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Determinants of the Acute-Phase Protein C-Reactive Protein in Myocardial Infarction Survivors: The Role of Comorbidities and Environmental Factors

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BACKGROUND: C-reactive protein (CRP), a sensitive marker of the acute-phase response, has been associated with future cardiovascular endpoints independently of other risk factors. A joint analysis of the role of risk factors in predicting mean concentrations and variation of high-sensitivity CRP (hsCRP) in serum has not been carried out previously.

METHODS: We used data from 1003 myocardial infarction (MI) survivors who had hsCRP measured monthly up to 8 times and multivariate mixed effects statistical models to study the role of time-variant and -invariant factors on the geometric mean of and the intraindividual variation in hsCRP concentrations.

RESULTS: Patients with ≥6.5% glycosylated hemoglobin (HbA1c) had 26.2% higher hsCRP concentrations (95% CI, 7.2%–48.6%) and 20.7% greater variation in hsCRP values ($P = 0.0034$) than patients with lower baseline Hb A_{1c} values (<6.5%). Similar but less pronounced differences were seen in patients with a self-reported diagnosis of type 2 diabetes. hsCRP concentrations showed less variation in patients who reported angina pectoris, congestive heart failure, or emphysema (−11.0%, −24.9%, and −41.6%, respectively, vs patients without these conditions) but greater variation in males and smokers (+24.8% and +27.3%, respectively, vs females and nonsmokers). Exposures in the 24 h before blood sampling, including exposure to environmental tobacco smoke, alcohol consumption, and extreme stress, did not have a major impact.

CONCLUSIONS: One or 2 hsCRP measurements may not be sufficient to adequately characterize different patient groups after MI with similar precisions. We found hsCRP concentrations to be especially variable in males, smokers, and patients with increased Hb A_{1c} values.

C-reactive protein (CRP),[14] a sensitive marker of the acute-phase response, has attracted increasing attention in recent years because many epidemiologic studies have shown consistent positive associations between high-sensitivity CRP (hsCRP) concentrations in the peripheral circulation and the risk of future cardiovascular events, independently of established risk factors. Associations have been found with angina pectoris *(1)* and "hard" coronary and cerebrovascular events in men and women *(2)*. Koenig et al. *(3)* reported an almost 3-fold increase in the risk of a first major coronary event for individuals in the highest quintile of the hsCRP distribution in a random sample of initially healthy men from the general population. These findings have led to an ongoing discussion on whether hsCRP should be measured routinely in individuals at risk of cardiovascular disease *(4)*. The CDC and the American Heart Association recently recommended that hsCRP be measured in individuals at intermediate risk (as defined by the Framingham Risk Score), with the assays to be performed on 2 samples from each

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[14] Nonstandard abbreviations: CRP, C-reactive protein; hsCRP, high-sensitivity CRP; BMI, body mass index; MI, myocardial infarction; ETS, environmental tobacco smoke; P-spline, penalized splines; Hb A_{1c}, glycosylated hemoglobin; CHF, congestive heart failure.

person, fasting or nonfasting, taken approximately 2 weeks apart. In the case of an hsCRP measurement >10 mg/L, indicating an acute inflammatory process, the measurement should be discarded and repeated 2 weeks later *(5)*. For routine screening, knowledge of basic determinants of hsCRP concentrations is essential. Several determinants have been studied intensively in the past, including nutrition *(6)*, medication *(2, 7–10)*, smoking *(11, 12)*, body mass index (BMI), and physical activity *(13–15)*; however, most of these studies relied on only 1 or 2 measurements per patient. Only a few studies have examined factors acutely affecting hsCRP concentrations *(13, 14, 16)* or the degree of within-patient variation in hsCRP concentration. We used data from a large European study of myocardial infarction (MI) survivors who had hsCRP measured up to 8 times in an attempt to conduct, for the first time, a joint analysis of the role of risk factors in predicting the mean hsCRP concentration and the intraindividual variation in hsCRP. Given that clinical practice may consider preventive measures based on a single hsCRP measurement, this study may contribute important additional information.

Materials and Methods

STUDY POPULATION

The AIRGENE study, a prospective longitudinal study of post-MI patients, was performed in 6 European cities—Athens (Greece), Augsburg (Germany), Barcelona (Spain), Helsinki (Finland), Rome (Italy), and Stockholm (Sweden). Candidates for the study were identified from population registries of MI patients [Augsburg—Cooperative Health Research in the Augsburg Region (KORA) *(17)*; Barcelona; Stockholm] or from administrative databases of hospital admissions (Athens, Helsinki, Rome). MI was defined according to the Joint European Society of Cardiology/American College of Cardiology Committee for the Redefinition of Myocardial Infarction *(18)*; the study design has been described in detail elsewhere *(19)*. In brief, the study recruited patients 35–80 years of age who had experienced an MI between 4 months and 6 years before the start of the study. Patients who had undergone interventional procedures <3 months before the beginning of the study or who had chronic inflammatory diseases were not included. Because AIRGENE initially was a study of the health effects of air pollution, the recruitment of current nonsmokers was preferred, but the inclusion of smokers in some of the centers was unavoidable. All study partners had the study protocol approved by their local human-studies 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.

CLINICAL MEASUREMENTS

Patients were invited to participate in 6 to 8 clinical visits at approximately monthly intervals between May 2003 and July 2004. At the first visit, the patient completed a baseline questionnaire regarding comorbidities, regular exercise, smoking history, exposure to environmental tobacco smoke (ETS), socioeconomic status, and alcohol intake. Data recorded regarding medication intake included brand names, doses, and intake pattern. Clinical measurements included blood pressure and BMI, and a serum sample was taken to assess baseline serum lipids, glycosylated hemoglobin (Hb A_{1c}) (an indicator of glucose control), and N-terminal pro-B-type natriuretic peptide (a marker of hemodynamic stress).

