Air Temperature and Inflammatory Responses in Myocardial Infarction Survivors

Alexandra Schneider,[a] *Demosthenes Panagiotakos,*[b] *Sally Picciotto,*[c] *Klea Katsouyanni,*[b] *Hannelore Löwel,*[a] *Bénédicte Jacquemin,*[d] *Timo Lanki,*[e] *Massimo Stafoggia,*[c] *Tom Bellander,*[f,g] *Wolfgang Koenig,*[h] *and Annette Peters*[a] *for the AIRGENE Study Group*

Background: Temperature changes have been associated with increased cardiovascular risk, but the role of inflammatory markers in this relationship is not well understood. The objective of this study was to analyze the association between air temperature and C-reactive protein, interleukin-6 and fibrinogen in postmyocardial infarction patients.

Methods: In a multicenter panel study, the 3 inflammatory blood markers were measured repeatedly. In total, 5813 blood samples in 1003 subjects were collected in 6 European cities representing different climates. Data on patient characteristics and disease history were gathered at the baseline visit. Meteorologic data were obtained from the city-specific network stations. The association was analyzed using a semiparametric model with random patient effects.

Results: A 10°C decrease in the 5-day-average of air temperature before the blood withdrawal was associated with a 4% increase in C-reactive protein (4.3% [95% confidence interval = 0.2% to 8.1%]). Correspondingly, an increase of interleukin-6 was observed for the same time window (3.3% [0.1% to 6.3%]) whereas fibrinogen showed an increase of 1.3% (0.2% to 2.4%) with a lag of 3 days.

Conclusion: A decrease in air temperature, particularly the average temperature of the last 5 days, was associated with an increase in both C-reactive protein and interleukin-6, whereas fibrinogen seemed to react to temperature changes after 3 days. In susceptible patients this might lead to an additional risk for cardiovascular events and suggests a biologic mechanism for the observed seasonal variation in death from ischemic heart disease and stroke in the elderly.

(*Epidemiology* 2008;19: 391–400)

In recent years, studies worldwide have indicated that variation in climatologic conditions is correlated with higher mortality and morbidity rates.[1–3] In particular, temperature and, to a lesser extent, relative humidity have been found to interfere with health and disease, especially with cardiovascular disease (CVD).[4,5] These results suggest that patients with ongoing CVD might benefit from possible precautions, especially during winter weather, as a means of reducing risk related to cold temperature exposures among the elderly.

However, the exact mechanisms that promote this association have not been fully understood. In a study of weather-related changes on the 24-hour blood pressure profile,[6] the investigators reported that mean 24-hour systolic blood pressure was lower on hot days and higher on cold days when compared with intermediate days, but no relationship was found between air temperature and heart rate. Furthermore, Jehn et al[7] studying men and women in the Dietary Approaches to Stop Hypertension (DASH) project, reported that the increase in cardiovascular mortality observed in winter could be attributed to an increase in blood pressure variability. In addition, low-grade systemic inflammation seems to play an essential role in the initiation, progression, and the final pathophysiological steps of atherosclerosis, plaque erosion, and eventually plaque rupture.[8] It also participates in the development of obesity, insulin resistance, and hypertension.[9–11] It has been suggested that in cold conditions fibrinogen as well as platelet count both are increased and promote clotting, while reduced plasma volume and increased blood viscosity during cold exposure also tend to promote thrombosis.[12]

However, the association between climatologic conditions, such as air temperature and humidity, and low-grade systemic inflammation has to our knowledge never been investigated, especially at population level, although seasonal variation has been demonstrated for fibrinogen. In this work we sought to evaluate whether air temperature was associated

Submitted 13 July 2007; accepted 10 December 2007; posted 1 April 2008.
From the [a]Helmholtz Zentrum Muenchen, German Research Center for Environmental Health, Institute of Epidemiology, Neuherberg, Germany; [b]Department of Hygiene and Epidemiology, University of Athens, Athens, Greece; [c]Local Health Authority Rome, Rome, Italy; [d]IMIM-Municipal Institute of Medical Investigation, Barcelona, Spain; [e]Environmental Epidemiology Unit, National Public Health Institute (KTL), Kuopio, Finland; [f]Institute of Environmental Medicine, Karolinska Institute, Stockholm, Sweden; [g]Department of Occupational and Environmental Health, Stockholm County Council, Stockholm, Sweden; and [h]Department of Cardiology, University of Ulm, Ulm, Germany.
The AIRGENE study was funded as part of the European Union's 5th Framework Program, key action number 4: "Environment and Health," contract number QLRT-2002-02236.
Supplemental material for this article is available with the online version of the journal at www.epidem.com; click on "Article Plus."
Correspondence: Alexandra Schneider, Institute of Epidemiology, Ingolstaedter Landstr. 1, 85764 Neuherberg, Germany. E-mail: alexandra.schneider@helmholtz-muenchen.de.

ISSN: 1044-3983/08/1903-0391
DOI: 10.1097/EDE.0b013e31816a4325

with inflammation indices, such as the acute phase proteins C-reactive protein and fibrinogen as well as the messenger interleukin-6 in myocardial infarction survivors from various parts of Europe within a repeated measurements framework. In addition we considered other weather parameters in sensitivity analyses to evaluate the role of temperature in the complex weather settings.

METHODS

Study Population

A prospective longitudinal study (AIRGENE) of post-myocardial infarction (MI) patients was performed in 6 European cities—Athens (Greece), Augsburg (Germany), Barcelona (Spain), Helsinki (Finland), Rome (Italy), and Stockholm (Sweden)—chosen so as to include a variety of geographic and climatic conditions.[13] Briefly, patients aged 35 to 80 years, who had MIs not longer than 6 years but at least 3 months ago, were recruited. The following patients were excluded: those who had undergone surgical procedures (percutaneous transluminal coronary angioplasty, bypass surgery) less than 3 months before the beginning of the study, those with chronic recurring inflammatory diseases (eg, Crohn's disease) and those who were taking anti-inflammatory medication that modifies the biomarkers were not included. Preferably, never-smokers and ex-smokers who had quit at least 3 months before the start of the study were recruited. The Southern centers also accepted light current smokers. Study protocols were approved at local human subjects committees, and written informed consent was obtained from all patients. All methods used in the study centers were conducted according to common standard operating procedures developed by the participating centers. A more detailed description of the patient recruitment and the studied panel can be found elsewhere.[13]

Clinical Measurements

The patients were scheduled for 6 to 8 clinical visits between May 2003 and July 2004. The visits were conducted every 4 to 6 weeks on the same day and at the same time of the week to minimize the impact of circadian variation and day of the week. If patients suffered from acute infections such as a cold or influenza in the 3 days before the clinical visit, examinations were postponed.

