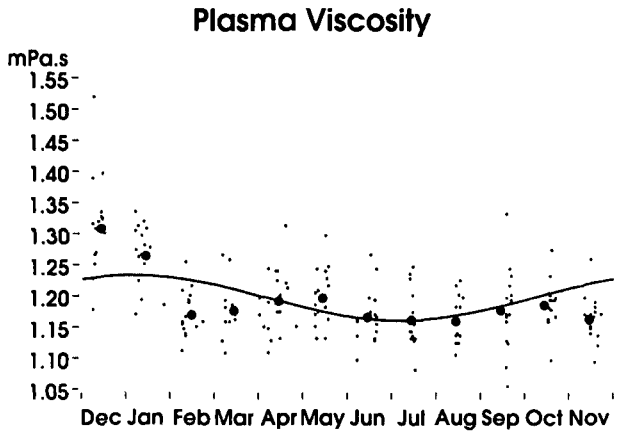

FIG 1. Seasonal variation of PV. 95% CI; ●, monthly means; •, individual observations; −, fitted curve.

seasonal difference together with its 95% CI, and the corresponding probability value.

Peak fitted levels are present during autumn and winter months (October to February). A strong variation is seen for PV (Fig 1) and RBC deformability (Fig 2). PV reaches a maximum fitted level in January, with a value of 1.23 mPa · s. It decreases during summer months, and the minimum is reached in July, with a value of 1.16 mPa · s ($P<.001$ for the seasonal difference). RBC deformability shows a large seasonal difference, with the peak level in November (14.5 AU) and the lowest level in April (13.1 AU) ($P<.001$). Seasonal differences in whole BV, hemoglobin, Hk, MCV, and platelet count are not as pronounced but still statistically significant. However, after standardization on an Hk of 45%, seasonal differences in whole BV decreased and became statistically insignificant for shear stresses of 100 and 2000 mPa. There was no statistically significant seasonal variation in RBC aggregation.

Acute-Phase Reactants

Seasonal variations for acute-phase reactants are shown in Table 2. Statistically significant seasonal variations are seen for fibrinogen (measured immunonephelometrically), α1-glycoprotein, RBC sedimentation rate, cortisol, and PAI-1. Maximum fitted values are found between February and April. Exceptions are RBC sedimentation rate and cortisol, which show a maximum in August. In addition, a weak seasonal difference is found for plasminogen, with a maximum value in August, although not statistically significant. Displacement of the slope from zero is large for α1-glycoprotein. Its fitted maximum level is reached in February (0.7 g/L) and reveals an impressive decline from May to August, with a minimum level of 0.4 g/L ($P<.001$ for the seasonal differences). Seasonal differences for fibrinogen measured nephelometrically (Fig 3) and PAI-1 are less pronounced, although still significant. No statistically significant seasonal changes are seen for fibrinogen measured by the Clauss method,[22] complement factor C4, ceruloplasmin, haptoglobin, and WBC count, although absolute differences in some of these parameters (complement factor C4 and ceruloplasmin) were sizable.

Measurements of α2-macroglobulin, CRP, interleukin-1α, interleukin-1β, and tumor necrosis factor-α did not provide conclusive results, probably attributable to the lack of sensitivity in the methods used.

Lipoproteins

Cholesterol and triglycerides are shown in Table 3. HDL and LDL cholesterol and triglycerides demonstrate a statistically significant seasonal variation. LDL cholesterol peaks in January (3.3 mmol/L) and reaches its lowest levels in July (3.0 mmol/L) ($P=.003$). HDL cholesterol reveals an inverse pattern. The peak value is seen in August (1.55 mmol/L) and the lowest value is reached in February (1.30 mmol/L) ($P<.001$). The result for triglycerides exhibits a peak in September. The lowest level is reached in April ($P<.001$).

No gender differences were seen in the seasonal variation of the parameters under study.

