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Association of European population levels of thrombotic and inflammatory factors with risk of coronary heart disease: the MONICA Optional Haemostasis Study†

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Aims Classical risk factors do not fully explain international differences in risk of coronary heart disease (CHD). We therefore measured thrombotic and inflammatory markers in a substudy of the WHO MONICA project and correlated these with CHD event rates.

Methods and results We measured levels of fibrinogen (clottable and nephelometric), von Willebrand factor (vWf), tissue plasminogen activator antigen, plasminogen activator inhibitor activity, fibrin D-dimer, plasma viscosity, C-reactive protein, and total cholesterol in 12 MONICA populations (listed at the end of this paper), all but one European. Men and women aged 45–64 years were studied from 10 countries. All samples were collected using a carefully standardized protocol, and analysed centrally. Results were available for 3996 subjects (nephelometric fibrinogen and viscosity), 2378 subjects (other thrombotic assays), and 1757 subjects (C-reactive protein and total cholesterol). Significant differences in levels of thrombotic and inflammatory factors exist in MONICA populations mainly from European countries. These differences persist after adjustment for age, smoking habit, and body mass index. Cross-sectional correlations between coronary event rates and these thrombotic/inflammatory markers were significant for vWF antigen in both sexes, nephelometric fibrinogen in men, and D-dimer in women.

Conclusion In particular, vWF, nephelometric fibrinogen, and D-dimer should be examined in further research as potential risk factors which may help explain differences in coronary risk between European populations.

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Introduction

The World Health Organization MONICA project has examined the relationship between secular trends in the classical risk factors for coronary heart disease (CHD) (hypercholesterolaemia, hypertension, cigarette smoking, and also body mass index) and secular trends in the incidence of CHD in 38 centres in 21 countries.[1,2] A recent major report from the MONICA investigators concluded that the classical risk factors only partly explained the population trends in CHD and that medical interventions and other factors may provide opportunities for improved prevention and treatment.[3] In cohort studies, classical risk factors frequently predict subsequent CHD rather weakly,[4] and during the past 20 years, interest has grown in thrombotic factors,[5] which have been shown to be strongly predictive of subsequent CHD in meta-analyses of prospective studies.[6–10] Evidence is strongest for fibrinogen,[5,6] plasma viscosity,[7] von Willebrand factor (vWF; an endothelial marker and an important co-factor in platelet adhesion and aggregation),[8] fibrin D-dimer (a marker of fibrin turnover),[9] and tissue plasminogen activator (t-PA) antigen (an endothelial and fibrinolytic marker),[10] each of which have been shown to be independent predictors of CHD in such meta-analyses. Recent interest has focussed on inflammatory markers such as C-reactive protein, which has been linked to CHD in prospective studies[11,12] and in meta-analyses.[6,13]

The aim of the present study was to correlate these risk markers with population levels of CHD risk in a multi-centre substudy of the MONICA project based on two central laboratories, each of which was responsible for a particular set of thrombotic assays. The study design was ecological and examined the associations between population levels of thrombotic risk factors in the substudy populations and the contemporary incidence rates for CHD in men and in women in these populations.

Subjects and methods

Methods

MONICA centres were invited to participate in this study as a component of the final MONICA surveys in each centre from 1991 onwards.

Study populations

In order to provide manageable numbers of samples, blood samples were only requested for the 45–64 year age group. The target sample sizes were 200 males and 200 females from each centre as proposed in the main MONICA study to obtain adequate statistical power to detect changes in the major coronary risk factors within centres over a 10 year period.[14] Selected demographic and personal data were requested for each participant including age, smoking habit, and body mass index.

Blood collection, separation, and analysis

A standard protocol for blood sampling was used in each study centre. Venous blood samples were obtained with minimum venous stasis using 21 gauge butterfly needles and K_2 EDTA vacutainers obtained from Sarstedt (UK). In most centres an additional trisodium citrate tube (Sarstedt, UK) was also collected, usually on a subsample (up to 100 males and 100 females), but, for the centres in Italy and Spain, all eligible subjects were sampled, since Mediterranean centres were under-represented. Unless collection materials were obtained (as specified) by the local investigators, all materials were centrally supplied to the study centres.

Plasma was separated within 6 h of collection and subsequently stored at −20 to −80°C until air freighted to Belfast for distribution to two central laboratories. EDTA samples were freighted to the Department of Haematology, Frenchay Hospital, Bristol, where plasma viscosity and plasma fibrinogen were measured using a Coulter Harkness viscometer[15,16] and a Thorpe nephelometer,[17,18] respectively. Plasma viscosity assays were performed locally in Augsburg and Glasgow, using identical viscometers, but samples were not available from these centres to measure nephelometric fibrinogen.

Other thrombotic assays were performed in citrated samples (as appropriate) in the University Department of Medicine, Glasgow Royal Infirmary, as described previously.[12,19] Because of limited resources, target sample sizes were selected by systematic one in two sampling from all centres except those in Italy and Spain in which all available samples were used. Enzyme-linked immunosorbent assays (ELISAs) were used for measurement of D-dimer (AGEN, Parsippany, NJ, USA), t-PA (Biopool, Umea, Sweden), and vWF antigen (Dako, Copenhagen, Denmark). PAI-1 (PA inhibitor-1) activity was measured using a chromogenic assay (Chromogenix, Stockholm, Sweden). Clottable fibrinogen was measured by an automated Clauss assay in a Coag-A-Mate X 2 coagulometer (Organon Teknika, Cambridge). C-reactive protein (high sensitivity nephelometry, Dade Behring) and total cholesterol were assayed using residual EDTA plasma at the University Department of Medicine, Glasgow Royal Infirmary, as described previously.[12] PAI-1 and vWF could not be performed on Augsburg samples due to lack of citrated plasma samples. We assayed both nephelometric and clottable fibrinogen because of our previous finding that nephelometric fibrinogen was a stronger predictor of CHD in the Caerphilly and Speedwell cohorts.[17]