Each clinical visit was scheduled at the same time of the day and on the same day of the week to minimize the impact of circadian and weekly variation. If patients had acute infections such as a cold or influenza during the 3 days preceding the scheduled visit, examinations were postponed or the blood samples were excluded from analyses.

The patient was asked to recall medication intake for the previous 7 days at each clinical visit and to complete a short questionnaire about time-varying variables in the previous 24 h, such as active and passive smoking, physical activity, perception of extreme stress or anger, consumption of alcohol and black or green tea, and the time of the latest meal before blood draw.

Venous blood samples for preparing EDTA-plasma for hsCRP measurement were drawn while the patient was sitting. Samples were cooled and stored at 4 °C for further processing within a maximum of 4 hours. EDTA-containing blood was centrifuged for 20 min at 2500*g* in a centrifuge precooled to 4 °C. Plasma aliquots were shipped on dry ice to the central laboratory in Ulm, Germany, and were stored at −80 °C until analysis. Blood samples were analyzed for hsCRP by latex-enhanced immunonephelometry on a BNII analyzer (Siemens). The interassay CVs for hsCRP were 4.3%, 6.2% and 4.5% at hsCRP concentrations of 1.17 mg/L, 2.38 mg/L, and 13.5 mg/L, respectively.

STATISTICAL ANALYSES

All statistical analyses were performed with the Statistical Analysis System (SAS) software package (Version 9.1 for Windows; SAS Institute).

We calculated hsCRP CVs as described by Bland and Altman *(20)* and Fraser and Harris *(21)*. We used the SAS MIXED procedure to compute estimates of between- and within-individual variances, assuming nested normal random-effects models. These compo-

nents of variation were then transformed into corresponding CVs, which were calculated as the square root of the respective variance-component estimates divided by the overall mean and then expressed as percentages.

Determinants of mean hsCRP concentrations. hsCRP data required log-transformation to fulfill the model assumption of residual normality; therefore, concentration results are given as the geometric mean. To estimate the effect of various determinants on the geometric means of hsCRP concentrations, we used mixed-effects models with random patient effects accounting for repeated measures. Because the half-life of hsCRP is 19 h *(22)* and therefore much shorter than the intervals between visits, we assumed a compound symmetry structure for the covariance matrix to model the correlation between repeated measures in each patient. Penalized splines (P-splines) in the additive mixed-model framework allowed for nonparametric exposure–response functions *(23)*.

We first built a confounder model (base model), which included preselected time-invariant patient characteristics, to permit the assumption of a normally distributed random patient intercept. We tested a wide range of variables known from the literature to have a possible influence on hsCRP, such as city, age, sex, and BMI. Linear variables were added linearly to the model. The decision on whether a specific factor remained in the model was based on the goodness-of-fit according to Akaike's information criterion.

In a second step, additional time-invariant variables not initially considered for the base model (such as reported diseases, regular medication intake, and smoking history) as well as time-varying variables, such as physical activity or alcohol consumption in the 24 h before the blood draw, were added to the base model, always one at a time. To avoid overcontrol, we removed pack-years of smoking from the base model when we analyzed smoking status, and we removed Hb A_{1c} for the analysis of diabetes. Variables that described a time difference, such as the time of the last meal before the blood sampling, were categorized into 4 intervals of 6 h each: 0–5 h, 6–11 h, 12–17 h, and 18–23 h before sampling. Results are given as the percent change in the geometric mean of the hsCRP concentration.

Determinants of hsCRP variation. To calculate differences in variation, we used the MIXED procedure in SAS with the "repeated/group= " statement, which calculates the within-patient variation, and a "random/group= " statement, which allows for different intercepts in the defined groups, representing the between-patient variation. A likelihood-ratio test was used to determine if the differences between the groups were statistically significant. Linear variables were categorized beforehand, usually with interquartile ranges. Results are given as variance estimates of log-transformed hsCRP concentrations, with between-individual and within-individual results presented separately (Fig. 1), and as the relative difference (in percent) in within-individual variation compared with the reference group (see tables).

To account for the large number of statistical tests, we corrected the significance level of the P value to 0.00125, which equals a Bonferroni correction for 40 variables.

Sensitivity analyses. We conducted sensitivity analyses for comorbidities that might be associated with the intake of certain medications and used a χ^2 test to evaluate possible associations between comorbidities and medication intake. If we found an association ($P \leq 0.05$), we adjusted the multivariable model for the respective medication to investigate whether the comorbidity effect was altered by including medication in the model. Moreover, we calculated a model that included most of the presented variables to identify those variables that led to the greatest increase in variation.

Results

STUDY POPULATION

In total, 1003 patients with at least 2 valid blood samples participated in the study. Of the 6068 collected samples, 255 had to be excluded because of acute infections or surgical procedures that occurred shortly before the clinic visit. Overall, 5813 plasma samples remained for analysis (see Table 1 in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol55/issue2).

Table 2 in the online Data Supplement summarizes the patient characteristics by center, and Table 3 in the online Data Supplement presents the patient characteristics according to sex. Mean hsCRP concentrations were highest in Barcelona and lowest in Helsinki; however, hsCRP concentrations were not exceptionally high on average. In 75 samples, the hsCRP concentration was lower than 0.16 mg/L, and these values were set at 0.16 mg/L. More details are given elsewhere *(19)*. The CV was 107% of the overall mean within individuals and 139% between individuals.