Discussion

We found seasonal variations for a variety of hemorheological and hemostatic parameters, as well as for acute-phase reactants. On the sinusoidal curve fitted to the data, PV reached its maximum level in January (1.23 mPa · s) and declined during summer months. Peak fitted values in winter were also seen for whole BV. The latter can be considered the result of seasonal variations in PV ($P<.001$ for the seasonal difference), RBC deformability ($P<.001$), and Hk ($P<.001$). Various studies demonstrated cardiovascular events to be correlated with Hk (reviewed in Reference 24). There is a twofold to sixfold increase in the risk of CHD and stroke when Hk levels raise above 50%. In the present study, we measured a mean Hk of 42.2% with a seasonal difference of 2.3%. Although this difference is statistically significant, peak values are below the level at which a substantial increase in CHD can be expected.

Seasonal variation was confirmed for fibrinogen, measured quantitatively by nephelometry ($P<.001$). The maximum level was reached in April, and the seasonal difference was 0.32 g/L. This amount of variation may be of considerable clinical relevance, as in the ECAT study, plasma fibrinogen concentrations of patients with coronary events compared with those without differed by the same magnitude (0.28 g/L).[9] This was also confirmed by data from the Caerphilly and Speedwell Collaborative Heart Disease Study.[12] The Bezafibrate Infarction Prevention Study Group demonstrated that an increase in

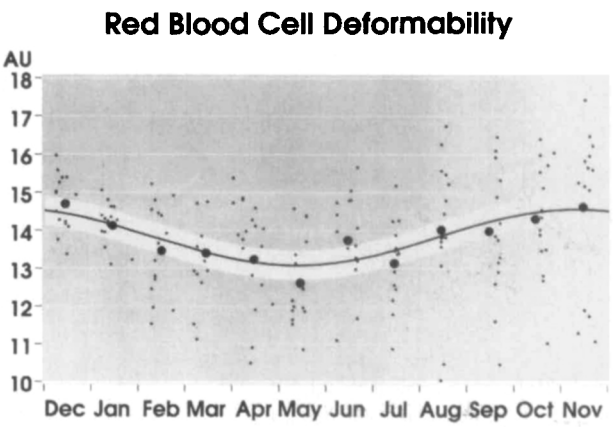

FIG 2. Seasonal variation of RBC deformability. 95% CI; ●, monthly means; •, individual observations; −, fitted curve.

TABLE 2. Seasonal Variation of Acute-Phase Reactants

Variable	Annual Mean	Month of Maximum	Seasonal Difference		
			Value	95% CI	*P*
Fibrinogen (nephelometry), g/L	2.19	April	0.32	0.15-0.49	<.001
Fibrinogen (Clauss), g/L	2.14	July	0.09	−0.08-0.25	.294
α1-Glycoprotein, g/L	0.55	February	0.29	0.24-0.35	<.001
ESR, mm/h	5.25	August	1.86	1.02-2.71	<.001
Cortisol, μg/dL	20.52	August	3.94	1.65-6.23	.001
PAI-1, ng/mL	4.92	March	2.68	0.99-4.37	.002
Plasminogen, %	95.96	August	4.64	0.64-8.63	.023
C4, mg/dL	20.95	October	1.40	−0.37-3.18	.121
WBC count, 10^9/L	5.96	June	0.19	−0.15-0.52	.264
Ceruloplasmin, mg/L	278.22	March	13.73	−13.68-41.14	.324
Haptoglobin, mg/dL	121.8	October	4.32	−10.38-19.02	.563

ESR indicates erythrocyte sedimentation rate.

fibrinogen of 0.75 g/L (equivalent to one SD) increased the risk of CHD by 29% in men.[25]

Fibrinogen measurement by the clotting rate–based assay of Clauss[22] did not reveal a statistically significant seasonal difference. This discrepancy may be explained by interindividual and intraindividual qualitative differences in fibrinogen. In blood, fibrinogen is degraded, which leads to a variety of degradation products with different molecular weights: high-molecular-weight, low-molecular-weight, and very–low-molecular-weight fibrinogen. DeMaat et al[26] showed that different fibrinogen assays have distinct sensitivities for the various fibrinogen forms, leading to different values in fibrinogen measurement.