Samples were collected during the period 1991–1996. The majority of samples were assayed within 12–18 months of collection and previous studies had indicated the stability of frozen samples during this period.[12] In both laboratories, quality control was maintained using duplicate samples and internal laboratory standards; additional duplicate samples whose identity was blinded to the participating laboratory were assayed for large population studies of cardiovascular risk factors.[19] The coefficient of variation of the blinded duplicate samples ranged from 2.3% in the case of plasma viscosity to 27% in the case of D-dimer antigen. As noted elsewhere,[20] the highly skewed distribution contributed to the large coefficient for D-dimer but the discrimination between subjects was excellent. In the Glasgow laboratory it was possible to run samples from up to three centres concurrently in order to reduce between-centre differences due to between-batch variations. C-reactive protein and cholesterol were assayed using residual EDTA samples at a later date in the Glasgow laboratory 2–9 years after initial collection. Previous studies have suggested long-term stability of these plasma components under similar storage conditions.[12,14]

Table 1 Age-standardized coronary event rates per 100 000 and smoking prevalence in MONICA populations during final surveys[a]

Centre	Men		Women	
	Event rate	% smokers	Event rate	% smokers
Finland, N. Karelia	522	26	101	12
Germany, Augsburg	251	30	65	21
Iceland	382	23	82	31
Italy, Friuli	240	29	45	22
Lithuania, Kaunas	522	35	89	4
Poland, Tarnobrzeg	476	54	110	21
Poland, Warsaw	605	52	159	34
Russia, Novosibirsk	490	60	127	6
Spain, Catalonia	230	41	39	15
UK, Belfast	603	29	175	25
UK, Glasgow	724	41	268	41

[a]The Hungarian MONICA centre did not provide validated coronary event data but did participate in this substudy.

Statistical methods

Essential demographic and personal data were obtained from each study centre and matched to the laboratory results. Unfortunately due to missing data, target numbers of usable results were not achieved in the majority of centres. Response rates for the main MONICA surveys, from which these samples were taken as a substudy, are reported elsewhere.[14] Variables used in this analysis are the key variables which correlate with both thrombotic factors, C-reactive protein, and CHD risk: age, smoking habit, and body mass index.[19,21] Distributions of each thrombotic variable were examined and a small number of values considered as extremely likely to result from biological degradation, laboratory, or clerical error, were removed. These mainly related to plasma viscosity values greater than three, mainly found in samples from Iceland. Ten values were excluded in total. C-reactive protein results for Lithuania were also excluded as they were clearly deviant and, after a detailed investigation, this was considered to be due to a biological problem with the serum sample. The distributions of D-dimer antigen and C-reactive protein showed strong positive skews and were logarithmically transformed. Results for these variables are reported as geometric means with interquartile (IQ) ranges.

Mean values were compared between the sexes using independent samples *t*-tests in a preliminary analysis. Further analyses were performed separately for each sex and mean values for each study centre were adjusted for the potential confounding variables: age, smoking habit, and body mass index. Adjusted means were calculated using the following values (M/F): age 54.8/54.6 years, smoking habit 41/20% current smokers, and body mass index 27.1/27.8 kg/m^2. If there was evidence of significant heterogeneity between centres in the relationship between a thrombotic or inflammatory variable and a confounder, then an interaction between centre and confounder was included in the regression model thus permitting the relationship between variable and confounder to differ from centre to centre. Plots of regression analysis residuals indicated the need for logarithmic transformation of D-dimer antigen and C-reactive protein but otherwise confirmed the basic regression assumptions.

A cross-sectional, ecological analysis was carried out using MONICA registration data for coronary events in each study centre.[22] Event rates were calculated by extrapolation from the published data to the survey study period for each population centre. Spearman correlation coefficients were calculated using adjusted population mean values for each thrombotic variable and event rates. Event rates used are shown in *Table 1* below, which also shows prevalence rates for smoking habit for the final MONICA surveys. Comprehensive details of the whole MONICA project are available elsewhere.[23]

Results

Twelve study centres from 11 countries participated, providing a total of 3996 EDTA and 2378 citrate plasma samples. Samples were obtained for 1942 males and 2054 females (EDTA) and 1163 males and 1215 females (citrate).

All thrombotic variables showed positive associations with age except PAI-1 in men, which showed decreasing activity with age. All variables, with the exception of vWF and PAI-1, showed a positive association with smoking habit, although the effects were not always consistent in men and in women. Body mass index showed positive associations with t-PA antigen, PAI-1 activity, nephelometric fibrinogen, plasma viscosity, and C-reactive protein in both sexes, and a positive association with clottable fibrinogen, D-dimer, and vWf in women only.

Because of the possibility of differences in demographic and personal characteristics between study populations, all results for individual centres were adjusted for age, smoking habit, and body mass index. Significant interactions suggested that there was centre-to-centre heterogeneity in the relationships between these three confounders and some of the thrombotic and inflammatory variables. For relationships with age, such heterogeneity was observed for D-dimer (men and women) and for plasma viscosity, nephelometric fibrinogen, PAI-1, clottable fibrinogen, and t-PA (women only). For relationships with body mass index, heterogeneity was observed for nephelometric fibrinogen (men and women), cholesterol (men only), and plasma viscosity (women only). For relationships with smoking habit, heterogeneity was observed in plasma viscosity, clottable fibrinogen, and C-reactive protein (women only). Where such heterogeneity was observed, the adjustments permitted a different relationship between variable and confounder in each centre. *Table 2* shows unadjusted and adjusted mean values for each of the study centres for males and for females for clottable fibrinogen, D-dimer, t-PA, PAI-1, and vWF.

