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Determinants of the acute phase protein CRP in myocardial infarction survivors: The role of co-morbidities and environmental factors

Thesis

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ZUSAMMENFASSUNG

Hintergrund: C-reaktives Protein (CRP), das klassische Akute Phase Protein, zeigt, unabhängig von anderen bekannten Risikofaktoren wie Body Mass Index (BMI), Alter und Cholesterinspiegel, einen Zusammenhang mit kardiovaskulären Ereignissen. Bei Patienten mit kardiovaskulärem Risikoprofil werden einer auf CRP basierenden Therapieentscheidung meist ein oder zwei CRP-Messungen zugrunde gelegt. Die Variabilität von CRP in bestimmten Untergruppen der Bevölkerung wurde jedoch bisher noch nicht im Detail betrachtet.

Ein Ziel dieser Arbeit war es, zeitunabhängige Einflussgrößen im Zusammenhang mit der mittleren CRP Konzentration und ihrer Variabilität in einem gemeinsamen Ansatz zu untersuchen. Zusätzlich wurden Parameter, die das CRP beeinflussen könnten, wie beispielsweise Passivrauchen, Alkoholkonsum oder extremer Stress in den 24 Stunden vor der Blutabnahme betrachtet. Weiterhin wurde der kurzfristige Einfluss von Außenluftschadstoffen auf die Höhe des CRPs untersucht.

Methoden: Diese Arbeit beruht auf Daten der AIRGENE Studie, die in sechs europäischen Städten durchgeführt wurde. Bei 1003 Herzinfarktpatienten wurde im Abstand von vier bis sechs Wochen bis zu achtmal hochsensitives (hs) CRP gemessen. Bei der ersten Untersuchung wurden Blutdruck und BMI gemessen, Blutproben entnommen und mittels eines Fragebogens Daten zu Gesundheitszustand, Medikation und Rauchstatus erfasst. Bei allen Untersuchungen wurde jeweils eine Blutprobe zur Messung von hs-CRP entnommen und Daten zu Einflussfaktoren auf das hs-CRP in den 24 Stunden vor der Blutabnahme gesammelt. In jeder Stadt wurden die Anzahl von ultrafeinen Partikeln, Schwebstaub mit einem Durchmesser von <10 μm (PM_{10}), Feinstaub mit einem Durchmesser von <2.5 μm ($PM_{2.5}$), gasförmige Schadstoffe sowie meteorologische Daten auf stündlicher Basis erfasst.

Ergebnisse: BMI zeigte sich als einer der stärksten Einflussfaktoren, mit höheren Mittelwerten für hs-CRP bei übergewichtigen und adipösen Patienten. Hinsichtlich des Alters fand sich ein U-förmiger Zusammenhang mit den niedrigsten hs-CRP Werten in der Gruppe der 50 bis 59 jährigen.

Die intra-individuelle Variabilität von hs-CRP war nur geringfügig niedriger als die inter-individuelle. Patienten mit Angina Pectoris, Emphysem oder Herzschwäche zeigten eine niedrigere Variabilität des CRP (-11,0; -24,9 bzw. -41,6% Variation) während der Mittelwert bei allen drei Diagnosen nicht beeinflusst schien. Für Patienten, deren Wert für glykosyliertes Hämoglobin (HbA1c) zu Beginn der Studie bei 6,5% oder höher lag, zeigten die Daten eine erhöhte hs-CRP Konzentration (geometrisches Mittel: 26,2; Konfidenzinterval: 7,2;48,6) sowie eine höhere Variabilität (20,7% Variation, p-Wert 0,0034). Ähnliche, wenn auch nicht so ausgeprägte Ergebnisse fanden sich für Patienten mit Typ 2 Diabetes.

Die Variabilität war zudem höher bei Männern und Rauchern (24.8 bzw. 27.3%) verglichen mit Frauen bzw. Nichtrauchern, mit geringerer mittlerer Konzentration bei Männern, aber einer höheren mittleren Konzentration bei Rauchern. Patienten, die Statine oder andere Blutfett senkende Medikamente einnahmen, zeigten signifikant niedrigere mittlere CRP Werte und zudem eine geringere Variabilität. Dagegen fand sich eine höhere Variabilität bei Patienten, die Hemmer des Angiontensin-konvertierenden Enzyms (ACE-Hemmer) einnahmen, während die mittlere CRP-Konzentration nicht beeinflusst schien. Von den untersuchten Variablen zum Verhalten in den 24 Stunden vor der Blutabnahme zeigte keine einen größeren kurzfristigen Effekt auf hs-CRP. Zudem wurde kein Zusammenhang zwischen Außenluftschadstoffen und der CRP-Konzentration gefunden.

Folgerungen: Diese Arbeit bestätigt und erweitert bereits publizierte Zusammenhänge zwischen Patientencharakteristiken sowie Medikation und der Höhe des CRP in Herzinfarktüberlebenden beiderlei Geschlechts. Die hohe Variabilität, speziell bei Männern, Rauchern und Personen mit erhöhten HbA1c Werten, deuten darauf hin, dass eine einzige CRP Messung als Basis für präventive Maßnahmen unter Umständen nicht ausreicht. Zudem ist vorstellbar, dass Individuen mit einer generell höheren CRP Konzentration und/oder einer höheren Variabilität, z.B. Patienten mit Typ 2 Diabetes, stärker auf Umweltfaktoren, wie beispielsweise Luftschadstoffe, reagieren. Der fehlende Zusammenhang zwischen Luftschadstoffen und CRP in diesen Daten lässt sich vermutlich auf die weit verbreitete Einnahme von Statinen in der untersuchten Personengruppe von Herzinfarktüberlebenden zurückführen.

SUMMARY

Background: C-reactive protein (CRP), a sensitive marker of the acute phase response, has been associated with future cardiovascular endpoints independent of other risk factors such as body mass index (BMI), age and cholesterol levels. Risk assessment in populations at risk of cardiovascular disease is usually based on one or two measurements. Variation of CRP among certain subgroups of the population, however, has not been examined in detail. The purpose of this thesis is to study associations between time-invariant patient characteristics and mean high sensitivity (hs)-CRP concentrations and parameters that influence the variation of hs-CRP in a joint analysis. Additionally, associations between parameters that might impact hs-CRP in the 24 hours before the blood draw, such as environmental tobacco smoke exposure, alcohol consumption or extreme stress or anger, were examined. Moreover, the short-term impact of ambient air pollution on hs-CRP was studied.

Methods: This thesis is based on AIRGENE, a multi centre study conducted in six European cities. Hs-CRP was measured repeatedly up to eight times every four to six weeks in 1,003 myocardial infarction (MI) survivors. At the first visit data on health status, medication intake and smoking history were collected by questionnaire. Blood pressure and BMI were measured and a blood serum sample was drawn. An additional blood sample was drawn at each visit for the determination of hs-CRP and data on life-style in the 24 hours before the visit were collected. In each city, hourly data on particle number concentrations, mass concentrations of particulate matter (PM) <10µm (PM_{10}) and <2.5µm ($PM_{2.5}$), gaseous pollutants and meteorological data were collected at central monitoring sites.

Results: BMI was one of the strongest determinants with higher geometric mean hs-CRP concentrations in overweight and obese patients. Regarding age, a U-shaped relationship with the lowest hs-CRP level in the group of 50 to 59 year olds was found. Variation of hs-CRP within patients was only slightly lower than between patients. Patients who reported the presence of angina pectoris, emphysema and congestive heart failure showed a lower variation (-11.0, -24.9 and -41.6% variation, respectively) while the geometric mean concentration seemed not to be affected. For patients with baseline glycosylised haemoglobin (HbA1c) levels of 6.5% and above, on the other hand, our data revealed higher hs-CRP (geometric mean: 26.2, confidence interval: 7.2; 48.6) and a higher variation (20.7% variation, p-value 0.0034). Results were similar, although not as pronounced, for the diagnosis of type 2 diabetes. Variation was also higher in males compared to females and smokers compared to non-smokers (24.8 and 27.3%, respectively) with a lower geometric mean concentration in males and a higher one in smokers. Patients reporting the intake of statins or other lipid-lowering drugs showed significantly lower hs-CRP and also less variation. Patients using angiotensine converting enzyme (ACE)-inhibitors

on the other hand, a higher variation was found while geometric mean concentrations seemed not to be associated with medication intake.

Life-style parameters in the 24 hours preceding blood draw did not have a major impact on hs-CRP. No association was seen between ambient air pollutants and hs-CRP concentrations.

Conclusion: This work confirms and extends published results on the association between patient characteristics and intake of medication and hs-CRP concentrations in a panel of male and female MI survivors. The higher variation found in males, smokers and subjects with elevated HbA1c concentrations indicates that basing preventive medical measures on a single measurement of hs-CRP might not be sufficient. It is conceivable that individuals with a generally higher level of inflammatory markers, and/or a higher variation, for example patients with diabetes, might react more strongly to environmental factors such as air pollution. The lack of association between air pollution and hs-CRP in these data is possibly due to a widespread intake of statins in this population of MI survivors.