At the first visit, a baseline questionnaire was administered regarding health status, pulmonary and cardiac symptoms, medication intake, and smoking history. Moreover, a blood pressure measurement was performed. Body mass index (BMI) was measured and a blood serum sample was drawn to assess glycosylized hemoglobin, a severity index for hyperglycemia and therefore a marker for the control of diabetes, N-Terminal proB-type natriuretic peptide, a marker for congestive heart disease,[14] and baseline serum lipids.

A 7-day recall on medication intake was obtained and venous ethylenediamine tetraacetic-plasma samples were collected for the determination of the inflammatory markers C-reactive protein, interleukin-6, and fibrinogen in the central laboratory in Ulm, Germany. Details on storage, handling and processing of the blood samples can be found in the paper by Peters et al.[13] Because of quality-control issues, fibrinogen could not be analyzed in Athens. Data on influenza virus occurrence during the winter months of the study period were obtained via medical surveillance networks in each city except for Athens.

Meteorologic Data

The meteorologic parameters air temperature (*temp*), relative humidity, dew point temperature (*dp*) and barometric pressure were obtained through the city-specific air monitoring networks and the meteorological services. Apparent temperature (*at*) was calculated by using the formula $at = -2.653 + (0.994 \times temp) + (0.0153 \times dp^2)$.[15,16] For valid 24-hour mean values, at least 75% of the hourly observations had to be present. Missing data on the aggregate level were replaced using an adapted formula based on the APHEA-Study.[13,17]

Moving averages of meteorologic parameters concurrent with the clinical examinations were used to characterize the population-average exposures, taking into account the time of the blood withdrawal. For each person and visit the individual 24-hour average exposure immediately preceding the clinical visit (lag 0) up to 4 days (lag 1–4) prior to the examination were calculated if more than 2/3 of the hourly measurements were available. In addition, the mean of lags 0 to 4 was calculated.

Furthermore, air pollution data (eg, hourly means of particles with aerodynamic diameter below 10 μm [PM_{10}] and ultrafine particles) from fixed monitoring sites representing urban background concentrations were collected. Moving 24-hour averages were calculated in analogy to the meteorological parameters.

Statistical Analyses

Data were analyzed using random effects models to account for repeated measures and for unobserved heterogeneity of the data. For the dependencies between the repeated measures in each patient, compound symmetry structure was assumed for the covariance matrix, as the half-lives of the markers (C-reactive protein: 19 hours, interleukin-6: 6 hours, fibrinogen: 4 to 6 days) were much shorter than the intervals between the visits. Penalized splines in the additive mixed models framework were used to allow for nonparametric exposure-response functions.[18] C-reactive protein and interleukin-6 needed to be log-transformed to fulfil the model assumptions of residual normality in simple additive models.

City-specific confounder models without meteorologic parameters were built for each blood marker separately, identifying the time-invariant patient characteristics associated with the mean level of C-reactive protein, fibrinogen and

interleukin-6 and leading to a normally distributed random patient intercept. In a first step, time-invariant factors were selected for all cities combined. Variables considered to impact the average blood marker concentrations included BMI, diabetes, pack-years of smoking, medication, physical activity, alcohol consumption, sex, and N-Terminal proB-type natriuretic peptide. As further possible indicators for blood marker levels, we also considered age, number of MIs, bronchitis (ever diagnosed), level of education, cholesterol (total and high density lipoprotein), glycosylized hemoglobin, blood pressure, symptoms of chronic obstructive pulmonary disease, self-reported health status, hypertension (ever diagnosed), arrhythmia (ever diagnosed), heart failure (ever diagnosed), and the presence of a cardiac pacemaker. In a second step for each city, a more parsimonious model was selected out of the formerly chosen all-cities variables and then time-varying risk factors were added. To assure an adjustment for season, long-term time trend was forced into all models. Moreover relative humidity was added to each model using the lag corresponding to the analyzed temperature lag. Additionally, time of the day and day of the week were included if this adjustment proved necessary. P-splines were used to model continuous confounding variables and were compared with linear terms and polynomials of degree 2 and 3. All decisions on goodness-of-fit were based on Akaike's Information Criterion. Details on the models can be found in the supplemental material, available with the online version of this article (eTable 1).

After completion of the confounder models, the effects of different air temperature and apparent temperature lags were estimated linearly as well as smoothly. In a further step, heterogeneity between centers was assessed according to the DerSimonian and Laird approach.[19] City-specific effect estimates were pooled subsequently with fixed or random effects (decision based on the homogeneity test result with 90%-confidence interval [CI] to be more conservative) by using meta-analysis methodology.[20,21] Data were analyzed using the statistical package SAS Version 9.1 (SAS Institute Inc., Cary, NC).

Sensitivity analyses were performed to explore the robustness of the models and the results. We used a more parsimonious model without adjustment for relative humidity. An additional adjustment for barometric pressure (corresponding lag to temperature) was tested. To account for seasonal variability, a dichotomous variable for cold season (October to March) and warm season (April to September) was introduced into the model, in addition to the trend variable. Categorical medication variables were selected using the same methods as for the other confounding variables, and these were added to the model of each city. Finally, a continuous variable for influenza occurrence during the study period was added as a smooth function to the core model of each city (except for Athens). A possible differing influence of hot and cold days (below the 10th percentile or above the 90th percentile of the city-specific air temperature distribution) was also checked by using indicator variables for such days and by analyzing smooth exposure-response-functions of air temperature. The influence of simultaneous immediate (lag 0) and cumulative temperature effects (mean of lag 0 to lag 3) in the model were analyzed. An additional sensitivity analysis was performed adjusting for air pollution effects. Effect modification was analyzed by sex, diabetic status (yes vs. no), BMI (underweight or normal vs. overweight or obese), and season (cold vs. warm).