Unadjusted mean values were significantly higher for women for D-dimer ($P < 0.0001$) and higher for men for t-PA ($P < 0.0001$) and vWF ($P = 0.006$). All biological samples showed a range of values across the population

Table 2 Mean levels of clottable fibrinogen, D-dimer, t-PA, PAI-1, and vWF in MONICA centres, unadjusted and adjusted for age, smoking habit, and body mass index (citrate sample)

Centre	No. of subjects	Clottable fibrinogen (g/L)		D-dimer (ng/mL)		t-PA (ng/mL)		PAI-1 (% pool)		vWF (IU/dL)	
		Mean (SD)	Adjusted mean (SE)	Geometric mean (IQ range)	Adjusted geometric mean	Mean (SD)	Adjusted mean (SE)	Mean (SD)	Adjusted mean (SE)	Mean (SD)	Adjusted mean (SE)
Men											
Finland, N. Karelia	73	3.28 (0.72)	3.32 (0.09)	88 (62,133)	89	8.0 (2.8)	7.7 (0.48)	62 (35)	58 (4.3)	136 (53)	134 (5.6)
Germany, Augsburg	93	–	–	95 (61,141)	95	10.8 (6.1)	10.8 (0.42)	–	–	–	–
Hungary, Budapest	78	3.55 (0.82)	3.53 (0.09)	99 (60,148)	97	13.8 (6.4)	13.3 (0.46)	92 (48)	88 (4.1)	175 (57)	172 (5.5)
Iceland	94	3.35 (0.83)	3.38 (0.08)	79 (54,111)	81	11.3 (3.6)	11.0 (0.45)	116 (50)	114 (4.0)	120 (41)	119 (5.3)
Italy, Friuli	184	3.14 (0.63)	3.19 (0.06)	70 (49,104)	72	9.8 (4.3)	10.0 (0.30)	119 (35)	120 (2.7)	127 (53)	128 (3.6)
Lithuania, Kaunas	42	3.22 (0.86)	3.19 (0.12)	83 (56,117)	78	9.0 (3.9)	8.8 (0.65)	77 (40)	80 (5.9)	142 (51)	136 (7.7)
Poland, Tarnobrzeg	85	3.44 (0.93)	3.43 (0.08)	105 (65,148)	108	10.6 (4.6)	11.3 (0.44)	65 (43)	70 (4.0)	152 (48)	154 (5.3)
Poland, Warsaw	80	3.36 (0.74)	3.34 (0.08)	93 (64,129)	93	10.7 (3.7)	10.3 (0.46)	88 (36)	81 (4.1)	137 (48)	139 (5.6)
Russia, Novosibirsk	104	2.96 (0.79)	2.88 (0.07)	94 (65,133)	91	9.0 (4.0)	9.1 (0.40)	64 (37)	68 (3.6)	132 (47)	131 (4.8)
Spain, Catalonia	137	3.05 (0.61)	3.05 (0.06)	53 (32,80)	53	10.2 (3.3)	10.0 (0.35)	106 (38)	103 (3.4)	125 (45)	125 (4.2)
UK, Belfast	98	3.34 (0.8)	3.34 (0.08)	94 (65,127)	93	8.6 (3.7)	8.9 (0.41)	75 (36)	79 (3.7)	140 (44)	140 (4.9)
UK, Glasgow	95	3.26 (0.94)	3.27 (0.08)	105 (66,146)	100	12.7 (4.5)	12.9 (0.43)	73 (45)	73 (3.8)	149 (54)	149 (5.0)
Overall mean	1163	3.24 (0.79)	–	83 (54,124)	–	10.4 (4.6)	–	89 (45)	–	137 (51)	–
Women											
Finland, N. Karelia	88	3.65 (0.68)	3.60 (0.09)	118 (79,171)	112	7.3 (3.1)	6.9 (0.36)	57 (24)	52 (3.7)	127 (48)	124 (4.9)
Germany, Augsburg	102	–	–	113 (74,167)	111	7.3 (3.2)	7.5 (0.33)	–	–	–	–
Hungary, Budapest	99	3.51 (0.83)	3.50 (0.08)	94 (65,133)	90	12.1 (4.8)	11.7 (0.33)	103 (45)	100 (3.5)	164 (61)	163 (4.6)
Iceland	75	3.35 (0.83)	3.36 (0.12)	108 (74,146)	111	9.1 (3.4)	8.6 (0.42)	99 (43)	99 (4.4)	116 (38)	117 (5.7)
Italy, Friuli	189	3.28 (0.65)	3.32 (0.06)	67 (49,97)	69	7.9 (3.4)	8.2 (0.25)	115 (33)	120 (2.5)	114 (39)	115 (3.3)
Lithuania, Kaunas	64	3.24 (1.04)	3.24 (0.11)	97 (69,125)	93	10.1 (4.1)	9.4 (0.44)	74 (38)	70 (4.6)	118 (55)	119 (6.1)
Poland, Tarnobrzeg	100	3.38 (0.72)	3.37 (0.08)	117 (78,161)	117	8.9 (3.7)	8.7 (0.33)	75 (35)	71 (3.5)	144 (37)	145 (4.6)
Poland, Warsaw	88	3.17 (0.72)	3.16 (0.08)	122 (91,159)	121	8.9 (2.9)	8.9 (0.35)	79 (32)	78 (3.6)	143 (38)	143 (4.9)
Russia, Novosibirsk	93	2.66 (0.87)	2.61 (0.08)	120 (78,156)	117	8.9 (3.6)	8.8 (0.35)	75 (42)	74 (3.6)	136 (47)	135 (4.8)
Spain, Catalonia	144	3.14 (0.61)	3.15 (0.07)	76 (47,109)	79	8.0 (2.5)	8.0 (0.27)	112 (38)	110 (3.4)	113 (38)	114 (3.8)
UK, Belfast	72	3.41 (0.78)	3.46 (0.09)	118 (72,146)	117	6.3 (3.0)	6.9 (0.39)	68 (21)	74 (4.1)	133 (42)	135 (5.5)
UK, Glasgow	101	3.58 (1.24)	3.46 (0.08)	118 (74,176)	117	10.8 (5.4)	10.6 (0.33)	77 (48)	78 (3.4)	152 (60)	152 (4.6)
Overall mean	1215	3.30 (0.84)	–	99.5 (66.0,145.5)	–	8.71 (3.91)	–	89 (42)	–	131.3 (48.5)	–