Considerable seasonal differences were demonstrated for acute-phase reactants like PAI-1 and α1-glycoprotein. Peak values were seen in February and March, respectively. The seasonal difference of PAI-1 level was 2.7 ng/mL. By comparison, Juhan-Vague et al[27] demonstrated a difference of 3.4 ng/mL in PAI-1 antigen levels between patients with myocardial infarction or coronary death compared with the event-free group. α1-Glycoprotein level rises strongly with the severity and extent of CHD. Mori et al[28] reported a difference in α1-glycoprotein of 0.12 g/L comparing patients in the lower quartile of the Gensini's score with the upper quartile. Our data showed a seasonal variation of 0.29 g/L, which was even larger than the difference reported between minor and severe CHD. In reference to the markers of the acute phase, cortisol levels were related inversely, with a maximum level seen in August. This finding may be explained by a cortisol-induced suppression of interleukin-6, which is known to be a potent trigger of the acute-phase reaction.[29] Other acute-phase markers like plasminogen, complement factor C4, and ceruloplasmin did not show statistically significant seasonal differences. In interpreting these results, however, it has to be considered that the study population was relatively small, which limits the statistical power of the results. This is evident, for example, when looking at the above-mentioned study of Mori et al,[28] in which ceruloplasmin differed by 30 mg/L between those with minor and those with severe CHD. In comparison, we observed a seasonal variation of 14 mg/L, which, was not statistically significant.

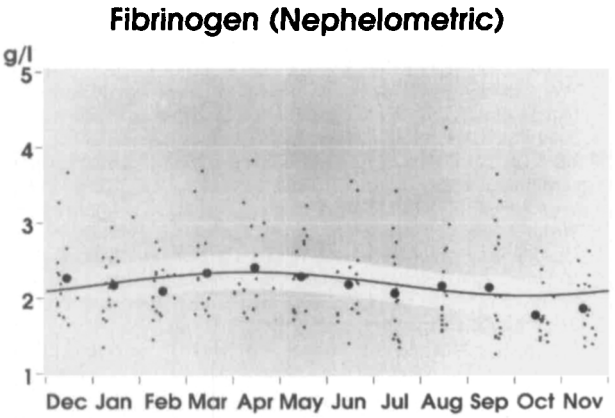

FIG 3. Seasonal variation of fibrinogen concentrations measured by nephelometry. 95% CI; ●, monthly means; •, individual observations; −, fitted curve.

The underlying statistical model to fit the data clearly has limitations because it does not seem to be fully appropriate for all variables. For example, monthly means of several variables were not within the 95%CI band.

In contrast to the present study, other studies measured inflammatory and hemostatic parameters in elderly people, and subjects with clinical signs of infection had not been excluded.[2,4,5,15,16,30] Significant seasonal variations of plasma fibrinogen concentrations, with an increase in winter months, have been reported by Stout and Crawford[16] and Woodhouse et al.[15] This was consistent with an increase in PV.[15] The authors attributed these findings to an increased incidence of upper respiratory tract infections in winter, during which fibrinogen is acting as an acute-phase protein. In the study of Woodhouse et al,[15] there was a strong association between fibrinogen and other markers of inflammation, such as neutrophil count, CRP, and α1-antichymotrypsin, as well as cough and coryza.

However, several longitudinal studies demonstrated an association between WBC count and myocardial infarction.[12,31-39] Furthermore, there is experimental evidence that inflammatory processes play an essential role in the pathogenesis of atherosclerosis.[40,41] In the ECAT study,[9] a positive association has been found between CRP and the incidence of myocardial infarction. CRP levels were higher in patients with unstable angina, compared with a group of patients with stable angina,[42] and high CRP levels were associated with a worse prognosis in patients with unstable angina.[43] However, the pathomechanism leading to elevated CRP and fibrinogen levels in CHD remains unclear.

TABLE 3. Seasonal Variations of Lipoproteins

Variable	Annual Mean	Month of Maximum	Seasonal Difference		
			Value	95% CI	P
HDL cholesterol, mmol/L	1.44	August	0.25	0.17-0.32	<.001
LDL cholesterol, mmol/L	3.16	January	0.29	0.09-0.47	.003
Total cholesterol, mmol/L	4.99	November	0.13	−0.06-0.33	.178
Triglycerides, mmol/L	0.86	September	0.19	0.04-0.28	<.001