RESULTS

Study Population

The study population comprised 1003 MI survivors who fulfilled the inclusion and exclusion criteria and who had results for at least 2 valid, repeated blood samples. Table 1 presents the baseline characteristics of the study participants. Nearly all patients were treated for hypertension and a majority of the patients were treated with lipid reducers, as well as antithrom-

TABLE 1. Baseline Characteristics of 1003 Myocardial Infarction Survivors From 6 European Cities (Helsinki, Stockholm, Augsburg, Rome, Barcelona, and Athens)

Characteristics	
No. blood samples[a]	5813
Male sex; %	78.6
Age (yrs); mean ± SD (range)	62.2 ± 9.5 (37 to 81)
Body mass index (kg/m^2); mean ± SD (range)	28.4 ± 4.2 (17.5 to 48.9)
First MI; %	85.0
Self-reported history[b]; %	
Angina pectoris	34.3
Arrhythmia	22.5
Congestive heart failure	10.4
Hypertension	51.0
Diabetes	19.7
Chronic renal disease	4.6
Asthma	4.7
Total cholesterol (mg/dL)[c]; mean ± SD (range)	184.4 ± 39.4 (91.1 to 390.0)
HDL cholesterol (mg/dL)[c]; mean ± SD (range)	50.2 ± 14.0 (22.0 to 119.3)
HbA1c[c]; mean ± SD (range)	5.5 ± 1.0 (2.8 to 10.5)
NT-proBNP (pg/mL); mean ± SD (range)	393.5 ± 716.0 (10.8 to 9308.0)
Medication; %	
Statins	84.5
Lipid-lowering medication	86.3
Antithrombotic medication	97.8

[a]For Athens no fibrinogen detection possible due to quality reasons.
[b]Ever diagnosed by a physician.
[c]Blood biomarkers determined at local laboratories.
BMI indicates body mass index (weight [kg]/height [m^2]); HDL, high-density lipoprotein; HbA1c, glycosylized hemoglobin; NT-proBNP, N-Terminal proB-type natriuretic peptide.

botic therapy to prevent future myocardial infarctions. A more detailed description of the patient characteristics can be found elsewhere.[13] Two-thirds of the observations (n = 3802) were made in the cold season (October to March) and one-third (n = 2011) in the warm season (April to September).

Meteorologic Parameters

The distributions of the 24-hour average concentrations of the meteorologic data are given in Table 2. Mean air temperature and apparent temperature increased from north to south, with Athens being the hottest city. Relative humidity was highest in Stockholm and Rome, whereas air pressure was highest in Augsburg. Relative humidity and barometric pressure showed very low correlation with the other meteorologic parameters. However, due to the above formula, apparent temperature was highly correlated with dew point temperature and air temperature (eTable 2).

Inflammatory Markers

Based on the questionnaire data at the clinical visits, 255 of 6068 collected blood samples had to be excluded due to acute infections (cold/flu, urinary tract infection, gastro-intestinal in-

TABLE 2. Description of Meteorologic and Air Pollution Parameters by City

	No.	Mean ± SD	Percentile		
			5th	Median	95th
Northern to Central Europe					
Helsinki (September 5, 2003 to June 2, 2004)					
Air temperature (°C)	272	3.1 ± 6.9	−7.6	3.1	14.7
Relative humidity (%)	272	76 ± 14	46	80	91
Barometric pressure (hPa)	269	1003.6 ± 11.8	985.0	1004.1	1023.0
Ultrafine particles (number count)	270	8534 ± 3796	3306	8220	15077
PM_{10} ($\mu g/m^3$)	268	17.1 ± 9.5	6.1	14.6	36.1
Stockholm (August 30, 2003 to June 24, 2004)					
Air temperature (°C)	301	4.7 ± 6.2	−4.9	4.8	15.1
Relative humidity (%)	301	82 ± 10	62	84	94
Barometric pressure (hPa)	301	1006.9 ± 11.9	987.6	1006.8	1026.7
Ultrafine particles (number count)	287	9748 ± 3869	4918	8998	17578
PM_{10} ($\mu g/m^3$)	286	17.8 ± 10.3	7.3	14.3	40.1
Augsburg (May 14, 2003 to February 24, 2004)					
Air temperature (°C)	285	10.2 ± 9.6	−3.4	10.3	25.1
Relative humidity (%)	285	69 ± 14	47	69	92
Barometric pressure (hPa)	285	1018.7 ± 6.9	1006.0	1019.8	1028.7
Ultrafine particles (number count)	198	11876 ± 6077	4835	10824	25135
PM_{10} ($\mu g/m^3$)	286	33.1 ± 13.4	12.9	32.7	56.6
Southern Europe					
Rome (September 20, 2003 to July 15, 2004)					
Air temperature (°C)	299	13.5 ± 6.1	3.7	13.1	23.9
Relative humidity (%)	299	80 ± 12	56	82	95
Barometric pressure (hPa)	299	1015.1 ± 7.4	1003.0	1015.3	1028.1
Ultrafine particles (number count)	277	35450 ± 17413	13799	31669	69226
PM_{10} ($\mu g/m^3$)	297	42.1 ± 16.1	21.3	39.1	76.1
Barcelona (August 30, 2003 to June 16, 2004)					
Air temperature (°C)	293	15.2 ± 4.6	9.1	14.5	23.2
Relative humidity (%)	293	67 ± 12	46	68	86
Barometric pressure (hPa)	275	1016.8 ± 7.5	1003.7	1017.4	1028.8
Ultrafine particles (number count)	245	18133 ± 10089	4323	16175	36526
PM_{10} ($\mu g/m^3$)	274	40.7 ± 24.1	14.3	33.8	88.7
Athens (September 8, 2003 to July 30, 2004)					
Air temperature (°C)	336	17.4 ± 7.0	6.4	16.6	29.3
Relative humidity (%)	339	67 ± 10.2	49	66	84
Barometric pressure (hPa)	339	1003.6 ± 6.3	992.5	1003.7	1014
Ultrafine particles (number count)	299	20590 ± 12051	7823	17041	47573
PM_{10} ($\mu g/m^3$)	325	38.5 ± 18.8	17.0	35.6	64.6

PM_{10} indicates particulate matter with aerodynamique diameter <10 μm.