Table 3 Mean levels of nephelometric fibrinogen and plasma viscosity in MONICA centres unadjusted and adjusted for age, smoking habit, and body mass index (EDTA sample)

Centre	Men					Women				
	No. of subjects	Nephelometric fibrinogen (g/L)		Plasma viscosity (mPa.s)		No. of subjects	Nephelometric fibrinogen (g/L)		Plasma viscosity (mPa.s)	
		Mean (SD)	Adjusted mean (SE)	Mean (SD)	Adjusted mean (SE)		Mean (SD)	Adjusted mean (SE)	Mean (SD)	Adjusted mean (SE)
Finland, N. Karelia	181	3.74 (0.67)	3.75 (0.07)	1.65 (0.09)	1.65 (0.01)	226	3.74 (0.68)	3.71 (0.05)	1.66 (0.08)	1.66 (0.01)
Germany, Augsburg	299	–	–	1.59 (0.08)	1.58 (0.01)	300	–	–	1.58 (0.07)	1.59 (0.02)
Hungary, Budapest	147	4.05 (0.90)	4.00 (0.07)	1.70 (0.12)	1.70 (0.01)	183	4.23 (0.88)	4.14 (0.06)	1.68 (0.11)	1.68 (0.01)
Iceland	94	3.79 (0.90)	3.76 (0.09)	1.66 (0.12)	1.66 (0.01)	75	3.38 (0.89)	3.28 (0.11)	1.61 (0.26)	1.65 (0.02)
Italy, Friuli	196	3.66 (0.69)	3.68 (0.06)	1.62 (0.09)	1.63 (0.01)	197	3.72 (0.68)	3.80 (0.06)	1.63 (0.09)	1.64 (0.01)
Lithuania, Kaunas	42	4.40 (0.62)	4.42 (0.16)	1.76 (0.09)	1.75 (0.02)	64	4.74 (0.71)	4.60 (0.10)	1.80 (0.10)	1.78 (0.02)
Poland, Tarnobrzeg	188	4.50 (0.95)	4.52 (0.06)	1.80 (0.12)	1.81 (0.01)	217	4.41 (0.78)	4.39 (0.06)	1.80 (0.10)	1.80 (0.01)
Poland, Warsaw	80	4.69 (1.09)	4.54 (0.10)	1.60 (0.1)	1.66 (0.01)	89	4.57 (0.93)	4.54 (0.08)	1.70 (0.14)	1.70 (0.01)
Russia, Novosibersk	199	3.96 (0.78)	3.95 (0.06)	1.70 (0.1)	1.70 (0.01)	180	4.14 (0.86)	4.18 (0.06)	1.73 (0.11)	1.73 (0.01)
Spain, Catalonia	159	3.36 (0.62)	3.34 (0.07)	1.63 (0.1)	1.63 (0.01)	196	3.67 (0.70)	3.68 (0.06)	1.66 (0.09)	1.66 (0.01)
UK, Belfast	285	4.14 (1.04)	4.17 (0.05)	1.62 (0.09)	1.62 (0.01)	252	3.91 (0.93)	4.06 (0.05)	1.62 (0.10)	1.64 (0.01)
UK, Glasgow	72	–	–	1.68 (0.10)	1.68 (0.01)	76	–	–	1.67 (0.11)	1.66 (0.01)
Overall mean	1942	3.98 (0.92)	–	1.67 (0.12)	–	2054	4.00 (0.87)	–	1.68 (0.13)	–

Table 4 Mean levels of C-reactive protein (CRP) and cholesterol in MONICA centres unadjusted and adjusted for age, smoking habit, and body mass index (residual EDTP sample)

Centre	Men					Women				
	No. of subjects	CRP (mg/L)		Cholesterol (mmol/L)		No. of subjects	CRP (mg/L)		Cholesterol (mmol/L)	
		Geometric mean (IQ range)	Adjusted geometric mean	Mean (SD)	Adjusted mean (SE)		Geometric mean (IQ range)	Adjusted geometric mean	Mean (SD)	Adjusted mean (SE)
Finland, N. Karelia	70	1.26 (0.76,2.48)	1.22	5.75 (0.86)	5.74 (0.13)	84	1.34 (0.60,3.22)	1.10	5.70 (0.86)	5.66 (0.11)
Germany, Augsburg	–	–	–	–	–	–	–	–	–	–
Hungary, Budapest	–	–	–	–	–	–	–	–	–	–
Iceland	90	1.38 (0.56,2.75)	1.35	5.83 (1.06)	5.90 (0.12)	66	1.31 (0.58,3.63)	1.35	5.79 (1.16)	5.89 (0.14)
Italy, Friuli	173	1.14 (0.56,2.41)	1.20	5.49 (1.08)	5.49 (0.08)	179	1.21 (0.55,2.48)	1.37	5.99 (0.96)	5.99 (0.08)
Lithuania, Kaunas	14	–	–	5.07 (0.29)	5.05 (0.29)	36	–	–	5.71 (1.07)	5.66 (0.18)
Poland, Tarnobrzeg	62	1.45 (0.70,2.83)	1.59	5.97 (1.30)	6.18 (0.14)	96	1.52 (0.69,3.16)	1.36	5.95 (1.09)	5.99 (0.10)
Poland, Warsaw	64	1.26 (0.47,3.39)	1.14	5.66 (0.92)	5.64 (0.13)	79	1.15 (0.60,2.44)	1.11	5.92 (1.07)	5.89 (0.11)
Russia, Novosibersk	102	1.34 (0.61,2.75)	1.29	5.20 (0.98)	5.17 (0.11)	80	1.30 (0.58,2.75)	1.27	5.87 (1.22)	5.86 (0.11)
Spain, Catalonia	74	1.32 (0.64,2.36)	1.25	4.77 (0.79)	4.74 (0.13)	62	1.34 (0.54,3.49)	1.33	5.19 (0.84)	5.23 (0.13)
UK, Belfast	119	1.57 (0.67,3.25)	1.61	5.75 (1.09)	5.78 (0.10)	118	1.06 (0.50,2.23)	1.25	5.97 (1.07)	5.98 (0.10)
UK, Glasgow	93	1.75 (0.94,3.67)	1.75	–	–	96	1.77 (0.76,3.78)	1.59	–	–
Overall mean	861	1.34 (0.63,2.75)	–	5.53 (1.08)	–	896	1.28 (0.59,2.80)	–	5.84 (1.05)	–