ASSOCIATION BETWEEN TIME-INVARIANT VARIABLES AND hsCRP

Base model. Table 1 shows the associations of patient characteristics with the geometric mean of the hsCRP concentration and its variation, as estimated jointly from the base model. Male participants had signifi-

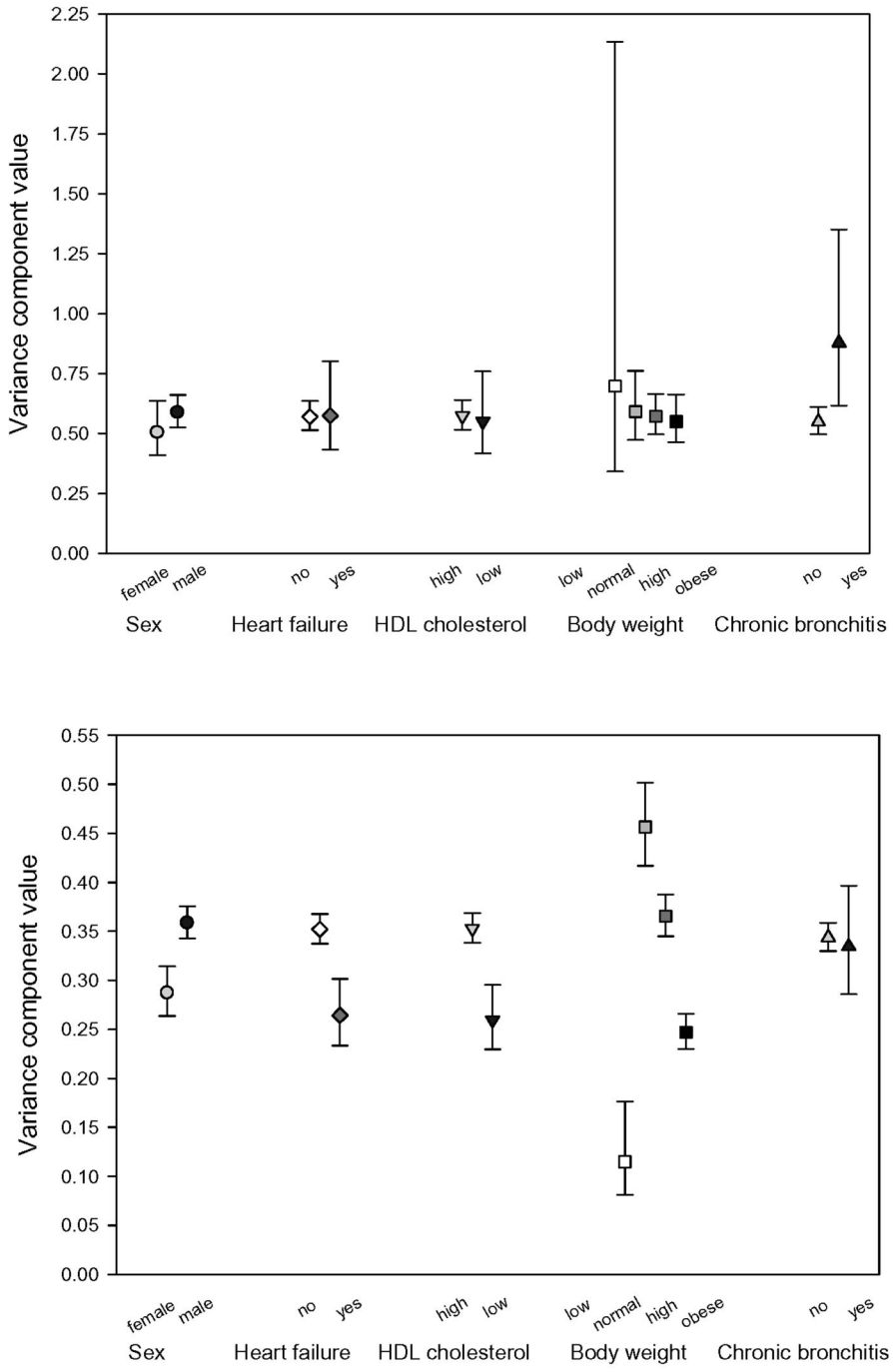

Fig. 1. Variation in mean hsCRP concentration between (upper panel) and within (lower panel) individuals for separate hsCRP measurements made over time.

Variance component values are presented according to patient characteristics (sex, CHF diagnosis, HDL cholesterol concentration, body weight, and chronic bronchitis). Error bars represent 95% confidence limits.

Table 1. Association of time-invariant variables with the geometric mean of and the variation in hsCRP concentration—multivariate base model.