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LIST OF ABBREVIATIONS:

ACE: angiontensine converting enzyme
AIC: Akaike's Information Criterion
AM: Arithmetic mean
ASA: acetyl salicylic acid
BMI: body mass index
CHF: congestive heart failure
CO: carbon monoxide
CRP: C-reactive protein
CV: coefficient of variation
GM: geometric mean
HbA1c: glycosylised haemoglobin
HDL: high density lipoprotein
HRT: hormone replacement therapy
hs: high sensitivity
IQR: interquartile range
IL-6: interleukin 6
LDL: low-density lipoprotein
MI: myocardial infarction
NO: nitrogen monoxide
NO_2: nitrogen dioxide
Nt-pro BNP: N-terminal proB-type natriuretic peptide
O_3: ozone
$PM_{2.5}$: mass concentration of particles less than 2.5 µm in diameter
PM_{10}: mass concentration of particles less than 10 µm in diameter
PNC: particle number concentration
SAA: serum amyloid A
SO_2: sulphur dioxide
UFP: ultrafine particles, number concentration of particles from 0.01 to 0.1 µm

1 INTRODUCTION

Cardiovascular disease is the main cause of death in developed countries. In 2005, an estimated 17.5 million people died from cardiovascular disease, representing 30% of all global deaths (WHO, 2009). In recent years, atherosclerosis, the precursor of cardiovascular disease, has been recognised as inflammatory disease, as inflammation plays a crucial role in plaque formation, progression and rupture. Interest has emerged in the potential of inflammatory biomarkers, especially C-reactive protein (CRP), for the prediction of cardiovascular risk in individuals (Koenig, 2005).

1.1 Inflammation

Inflammation is a protective reaction by the body with the aim of limiting tissue damage, eliminating foreign substances and starting repair mechanisms so that the body can return to its normal functions. An inflammatory process starts when tissue is destroyed by infections or injuries. Whenever the first barrier of defence, such as skin, mucosa or cilia on the lung is broken, foreign substances enter the body and the inflammatory process is activated. Inflammatory mediators, such as cytokines and chemokines, lead to an enhancement of the inflammatory response. Their main task is the recruitment of inflammatory cells such as polymorphe granulocytes, monocytes and thrombocytes at the site of foreign substances or cell damage (Thomas, 1998).

The first step in inflammation is called acute phase response. In a narrower sense, it is regarded as the change in the concentration of a large number of plasma proteins which reflect the gene-expression of secretory proteins during an inflammatory response. The acute phase response happens in the first few hours of an inflammatory response and can be triggered by microbiological agents, cytotoxic antibodies or mechanical trauma. Involved proteins can be divided into negative acute phase proteins, like albumin, transferrin and alpha-fetoprotein, which decrease during an acute phase response, and positive acute phase proteins, which increase. Coeruloplasmin, for example, increases up to 50%, fibrinogen two to five-fold and serum

amyloid A (SAA) and CRP up to 1000-fold during an acute inflammation. CRP was one of the first acute phase proteins to be described and represents one of the most extensively studied proinflammatory molecules (Thomas, 1998).

1.2 CRP, the classical acute phase protein

CRP was first described by Tillet and Francis in 1930 (Tillet et al., 1930) as a substance in the serum of patients with acute inflammation that reacted with the C polysaccharide of streptococcus pneumoniae. It is a member of the pentraxin family of proteins, due to its disc-like pentrametric structure of five identical sub-units. The pentraxins are calcium and phospholipid binding proteins with immuno-defence properties.

CRP is almost exclusively synthesised in the liver upon stimulation through interleukin-6 (IL-6), the key cytokine that stimulates the synthesis of all major acute phase proteins (Woods et al., 2000). However, recent evidence has suggested that it may also be produced locally in vascular smooth muscle cells and macrophages of atherosclerotic lesions (Calabro et al., 2003; Yasojima et al., 2001). The normal synthesis rate of 1 to 10 mg per day can increase up to more than 1g per day during acute inflammation. It is assumed that CRP is vital, as so far no genetic CRP defect has been described (Vigushin et al., 1993). CRP has a half life of 19 hours (Koenig et al., 2003) which is identical under all conditions so that the synthesis rate of CRP is the sole determinant of its plasma concentration (Vigushin et al., 1993).

The role of CRP is to clear a large number of exogene and endogene substances from the blood. Endogene substances comprise products of used or necrotic cells, e.g. cell membranes which, in the presence of calcium, are bound to CRP. This binding only takes place if the normal lipid layer structure of a cell is destroyed, and the internal phospholipids of the membrane are presented. Exogene substances include cell walls of bacteria, fungi and parasites. After binding, various biological systems are activated which leads to an elimination of the CRP-ligand-complex through macrophages in tissue, blood and spleen.

1.3 CRP as predictor for cardiovascular disease

Increasing evidence from basic research suggests that atherosclerosis is an inflammatory disease (Koenig, 2005) and established risk factors, like smoking, hypercholesterolemia and hypertension only explain about half of the incidence of myocardial infarction (MI), angina pectoris and stroke (Braunwald, 1997; Koenig et al., 1998). Therefore, interest has emerged in the potential of inflammatory biomarkers for the prediction of cardiovascular risk (Koenig, 1999; Koenig, 2005). CRP, as measured by high sensitivity (hs) assays, has appeared as a reliable and independent predictor of incident cardiovascular events (Koenig et al., 2006; Ridker et al., 1998c; Ridker et al., 1998b; Ridker et al., 1998a), although there are also critical voices (Pepys, 2005). A recent recommendation by the Centres for Disease Control and Prevention and the American Heart Association states that of all inflammatory markers currently used, CRP should be the analyte of choice due to its stability, assay precision, accuracy and availability. It suggests measuring CRP in those at intermediate risk according to the Framingham Risk Score (Jones et al., 1990). The assay should be performed twice, fasting or non-fasting, about two weeks apart. In case of CRP >10mg/L, indicating an acute inflammatory process, the respective measurement should be discarded and repeated two weeks later (Pearson et al., 2003).

In the past years, various epidemiological studies showed consistent and independent associations between the CRP concentration and diverse future cardiovascular endpoints in diseased and initially healthy populations, independent of established risk factors.

In a large group of patients with stable angina pectoris an approximately two-fold increase in the risk of coronary events was observed in patients in the highest quintile of the CRP distribution compared to those in the lower ones (Haverkate et al., 1997). Additionally, Liuzzo et al. (1994) found that patients with unstable angina pectoris and CRP concentrations above 3.0 mg/L experienced more ischaemic periods, required revascularisation more frequently and developed an acute MI more often than those with lower concentrations. Berk et al. (1990) reported from a prospective study of about 70 participants that 90% of unstable angina pectoris patients exhibit an

elevation of CRP concentrations compared to 13% of patients with stable angina. A meta analysis of 22 population-based studies, including a total of over 7000 patients with incident coronary events, found a modest but statistically significant association (Danesh et al., 2004).

Regarding apparently healthy populations Koenig et al. (1999) reported an almost three-fold increase in risk for a first major coronary event in 936 initially healthy men from a random sample of the general population. Moreover, data from the Physicians' Health Study (Ridker et al., 1998b) showed a positive association between CRP and the development of symptomatic peripheral arterial occlusive disease and CRP was also found to be predictive of strokes in men (Ridker et al., 1997) and of coronary and cerebrovascular events in women (Ridker et al., 2000). In additional results from the Physicians' Health Study (Ridker et al., 1998c) the relative risks of future MI among men with high concentrations of both CRP and total cholesterol were more than three times greater than the individual risk associated with isolated elevation of CRP and more than twice as great as the risk associated with total cholesterol alone. This finding supports the value of adding an inflammatory marker, especially CRP, to the conventional risk factor profile.

1.4 Patient characteristics that affect hs-CRP concentrations

With CRP gaining importance as a marker for future cardiovascular events, interest has developed in patient characteristics as well as life-style factors associated with reduced or elevated systemic inflammatory activity.

Some studies have shown that age and sex determine the concentration of CRP with a positive linear relationship between CRP and age (Garcia-Lorda et al., 2006; Hutchinson et al., 2000; Sung, 2006), but a lack of association has also been reported (Greenfield et al., 2004). Regarding sex, men tend to have lower CRP concentrations, (Frohlich et al., 2003; Geffken et al., 2001; Hutchinson et al., 2000; Imhof et al., 2004) however some authors report no difference of the CRP concentration by gender (Garcia-Lorda et al., 2006). Furthermore, various measures of body composition, such as body mass index (BMI) or waist to hip ratio have been positively associated

with increasing CRP concentrations (Garcia-Lorda et al., 2006; Greenfield et al., 2004; Mora et al., 2006; Sung, 2006; Thorand et al., 2006; Verdaet et al., 2004).

A significant amount of research has been conducted regarding the impact of life-style or life-style changes on the concentration of CRP, among them smoking, physical activity and nutrition. Regarding cigarette smoking, most studies show lower CRP concentrations in non-smokers and intermediate concentrations in ex-smokers compared to current smokers (Frohlich et al., 2003; Garcia-Lorda et al., 2006; Geffken et al., 2001; Greenfield et al., 2004; Ikonomidis et al., 1999; Ikonomidis et al., 2005; Verdaet et al., 2004; Wannamethee et al., 2005) and a positive association with smoking duration (Frohlich et al., 2003) and pack-years of smoking (Frohlich et al., 2003; Wannamethee et al., 2005). Reduction of excess body weight and regular endurance exercise also positively alter the acute phase response (Ditschuneit et al., 1995; Esposito et al., 2003; Fontana et al., 2007; Mora et al., 2007; Selvin et al., 2007). However, some authors state that leisure time physical activity is not an independent predictor of CRP but associated with lower body weight in those who exercise (Davis et al., 2007; Fontana et al., 2007; Geffken et al., 2001; Simpson et al., 2005; Verdaet et al., 2004).