Table 5 Spearman rank correlations between MONICA centre coronary event rates and adjusted population means of individual risk factors[a]

	Nephelometric fibrinogen	Plasma viscosity	Clottable fibrinogen	D-dimer (log)	t-PA	PAI-1	vWF	C-reactive protein (log)	Total cholesterol
Males	0.75*	0.32	0.22	0.50	−0.04	−0.42	0.70*	0.28	0.17
Females	0.53	0.34	0.33	0.87**	0.34	−0.38	0.89**	−0.07	0.30

No correction was made for multiple comparisons. $^{*}P < 0.05$, $^{**}P < 0.001$.
[a]Number of centres shown in *Tables 2–4* (Hungarian centre excluded).

samples studied. *Table 3* shows the unadjusted and adjusted mean values for nephelometric fibrinogen and plasma viscosity for men and for women.

Mean values for women were slightly higher than those for men; this was statistically significant for plasma viscosity ($P = 0.005$). A range of values was noted across the populations.

Table 4 shows the corresponding results for C-reactive protein and total cholesterol.

C-reactive protein levels were non-significantly higher in men and cholesterol levels were significantly higher in women ($P < 0.0001$).

Coronary event rates were compared with the adjusted mean values calculated for each centre using Spearman rank correlation coefficients. These results are shown in *Table 5*.

Four of these correlations achieve formal statistical significance: vWF antigen for both sexes, D-dimer for women, and nephelometric fibrinogen for men. No significant correlations of coronary event rates were observed for plasma viscosity, clottable fibrinogen, t-PA, PAI-1, C-reactive protein, or total cholesterol. Except in the case of t-PA, other correlations show similar results in men and women. The scatterplots for nephelometric fibrinogen, vWF, and D-dimer are shown in *Figure 1*. Adjusted centre mean values are shown.

Discussion

In a large population sample of British men, we have previously reported the influence of lifestyle factors on variation in these thrombotic factors, measured in the same laboratories.[19] For C-reactive protein, body mass index is a major determinant.[21] All variables showed marked and statistically significant positive trends with age, with the exception of PAI-1 activity in men. Similarly, with the exception of vWF antigen and PAI-1, these variables showed positive associations with smoking habit. Finally, five variables showed positive associations with body mass index: t-PA, PAI-1, nephelometric fibrinogen, plasma viscosity, and C-reactive protein in both sexes.

Other 'lifestyle' variables such as alcohol consumption, leisure-time physical activity, use of prescribed medicines, and, for t-PA and plasma viscosity only, the time of blood sampling, also showed associations with these thrombotic variables,[19] but were not measured in all the relevant MONICA populations in the current study. Similarly, menopausal status has been shown to be associated with modest changes in certain thrombotic variables such as fibrinogen but data on menopausal status was unavailable from the majority of centres.[23,24]

In this large international sample of men and women from 12 MONICA populations, the thrombosis-related variables showed the expected relationships with age, smoking habit, and body mass index in men, but not consistently in women; for example, PAI-1 activity showed only a small, non-significant rise in activity with age. Mean body mass indices were similar in the populations studied (data not shown) but alcohol intake and type of alcohol may differ among the populations. Data from another British study showed that alcohol consumption is associated with t-PA, PAI-1, and the two measures of fibrinogen;[19] but the data are confined largely to the associations with beer consumption and there is, to our knowledge, little information on the associations of thrombotic variables with wine or spirit consumption.[19]

In general, we observed that the mean levels of these variables between these study populations are weakly correlated with the coronary event rates calculated from the relevant MONICA registration data. After the population means were adjusted for age, smoking habit, and body mass index, associations were statistically significant for vWF in both sexes, and for fibrin D-dimer and nephelometric fibrinogen for women and men, respectively. The overall correlation between nephelometric and clottable fibrinogen was only 0.51 and nephelometric fibrinogen showed a closer association with coronary event rates than clottable fibrinogen, which is consistent with our findings for incident CHD in the Caerphilly and Speedwell cohorts.[17] PAI-1 activity showed a consistent, but non-significant, inverse association with coronary event rates but the association with incident CHD in prospective population studies was not significant in a recent meta-analysis.[10]

Coronary registration was incomplete for the Hungarian centre, but mean levels of haemostatic factors in both men and women tended to be above the overall mean, reflecting the high mortality from CHD in Hungary.[25]

It should be noted that these correlations between coronary event rates prevalent at the time of the surveys and the population levels of thrombotic factors may be underestimates, due to their biological