The "inflammatory and thrombogenic state" could be due to acute and chronic infections. Some authors have found an association between infectious agents such as *Chlamydia pneumoniae*, *Helicobacter pylori*, herpes simplex virus, and cytomegalovirus and CHD.[44-48] *Chlamydia pneumoniae* has been detected in atherosclerotic plaques of coronary arteries.[49]

In the present study, subjects with acute and chronic infections had been excluded. Furthermore, no seasonal variation in WBC count was seen, which supports the notion that infections were highly unlikely among the subjects during the 1-year period. Despite this, seasonal changes, with elevated levels in winter, were demonstrated for a variety of hemostatic and hemorheological parameters. This has to be attributed to so far unknown factors. Acute and chronic infections may augment basal seasonal changes.

Seasonal changes in LDL concentration, with peak levels in winter months, have been demonstrated by others.[4,5] Robinson et al[30] showed seasonal variations of total cholesterol levels, with a 3% to 5% increase in winter in a large sample of subjects. Seasonal difference of LDL cholesterol in our study was of the same magnitude. Declining HDL cholesterol levels in winter and an increase in summertime have not been reported yet. Previous studies have shown elevated HDL levels in winter.[4,5] In the present study, triglyceride level peaks in September. Gordon et al[5] measured the highest triglyceride levels in autumn, although the values were distributed in an irregular pattern, and Woodhouse et al[4] measured the highest triglyceride levels in January.

Morbidity and mortality from cardiovascular diseases are clearly elevated during winter months. Data from England and Wales suggest a 30% increase in death from these causes during wintertime among elderly people.[1] Data from the MONICA Augsburg coronary event register[50] demonstrate a similar pattern: an impressive increase in the incidence of acute myocardial infarction during winter. Since an occlusive thrombus presents the final pathophysiological hallmark of acute myocardial infarction, parameters influencing blood rheology and blood coagulation are most likely to play an important role. Whole BV is determined by a variety of parameters, including age, sex, nutrition, temperature, and time of day. Further determinants are RBC flexibility, RBC aggregation, Hk, and PV.[51] Several high-molecular, asymmetrical proteins, especially fibrinogen, are the major contributors to PV. In addition, immunoglobulin M, α2-macroglobulin, haptoglobin, and lipoproteins play a role.

In the WHO-MONICA project, regional differences in PV between the Glasgow (mean PV 1.327 mPa · s) and Augsburg (mean PV 1.261 mPa · s) populations were consistent with a two (men) to four (women) times higher coronary event rate in the West of Scotland population.[52] Resch et al[53] found that patients with a second event of stroke or myocardial infarction had higher BV and fibrinogen levels than control subjects, and the Caerphilly and Speedwell Collaborative Heart Disease Studies[12] showed that fibrinogen and PV were strong independent risk factors for CHD. Several other studies have confirmed the association of fibrinogen levels and CHD.[10-14] It is therefore conceivable that elevated fibrinogen levels in winter, as demonstrated in this study and in others,[15,16] correlate with increased PV and thus may contribute to raised mortality from CHD during winter months.

However, the fibrinogen curve did not strictly parallel the graph of PV, where maximum values were shown in January. One possible explanation for this finding is that PV is also markedly influenced by temperature.[51,54] This finding may help to explain the relationship between myocardial infarct deaths and decreasing temperatures that had been demonstrated in three recent studies.[55-57] Classical cardiovascular risk factors seem to be aggravated by cold ambient temperature. Woodhouse et al[2] found that a 1°C decrease in living room temperature was associated with a rise of 1.3 mm Hg in systolic blood pressure and a rise of 0.6 mm Hg in diastolic blood pressure. Robinson et al[30] demonstrated a 3% to 5% increase of cholesterol levels in winter. Levels were strongly and negatively correlated with mean air temperature. This was confirmed in the large Caerphilly and Speedwell Prospective Heart Disease Study[58] in which a fall in temperature of 16°C was associated with higher blood pressures. In addition, fibrinogen and platelet count were significantly inversely associated with air temperature.

In conclusion, the present study shows that a variety of factors influencing blood flow properties are significantly altered in winter and thus may contribute at least in part to the higher incidence of CHD during these months.