The impact of nutrition on CRP has been studied in different ways. Whole diet styles, such as a Mediterranean or anti-oxidant diet have been examined in addition to single nutrient factors such as alcohol, black and green tea or certain vitamins. While some authors state that CRP concentrations are only marginally associated with individual dietary factors (Fredrikson et al., 2004) others report that a so called Mediterranean diet which is high in antioxidants like fruit, vegetables, fibre and red wine is inversely correlated with plasma CRP (Albert et al., 2003; Brighenti et al., 2005; Chrysohoou et al., 2004; Imhof et al., 2001; Imhof et al., 2004; Lopez-Garcia et al., 2004; Pitsavos et al., 2007). These results regarding a Mediterranean diet could be confirmed in analyses of the AIRGENE population (Panagiotakos et al., 2009). Of single nutrients, alcohol intake has been examined extensively, and most authors report a U-shaped function between alcohol intake and CRP (Albert et al., 2003; Greenfield et al., 2004; Imhof et al., 2001; Imhof et al., 2004).

Most of the studies cited above are based on only one or two measurements per patient and only few studies have examined the degree of variation in repeated CRP measurements in the same subjects within a period of weeks or months with conflicting results (Bogaty et al., 2005; Clark et al., 1993; Macy et al., 1997; Ockene et al., 2001).

1.5 Short term impact on CRP

The body of literature regarding parameters that impact CRP hours before the blood withdrawal is very limited. Only sports medicine has examined the effect of extremely strenuous activities on inflammatory markers, among them CRP, with clear increases in CRP concentrations shortly after exertion (Davis et al., 2007; Kim et al., 2007; Margeli et al., 2005; Simpson et al., 2005). However, other parameters, such as nutrition or extreme anger, seem not yet to have been considered.

1.6 Possible biological mechanisms

The rupture of an atherosclerotic plaque as the final pathophysiological mechanism leading to acute ischaemic syndromes is now universally accepted. The classical “response-to-injury” hypothesis postulates that endothelial dysfunction represents the initial step in atherogenesis (Koenig, 1999). Endothelial dysfunction can be induced by haemodynamic forces (shear stress), by a variety of vasoactive substances, by mediators from blood cells and directly by cigarette smoke, atherogenic diet, elevated glucose levels and oxidised or enzymatically modified low-density lipoprotein (LDL) cholesterol (Rubanyi, 1993). At the site of an endothelial injury, invading inflammatory cells which produce numerous proinflammatory factors promote and amplify both local and systemic inflammation (Trepels et al., 2006). Moreover, dysfunctional endothelial cells produce adhesion molecules that, in turn, interact with inflammatory cells (Koenig et al., 2007). Pathological studies show the abundant presence of inflammatory cells at the site of rupture or superficial erosion preferably in the shoulder of an atheromatous plaque cap. As in other types of tissue injury, acute MI also generates an acute phase response resulting in subsequent systemic increases in a number of inflammatory reactants (De Servi et al., 2005;

Rosenson, 1993). The deposition of CRP in the infarcted region, co-localising with activated fragments of the complement system, the unspecific immune defence system of the body, indicates that complement activation may enhance local inflammation during acute MI (Lagrand et al., 1997). This can lead to subsequent extension of tissue damage in the myocardium.

Initially, CRP was merely regarded as an innocent bystander in the atherosclerotic process, and there is still no full agreement amongst the scientific community (Pepys, 2005), but current evidence argues for the contribution of CRP to the pathogenesis of atherothrombosis (Koenig, 1999; Koenig et al., 2007). It has, for example, been shown that CRP binds to the plasma membranes of damaged cells and that aggregated CRP selectively binds LDL and very-low-density lipoprotein from whole plasma, thereby possibly participating in their atherogenic accumulation. Furthermore, complexed CRP activates the complement system via the classical pathway and can be pro-inflammatory (Koenig, 1999). Other proteins augmented during the acute phase response include pro-coagulatory proteins such as fibrinogen and plasminogen activator inhibitor-1. Thus, inflammation can promote thrombus formation and enhance clot stability by inhibiting endogeneous fibrinolysis (Libby et al., 2001). CRP has also been found to stimulate tissue factor production by macrophages in vitro. Tissue factor is the main initiator of coagulation in vivo, and its local concentration in the arterial wall is clearly related to coronary thrombotic events. Therefore, the capacity of CRP to enhance tissue factor production suggests another possible causal link between raised CRP values and coronary events (Koenig, 1999). Moreover, recent experimental studies suggest that CRP might decrease endothelial nitric oxide (NO) synthase expression and increase the production of reactive oxygen species, thereby directly interfering with bioavailability of endothelial nitric oxide (Fichtlscherer et al., 2004). Fichtlscherer et al. (2004) showed in 75 patients with stable coronary artery disease, that low grade inflammation as measured by CRP levels is associated with impaired systemic bioavailability of nitric oxide. Endothelium derived nitric oxide is an endogenous vasodilator that also has anti-inflammatory properties (Libby et al., 2002). Nitric oxide also inhibits platelet aggregation (Loscalzo et al., 1995), and platelet activation and recruitment are tightly regulated

by endothelium derived nitric oxide. Therefore, an impaired systemic bioavailability of nitric oxide might contribute to the transition from stable coronary artery disease to acute coronary syndromes.

1.7 Response to environmental parameters

Heavy physical exertion (Albert et al., 2000; Mittleman et al., 1993), extreme anger (Mittleman et al., 1995) and cocaine or marijuana use (Mittleman et al., 1999; Mittleman et al., 2001) have been reported as triggers for an MI. In addition, it has been shown that environmental factors such as noise (Babisch, 2006; Ising et al., 2004; Willich et al., 2006), meteorological variables (Medina-Ramon et al., 2006; Medina-Ramon et al., 2007; Morabito et al., 2005; Sarna et al., 1977) and air pollution (Brook, 2007; Lanki et al., 2006; Mills et al., 2009; O'Toole et al., 2008; Peters et al., 2001a) are associated with an elevated risk for adverse cardiovascular events. It is conceivable that individuals with special characteristics react more strongly to environmental parameters than others. A generally higher level of inflammatory markers, and/or a higher variation in inflammation might represent one explanation, as persistently elevated concentrations and also acute changes in levels of inflammatory markers have been associated with an increased risk of cardiovascular events in cohort studies (Koenig et al., 1999; Ridker et al., 1997). In addition, this might be one possible link for the reported associations between air pollution and adverse cardiovascular outcomes, since particle induced systemic inflammation is one hypothesised pathway between air pollution and cardiovascular disease (Peters et al., 1997; Pope et al., 2004; Seaton et al., 1999). Furthermore, studies on individuals with diabetes and MI show an enhanced susceptibility for air pollution related conditions. This might be the result of increased inflammatory processes in these people relating to their underlying disease (Bateson et al., 2004; Brook et al., 2008; Goldberg et al., 2001; O'Neill et al., 2007; Zanobetti et al., 2001). Moreover, a decrease in temperature has been shown to be associated with elevated inflammatory markers, among them CRP in the AIRGENE population (Schneider et al., 2008).

Clinical trials indicate that statins and aspirin may be able to modify an inflammatory response. In particular, some statins have recently been associated with decreased CRP concentrations (Albert et al., 2001a; Dubowsky et al., 2006; Ridker et al., 2001; Ridker et al., 2008; Rosenson et al., 2003). CRP lowering effects were also found with acetyl salicylic acid (ASA) (Ikonomidis et al., 1999) and angiontensine converting enzyme (ACE) inhibitors (Soriano et al., 2007) while hormone replacement therapy (HRT) seems to increase CRP concentrations (Cushman et al., 1999; Ridker et al., 2000).

2 HYPOTHESES

Despite the vast body of publications, many issues regarding CRP are not completely resolved. This thesis uses data from a large European study on MI survivors, whose plasma hs-CRP concentrations were measured up to eight times

1. a) to confirm known associations between time-invariant patient characteristics and hs-CRP concentrations,

   b) to extend the number of the characteristics published so far and

   c) to study the influence of the same parameters on the variation of hs-CRP hypothesising that the diagnosis of certain diseases would be related to higher hs-CRP concentrations and a more pronounced variation.
2. to examine the associations between hs-CRP and time-varying life-style parameters in the 24 hours before the blood draw, hypothesising that the consumption of tea and alcohol would lead to lower hs-CRP concentrations, while extreme stress or anger, active smoking and physical activity would show a positive association and
3. to examine the association of hs-CRP concentrations and environmental parameters, especially particulate air pollutants, assuming a positive linear relationship.

MI survivors might react differently compared to the normal healthy population due to their preceding disease. However, MI survivors were chosen as study subjects as they have been shown to be especially susceptible (Hellermann et al., 2003; McGovern et al., 2001; Rosamond et al., 1998) and therefore may require particular protection and public health actions.