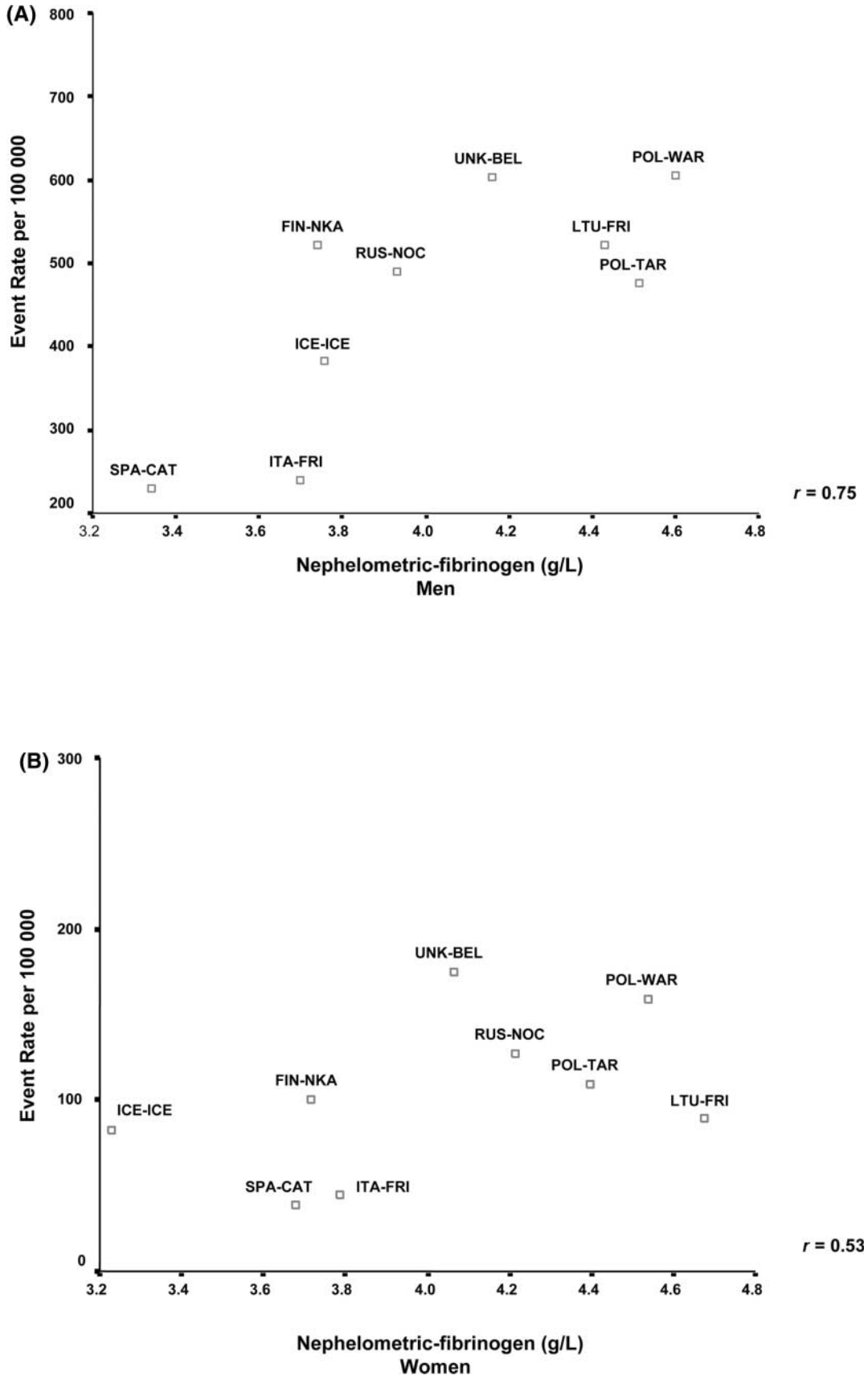


Figure 1 Scatterplots showing correlations between coronary event rates and population levels of nephelometric fibrinogen, vWF, and D-dimer in MONICA centres (*A*)–(*F*). Number of centres shown in *Tables 2–4* (Hungarian centre excluded).