TABLE 3. Description of Inflammatory Markers (Mean Per Patient) for All Cities Combined

Total	No. Patients	Mean ± SD	Percentile			Spearman Correlation Coefficient		
			5th	Median	95th	C-reactive Protein	Interleukin-6	Fibrinogen
C-reactive protein (mg/L)	1003	2.60 ± 3.21	0.35	1.58	7.81			
Interleukin-6 (pg/mL)	1003	3.03 ± 3.07	1.18	2.36	6.18	0.54		
Fibrinogen (g/L)[a]	895	3.58 ± 0.70	2.59	3.51	4.75	0.54	0.49	

[a]For fibrinogen, data from Athens were excluded due to quality reasons.

TABLE 4. Pooled Effects of Air Temperature on Blood Parameters Per 10°C Temperature Increase, Additionally Adjusted for Relative Humidity (Main Model)[a]

Time Prior to Blood Draw	C-reactive Protein (All Cities)	Interleukin-6 (All Cities)	Fibrinogen (All Cities Except Athens)
	% Change of Geometric Mean[b] (95% CI)	% Change of Geometric Mean[b] (95% CI)	% Change of Arithmetic Mean[c] (95% CI)[b]
Lag 0	−2.74 (−6.15 to 0.79)	−1.69 (−4.32 to 1.00)	−0.42 (−1.50 to 0.65)
Lag 1	−4.05 (−7.51 to −0.46)	−2.07 (−4.76 to 0.69)	−0.75[d] (−2.17 to 0.67)
Lag 2	−2.40 (−5.96 to 1.30)	−1.54 (−4.24 to 1.24)	−0.59 (−1.74 to 0.56)
Lag 3	−2.95 (−6.38 to 0.59)	−2.12 (−4.97 to 0.82)	−1.32 (−2.42 to −0.22)
Lag 4	−3.49 (−6.61 to −0.26)	−2.14 (−4.94 to 0.75)	−0.79[d] (−1.77 to 0.20)
Mean lag 0–4	−4.26 (−8.12 to −0.24)	−3.28 (−6.34 to −0.13)	−1.17[d] (−2.58 to 0.23)

[a]Details on the model can be found in the online supplemental material available with the electronic version of this article at www.epidem.com; click on ArticlePlus (eTable 1).
[b]Geometric mean is the antilog of arithmetic mean of log-transformed variable; %-change is the antilog of effect estimate obtained from regression minus 1.
[c]%-Change is the effect estimate obtained from regression divided by arithmetic mean of fibrinogen.
[d]Randomly pooled effect estimates, otherwise fixed effect pooling (based on χ^2-test for homogeneity between city-specific effect estimates).

fection, or acute respiratory infection) or surgical procedures (including severe dental intervention) 3 days before the clinical visit, which could have severely altered the concentrations of the inflammatory markers. Overall, 5813 plasma samples for inflammatory marker determination were available for analyses.

The description of the 3 inflammatory markers across all cities can be found in Table 3. The geometric means over all observations of C-reactive protein, interleukin-6, and fibrinogen were 1.39 mg/L, 2.33 pg/mL, and 3.51 g/L, respectively. The 3 markers showed a moderate correlation, with the Spearman correlation coefficient ranging between 0.42 and 0.52 for all single measurements using the data of all cities combined. The correlation between the mean values per patient are presented in Table 3. A more detailed description of the city-specific blood markers and their correlation can be found elsewhere.[13,22]

Regression Analyses

Pooled results for the regression of the 3 blood markers using the model with adjustment for relative humidity (main model) are summarized in Table 4. Due to the high correlation of air temperature and apparent temperature (data not shown) their effect estimates were quite similar, with the estimates for apparent temperature being a little smaller. Therefore, only the influence of air temperature will be discussed in more detail.

The increases of C-reactive protein-levels with a decrease in air temperature were similar for all analyzed lags. A decrease of 10°C on average led to an increase of 3% to 4% in the geometric mean of C-reactive protein. The strongest effects were found for lag 1, lag 4, and the 5-day-average. As with the C-reactive protein results, interleukin-6 was negatively associated with air temperature in the main model, with around 2% change in geometric mean. The strongest effect was seen for the 5-day-average. In contrast, fibrinogen seemed to react mainly with a lag of 3 days but also showed an increase of around 1% of the arithmetic mean with a decrease in air temperature for all other lags. The city-specific effect estimates and the pooled effect estimate for lag 1 and the 5-day-average for all 3 blood markers are presented in Figure 1.

Sensitivity Analyses

The effect estimates of the analysis without adjustment for relative humidity (basic model) showed the same pattern,

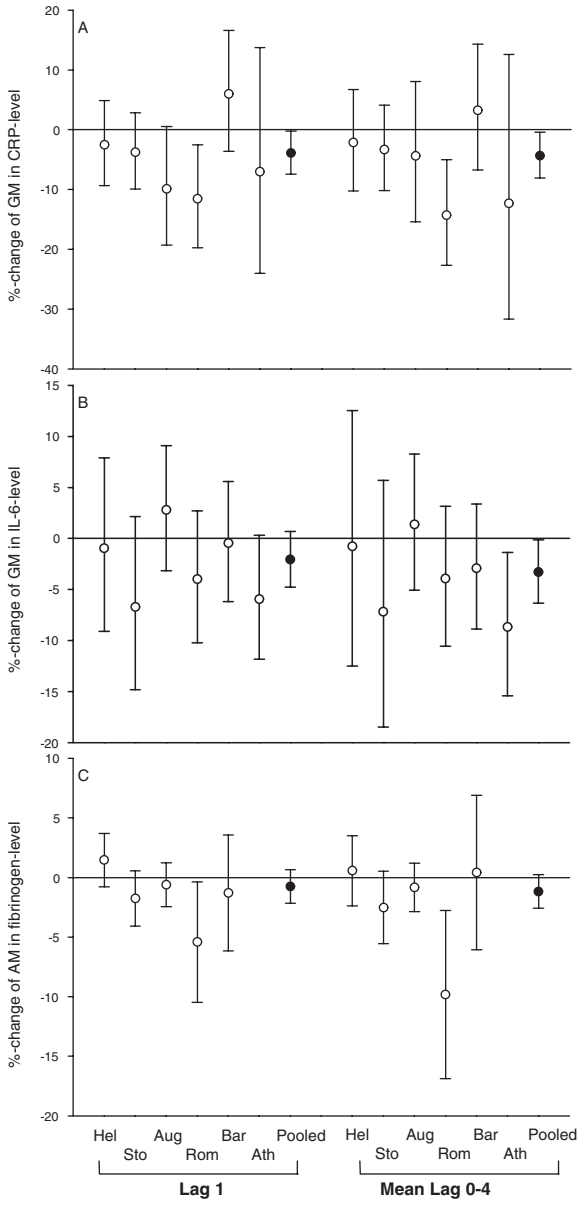

FIGURE 1. Air temperature effect estimates (%-change of geometric [GM] or arithmetic mean [AM]) of inflammatory markers per 10°C temperature increase, with 95% CIs (vertical bars). For (A) C-reactive protein and (B) interleukin-6, geometric mean; for (C) fibrinogen, arithmetic mean (no data on fibrinogen for Athens due to quality-control issues).