3 METHODS

3.1 Study population

A prospective longitudinal study of post MI patients was performed in six 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 patients with MI or from administrative data-bases of hospital admissions. The study design is described in detail elsewhere (Peters et al., 2007) (Appendix I). In brief, patients aged 35 to 80, preferably current non-smokers, who had experienced an MI between four months and six years before the start of the study were recruited. Patients with MI or interventional procedures less than three months before the beginning of the study, or chronic inflammatory diseases were not included. All partners had the study protocol approved by their local human subjects committees and written informed consent was obtained from all patients. All methods used in the study centres were conducted according to common standard operating procedures.

3.2 Clinical measurements

Patients were invited to participate in six to eight clinical visits between May 2003 and July 2004. The visits were scheduled every four to six weeks on the same weekday and at the same time of the day to minimize the impact of weekly and circadian variation. At the first visit a baseline questionnaire regarding health status, medication intake and smoking history was completed. Blood pressure and BMI were measured and a blood serum sample was drawn. At each clinical visit a short questionnaire on medication intake and life-style parameters in the past 24 hours, such as smoking, exposure to environmental tobacco smoke (ETS), physical activity, extreme stress or anger and consumption of tea and alcohol was completed.

Venous EDTA-plasma samples were drawn for the determination of hs-CRP while sitting. Samples were cooled and stored at 4°C until further processing. Plasma aliquots were shipped on dry ice to the central laboratory in Ulm, Germany, and analysed by means of latex enhanced immunonephelometry (Dade Behring Marburg GmbH, Marburg, Germany). If the hs-CRP

concentration was lower than the detection limit of 0.16 mg/L values were set to 0.16 mg/L. Details are given in (Ruckerl et al., 2007) (Appendix II). Blood samples of patients who suffered from acute infections during the three days before the scheduled visit were excluded from analyses.

3.3 Air pollution and meteorological data

Air pollution data were collected from fixed monitoring sites representing urban background concentrations. Hourly means of mass concentration of particles less than 10µm and less than 2.5µm in diameter (PM_{10} and $PM_{2.5}$, respectively), gaseous air pollutants (carbon monoxide (CO), sulphur dioxide (SO_2), ozone (O_3), nitrogen monoxide (NO), nitrogen dioxide (NO_2)) and meteorological variables (air temperature, relative humidity, barometric pressure) were obtained through the city-specific air monitoring networks and the meteorological services. Particle number concentration (PNC) measurements as indicator for ultrafine particles (UFP) were performed in all centres. Individual moving 24-hour averages of ambient concentrations of air pollutants and meteorogical variables were used to characterize the exposures for each person immediately preceding the clinical visit (lag 0) up to four days (lag1-lag4). In addition, the mean of lags 0 to 4 was calculated to account for cumulative effects. Details can be found in (Ruckerl et al., 2007) (Appendix II).

3.4 Study design

The chosen study design of time series analyses uses repeated measurements per subject collected over a period of several months. Some of the measured exposure variables vary over the course of the study and can then be associated with concurrent hs-CRP concentrations. Figure 1 pictures the principle of the repeated measurement design in association with air pollution as an example.

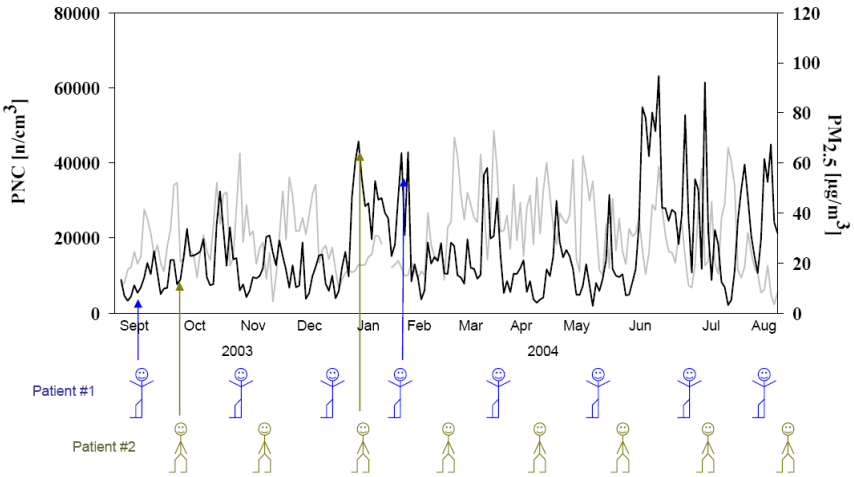

Figure 1. Principle of the repeated measurement design: Some of the examinations are conducted on days with low air pollution, others on days with high air pollution. The example shows eight repeated measurements in two patients and air pollution data from Barcelona, summer 2003 to summer 2004; — PNC, particle number concentration; — $PM_{2.5}$, mass concentration of particles less than 2.5µm in diameter

3.5 Statistical analyses

All statistical analyses were performed using the Statistical Analysis System (SAS) software package (Version 9.1 for Windows, SAS-Institute Inc., Cary, NC, USA).

Hs-CRP needed to be log-transformed to fulfil the model assumption of residual normality; therefore, concentration results are given as the geometric means. Data was analysed using mixed effects models with random patient effects accounting for repeated measures. Penalised splines (P-splines) in the additive mixed model framework were used to allow for non-parametric exposure-response functions (Greven et al. 2006). As the half life of hs-CRP is much shorter than the intervals between visits, a compound symmetry structure for the covariance matrix was assumed to model the correlation between repeated measures in each patient. All decisions on goodness-of-fit were based on Akaike's Information Criterion (AIC). To account for the large number of statistical tests, the significance-level of the p-value was corrected to 0.00125 which equals a Bonferroni correction for 40 tests.

3.5.1 Analysis of hs-CRP concentration and variation

A confounder model (base model) was built first (Table 1a), which included pre-selected time-invariant patient characteristics to permit the assumption of a normally distributed random patient

intercept. A wide range of variables known from literature to have a possible influence on hs-CRP such as city, age, gender and BMI were tested on the repeated measurements of the log transformed hs-CRP values. Linear variables were added linearly to the model.

The idea of relating a single baseline measurement of a time-invariant variable to repeated measurements rather than an average of these measurements or just the result of the first hs-CRP measurement collected at the same point in time might initially seem unnecessarily complicated. However, this approach has several advantages.

First of all, it increases the power if several CRP measurements per person are used rather than one. Additionally, the variability and the differing numbers of hs-CRP measurements per patient are accounted for. Possible alternatives would be to use only the first hs-CRP measurement or a median or mean value of all the hs-CRP measurements of one patient. However, all these approaches lead to an unnecessary loss of information. Moreover, the mean is considered to be very sensitive to outliers and the number of measurements varies between two and eight, and therefore a weighted mean or median would be needed. Also, as soon as time-variant variables such as season or trend are added to the model, repeated hs-CRP measurements per person are needed as only they reflect the changes over time in the outcome variable. Finally, the variability in various patient groups can only be analysed with repeated measurements. For a consistent interpretation, a common approach for all these analyses is necessary.

Table 1a: Variables of the base model including pre-selected time-invariant patient characteristics

Categorical	Linear	Cubic
City, HbA1c[a], sex, number of MI[b]	BMI[c], log(NT-proBNP[d]), packyears of smoking, total cholesterol	age

[a]HbA1c: glycosylised haemoglobin, [b]MI: myocardial infarction, [c]BMI: Body Mass Index, [d]NT-proBNP: N-terminal proB-type natriuretic peptide

In a second step, further time-invariant variables such as reported diseases, regular medication intake and smoking history were added to the base model, always one at a time. To avoid overcontrol, pack-years of smoking were removed from the base model when analysing smoking status and HbA1c was removed for the analysis of diabetes.

The associations of time-invariant patient characteristics are given as percent change from geometric mean (GM) of hs-CRP together with 95% confidence intervals (CI).

Crude coefficients of variation (CV) for hs-CRP were estimated using the UNIVARIATE procedure. Differences in variation for the fully adjusted model were calculated with the MIXED procedure in SAS using 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 significant. Linear variables were categorised, and single variables were then added to the base model. Results are given as variation estimates of log transformed hs-CRP between (panel A) and within (panel B) subjects in the figures and relative difference (%) in within subject variation compared to the reference group in the tables.

Sensitivity analyses for co-morbidities that might be associated with an intake of certain medication were conducted. A chi²-test was used to evaluate possible associations between co-morbidities and medication intake. If an association was found (p-value ≤ 0.05) the multivariable model was adjusted for the respective medication to investigate whether the co-morbidity effect was altered by including medication in the model. To explore the various aspects of different smoking variables and ETS exposure in more detail, additional analyses were conducted in- and excluding several smoking variables from the model (Ruckerl et al., 2009)(Appendix II).

As data on baseline LDL cholesterol were only available for men, additional analyses for 770 men who had complete data on triglycerides, high density lipoprotein (HDL)-, LDL- and total cholesterol were performed using the methods described above. Sex was deleted from the base model for these analyses as data were limited to men. Different models were calculated as shown in table 1b. For some models (models 1 b to d) total cholesterol was also removed. For the first model (model 1), cholesterol parameters were added singularly to the adapted base model. Additional models including total cholesterol and HDL (model 2), total cholesterol and LDL (model 3), total cholesterol, HDL and LDL (model 4) and total cholesterol and triglycerides (model 5) were calculated. Additionally, a model that included most of the presented variables at

the same time was calculated to identify those variables that led to the greatest increase in variation.