Variable	n	Change from GM,[a] %	95% Confidence limits, %		*P*, mean	Variation (difference from reference group), %	*P*, variation
			Lower	Upper			
City							
Athens	108	−29.54	−43.46	−12.19	0.002	12.4	0.0007[b]
Augsburg	200	−25.95	−36.83	−13.19	0.0002	19.8	
Barcelona	169	9.80	−7.61	30.48	0.29	14.9	
Helsinki	195	−26.44	−37.27	−13.75	0.00016[b]	6.5	
Rome	134	−13.52	−27.74	3.50	0.11	4.1	
Stockholm	197	Ref				Ref	
Sex							
Male	788	−13.28	−23.78	−1.34	0.03	24.8	<0.0001[b]
Female	215	Ref				Ref	
Age, years[c]							
<50	115	28.07	6.26	54.35	0.009	25.5	<0.0001[b]
50–59	271	Ref				Ref	
60–69	348	18.13	3.39	34.97	0.014	27.8	
≥70	269	28.26	10.45	48.95	0.001	37.2	
BMI[d,e]							
Linear: per 5-kg/m^2 increase	999	37.80	29.69	46.43	<0.0001[b]	—	
Obese	316	86.02	59.97	116.31	<0.0001[b]	−45.8	<0.0001[b]
Overweight	483	33.76	16.51	53.57	<0.0001[b]	−19.9	
Normal	189	Ref				Ref	
Underweight	11	−33.83	−59.49	8.10	0.099	−74.8	
Number of MIs							
≥2	150	13.10	−2.15	30.73	0.010	18.5	0.0027
1	853	Ref				Ref	
Smoking[c,e,f]							
Linear: per 25–pack-year increase	1002	16.31	9.65	23.38	<0.0001[b]	—	
>30.75 Pack-years	228	48.04	26.94	72.64	<0.0001[b]	1.1	0.082
≤30.75 Pack-years	470	16.45	3.17	31.44	0.014	5.0	
Never smoker	304	Ref				Ref	
Hb A_{1c}[c]							
High (≥6.5%)	108	26.24	7.23	48.61	0.005	20.7	0.0034
Low (<6.5%)	868	Ref				Ref	
Log-transformed NT-proBNP[c,e]							
Linear: per 2.7-ng/L increase	995	38.43	20.61	58.89	<0.0001[b]		
≥5.98 (ng/L)	498	26.58	7.69	49.41	0.004	−4.4	<0.0001[b]
4.47–5.97 (ng/L)	497	3.26	−9.53	17.86	0.64	34.2	
<4.47 (ng/L)		Ref				Ref	
Total cholesterol[c,e]							
Per 1.03-mmol/L increase	998	15.53	9.60	21.79	<0.0001[b]	—	
High (>6.46 mmol/L)	60	30.07	15.44	46.55	<0.0001[b]	−6.1	0.052
At risk (5.17–6.46 mmol/L)	249	23.85	−0.11	53.56	0.051	8.7	
Low (<5.17 mmol/L)	689	Ref				Ref	

[a] GM, geometric mean; Ref, reference; NT-proBNP, N-terminal pro–B-type natriuretic peptide.
[b] Statistically significant after adjusting for multiple testing ($\alpha = 0.00125$).
[c] Measured at baseline.
[d] BMI classification according to the WHO (2000).
[e] Base model including the linear variable.
[f] Categories correspond to interquartile ranges.

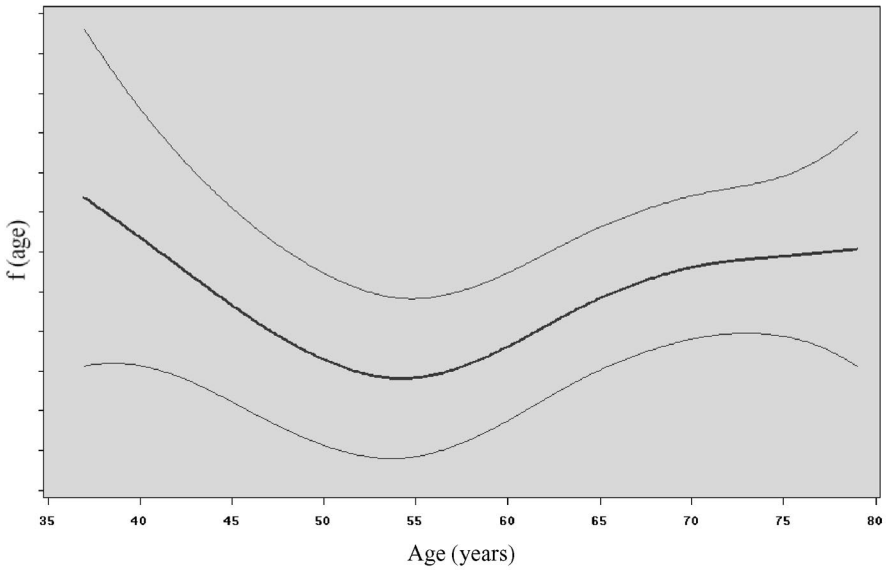

Fig. 2. Smooth spline of log hsCRP concentration [f(age)] on patient age in 1003 MI patients from the AIRGENE study, after adjustment for all of the variables in the base model.

cantly lower hsCRP concentrations than female participants but had greater variation over time in log hsCRP concentration (Fig. 1). This difference was less pronounced after controlling for the intake of hormone-replacement medications in women. We found a U-shaped relationship for age with the lowest hsCRP concentration in the age group of 50–59 years (Fig. 2), whereas most other associations were linear. A separate analysis showed that this effect was mainly driven by the results for men; women had a positive linear association between hsCRP concentration and age (data not shown). In contrast, hsCRP variation was greatest in the oldest patient group. Overweight and obese patients *(24)* had higher hsCRP concentrations than participants with normal weights, but the concentrations in these patients were less variable (Fig. 1). Hb A_{1c} concentrations >6.5% were positively associated with the geometric mean of and the variation in hsCRP concentration, whereas a diagnosis of type 2 diabetes was positively associated with the variation but not with the geometric mean (Table 2). hsCRP concentrations were also positively associated with higher concentrations of N-terminal pro–B-type natriuretic peptide and total cholesterol.

Additional time-invariant variables and hsCRP. Tables 2 and 3 summarize the associations of hsCRP concentration with disease history, lifestyle, and medication intake. A family history of MI was associated with slightly higher hsCRP concentrations. On the other hand, hsCRP concentrations showed less variation in patients who reported angina pectoris, congestive heart failure (CHF), emphysema, or a family history of MI (Fig. 1), and these results remained statistically significant after adjusting for multiple testing. Time since last MI did not show any association with the geometric mean of or the variation in hsCRP concentration (Table 2).