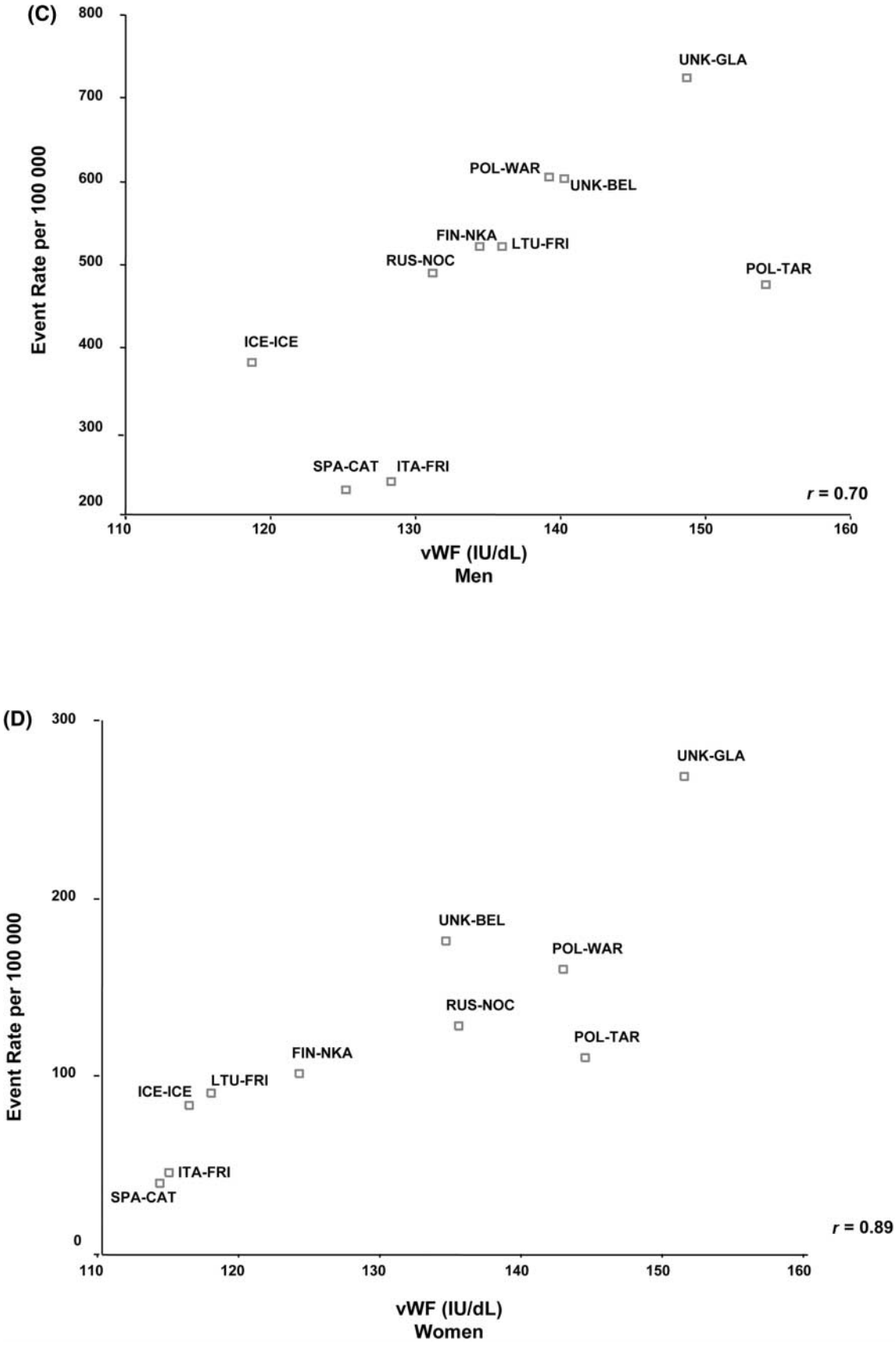
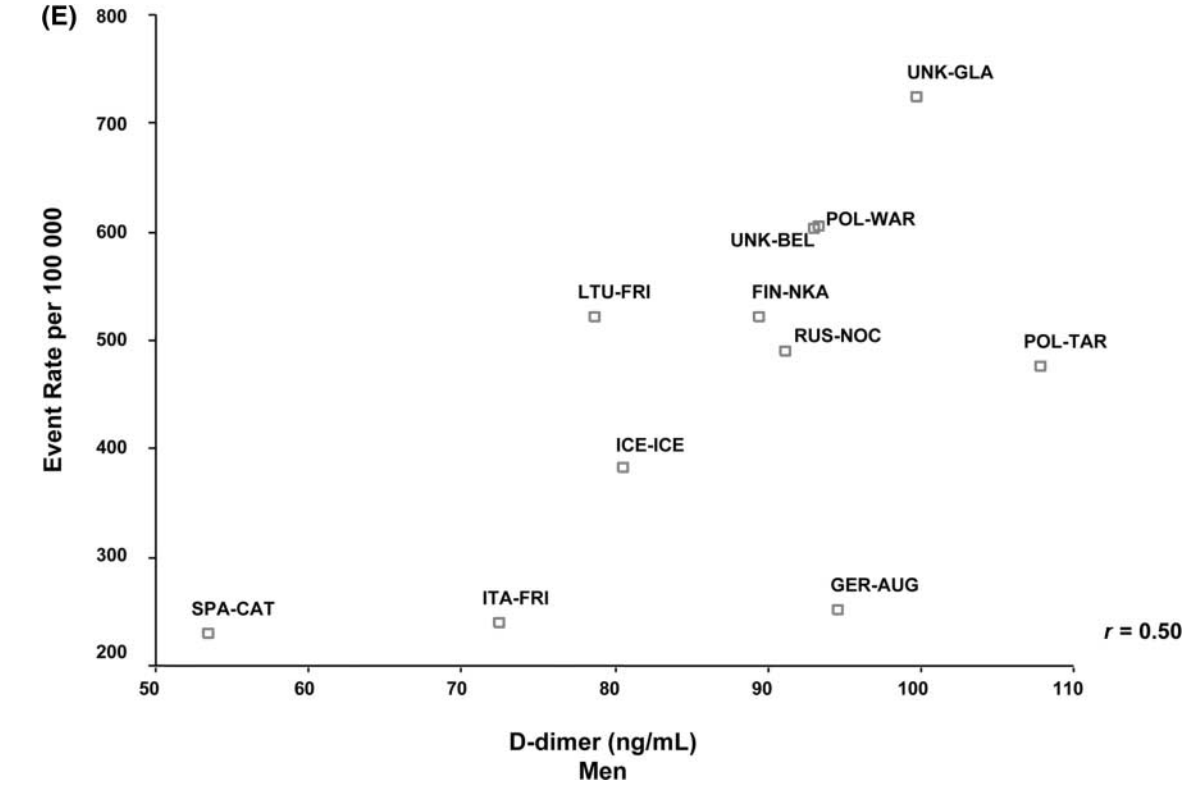


Figure 1 Continued.