Table 1b: Variables included in the subgroup analyses for 770 men – adjusted for the variables of the base model except for sex (x: included in the model --: not included in the model)

Model	Total cholesterol	HDL[a] cholesterol	LDL[b] cholesterol	Triglycerides
1 a	x	--	--	--
1 b	--	x	--	--
1 c	--	--	x	--
1 d	--	--	--	x
2	x	x	--	--
3	x	--	x	--
4	x	x	x	--
5	x	--	--	x

[a]HDL: high density lipoprotein, [b]LDL: low density lipoprotein

3.5.2 Analysis of life-style parameters and hs-CRP

Life-style parameters were added to the base model, always one at a time. Variables that described a time difference, such as time of the last meal before the blood withdrawal, were categorised into six hour categories (0 to 5 hours, 6 to 11 hours, 12 to 17 hours and 18 to 23 hours). Results are given as percent change from GM of hs-CRP together with 95% CI.

3.5.3 Analysis of air pollution parameters and hs-CRP

For the analysis of air pollution data, firstly city specific effects were calculated using a more parsimonious model selected out of the base model for each city. Additional meteorological variables were forced into all models to assure sufficient adjustment for season and meteorological impact (Table 1c). In a second step, single air pollutants were added to the model and effects estimated linearly. Finally, city-specific effect estimates were combined using meta-analysis methodology (Van Houwelingen et al. 2002). Additionally, it was examined whether an HbA1c level of more than 6.5% modified the effects of air pollution on blood markers. Results of the air pollution analysis are based on an increase in air pollution concentrations from the first to the third quartile (interquartile range, IQR) and are presented as percent change from GM of hs-CRP together with 95% CI. For details see (Ruckerl et al., 2007) (Appendix III).

Table 1c: Variables of the confounder models for the estimation of air pollution effects, by city

City	Time invariant variables	Time variant variables/meteorology
Athens	BMI, log(NT-proBNP), systolic blood pressure	trend, apparent temperature (mean of lags 0 to 1), hour of visit
Augsburg	BMI, log(NT-proBNP), total cholesterol, chronic bronchitis, packyears of smoking	trend, apparent temperature (lag 0), relative humidity (mean of lags 0 to 1), hour of visit
Barcelona	BMI, log(NT-proBNP), chronic bronchitis, cholesterol, packyears of smoking	trend, apparent temperature (mean of lags 0 to 1)
Helsinki	age, BMI, hypertension, packyears of smoking, sex, systolic blood pressure, HbA1c	trend, apparent temperature (mean of lags 0 to 1)
Rome	BMI, log(NT-proBNP), total cholesterol	trend, apparent temperature (mean of lags 0 to 3)
Stockholm	age, BMI, log(NT-proBNP), total cholesterol, packyears of smoking, sex, HbA1c	trend, apparent temperature (mean of lags 0 to 1)

4 RESULTS

In total, 1,003 MI survivors who had at least two valid blood samples were taken for the analyses. Of 6,068 samples collected, 255 had to be excluded due to acute infections or surgical procedures within three days before the clinic visit. In 75 of the samples from Helsinki, Stockholm, Augsburg and Rome the hs-CRP concentration was lower than the detection limit. Overall, 5,813 plasma samples remained for analysis. The number of visits per patient are shown in Table 2. Thirty-eight had only two usable visits; however, more than half of the participants reached the aspired number of six visits.

Table 2: Number of patients and visits in AIRGENE

Number of visits	Number of patients N (%)	Total number of visits N (%)
2	38 (3.8)	76 (1.3)
3	48 (4.8)	144 (2.5)
4	40 (4.0)	160 (2.8)
5	82 (8.2)	410 (7.1)
6	598 (59.6)	3588 (61.7)
7	141 (14.1)	987 (17.0)
8	56 (5.6)	448 (7.7)
total	**1003 (100)**	**5813 (100)**

Baseline characteristics of the study population in total and separated by gender are presented in Table 3, characteristics by city are shown in Table 4. Regarding the whole study population, approximately two thirds of the participants were male. Half of the population reported hypertension, one third angina pectoris and about 20% were diabetics. Average BMI was high, with a very wide range from 17 kg/m² to almost 49 kg/m². Two thirds of the population were occasional or former smokers. As expected in a panel of MI survivors, medication intake was high, especially of anti-platelet medication, statins and beta blockers.

A comparison between male and female participants shows that the women in the study population were on average significantly older than the men. Considering medical history both groups were equal, with a slightly higher percentage of women reporting hypertension. There were fewer ex- or occasional smokers among women. Medication intake varied only slightly,

with a higher intake of diuretics in women, which is probably a result of the higher percentage of hypertensives in this group.

Regarding the study populations of the different cities, more women participated in the Northern European centres Stockholm and Helsinki compared to the other centres. Participants had a similar age range; however, a higher proportion of young men were recruited in Athens. A history of angina pectoris was more frequent in Athens and Stockholm. Hypertensive and diabetic patients were distributed equally among study centres. The best scores with respect to total cholesterol and HDL to total cholesterol ratio were observed in Barcelona and Stockholm, while the lipid profiles were most disadvantageous in Athens. Only in Augsburg it was possible to recruit exclusively current non-smokers, however the number of smokers were also very low in Stockholm and Helsinki. Participants from Athens showed the highest percentage of smokers. A history of smoking among current non-smokers was more prevalent in the southern European centres, particularly in Athens and Barcelona. In all the cities, the majority of the patients were treated with beta-blockers, ACE inhibitors, and lipid-lowering drugs, as well as antithrombotic therapy (aspirin) to prevent recurrent myocardial infarctions. Treatment was less vigorous in Athens.

Arithmetic mean, median and geometric mean of hs-CRP were not exceptionally high in the study population (Table 3, Figures 2a and 2b).

Table 3: Baseline characteristics of 1,003 myocardial infarction survivors in total and by gender

	Total N=1003	Male N=788	Female N=215	p-Value
Age [yrs][a]	62.2 (37 – 81)	61.4 (37-79)	65.1 (38-81)	<0.0001[2]
MI in family history [%]				0.0014[1]
Yes (mother and/or father)	35.2	33.1	42.8	0.0085[1]
No	54.5	57.5	43.7	0.0003[1]
Information incomplete	10.3	9.4	13.5	0.0794[1]
Self-reported medical history [%][b]				
Angina pectoris	34.3	33.9	35.8	0.6054[1]
Arrhythmia	22.5	21.6	26.1	0.1641[1]
Hypertension	51.0	49.4	56.7	0.0551[1]
Diabetes	19.7	20.2	18.1	0.5057[1]
Body mass index [kg/m²][a]	28.4 (17.5 – 48.9)	28.5 (17.6-48.9)	28.1 (17.5-41.6)	0.2233[2]
Systolic blood pressure [mmHg][a]	134.3 (81.0-209.0)	134.7 (84.0-202.0)	133.1 (81.0-209)	0.3665[2]
Diastolic blood pressure [mmHg][a]	79.1 (45.0-126.0)	80.0 (52.0-126.0)	75.9 (45.0-119.0)	<0.0001[2]
Total cholesterol [mg/dl][a]	184.4 (91.1–390.0)	183.0 (91.1-390.0)	189.4 (107.0-324.7)	0.0377[2]
HDL cholesterol [mg/dl][a]	50.2 (22.0–119.3)	47.6 (22.0-105.4)	59.9 (30.0-119.3)	<0.0001[2]
HbA1c[c] > 6.5% [%]	10.6	10.9	9.4	0.5319[1]
Smoking status [%]				<0.0001[1]
Never smoker	26.6	20.1	50.7	0.0107[1]
Ex or occasional smoker	65.5	70.9	45.6	<0.0001[1]
Current smoker	7.9	9.0	3.7	0.0107[1]
Packyears (cigarettes only)[a]	18.7 (0-205.2)	21.3 (0-205.2)	8.9 (0-62.5)	<0.0001[2]
Alcohol intake [%][d]				<0.0001[1]
None	16.5	13.2	28.4	<0.0001[1]
Moderate	70.4	73.0	60.9	0.0006[1]
Heavy	13.1	13.4	10.7	0.2411[1]
Medication (%)				
Beta-blockers	84.3	84.3	84.2	0.9778[1]
ACE-inhibitors	60.4	60.4	60.5	0.9875[1]
Calcium channel blockers	18.3	16.8	24.2	0.0125[1]
Diuretics	27.6	25.0	37.2	0.0004[1]
Statins	83.9	84.6	80.9	0.1896[1]
Anti-platelet medication	97.4	97.5	97.2	0.8363[1]
CRP [mg/L][e,f]				
Arithmetic mean (range)	2.6 (0.16-37.4)	2.6 (0.16-37.4)	2.7 (0.16-30.2)	0.4311[2]
Median	1.6	1.5	1.7	0.1336[2]
Geometric mean	1.4	1.3	1.6	0.1505[3]

[a]mean, with range in brackets, [b]ever physician diagnosed; [c]HbA1c: glycosylised haemoglobin; [d]none": 0g/day, "moderate": <20g/day for women and <40 g/day for men, "heavy": ≥20g/day for women and ≥40g/day for men; [e]values of CRP below 0.16 could not be measured and were set to 0.16; [f]values were calculated as means (median) of patient means (median) and might therefore differ slightly from table 5a
[1]Chi-Square-Test, [2]t-Test , [3]ANOVA using log-transformed values