Habitual physical activity did not influence hsCRP concentrations; however, the variation in hsCRP concentration seemed to be higher in inactive people and lower in those who were partially active, compared with regularly active study participants. HDL cholesterol was inversely related to the geometric mean of the hsCRP concentration; greater variation in hsCRP concentration was noted in patients with increased HDL cholesterol concentrations (Table 3).

Patients reporting the intake of statins or other lipid-lowering drugs had lower hsCRP concentrations and less variation. On the other hand, patients taking angiotensin-converting enzyme inhibitors had greater variation in hsCRP concentrations, whereas the geometric mean was negatively associated with medication intake (Table 3). Use of acetylsalicylic acid or Ca^{2+}-channel blockers did not affect the geometric mean of or the variation in hsCRP concentration.

Table 4 summarizes the results for different smoking-related variables. Twenty-five pack-years of smoking produced an increase of approximately 16% in the

Table 2. Association of disease history with the geometric mean of and the variation in hsCRP concentration, adjusted for the variables of the base model.

Variable	n	Change from GM,[a] %	95% Confidence limits, %		*P*, mean	Variation (difference from reference group), %	*P*, variation
			Lower	Upper			
Type 2 diabetes (excluding Hb A_{1c} from the model)[b]							
Yes	198	4.22	−8.50	18.70	0.53	11.57	0.0052
No	805	Ref				Ref	
Angina pectoris[b]							
Yes	344	2.54	−8.08	14.39	0.65	−11.0	<0.0001[c]
No	658	Ref				Ref	
CHF[b]							
Yes	104	2.83	−13.66	22.48	0.75	−24.9	<0.0001[c]
No	899	Ref				Ref	
Emphysema[b]							
Yes	23	17.19	−16.64	64.75	0.36	−41.6	0.00024[c]
No	980	Ref				Ref	
Family history of MI							
≥1 Parent	353	12.49	0.48	25.94	0.04	−20.5	<0.0001[c]
No	547	Ref				Ref	
Time since last MI							
Per increase in 1 year	1003	−0.22	−4.76	4.54	0.93	—	
≥3 years	266	13.70	−17.04	55.83	0.42	6.51	0.15
2.9–1.5 years	481	8.75	−9.37	30.49	0.37	−0.38	
<1.5 years	256	Ref				Ref	
Stroke[b]							
Yes	62	−2.77	−21.78	20.86	0.80	1.6	0.094
No	941	Ref				Ref	
Hypertension[b]							
Yes	511	−8.32	−17.18	1.48	0.093	6.3	0.061
No	492	Ref				Ref	
Chronic bronchitis[b]							
Yes	67	36.47	10.97	67.81	0.003	−2.7	0.65
No	936	Ref				Ref	
Asthma[b]							
Yes	47	16.81	−7.58	47.64	0.19	−9.5	0.15
No	956	Ref				Ref	

[a] GM, geometric mean; Ref, reference.
[b] Ever physician-diagnosed.
[c] Statistically significant after adjusting for multiple testing ($\alpha = 0.00125$).

geometric mean of the hsCRP concentration, and inclusion of smoking status in the model had little effect on this result. Including pack-years of smoking, however, removes the borderline effect for ex-smokers that we found in the model that does not include pack-years of smoking. Examination of the effects of smoking and ETS exposure revealed a heterogeneous picture. Current regular smokers and nonsmokers who reported regular ETS exposure had higher hsCRP concentrations, whereas occasional smokers seemed to have lower hsCRP

Table 3. Association of lifestyle factors and medication intake with the geometric mean of and the variation in hsCRP concentration, adjusted for the variables of the base model.

Variable	n	Change from GM,[a] %	95% Confidence limits, %		*P*, mean	Variation (difference from reference group), %	*P*, variation
			Lower	Upper			
Health status							
Excellent/good	592	10.46	−1.13	23.39	0.08	6.5	0.067
Moderate	342	Ref				Ref	
Poor/very poor	68	33.06	7.71	64.36	0.008	−11.2	
Physical activity							
Inactive	219	7.06	−7.16	23.46	0.35	7.0	0.0011[b]
Partly or irregularly active	280	3.09	−8.51	16.16	0.62	−13.1	
Regularly active/trained	504	Ref				Ref	
HDL cholesterol (adjusted for total cholesterol)[c]							
Per increase in 0.39 mmol/L	998	−8.15	−13.90	−2.03	0.0010[b]	—	
>0.91 mmol/L	884	−16.78	−29.73	−1.45	0.033	36.0	<0.0001[b]
≤91 mmol/L	114	Ref				Ref	
Lipid-lowering drugs (all)							
Yes	858	−11.51	−20.21	−1.87	0.021	−19.25	<0.0001[b]
No		Ref				Ref	
Statins							
Yes	841	−11.17	−19.81	−1.61	0.023	−19.33	<0.0001[b]
No		Ref				Ref	
ACE inhibitors							
Yes	606	−15.29	−22.96	−6.85	0.0006[b]	19.21	0.58
No		Ref				Ref	
Systemic antiinflammatory medication							
Yes	234	−9.34	−17.91	0.12	0.05	28.23	<0.0001[b]
No		Ref				Ref	
Acetylsalicylic acid							
Yes	878	−1.52	−12.59	10.95	0.80	−7.34	<0.0001[b]
No		Ref				Ref	
Diuretics							
Yes	277	12.59	1.94	24.36	0.020	0.26	<0.0001[b]
No		Ref				Ref	
Ca^{2+}-channel blockers							
Yes	184	2.22	−9.49	15.43	0.72	−2.07	0.014
No		Ref				Ref	
Beta-blockers							
Yes	845	−0.60	−12.16	12.49	0.92	−10.52	0.18
No		Ref				Ref	
Hormone-replacement therapy (women only)							
Yes	28	18.65	−8.83	54.41	0.20	−15.23	0.014
No		Ref				Ref	

[a] GM, geometric mean; Ref, reference; ACE, angiotensin-converting enzyme.
[b] Statistically significant after adjusting for multiple testing ($\alpha = 0.00125$).
[c] Measured at baseline.