but were a little smaller and the confidence intervals included the null (Table 5). The additional adjustment for barometric pressure in the main model did not lead to any changes in the effect estimates. When adjusted for the influence of season, the increase in the inflammatory marker levels became even stronger, with a percent change of around 5% to 6% in the geometric mean of C-reactive protein. A more detailed analysis of the seasonal pattern of the inflammatory markers can be found in the online supplemental material (eTable 3 and eFigure 1). Introduction of smooth influenza data did not change the value of the effect estimates for C-reactive protein but led to loss in accuracy of the estimation probably due to the heterogeneity of the city-specific effect estimates. But when influenza was included as a confounder in the interleukin-6 model, all analyzed temperature lags showed strong effects on the inflammatory marker with higher effect estimates when compared with the main model. Using the Akaike's Information Criterion-selected medication categories as additional confounder variables, the pooled effect estimates remained robust, with more heterogeneity in the city-specific estimates and therefore a smaller increase in blood marker level. Only fibrinogen showed a more than doubled effect estimate in association with the mean temperature of the last 5 days when adjusted for medication.

The adjustment for the immediate effect of ultrafine particles on interleukin-6 led to negative effect estimates that remained negative, to with a loss in accuracy mainly due to around 750 missing observations in ultrafine particle measurements during the study period. Adjustment for the 5-day-average of PM_{10} in the fibrinogen model resulted in robust effect estimates, as only around 150 observations were missing (data not shown). However, the different changes in the confounder model showed that the observed increases in levels of C-reactive protein, interleukin-6, and fibrinogen remained robust and were not particularly dependent on the chosen model.

The analyses with special indicators for hot and cold days, as well as the simultaneous modeling of immediate and cumulative temperature influences, did not reveal any new information (data not shown). When looking at the smooth exposure-response functions, temperature effects were mainly linear, with a very slightly U-shaped curve for Rome C-reactive protein and fibrinogen data, as well as for Barcelona fibrinogen data. When analyzing interaction of temperature with sex, diabetic status, BMI, and season, no effect modification was apparent. The effect estimates were a little larger in size for men, for nondiabetics, for underweight or normal patients, and for the cold season—but also more heterogeneous between cities, resulting in broad confidence intervals (online supplemental material [eFigure 2]).

DISCUSSION

The analyses revealed that a decrease in air temperature, particularly the average temperature of the last 5 days, leads to increased levels of C-reactive protein and interleukin-6, whereas fibrinogen seems to react to temperature changes after 3 days. In susceptible patients this might lead to an additional risk for cardiovascular events and suggests a biologic mechanism for the observed seasonal variation in death from ischemic heart disease and stroke. Our results indicate the stimulation of the interleukin-6 production with a decrease in temperature over the last 5 days, which is the first

TABLE 5. Sensitivity Analyses of the Pooled Effects of Temperature on Blood Parameters Per 10°C Temperature Increase[a]

	C-reactive Protein (All Cities)	Interleukin-6 (All Cities)	Fibrinogen (All Cities Except Athens)
Time Prior to Blood Draw	**% Change of Geometric Mean[b] (95% CI)**	**% Change of Geometric Mean[b] (95% CI)**	**% Change of Arithmetic Mean[c] (95% CI)[b]**
Basic model (unadjusted for time-varying variables)			
Lag 1	−3.11[d] (−6.36 to 0.26)	−1.46 (−4.10 to 1.26)	−0.76[d] (−2.27 to 0.74)
Mean lag 0–4	−3.32 (−6.65 to 0.13)	−2.17 (−5.23 to 0.98)	−0.94[d] (−1.98 to 0.10)
Adjusted for relative humidity and barometric pressure			
Lag 1	−4.04 (−7.54 to −0.42)	−2.26 (−5.08 to 0.65)	−0.60 (−1.67 to 0.47)
Mean lag 0–4	−4.44 (−8.34 to −0.37)	−3.13 (−6.23 to 0.06)	−0.99[d] (−2.26 to 0.28)
Adjusted for relative humidity and season			
Lag 1	−5.31 (−9.60 to −0.83)	−2.66[d] (−7.54 to 2.48)	−1.48[d] (−3.93 to 0.97)
Mean lag 0–4	−6.17 (−10.90 to −1.20)	−4.31[d] (−9.03 to 0.65)	−2.61[d] (−5.48 to 0.25)
Adjusted for relative humidity and influenza			
Lag 1	−4.26[d] (−8.62 to 0.30)	−3.63 (−7.21 to 0.09)	−0.33 (−1.81 to 1.16)
Mean lag 0–4	−4.65[d] (−9.81 to 0.81)	−5.88 (−10.31 to −1.23)	−1.24 (−3.24 to 0.76)
Adjusted for relative humidity and medication[c]			
Lag 1	−3.87[d] (−8.86 to 1.38)	−1.99 (−4.67 to 0.76)	−0.78[d] (−3.33 to 1.78)
Mean lag 0–4	−3.72 (−7.58 to 0.31)	−3.04 (−6.09 to 0.11)	−2.11[d] (−4.77 to 0.55)

[a]Details on the model can be found in the online supplemental material available with the electronic version of this article at www.epidem.com; click on ArticlePlus (eTable 1).
[b]Geometric mean is the antilog of arithmetic mean of log-transformed variable; %-change is the antilog of effect estimate obtained from regression minus 1.
[c]%-Change is the effect estimate obtained from regression divided by arithmetic mean of fibrinogen.
[d]Randomly pooled effect estimates, otherwise fixed effect pooling (based on χ^2-test for homogeneity between city-specific effect estimates).

step in the stimulation of acute phase proteins in the liver. The acute phase proteins C-reactive protein and fibrinogen reacted correspondingly.