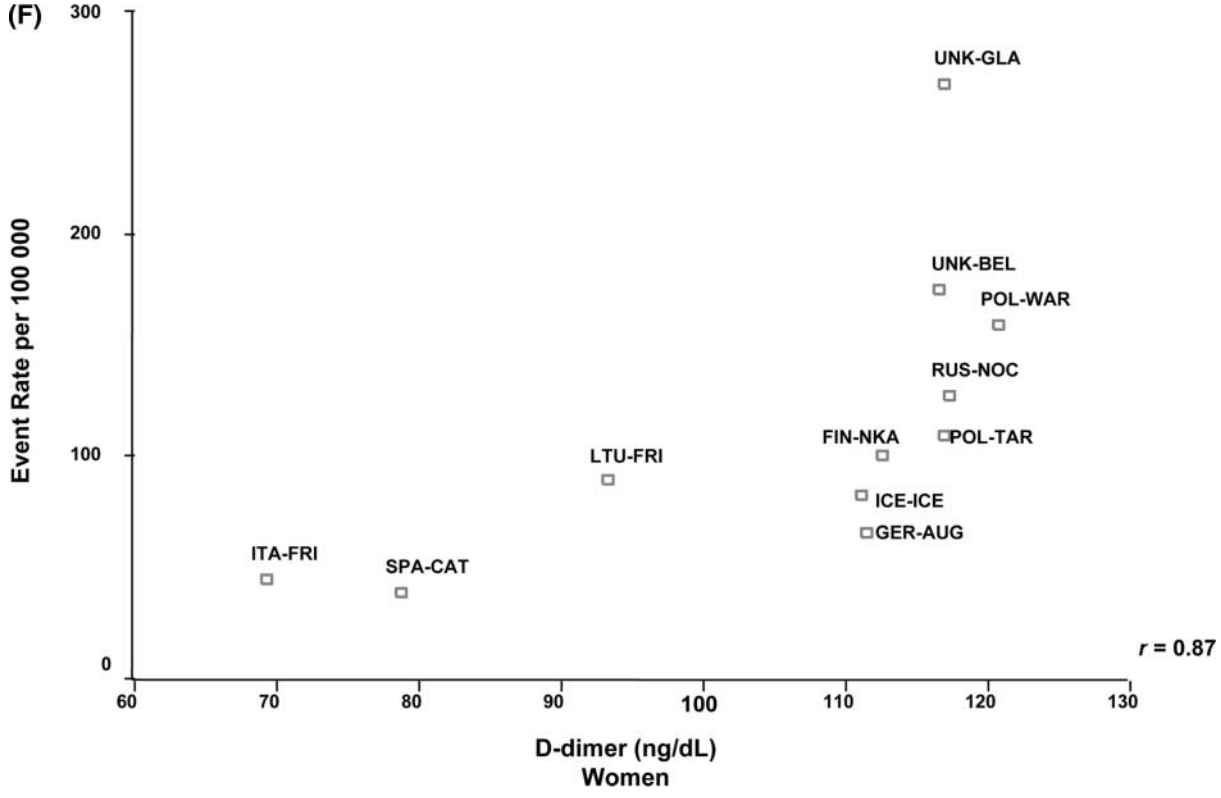


Figure 1 Continued.

variability.[6] Also, they are much stronger than those between total cholesterol and coronary event rates (0.17 and 0.30 for males and females, respectively, *Table 5*). One possible explanation for this is that short-term CHD incidence, given a certain level of cholesterol in the population, may be more strongly predicted by smoking habit, which would be more strongly related to thrombotic factors. However, the prevalence of smoking habit in the MONICA final surveys correlated only weakly with coronary event rates (0.18 and 0.39, in men and women, respectively). Only one population in this study had relatively low values for plasma cholesterol consistently in men and women (Catalonia, Spain) and further statistical analysis was not considered worthwhile.

One potential source of bias is due to seasonal variation in thrombotic measurements such as fibrinogen in plasma reported particularly in elderly populations.[26] However, the ARIC study reported that there was no significant seasonal variation in middle-aged American men and women and no MONICA centre exclusively surveyed during winter months, which minimized any possible seasonal bias.[27]

While there is increasing evidence from prospective studies (and meta-analyses) that several thrombotic factors are associated with increased risk of CHD,[5–10,16] there are few previous studies relating these factors to international differences in CHD risk. Such studies have suggested associations of international CHD risk with fibrinogen[28,29] and plasma viscosity.[30] We report for the first time that CHD risk is associated with plasma levels of vWF (in both sexes), nephelometric fibrinogen (in men), and fibrin D-dimer (in women). We suggest that thrombotic factors (which have been adjusted for age, smoking habit, and body mass index in each population) appear more strongly associated with the population risk of CHD in this study than total cholesterol or smoking habit. Although additional lifestyle or genetic factors may be responsible for these differences in risk between populations, it is also possible that mean population levels of thrombotic risk factors are causal determinants of CHD risk. Our study results are consistent with those recently reported from the PRIME study,[31] in which fibrinogen alone accounted for 30% of the excess risk of CHD in Belfast compared with France, while all the classical risk factors together explained only 25%. Although large randomized controlled trials of reducing plasma levels of fibrinogen or vWF are awaited, there is evidence that reducing the high rate of fibrin turnover (as measured by plasma fibrin D-dimer) by warfarin therapy[32,33] or cardioversion[34] reduces thrombotic risk in patients with atrial fibrillation. Further international comparisons, prospective studies, and experimental studies are proposed to clarify the role of thrombotic variables in CHD risk.

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Appendix: Collaborating centres

Finland

North Karelia—KTL–National Public Health Institute, Helsinki: V. Salomaa. Finnish Red Cross, BTS, Department of Haemostasis, Helsinki: V. Rasi, E. Vahtera.

Germany

Augsburg—University of Ulm: W. Koenig, H.W. Hense; GSF–Institute for Epidemiology, Munich: A. Döring.

Hungary

Budapest—Institute of Cardiology, Budapest: J. Duba.

Iceland

Heart Preventive Clinic, Reykjavik: N. Sigfusson.

Italy

Friuli—Cardiovascular Prevention Centre, Udine: D. Vanuzzo, L. Pilotto.

Lithuania

Kaunas—Medical University, Institute of Cardiology: J. Bluzhas, S. Domarkiene, A. Tamosiunas, R. Reklaitiene.

Poland

Tarnobrzeg—Institute of Public Health, Jagiellonian University, Krakow: A. Pajak. Warsaw—National Institute of Cardiology, Warsaw, Department of Cardiovascular Epidemiology and Prevention: S.L. Rywick, G. Broda, M. Polakowska, P. Kurjata.

Russia

Novosibersk—Institute of Internal Medicine: U. Nikitin.

Spain

Catalonia—Institute of Health Studies, Department of Health, Barcelona: S. Sans.

United Kingdom

Belfast—The Queen's University of Belfast, Northern Ireland: A. Evans, E. McCrum, D. O'Reilly, A. Scott,

J. Yarnell. Glasgow—University of Dundee/Glasgow Royal Infirmary: H. Tunstall-Pedoe, C. Morrison.

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