References

1. Curwen M. Excess winter mortality: a British phenomenon? *Health Trends*. 1990/91;22:169-175.
2. Woodhouse PR, Khaw KT, Plummer M. Seasonal variation of blood pressure and its relationship to ambient temperature in an elderly population. *J Hypertens*. 1993;11:1267-1274.
3. Brennan PJ, Greenberg G, Miall WE, Thompson SF. Seasonal variation in arterial blood pressure. *Br Med J*. 1982;285:919-923.
4. Woodhouse PR, Khaw KT, Plummer M. Seasonal variation of serum lipids in an elderly population. *Age Ageing*. 1993;22:273-278.
5. Gordon DJ, Hyde J, Trost DC, Whaley FS, Hannan PJ, Jacobs DR, Ekelund LG. Cyclic seasonal variation in plasma lipid and lipoprotein levels: the Lipid Research Clinics Coronary Primary Prevention Trial placebo group. *J Clin Epidemiol*. 1988;41:679-689.
6. Jarrett RJ, Murrells TJ, Shipley MJ, Hall T. Screening blood glucose values: effects of season and time of day. *Diabetologia*. 1984;27:574-577.
7. Mizuno K, Satomura K, Miyamoto A, Arakawa K, Shibuya T, Arai T, Kurita A, Nakamura H, Ambrose JA. Angioscopic evaluation of coronary-artery thrombi in acute coronary syndromes. *N Engl J Med*. 1992;326:287-291.