Table 4: Baseline characteristics of 1,003 myocardial infarction survivors by city

	Helsinki N=195	Stockholm N=197	Augsburg N=200	Rome N=134	Barcelona N=169	Athens N=108	p-Value
Sex = male [%]	68.7	70.6	82.0	86.6	83.4	87.0	<0.0001[1]
Age [yrs][a]	64.6 (45-78)	64.0 (38-76)	61.9 (39-76)	62.7 (39-79)	62.1(37-81)	54.7 (38-75)	<0.0001[5]
MI in family history [%]							<0.0001[1]
Yes (mother and/or father)	47.7	44.7	30.5	29.1	20.7	34.3	<0.0001[1]
No	41.0	44.2	58.0	64.2	70.4	54.6	<0.0001[1]
Information incomplete	11.3	11.2	11.5	6.7	8.9	11.1	0.7010[1]
Self-reported history [%][b]							
Angina pectoris	39.0	47.7	21.0	27.6	29.6	41.7	<0.0001[1]
Arrhythmia	31.3	20.8	24.0	23.1	13.0	21.3	0.0029[1]
Hypertension	51.3	49.7	51.0	55.2	46.2	54.6	0.73[1]
Diabetes	21.0	18.3	17.5	17.2	23.7	21.3	0.63[1]
Body mass index [kg/m²][a]	28.6 (19.1-48.9)	27.6 (17.5-43.2)	28.7 (19.1-48.4)	27.7 (19.0-39.4)	28.8 (19.3-43.5)	28.8 (20.8-46.3)	0.0039[5]
Systolic Blood Pressure [mmHg][a]	139.9 (93.0-209)	137.6 (97.0-196)	128.4 (84.0-198)	134.7 (95-188)	129.5 (81-196)	136.1 (100-190)	<.0001[5]
Diastolic Blood Pressure [mmHg][a]	79.5 (52.0-112.0)	80.4 (53.0-112.0)	78.1 (47.0-112.0)	77.6 (54.0-114.0)	77.4 (45.0-126.0)	82.3 (60.0-122.0)	0.0010[5]
Total cholesterol [mg/dl][a]	182.2 (91.1-291.9)	173.4 (96.7-324.7)	181.0 (107.0-316.0)	190.6 (120.0-321.0)	193.2 (119.0-390.0)	195.4 (92.0-293.0)	<0.0001[5]
HDL cholesterol [mg/dl][a]	54.0 (22.0-119.3)	53.7 (30.9-116.0)	47.9 (24.0-98.0)	43.7 (25.0-87.0)	52.7 (28.0-105.0)	46.1(24.0-87.0)	<0.0001[5]
HbA1c[c] > 6.5% [%]	15.9	6.6	9.5	8.2	10.7	14.8	0.010[1]
Smoking status [%]							<0.0001[4]
Never smoker	39.5	29.9	31.0	25.4	14.2	10.2	<0.0001[1]
Ex or occasional smoker	59.0	69.5	69.0	65.7	72.8	51.9	0.0019[1]
Current smoker	1.5	0.5	0	9.0	13.0	38.0	<0.0001[4]
Packyears (cigarettes only)[a]	9.1 (0-65.0)	12.2 (0-73.8)	15.1 (0-205.2)	21.8 (0-171.8)	28.1 (0-192.3)	35.6 (0-174.0)	<0.0001[3]
Alcohol intake [%][d]							<0.0001[1]
None	14.4	7.1	14.5	19.4	17.2	36.1	<0.0001[1]
Moderate	76.9	80.7	65.0	67.9	69.2	53.7	<0.0001[1]
Heavy	8.7	12.2	19.5[1]	12.7	13.6	10.2	0.037[1]
Medication							
Beta-blocker	95	91	92	75	75	64	< 0.001[1]
ACE-inhibitors	50	51	72	81	59	52	< 0.001[1]
Calcium channel blockers	14	22	15	26	21	29	0.0055[1]
Diuretics	23	25	44	33	23	10	< 0.001[1]
Statins	83	88	89	79	85	73	0.0024[1]
Anti-platelet medication	97	97	99	95	98	93	0.06[1]
CRP [mg/L][e]							
Arithmetic mean (range)	1.98 (0.16-12.15)	2.86 (0.16-37.44)	2.26 (0.16-24.65)	2.56 (0.16-15.33)	3.52 (0.33-30.16)	2.52 (0.23-24.25)	0.0001[5]
Median	1.37	1.59	1.40	1.62	2.17	1.49	<0.0001[2]
Geometric mean	1.18	1.41	1.18	1.34	1.98	1.34	<0.0001[6]

[a]mean, with range in brackets, [b]ever physician diagnosed; [c]HbA1c: glycosylised haemoglobin; [d]none": 0g/day, "moderate": <20g/day for women and <40 g/day for men, "heavy": ≥20g/day for women and ≥40g/day for men; [e]values of CRP below 0.16 could not be measured and were set to 0.16; [f]values were calculated as means (median) of patient means (median) and might therefore differ slightly from table 5a
[1]Chi-Square-Test, [2]Kruskal-Wallis-Test , [3]Median-Test, [4]Fisher´s exact test, [5]ANOVA, [6]ANOVA using log-transformed values

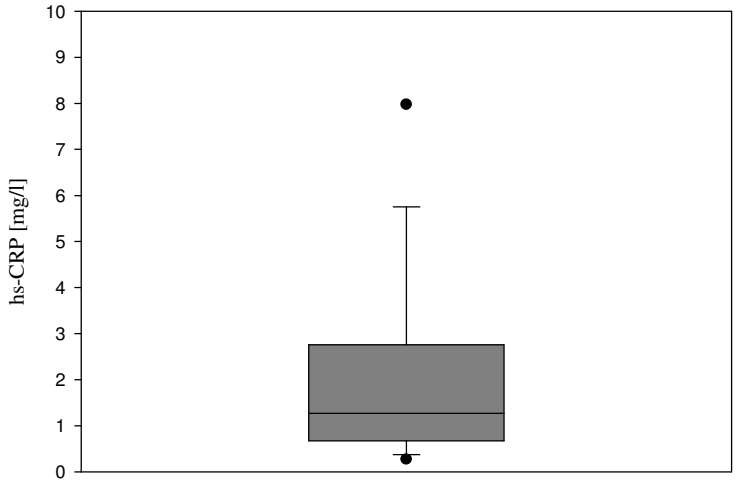

Figure 2a. Hs-CRP values of the repeated measurements of the AIRGENE population. Boxplot including 95th and 5th percentiles

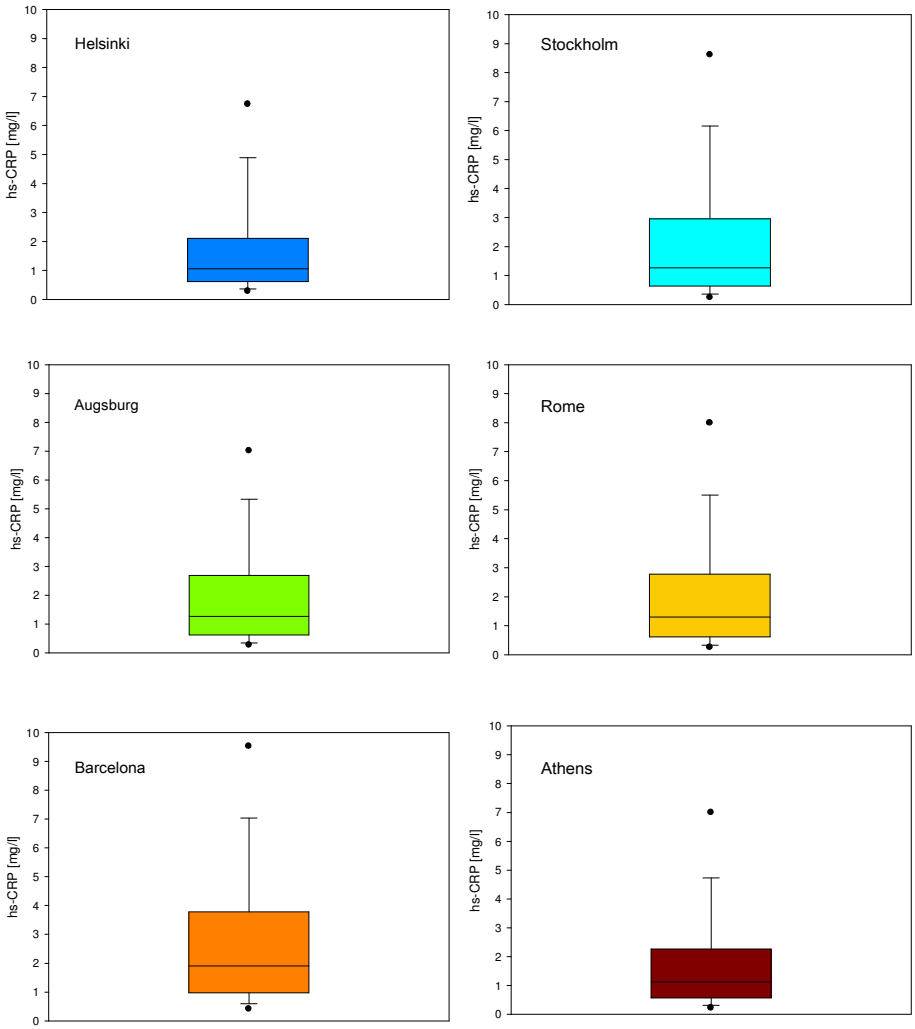

Figure 2b. Hs-CRP values of the repeated measurements of the AIRGENE population by city. Boxplots including 95th and 5th percentiles

Tables 5a shows the arithmetic and geometric mean hs-CRP concentrations of the variables of the base model. The mean concentration in Barcelona was 3.4 mg/L, while concentrations for all other cities ranged between 2.0 and 2.8. Values were on average higher for women. Age showed a U-shaped function, with the lowest hs-CRP concentration for the age group 50 to 59. A clear linear relationship was seen for BMI, ranging from 1.0 for underweight to 2.9 for obese. Hs-CRP concentrations were also higher in smokers than in non-smokers, and showed a dose-response relationship regarding packyears of smoking. Patients with high HbA1c levels or high N-terminal proB-type natriuretic peptide (NT-pro BNP) levels had also higher hs-CRP concentrations. For total cholesterol on the other hand, patients with an intermediate level showed the highest hs-CRP concentration, while those over 250gm/dl and those below 200mg/dl were lower and almost similar regarding their arithmetic mean concentrations.