To our knowledge no studies have looked at low-grade systemic inflammation and air temperature at the population level, although many studies have observed the influence of weather changes on hospitalization and mortality. Our findings are in agreement with some previous research without controlling for air pollution. In particular, Danet et al[23] have reported that a 10°C decrease in air temperature is associated with a 13% increase in daily rates of MI and coronary deaths. Similarly, a 1°C decrease in mean air temperature yielded a 5% increase in Acute Coronary Syndrome, in Athens, Greece.[24] These results are not completely in accordance with the U-shaped relation between temperature and mortality from coronary artery disease that are reported from a study in Taiwan.[25] Below the range of 26°C to 29°C that corresponded to a minimum of deaths, the risk increased by 2.8% per 1°C reduction. As an explanation for the absence of the ascending limb of the U-shaped curve in a more temperate climate, Fries et al[26] have proposed that thermal stress caused by the atmospheric conditions may be too weak to influence the incidence of MI. Braga et al[27] found that in colder cities both high and low temperatures were associated with increased CVD deaths, with the effect of cold temperatures persisting for days, whereas the effect of high temperatures was restricted to the day of the death or the day before, and included some harvesting. In comparison the hot-day effect was one-fifth the cold-day effect. In hot cities neither hot nor cold temperatures had much effect on CVD deaths. However, there have also been many studies on extreme high temperatures or heat waves and increased mortality.[28–30] In this context it is important to note that in the AIRGENE study the entire summer was included only in Augsburg, Germany.

Several mechanisms are thought to be involved in the impact of air temperature on CVD. Environmental temperature has an inverse relation with blood pressure.[6,31,32] An increased blood pressure decreases the ratio of myocardial oxygen supply to demand and may lead to myocardial ischemia, particularly in a vulnerable myocardium. Moreover, pulse pressure increases and this may result in arterial dissection and Acute Coronary Syndrome. Apart from the above, fibrinogen is inversely related to air temperature, but part of the rise may be the result of seasonal respiratory infections.[32–36] However, in the presented study clinical visits have been postponed, observations have been excluded when patients showed any signs of infections, and adjustment for influenza was included to ensure no such influence on the inflammatory markers. In cold conditions the plasma concentrations of certain clotting factors, platelet count and their in vitro aggregation are all increased and promote clotting.[33,37–40] Furthermore, reduced plasma volume and increased blood

viscosity during cold exposure also tend to promote thrombosis.[37,40] The role of these factors in atherogenesis is uncertain but cold can also adversely alter plasma lipid concentrations leading to a potential chronic atherogenic effect.[32,33,35] In addition, C-reactive protein-levels seem to be seasonally dependent, with higher values during winter and spring[41] and with cardiovascular events more prominent during the cold season. The acute changes in processes involved in atherothrombosis may acutely increase the risk of an event. Elevated single measurements of these risk factors, such as C-reactive protein, have been implicated in increasing the risk of cardiovascular events in cohort studies.[42,43] Therefore, these recurrent acute changes during winter months may contribute to the progression of atherosclerosis. On the other hand, the relationship between temperature and Acute Coronary Syndrome may be indirectly causal. During winter months there may be increased indoor smoking, increased body weight due to a higher fat intake, or lower levels of physical activity and a higher rate of loneliness.

The Eurowinter Survey by Keatinge et al[44] made clear that especially the elderly are an extremely vulnerable population group with regard to low ambient and room temperatures. Given the fact that the association between temperature and Acute Coronary Syndrome may be attributed to pathophysiological factors, it would be wise to take sufficient precautions against cold, such as adequate indoor heating, sufficient protective clothing, and the adoption of a healthier lifestyle during winter.

Strengths and Limitations

The large cohort of vulnerable post MI patients with up to 8 consecutive measurements of inflammatory markers levels is the main strength of this study. As increased concentrations in C-reactive protein, interleukin-6, and fibrinogen have been associated with the risk of CVD and mortality,[45–48] it is particularly necessary to study high-risk groups. However, this is also the reason why the studied patients were heavily medicated, in particular statins were taken by almost all subjects regularly. In addition, the multiethnic origin of the participants, the large range of European climates and the severe adjustments for confounding are strengths of this paper. We selected as our main model the more conservative model without adjustment for season because we wanted to avoid an overestimation of the temperature effect due to induced confounding as long-term time trend, temperature and season all compete for the same effect in the model.

Air pollution is a factor that has been associated with CVD mortality and morbidity, and it correlates well with air temperature. It is still unclear whether air pollutants are confounders or effect modifiers of the temperature-biomarker association. However, Ruckerl et al[22] have shown an effect of ultrafine particles on interleukin-6 in the first 24 hours after air pollution exposure and an association of the 5-day-average of PM_{10} with fibrinogen in this same study population. Therefore, we additionally adjusted for particulate matter to disentangle the effects of temperature and air pollution on the inflammatory markers.

Nevertheless one limitation is that only outdoor temperature was measured while study subjects probably spent a lot of time indoors. However, as the number of cardiovascular events is increased in winter, it is very likely that the effect of cold temperature on inflammatory markers is linked to short exposures to outdoor temperatures. In the cities with warmer climates, the housing conditions and lower personal adjustment to cold temperatures might contribute to the increase of cardiovascular events in winter. This might also explain the strong effects on inflammatory markers in Rome as observed in this study (Fig. 1). Another limitation of the study is the relatively small number of observations in the really hot months of the year. This might be one reason why we have not found a U-shaped response curve, as has sometimes seen for the association between air temperature and mortality.[25,49,50] Another reason for the mainly linear associations in this study might be that the study population consisted of vulnerable people, but not extremely sick people—because the latter would not have been able to come to the study centers for their visits, whereas mortality studies depend by their nature on very sick people.

The present work suggests that a decrease in air temperature is associated with an increase in inflammatory markers, which might lead to the promotion of atherosclerosis. Our findings offer evidence for temperature exposure playing a role in seasonal patterns of cardiovascular morbidity and mortality by examining markers of intermediate processes. Therefore, we added pathophysiologic plausibility for the effect of environmental conditions on the cardiovascular system that could be investigated in future prospective cohort studies.