Table 4. Association of smoking and ETS exposure with the geometric mean of and the variation in hsCRP concentration, adjusted for the variables of the base model.

Variable		n	Change from GM,[a] %	95% Confidence limits, %		*P*, mean	Variation (difference from reference group), %	*P*, variation
				Lower	Upper			
Pack-years of smoking: excluding smoking status from the model	Linear: per increase of 25 pack-years	1002	16.31	9.65	23.38	<0.0001[b]	—	
Pack-years of smoking: including smoking status in the model	Linear: per increase of 25 pack-years	1002	14.88	7.59	22.67	<0.0001[b]	—	
Smoking status: excluding pack-years of smoking from the model	Current smoker (regular/occasional)		16.33	−5.91	43.85	0.16	10.9	0.095
	Ex-smoker	627	19.67	5.97	35.16	0.004	−2.4	
	Never smoker	277	Ref				Ref	
Smoking status: including pack-years of smoking in the model	Current smoker (regular/occasional)	99	4.59	−15.75	29.83	0.68	10.9	0.116
	Ex-smoker	627	5.97	−7.32	21.16	0.40	−2.2	
	Never smoker	277	Ref				Ref	
Smoking status and ETS exposure: excluding pack-years of smoking from the model	Current regular smoker	72	23.68	−2.19	56.38	0.08	27.3	<0.0001[b]
	Occasional smoker	27	−27.94	−47.63	−0.86	0.04	−24.6	
	Not current smoker, constant ETS exposure	136	9.57	−6.31	28.14	0.25	−9.9	
	Not current smoker, no constant ETS exposure	767	Ref				Ref	
Smoking status and ETS exposure: including pack-years of smoking in the model	Current regular smoker	72	15.23	−8.79	45.59	0.23	26.8	<0.0001[b]
	Occasional smoker	27	−21.20	−42.66	8.29	0.14	−25.0	
	Not current smoker, constant ETS exposure	136	6.50	−8.81	24.38	0.43	−10.3	
	Not current smoker, no constant ETS exposure	767	Ref				Ref	

[a] GM, geometric mean; Ref, reference.
[b] Statistically significant after adjusting for multiple testing ($\alpha = 0.00125$).

concentrations than nonsmokers not regularly exposed to cigarette smoke. The results were not statistically significant, however, especially when pack-years of smoking was included in the model. The numbers of participants were low in several of the groups (Table 4).

ASSOCIATION BETWEEN TIME-VARYING VARIABLES AND hsCRP

Time-varying variables had either no or a small influence on hsCRP concentration (Fig. 3A). Recent alcohol consumption and extreme stress or anger were associated with lower geometric-mean hsCRP