In terms of diagnosed diseases, slightly higher hs-CRP concentrations in patients with emphysema or stroke were found. Clearly higher hs-CRP was seen in patients with chronic bronchitis and asthma (Table 5b).

Concerning smoking and ETS exposure, current regular smokers showed the highest hs-CRP concentrations, followed by patients who are constantly exposed to ETS. Surprisingly, occasional smokers had the lowest hs-CRP concentrations; however the number of subject in this group was very small. Analyses also showed higher hs-CRP concentrations in patients with bad or very bad health status and those who claim to be inactive.

In respect of medication intake, patients reporting the intake of serum lipid reducers, statins, ASA and beta blockers had lower hs-CRP concentrations, while in patients on calcium channel blockers the concentration was higher (Table 5c).

Table 5a: Arithmetic mean, geometric mean* and unadjusted coefficients of variation† of hs-CRP – variables of the base model

Variable		Arithmetic mean of hs-CRP [mg/L]		Geometric mean of hs-CRP [mg/L]		Coefficient of variation of hs-CRP	n
		mean	std	mean	std	mean	
City	Athens	2.6	5.3	1.3	2.9	55.9	108
	Augsburg	2.2	4.5	1.2	2.8	56.8	200
	Barcelona	3.4	5.7	2.0	2.7	59.3	169
	Helsinki	2.0	3.1	1.2	2.6	53.6	195
	Rome	2.5	4.0	1.3	2.9	52.3	134
	Stockholm	2.8	5.1	1.4	3.0	52.0	197
Sex	Male	2.5	4.5	1.3	2.9	56.8	788
	Female	2.8	5.1	1.6	2.8	48.3	215
Age [years]	< 50	2.7	4.8	1.5	2.9	56.1	115
	50-59	2.0	3.3	1.1	2.7	50.2	271
	60-69	2.8	5.2	1.4	3.0	55.6	348
	≥ 70	2.9	5.0	1.6	2.8	58.5	269
BMI[a]	obese	2.9	3.6	1.8	2.5	49.0	999
	overweight	2.6	5.2	1.3	2.9	56.2	316
	normal	2.2	4.9	1.0	2.9	63.4	483
	underweight	1.0	1.1	0.7	2.6	29.5	189
Number of MI	2 or more	3.1	4.5	1.7	2.9	60.2	150
	1	2.5	4.7	1.3	2.8	54.1	853
Smoking [packyears][b]	> 30.75	3.5	5.7	1.9	2.9	55.8	228
	≤ 30.75	2.5	4.8	1.3	2.9	55.4	470
	Never smokers	2.1	3.2	1.2	2.7	53.8	304
HbA1c level[cd]	High (≥6.5%)	3.2	4.5	1.9	2.8	56.6	108
	Normal (<6.5%)	2.5	4.5	1.3	2.8	54.4	868
Log NT-pro BNP[e] **[pg/ml]**	≥ 5.98	3.3	6.2	1.7	3.0	63.0	245
	5.97 - 5.12	2.5	4.1	1.3	3.0	51.2	252
	5.11 - 4.47	2.2	3.4	1.3	2.6	51.5	250
	< 4.47	2.4	4.5	1.3	2.8	54.3	256
Total cholesterol level[c]	High (>250mg/dl)	2.5	3.8	1.5	2.7	53.5	60
	At risk (200-250mg/dl)	3.2	5.7	1.7	2.9	57.1	249
	Normal (<200mg/dl)	2.4	4.3	1.3	2.8	54.3	689

[a]BMI classification according to WHO (2000): obese ≥30 kg/m², overweight ≥25<30 kg/m², normal weight ≥20<25 kg/m², underweight <20 kg/m²; [b]categories correspond interquartile ranges; [c]measured at baseline; [d]HbA1c: glycosylised haemoglobin; [e]NT-pro BNP: N-terminal proB-type natriuretic peptide, categories: quartiles

*arithmetic and geometric mean based on all repeated measurements taken together and might therefore differ slightly from tables 3 and 4; †coefficient of variation based on repeated measurements by subject

Table 5b: Arithmetic mean, geometric mean* and unadjusted coefficients of variation† of hs-CRP – disease history

Variable		Arithmetic mean of hs-CRP [mg/L]		Geometric mean of hs-CRP [mg/L]		Coefficient of variation of hs-CRP	n
		mean	std	mean	std	mean	
Type 2 Diabetes[a]	Yes	2.8	4.2	1.6	2.8	57.2	198
	No	2.5	4.8	1.3	2.9	54.4	805
Angina pectoris[a]	Yes	2.7	4.6	1.5	2.9	52.4	344
	No	2.5	4.7	1.4	2.8	56.3	658
Congestive heart failure[a]	Yes	2.8	3.9	1.7	2.7	48.3	104
	No	2.6	4.7	1.4	2.9	55.7	899
Emphysema[a]	Yes	3.0	3.7	1.8	2.8	42.1	23
	No	2.6	4.7	1.4	2.9	55.3	980
Family history of MI[b]	≥ 1 parent	2.6	4.6	1.4	2.8	53.2	353
	No	2.6	4.9	1.4	2.9	56.1	547
Stroke[a]	Yes	3.0	4.9	1.7	2.8	54.0	62
	No	2.6	4.6	1.4	2.9	55.0	941
Hypertension[a]	Yes	2.6	4.4	1.4	2.9	55.5	511
	No	2.6	5.0	1.4	2.9	54.4	492
Chronic bronchitis[a]	Yes	4.3	7.5	2.3	3.2	51.7	67
	No	2.5	4.4	1.3	2.8	55.2	936
Asthma[a]	Yes	3.1	3.8	1.79	3.02	49.4	47
	No	2.6	4.7	1.37	2.85	55.2	956

[a]ever physician diagnosed; [b]MI: myocardial infarction;
*arithmetic and geometric mean based on all repeated measurements taken together;
†coefficient of variation based on repeated measurements by subject

Table 5c: Arithmetic mean, geometric mean* and unadjusted coefficients of variation† of hs-CRP – life-style variables and medication intake

Variable		Arithmetic mean of hs-CRP [mg/L]		Geometric mean of hs-CRP [mg/L]		Coefficient of variation of hs-CRP	n
		mean	std	mean	std	mean	
Smoking status and ETS exposure excluding packyears of smoking from the model	Current regular smoker	3.4	5.9	1.8	3.1	58.8	72
	Occasional smoker	1.8	2.6	1.0	2.9	50.1	27
	No current smoker, constant ETS exposure	3.2	5.3	1.5	3.3	52.3	136
	No current smoker, no constant ETS exposure	2.4	4.5	1.4	2.8	55.3	767
Health status	Excellent/good	2.5	4.5	1.4	2.8	54.9	592
	Moderate	2.5	5.0	1.3	3.1	56.5	342
	Bad/very bad	3.4	4.5	1.9	2.8	48.1	68
Physical activity	Inactive	3.3	5.6	1.8	2.9	56.8	219
	Partly/Irregularly active	2.5	4.5	1.3	2.9	52.4	280
	Regularly active/trained	2.4	4.2	1.3	2.8	55.5	504
HDL cholesterol[a] [mg/dl] adjusted for total cholesterol	> 35	2.6	4.6	1.4	2.9	55.4	884
	≤ 35	2.7	5.0	1.5	2.7	51.0	114
Serum lipid reducers	Yes	2.5	2.5	1.3	2.9	54.7	858
	No	3.0	4.5	1.71	2.8	56.3	
Statins	Yes	2.5	4.7	1.3	2.9	54.8	841
	No	3.0	4.4	1.7	2.8	55.9	
ACE[b]-inhibitors	Yes	2.6	4.6	1.4	2.9	56.9	606
	No	2.6	4.7	1.4	2.8	52.1	
Systemic anti-inflammatory medication	Yes	2.7	6.0	1.5	2.7	57.6	234
	No	2.6	4.5	1.4	2.9	54.2	
Acetylsalicylic acid	Yes	2.5	4.7	1.3	2.9	54.8	878
	No	3.1	4.7	1.7	2.8	56.5	
Diuretics	Yes	2.9	4.8	1.7	2.8	54.5	277
	No	2.5	4.6	1.3	2.9	55.1	
Ca^{2+}-channel blockers	Yes	3.0	4.7	1.6	3.0	54.2	184
	No	2.5	4.6	1.4	2.8	55.1	
Beta-blocker	Yes	2.5	4.4	1.4	2.9	54.0	845
	No	2.9	5.7	1.6	2.9	59.8	

[a]measured at baseline, [b]angiotensin converting enzyme
*arithmetic and geometric mean based on all repeated measurements taken together;
†coefficient of variation based on repeated measurements by subject

2711 of the examinations were conducted in the morning, before noon, and 3102 in the afternoon. Mean concentrations did not show a clear diurnal pattern (Figure 3a) and also also hardly varied with season (Figures 3b and 3c).