8. Merlini PA, Bauer KA, Oltrona L, Ardissino D, Cattaneo M, Belli C, Mannucci PM, Rosenberg RD. Persistent activation of coagulation mechanism in unstable angina and myocardial infarction. *Circulation*. 1994;90:61-68.
9. Thompson SG, Kienast J, Pyke SDM, Haverkate F, van de Loo JCW. Hemostatic factors and the risk of myocardial infarction or sudden death in patients with angina pectoris. *N Engl J Med*. 1995; 332:635-641.
10. Meade TW, Brozovic M, Chakarabarti RR, Haines AP, Imeson JD, Mellows S, Miller GJ, North WRS, Stirling Y, Thompson SG. Haemostatic function and ischaemic heart disease: principal results of the Northwick Park Heart Study. *Lancet*. 1986;2:533-537.
11. Kannel WB, Wolf PA, Castelli PA, D'Agostino RB. Fibrinogen as a risk of cardiovascular disease: the Framingham study. *JAMA*. 1987;258:1183-1186.
12. Yarnell JWG, Baker IA, Sweetnam PM, Bainton D, O'Brien JR, Whitehead PJ, Elwood PC. Fibrinogen, viscosity, and white blood cell count are major risk factors for ischemic heart disease: the Caerphilly and Speedwell Collaborative Heart Disease Studies. *Circulation*. 1991;83:836-844.
13. Balleisen L, Schulte H, Assmann G, Epping PH, van de Loo J. Coagulation factors and the progress of coronary heart disease. *Lancet*. 1987;2:461.
14. Cremer P, Nagel D, Böttcher B, Seidel D. Fibrinogen: ein koronarer Risikofaktor. *Diagn Labor*. 1992;42:28-35.
15. Woodhouse PR, Khaw KT, Plummer M, Foley A, Meade TW. Seasonal variations of plasma fibrinogen and factor VII activity in the elderly: winter infections and death from cardiovascular disease. *Lancet*. 1994;343:435-439.
16. Stout RW, Crawford V. Seasonal variations in fibrinogen concentrations among elderly people. *Lancet*. 1991;338:9-13.
17. Saradeth T, Resch KL, Maier A, Ernst E. Physiological variations of fibrinogen levels in healthy volunteers. *Perfusion*. 1991;12:443. Abstract.
18. Cook NS, Ubben D. Fibrinogen as a major risk factor in cardiovascular disease. *Trends Pharmacol Sci*. 1990;11:444-451.
19. deMaat MPM, Kofflard M, Pietersma A, Sluiter W, Kluft C. Coronary artery disease and inflammation: association of coronary artery disease with fibrinogen, C-reactive protein, cytokines and smoking. In: deMaat MPM. *Regulation and Modulation of the Plasma Fibrinogen Level*. Rotterdam, Netherlands: University of Rotterdam; 1995:89-101. Thesis.
20. Ernst E. *Hämorheologie, Theorie, Klinik, Therapie*. New York, NY: Schattauer Verlag; 1989:3-12.
21. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem*. 1972;18:499-502.
22. Clauss A. Gerinnungsphysiologische Schnellmethode zur Bestimmung des Fibrinogens. *Acta Haematol*. 1957;17:237-246.
23. SAS Institute Inc. *SAS/STAT Software: Changes and Enhancements Through Release 6.11*. Cary, NC: SAS Institute Inc; 1996.
24. Ernst E. Haematocrit and cardiovascular risk. *J Intern Med*. 1995; 237:527-528.
25. Benderly M, Graff E, Reicher-Reiss H, Behar S, Brunner D, Goldbourt U. Fibrinogen is a predictor of mortality in coronary heart disease patients. *Arterioscler Thromb Vasc Biol*. 1996;16:351-356.
26. deMaat MPM, Kameling SWA, Kluft C. The sensitivity and specificity of some clotting rate and immunological fibrinogen assays for high, low and low molecular weight forms of fibrinogen. In: deMaat MPM. *Regulation and Modulation of the Plasma Fibrinogen Level*. Rotterdam, Netherlands: University of Rotterdam; 1995:47-59. Thesis.
27. Juhan-Vague I, Pyke SDM, Alessi MC, Jespersen J, Haverkate F, Thompson SG. Fibrinolytic factors and the risk of myocardial infarction or sudden death in patients with angina pectoris. *Circulation*. 1996;94:2057-2063.
28. Mori T, Sasaki J, Kawaguchi H, Handa K, Takada Y, Matsunaga A, Kono S, Arakawa K. Serum glycoproteins and severity of coronary atherosclerosis. *Am Heart J*. 1995;129:234-238.
29. Akira S, Taga T, Kishimoto T. Interleukin-6 in biology and medicine. *Adv Immunol*. 1993;54:1-78.
30. Robinson D, Bevan EA, Hinohara S, Takahashi T. Seasonal variation in serum cholesterol levels: evidence from the UK and Japan. *Atherosclerosis*. 1992;95:15-24.
31. Weijenberg MP, Feskens EJM, Kromhout D. White blood cell count and the risk of coronary heart disease and all-cause mortality in elderly men. *Arterioscler Thromb Vasc Biol*. 1996;16:499-503.
32. Ernst E, Hammerschmidt DE, Bagge U, Matrai A, Dormandy JA. Leukocytes and the risk of ischemic diseases. *JAMA*. 1987;257: 2318-2324.
33. Ensurd K, Grimm R. The white blood cell count and risk for coronary heart disease. *Am Heart J*. 1992;124:207-213.