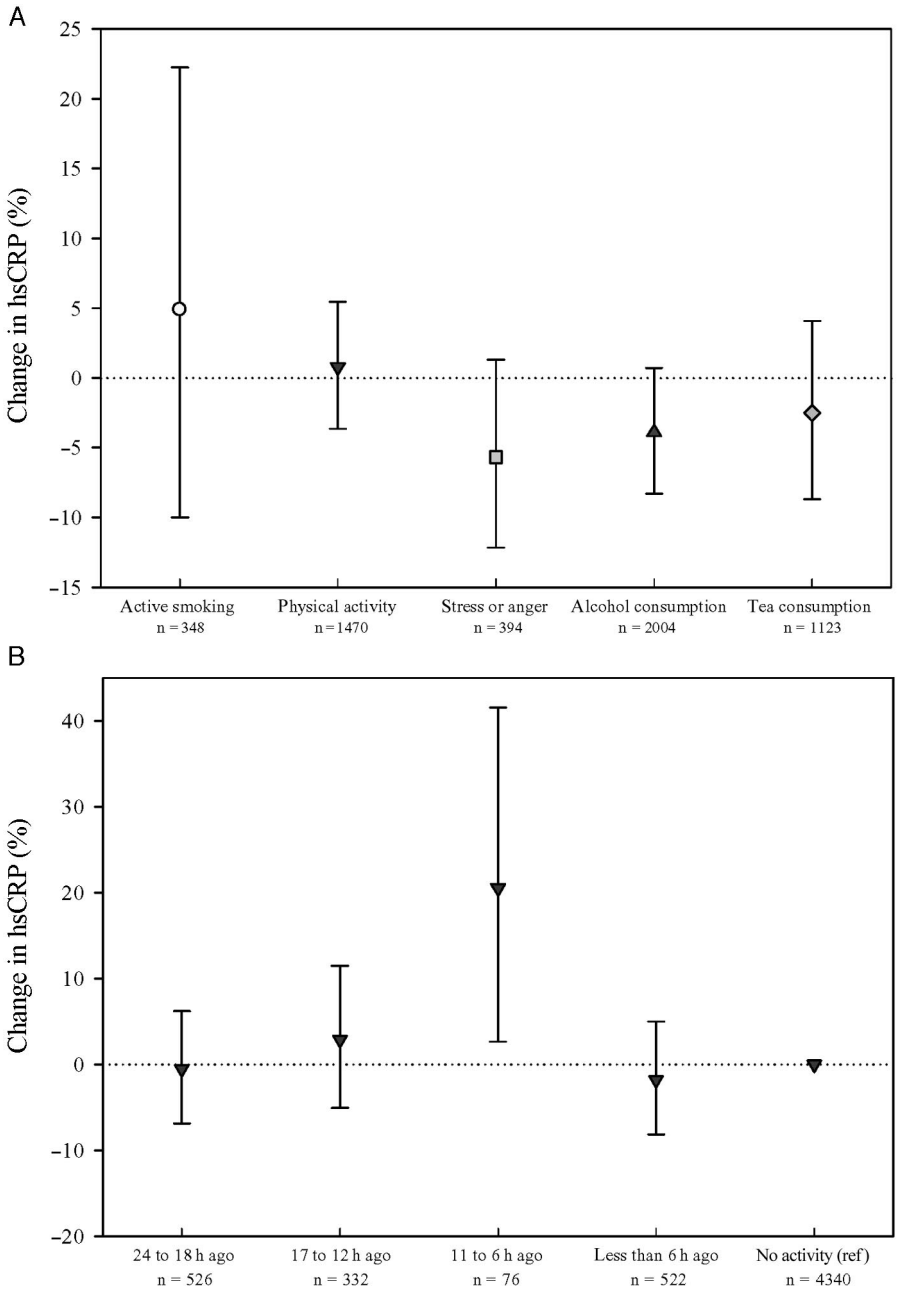

Fig. 3. Association between log hsCRP concentration and various variables (5813 visits in 1003 AIRGENE patients).
(A), Associations between log hsCRP concentration and lifestyle factors 24 h before blood draw. (B), Associations between log hsCRP concentration and physical activity in 6-h intervals before blood draw. ref, reference. Error bars represent 95% confidence limits.

concentrations, but the results were not statistically significant. Whereas physical activity over the previous 24 h showed no association with hsCRP concentration (Fig. 3A), physical activity between 6 and 11 hours before blood draw was associated with increased hsCRP concentrations (Fig. 3B). For the other time-varying variables, no such time-specific effects were seen.

SENSITIVITY ANALYSES

Additional adjustment for medication did not change the results for comorbidities (data not shown).

With very few exceptions, the results for the variation model that included all variables at the same time did not differ much from those described in the presented tables. The results revealed the largest increases in hsCRP variation for patients with HDL cholesterol concentrations >0.91 mmol/L, an older age (especially >70 years), male sex, a log B-type natriuretic peptide concentration of ≥5.98 (ng/L), and intake of anti-inflammatory medication (data not shown).

Discussion

We investigated repeated measurements of hsCRP in a population of MI survivors and found that the variation in hsCRP concentration within patients over time was only slightly less than the variation between patients. Moreover, our data revealed that certain subgroups had higher geometric-mean hsCRP concentrations and/or greater variation in the hsCRP concentration, but higher geometric means and greater variation did not necessarily occur together. Obese and overweight patients and certain age groups had higher hsCRP concentrations but less variation in concentration. We also found that patients who reported angina pectoris, emphysema, or CHF had less variation in hsCRP concentration, whereas the geometric-mean concentration did not seem to be affected. On the other hand, for patients with impaired glucose control, as indicated by increased baseline Hb A_{1c} concentrations (≥6.5%), we found a higher hsCRP concentration and greater hsCRP variation. We saw similar but less pronounced differences for the diagnosis of type 2 diabetes. Short-term exposures in the 24 h preceding blood draw, such as ETS exposure, alcohol consumption, or extreme stress or anger, did not have a major impact on hsCRP concentration. This study examined MI patients only, and therefore the results may not be entirely generalizable to a population without cardiovascular disease.

PATIENT CHARACTERISTICS THAT AFFECT hsCRP CONCENTRATION AND ITS VARIATION

A variety of studies have examined determinants of hsCRP concentrations. Although some investigators did not report any sex differences *(25)*, others found lower concentrations in men *(6, 7, 11, 26)*, in line with our results. Hutchinson et al. *(26)* hypothesized that the sex difference might be due to estrogen intake in women, and a study of diabetic women has shown significantly higher hsCRP concentrations in patients receiving hormone-replacement therapy *(7)*. Our data revealed that intake of hormone-replacement medications had a slightly positive but nonsignificant association with hsCRP concentration (Table 3), a result that is consistent with this hypothesis.

As for the influence of age on hsCRP concentrations, some authors have found a positive linear relationship *(26)*, but a lack of an association has also been reported *(12)*. As far as we know, a U-shaped function, as seen in our data, has not previously been reported. This observation could be due to the way the relationships were modeled and/or to the fact that our data were based on MI survivors, whereas most studies have been conducted with participants from the general population.

Consistent with our results, others have reported positive associations of hsCRP concentration with increased BMI and obesity *(12, 15, 25)*, for smokers compared with nonsmokers *(11, 25)*, and for individuals with low HDL cholesterol concentrations *(12, 25)*. Several studies have shown that statin therapy *(9, 10)* and treatment with angiotensin-converting enzyme inhibitors *(27)* reduce circulating hsCRP concentrations, results that are in line with our findings. Moreover, hsCRP–lowering effects have also been seen with acetylsalicylic acid *(8)*. Although our findings were consistent with a small reduction in hsCRP concentration due to acetylsalicylic acid, these associations were not statistically significant.

To our knowledge, none of the previously published studies examined variation in hsCRP concentration over time among different subgroups or with respect to possible determinants. Interestingly, we found that an increase of and greater variation in hsCRP concentration were not necessarily related. Individuals who reported angina pectoris, CHF, or emphysema had less variation in hsCRP concentration compared with participants who did not report any of these disorders. These findings remained stable for CHF and emphysema, even after adjustment for multiple testing and associated medication intake. Emphysema is often caused by smoking *(28)*, and our data showed that >80% of the emphysema patients were past or current smokers. Because emphysema and early-stage CHF do not necessarily include an inflammatory component, it is also conceivable that the lower variation in hsCRP concentration in these patients is merely a marker for a different mechanism, such as an underlying genetic component. Studies of twins have demonstrated a substantial genetic contribution to baseline hsCRP concentrations *(29)*, and genetic analyses of the AIRGENE data set revealed that minor alleles of several variants of selected candidate genes were significantly associated with intraindividual variation in hsCRP concentration *(30)*.