ACKNOWLEDGMENTS

The AIRGENE study group comprises the following partners: Helmholtz Zentrum Muenchen, German Research Center for Environmental Health, Institute of Epidemiology (Neuherberg, Germany): A. Peters (PI), M.-A. Bind, I. Brueske-Hohlfeld, H. Chavez, J. Cyrys, U. Geruschkat, H. Grallert, S. Greven, A. Ibald-Mulli, T. Illig, H. Kirchmair, S. von Klot, M. Kolz, M. Marowsky-Koeppl, M. Mueller, R. Rueckerl, A. Schaffrath Rosario, A. Schneider, H.-E. Wichmann; Institute of Health Economics and Health Care Management (Neuherberg, Germany): R. Holle, H. Nagl; KORA Study Center (Augsburg, Germany): I. Fabricius, C. Greschik, F. Günther, M. Haensel, U. Hahn, U. Kuch, C. Meisinger, M. Pietsch, E. Rempfer, G. Schaich, I. Schwarzwälder, B. Zeitler; KORA Myocardial Infarction Registry (Augsburg, Germany): H. Loewel; University of Ulm, Department of Cardiology (Germany): W. Koenig (PI), N. Khuseyinova, G. Trischler; Local Health Authority RME, Department of Epidemiology ASL RME (Rome, Italy): F. Forastiere (PI), P. Compagnucci, F. Di Carlo, M. Ferri, A. Montanari, C. Perucci, S. Picciotto, E. Romeo, M. Stafoggia; Catholic University (Rome, Italy): R.

Pistelli, L. Altamura, M.R. Andreani, F. Baldari, F. Infusino, P. Santarelli; Hospital San Filippo Neri: A.P. Jesi; Italian National Institutes of Health (Rome, Italy): G. Cattani, A. Marconi; National Public Health Institute (KTL) (Kuopio/ Helsinki, Finland): J. Pekkanen (PI), M. Alanne, H. Alastalo, T. Eerola, J. Eriksson, T. Kauppila, T. Lanki, P. Nyholm, M. Perola, V. Salomaa, P. Tiittanen; Helsinki University Central Hospital: K. Luotola; Institute of Environmental Medicine, Karolinska Institute (Stockholm, Sweden); Department of Occupational and Environmental Health, Stockholm County Council (Stockholm, Sweden); Department of Cardiology, Stockholm South Hospital (Stockholm, Sweden): T. Bellander (PI), N. Berglind, K. Bohm, R. Härdén, E. Lampa, P. Ljungman, F. Nyberg, B. Ohlander, G. Pershagen, M. Rosenqvist, T. Larsdotter Svensson, E. Thunberg, G. Wedeen; Municipal Institute of Medical Research (Barcelona, Spain): J. Sunyer (PI), M. Covas, M. Fitó, M. Grau, B. Jacquemin, J. Marrugat, L. Muñoz, G. Perelló, E. Plana, C. Rebato, H. Schroeder, C. Soler; University of Athens Medical School, Department of Hygiene and Epidemiology (Athens, Greece): K. Katsouyanni (PI), A. Chalamandaris, K. Dimakopoulou, D. Panagiotakos; Department of Cardiology (Athens, Greece): C. Stefanadis, C. Pitsavos, C. Antoniades, C. Chrysohoou, J. Mitropoulos; University of Helsinki, Department of Physical Sciences (Helsinki, Finland): M. Kulmala (PI), P. Aalto, P. Paatero.