34. Hansen LK, Grimm RH, Neaton JD. The relationship of white blood cell count to other cardiovascular risk factors. *Int J Epidemiol*. 1990;19:881-888.
35. Grimm RH, Neaton JD, Ludwig W. Prognostic importance of the white blood cell count for coronary, cancer, and all-cause mortality. *JAMA*. 1985;254:1932-1937.
36. Lowe GDO, Machado SG, Krol WF, Barton BA, Forbes CD. White blood cell count and haematocrit as predictors of coronary recurrence after myocardial infarction. *Thromb Haemost*. 1985;54:700-703.
37. Kannel WB, Anderson K, Wilson PWF. White blood cell count and cardiovascular disease: insights from the Framingham study. *JAMA*. 1992;267:1253-1256.
38. Gillum RF, Ingram DD, Makuc DM. White blood cell count, coronary heart disease, and death: the NHANES I epidemiologic follow-up study. *Am Heart J*. 1993;125:855-863.
39. Friedman GD, Tekawa I, Grimm RH, Manolio T, Shannon SG, Sidney S. The leukocyte count: correlates and relationship to coronary risk factors: the CARDIA Study. *Int J Epidemiol*. 1990;19:889-893.
40. Munro JM, Cotran RS. The pathogenesis of atherosclerosis: atherogenesis and inflammation. *Lab Invest*. 1988;58:249-261.
41. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990's. *Nature*. 1993;362:801-809.
42. Berck BC, Weintraub WS, Alexander RW. Elevation of C-reactive protein in "active' coronary artery disease. *Am J Cardiol*. 1990;65:168-172.
43. Liuzzo G, Biasucci LM, Gallimore JR, Grillo RL, Rebuzzi AG, Pepys MB, Maseri A. The prognostic value of C-reactive protein and serum amyloid A protein in severe unstable angina. *N Engl J Med*. 1994;331:417-424.
44. Saikku P, Mattila K, Nieminen MS, Huttunen JK, Leinonen M, Ekman MR, Mäkelä PH, Valtonen V. Serological evidence of an association of a novel *Chlamydia*, TWAR, with chronic coronary heart disease and acute myocardial infarction. *Lancet*. 1988;2:983-985.
45. Saikku P, Leinonen M, Tenkanen L, Linnanmäki E, Ekman MR, Manninen V, Mänttäri M, Frick MH, Huttunen JK. Chronic *Chlamydia pneumoniae* infection as a risk factor for coronary heart disease in the Helsinki Heart Study. *Ann Intern Med*. 1992;116:273-278.
46. Thom DH, Wang S-P, Grayston JT, Siscovick DS, Stewart DK, Kronmal RA, Weiss NS. *Chlamydia pneumoniae* strain TWAR antibody and angiographically demonstrated coronary artery disease. *Arterioscler Thromb*. 1991:11:547-551.
47. Patel P, Mendall MA, Carrington D, Strachan DP, Leatham E, Molineaux N, Levy J, Blakeston C, Seymour CA, Camm AJ, Northfield TC. Association of *Helicobacter pylori* and *Chlamydia pneumoniae* infections with coronary heart disease and cardiovascular risk factors. *Br Med J*. 1995;311:711-714.
48. Yamashiroya HM, Ghosh L, Yang R, Robertson AL. *Herpesviridae* in the coronary arteries and aorta of young trauma victims. *Am J Pathol*. 1988;130:71-79.
49. Kuo C-C, Shor A, Campbell LA, Fukushi H, Patton DL, Grayston JT. Demonstration of *Chlamydia pneumoniae* in atherosclerotic lesions of coronary arteries. *J Infect Dis*. 1993;167:841-849.
50. Löwel H, Janku D, Eberle E, Lewis M, Hörmann A, Koenig W, Gostomzyk J, Keil U. *Data-Book Coronary Event Register, Region Augsburg: MONICA Project 1985*. Munich, Germany: GSF Bericht 39/80, Gesellschaft für Strahlen und Umweltforschung; 1990.
51. Lowe GDO. Rheological influences on thrombosis. *Baillieres Clin Heamatol*. 1994;7:573-589.
52. Koenig W, Sund M, Lowe GDO, Lee AJ, Resch KL, Tunstall-Pedoe H, Keil U, Ernst E. Geographical variations in plasma viscosity and relation to coronary event rates. *Lancet*. 1994;344:711-714.
53. Resch KL, Ernst E, Matrai A, Paulsen HF. Fibrinogen and viscosity as risk factors for subsequent cardiovascular events in stroke survivors. *Ann Intern Med*. 1992;117:371-375.
54. Keatinge WR, Coleshaw SRK, Cotter F, Mattock M, Murphy M, Chelliah R. Increases in platelet and red cell counts, blood viscosity, and arterial pressure during mild surface cooling: factors in mortality from coronary and cerebral thrombosis in winter. *Br Med J*. 1984;289:1405-1408.
55. Frost DB, Auliciems A, deFreitas C. Myocardial infarct death and temperature in Auckland, New Zealand. *Int J Biometeorol*. 1992; 36:14-17.
56. Frost DB, Auliciems A. Myocardial infarct death, the population at risk, and temperature habituation. *Int J Biometeorol*. 1993;37:46-51.
57. Enquselassie F, Dobson AJ, Alexander HM, Steele PL. Seasons, temperature and coronary disease. *Int J Epidemiol*. 1993;22:632-636.
58. Elwood PC, Beswick A, O'Brien JR, Renaud S, Fifield R, Limb ES, Bainton D. Temperature and risk factors for ischaemic heart disease in the Caerphilly prospective study. *Br Heart J*. 1993;70:520-523.