Whether different factors affect each other and, if so, how they do remain speculative. It is possible that a

combination of variables amplifies hsCRP variation, although it is also conceivable that certain combinations of factors can reduce such variation. Additionally, factors that are associated with high variation could just be indicators for a different mechanism. For example, the increase in variation associated with medication intake seen in our data might be a direct effect of the medication itself; however, it is more likely that the high variation is due to the underlying disease that led to the prescription of the drug.

RESPONSE TO ENVIRONMENTAL FACTORS

It is still unclear why some patients develop cardiovascular disease or experience an MI due to certain triggers, whereas others do not. Heavy physical exertion *(31)* and extreme anger *(32)* have been reported as causes for an acute MI. In addition, environmental stimuli such as tobacco smoke *(33)* and air pollution *(34, 35)* are associated with an increased risk for adverse cardiovascular events. It is conceivable that individuals with special characteristics react in a more pronounced way to environmental factors than others. A generally higher concentration of inflammation markers, and/or greater variation in inflammation might offer one possible explanation.

We found that patients with increased Hb A_{1c} concentrations and patients with self-reported type 2 diabetes have greater variation in hsCRP concentration, even in this relatively homogeneous population of MI survivors. It is plausible, but quite speculative, that these subgroups also had a stronger reaction (e.g., a more pronounced inflammatory response) to environmental factors. Studies of diabetic patients *(7)* have shown considerably higher mean hsCRP concentrations than our population, which consisted of only about 20% diabetic individuals. Persistently increased hsCRP concentrations as well as acute changes in concentrations of inflammation markers have been associated in cohort studies with an increased risk of cardiovascular events *(2, 3)*. This observation might represent a possible link for the reported associations of air pollution and passive smoking with adverse cardiovascular outcomes, because particle-induced systemic inflammation is one of the hypothesized pathways *(33, 36)*. Individuals with certain diseases, such as diabetes and MI, have been demonstrated to have an enhanced susceptibility for air pollution–related conditions, possibly due to a disease-induced increased inflammatory burden *(37)*. We did not see higher hsCRP concentrations in diabetic patients, but we did observe greater variation in hsCRP concentration compared with nondiabetic patients. Furthermore, patients with increased Hb A_{1c} concentrations ($\geq$6.5%) had higher hsCRP concentrations and greater hsCRP variation. High Hb A_{1c} concentrations seem to reflect uncontrolled rather than undiagnosed diabetes, because 89% of the participants with Hb A_{1c} values $>$6.5% reported a diagnosis of diabetes. On the other hand, only half of the AIRGENE population with diagnosed diabetes met the Hb A_{1c} criterion of $\geq$6.5%. This finding might indicate that metabolically stable diabetic patients are at less risk compared with patients with unstable diabetes.

Our study is in line with others *(10)* in showing a clear negative association between statin intake and hsCRP concentration. We hypothesize that the intake of statins attenuates the impact of environmental variables, and therefore statin therapy in addition to following recommended guidelines might be beneficial in certain particularly susceptible subgroups to avoid adverse cardiovascular effects of environmental stimuli. More research in this area is clearly needed, however.

SHORT-TERM INFLUENCES ON hsCRP

Several studies have demonstrated that regular moderate to vigorous exercise leads to a decrease in hsCRP concentrations, although the results are conflicting and some authors have attributed the detected negative association to a lower BMI in the individuals who exercise rather than to a direct effect of physical activity on inflammation markers *(38)*. Short-term effects, however, have been studied only in individuals whose activities must be considered extreme, even for professional athletes *(16)*. Although our population of MI survivors were expected to perform in only light sporting activities, we found a transient increase in hsCRP concentration 6 to 11 hours after physical activity that quickly returned to baseline concentrations. A study of postmenopausal women did not observe any increase in hsCRP concentration at 1 h or 24 h after exercise, compared with baseline concentrations *(13)*. In addition, hsCRP concentrations measured immediately and 48 h after a 7-km hill race did not differ from baseline concentrations *(14)*. These conflicting results might be explained by different time frames and differences in exercise intensities. A study of the time course of hsCRP concentration after surgical procedures showed a rapid increase starting 6 to 8 hours after the operation, with the highest peak at about 48 h and the concentration returning to baseline between 72 h and 144 h after the surgical intervention *(39)*.

Regarding tea and alcohol intake, no publication has addressed the effects on hsCRP within 24 h after consumption. We found a slight decrease in hsCRP in association with tea and alcohol intake; however, whether this result reflects regular consumption or an immediate reaction is difficult to determine. A decrease in hsCRP concentration after regular consumption of black tea *(40)* and moderate amounts of alcohol *(6)* has been shown.

Conclusion

This study is the first to measure within-patient variation in hsCRP concentration in a large study population. We confirmed and extended published results on the association of patient characteristics and intake of medications with hsCRP concentrations in male and female MI survivors. Short-term influences, however, did not seem to impact hsCRP concentrations. Males, elderly individuals, smokers, and patients with increased Hb A_{1c} concentrations had greater intraindividual variation in repeated measurements of hsCRP. In patients with manifest cardiovascular disease, in particular after MI, several hsCRP measurements may be necessary to adequately characterize their risk, especially in defined subgroups. Whether this variation also makes these patients more susceptible to adverse environmental variables needs further investigation.

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