REFERENCES

1. Colwell RR, Epstein PR, Gubler D, et al. Climate change and human health. *Science*. 1998;279:968–969.
2. McMichael AJ. The urban environment and health in a world of increasing globalization: issues for developing countries. *Bull World Health Organ*. 2000;78:1117–1126.
3. Hajat S, Kovats RS, Atkinson RW, et al. Impact of hot temperatures on death in London: a time series approach. *J Epidemiol Community Health*. 2002;56:367–372.
4. Koken PJ, Piver WT, Ye F, et al. Temperature, air pollution, and hospitalization for cardiovascular diseases among elderly people in Denver. *Environ Health Perspect*. 2003;111:1312–1317.
5. Barnett AG, Dobson AJ, McElduff P, et al. Cold periods and coronary events: an analysis of populations worldwide. *J Epidemiol Community Health*. 2005;59:551–557.
6. Modesti PA, Morabito M, Bertolozzi I, et al. Weather-related changes in 24-hour blood pressure profile: effects of age and implications for hypertension management. *Hypertension*. 2006;47:155–161.
7. Jehn M, Appel LJ, Sacks FM, et al. The effect of ambient temperature and barometric pressure on ambulatory blood pressure variability. *Am J Hypertens*. 2002;15:941–945.
8. Koenig W, Rosenson RS. Acute-phase reactants and coronary heart disease. *Semin Vasc Med*. 2002;2:417–428.
9. Ridker PM, Morrow DA. C-reactive protein, inflammation, and coronary risk. *Cardiol Clin*. 2003;21:315–325.
10. Willerson JT, Ridker PM. Inflammation as a cardiovascular risk factor. *Circulation*. 2004;109:II2–II10.
11. Smith SC Jr., Anderson JL, Cannon RO III, et al. CDC/AHA workshop on markers of inflammation and cardiovascular disease: application to clinical and public health practice: report from the clinical practice discussion group. *Circulation*. 2004;110:e550–e553.
12. Wilmshurst P. Temperature and cardiovascular mortality. *BMJ*. 1994; 309:1029–1030.
13. Peters A, Schneider A, Greven S, et al. Air pollution and inflammatory response in myocardial infarction survivors: gene-environment-interactions in a high-risk group. Study design of the AIRGENE study. *Inhal Toxicol*. 2007;19(suppl 1):161–165.
14. de Lemos JA, McGuire DK, Drazner MH. B-type natriuretic peptide in cardiovascular disease. *Lancet*. 2003;362:316–322.
15. Steadman RG. The assessment of sultriness, Part 1: a temperature-humidity index based on human physiology and clothing science. *J Appl Meteorol*. 1979;18:861–873.
16. Kalkstein LS, Valimont KM. An evaluation of summer discomfort in the United States using a relative climatological index. *Am Meteorol Soc*. 1986;67:842–848.
17. Katsouyanni K, Schwartz J, Spix C, et al. Short term effects of air pollution on health: a European approach using epidemiologic time series data: the APHEA protocol. *J Epidemiol Community Health*. 1996;50(suppl 1):S12–S18.
18. Greven S, Küchenhoff H, Peters A. Additive mixed models with P-Splines. In: Hinde J, Einbeck J, Newell J, editors. *Proceedings of the 21st International Workshop on Statistical Modelling, Galway, Ireland, July 3–7, 2006*. Amsterdam: Statistical Modelling Society; 2006. pp. 201–207.
19. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials*. 1986;7:177–188.
20. Normand SL. Meta-analysis: formulating, evaluating, combining, and reporting. *Stat Med*. 1999;18:321–359.
21. van Houwelingen HC, Arends LR, Stijnen T. Advanced methods in meta-analysis: multivariate approach and meta-regression. *Stat Med*. 2002;21:589–624.
22. Ruckerl R, Greven S, Ljungman P, et al. Air pollution and inflammation (IL-6, CRP, fibrinogen) in myocardial infarction survivors. *Environ Health Perspect*. 2007;115:1072–1080.
23. Danet S, Richard F, Montaye M, et al. Unhealthy effects of atmospheric temperature and pressure on the occurrence of myocardial infarction and coronary deaths. A 10-year survey: the Lille-World Health Organization MONICA project (Monitoring trends and determinants in cardiovascular disease). *Circulation*. 1999;100:E1–E7.
24. Panagiotakos DB, Chrysohoou C, Pitsavos C, et al. Climatological variations in daily hospital admissions for acute coronary syndromes. *Int J Cardiol*. 2004;94:229–233.
25. Pan WH, Li LA, Tsai MJ. Temperature extremes and mortality from coronary heart disease and cerebral infarction in elderly Chinese. *Lancet*. 1995;345:353–355.
26. Fries R, Jung J, Ozbek C, et al. Influence of thermal stress on the incidence of acute myocardial infarction in a temperate climate. *Cardiology*. 1998;90:67–71.
27. Braga AL, Zanobetti A, Schwartz J. The effect of weather on respiratory and cardiovascular deaths in 12 U.S. cities. *Environ Health Perspect*. 2002;110:859–863.
28. Tan J, Zheng Y, Song G, et al. Heat wave impacts on mortality in Shanghai, 1998 and 2003. *Int J Biometeorol*. 2007;51:193–200.
29. Keatinge WR, Donaldson GC, Cordioli E, et al. Heat related mortality in warm and cold regions of Europe: observational study. *BMJ*. 2000;321: 670–673.
30. Hajat S, Armstrong B, Baccini M, et al. Impact of high temperatures on mortality: is there an added heat wave effect? *Epidemiology*. 2006;17: 632–638.
31. Kunes J, Tremblay J, Bellavance F, et al. Influence of environmental temperature on the blood pressure of hypertensive patients in Montreal. *Am J Hypertens*. 1991;4:422–426.
32. Elwood PC, Beswick A, O'Brien JR, et al. Temperature and risk factors for ischaemic heart disease in the Caerphilly prospective study. *Br Heart J*. 1993;70:520–523.
33. Woodhouse PR, Khaw KT, Plummer M, et al. Seasonal variations of plasma fibrinogen and factor VII activity in the elderly: winter infections and death from cardiovascular disease. *Lancet*. 1994;343:435–439.
34. Horan JT, Francis CW, Falsey AR, et al. Prothrombotic changes in hemostatic parameters and C-reactive protein in the elderly with winter acute respiratory tract infections. *Thromb Haemost*. 2001;85:245–249.
35. Mavri A, Guzic-Salobir B, Salobir-Pajnic B, et al. Seasonal variation of some metabolic and haemostatic risk factors in subjects with and without coronary artery disease. *Blood Coagul Fibrinolysis*. 2001;12:359–365.
36. Mercer JB, Osterud B, Tveita T. The effect of short-term cold exposure on risk factors for cardiovascular disease. *Thromb Res*. 1999;95:93–104.
37. Keatinge WR, Coleshaw SR, Cotter F, et al. 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 (Clin Res Ed)*. 1984;289:1405–1408.
38. Thakur CP, Anand MP, Shahi MP. Cold weather and myocardial infarction. *Int J Cardiol*. 1987;16:19–25.
39. Yeh CJ, Chan P, Pan WH. Values of blood coagulating factors vary with ambient temperature: the Cardiovascular Disease Risk Factor Two-Township Study in Taiwan. *Chin J Physiol*. 1996;39:111–116.
40. Neild PJ, Syndercombe-Court D, Keatinge WR, et al. Cold-induced increases in erythrocyte count, plasma cholesterol and plasma fibrinogen of elderly people without a comparable rise in protein C or factor X. *Clin Sci (Lond)*. 1994;86:43–48.
41. Sung KC. Seasonal variation of C-reactive protein in apparently healthy Koreans. *Int J Cardiol*. 2006;107:338–342.
42. Ridker PM, Cushman M, Stampfer MJ, et al. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N Engl J Med*. 1997;336:973–979.
43. Koenig W, Sund M, Frohlich M, et al. C-Reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men: results from the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) Augsburg Cohort Study, 1984 to 1992. *Circulation*. 1999;99:237–242.
44. Keatinge WR, Donaldson GC, Bucher K, et al. Winter mortality in relation to climate. *Int J Circumpolar Health*. 2000;59:154–159.
45. Koenig W, Khuseyinova N. Biomarkers of atherosclerotic plaque instability and rupture. *Arterioscler Thromb Vasc Biol*. 2007;27:15–26.
46. Ridker PM, Hennekens CH, Buring JE, et al. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med*. 2000;342:836–843.
47. Espinola-Klein C, Rupprecht HJ, Bickel C, et al. Inflammation, atherosclerotic burden and cardiovascular prognosis. *Atherosclerosis*. 2007; 195:e126–e134.
48. Harris TB, Ferrucci L, Tracy RP, et al. Associations of elevated interleukin-6 and C-reactive protein levels with mortality in the elderly. *Am J Med*. 1999;106:506–512.
49. Ballester F, Corella D, Pérez-Hoyos S, et al. Mortality as a function of temperature. A study in Valencia, Spain, 1991–1993. *Int J Epidemiol*. 1997;26:551–561.
50. Nayha S. Cold and the risk of cardiovascular diseases. A review. *Int J Circumpolar Health*. 2002;61:373–380.