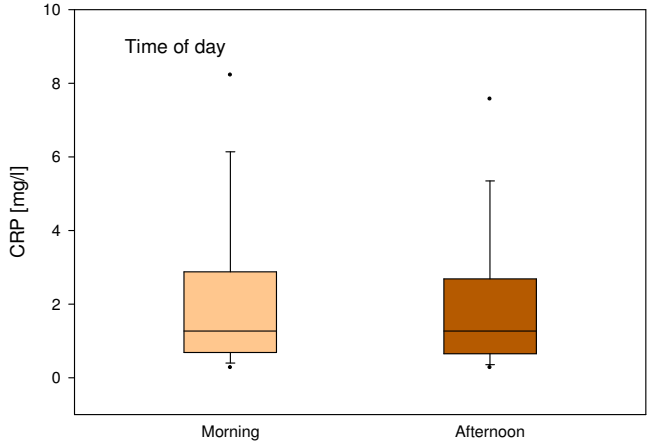

Figure 3a. Hs-CRP values of the repeated measurements of the AIRGENE population by time of the day.
(Morning: before 12.00 a.m., afternoon: after 12.00 a.m.)
Boxplots including 95th and 5th percentiles

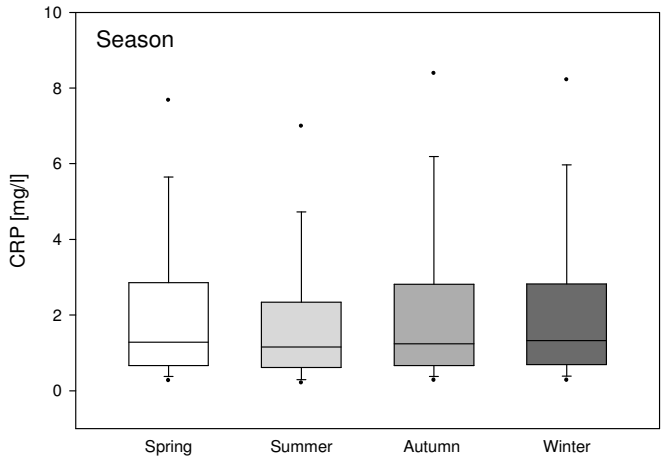

Figure 3b. Hs-CRP values of the repeated measurements of the AIRGENE population by season.
(Spring=March to May, Summer=June to August, Autumn=September to November, Winter=December to February). Boxplots including 95th and 5th percentiles

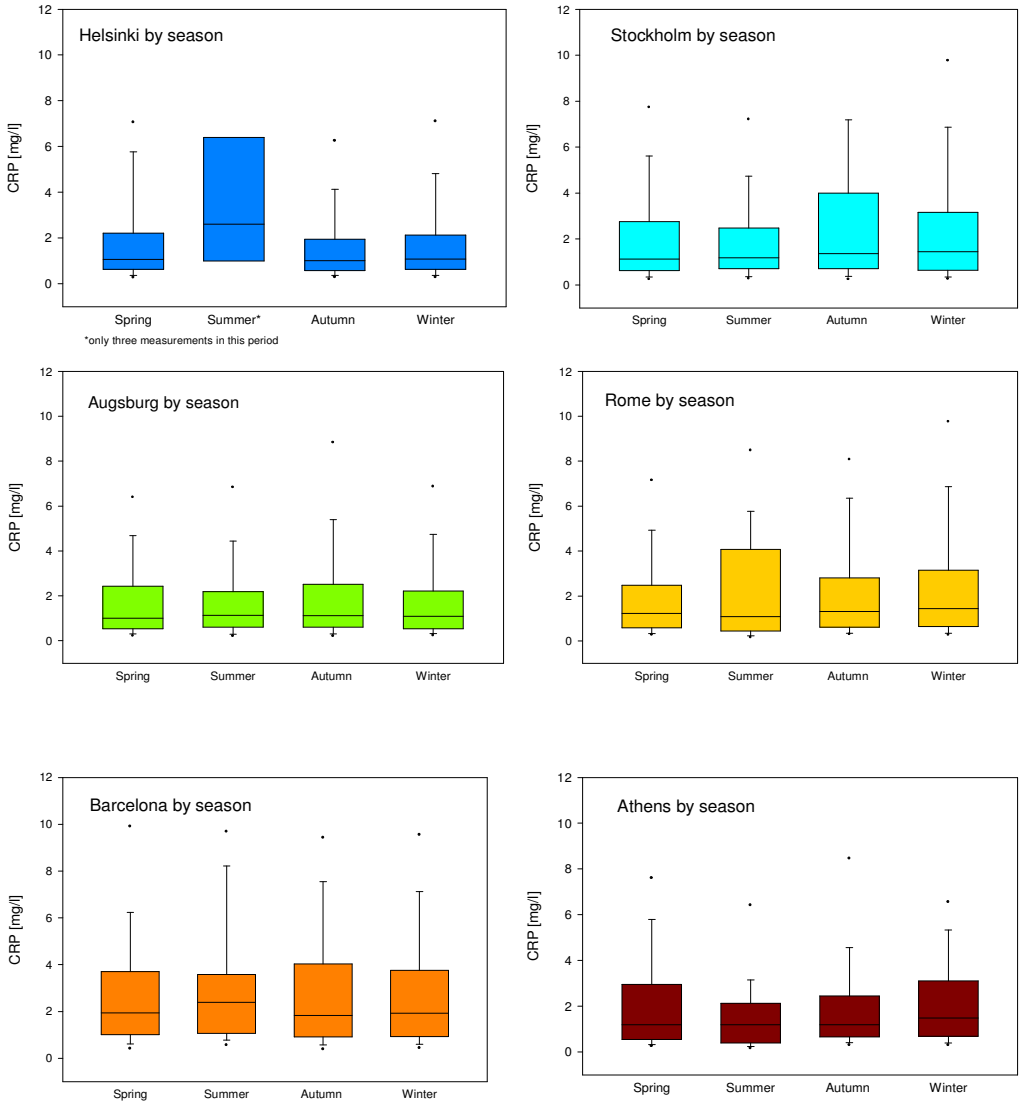

Figure 3c. Hs-CRP values of the repeated measurements of the AIRGENE population by city and season.
(Spring=March to May, Summer=June to August, Autumn=September to November, Winter=December to February). Boxplots including 95th and 5th percentiles

4.1 Patient characteristics that affect hs-CRP concentration and within patient variation

With few exceptions, the unadjusted CVs of different subgroups of the AIRGENE population as shown in Tables 5a to 5c are similar to those of the adjusted model (tables 6a to 6c). The associations between the concentration and variation of hs-CRP and patient characteristics as derived jointly from the base model are given in Table 6a. Regarding age, a U-shaped relationship with the lowest hs-CRP level in the group of 50 to 59 year olds was found (Figure 4). A separate analysis showed that this effect was mainly driven by men, whereas women showed a positive linear association between hs-CRP and age (data not shown).

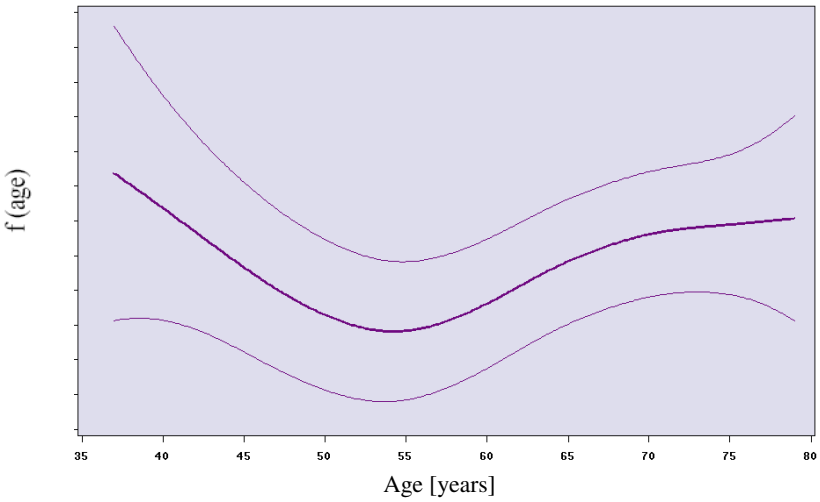

Figure 4. Function of log hs-CRP and age adjusted for the variables in the base model

Males had significantly lower hs-CRP concentrations compared to the female participants, however a higher variation in hs-CRP (Figure 5a). Bodyweight expressed as BMI was one of the strongest determinants with higher geometric mean hs-CRP concentrations in overweight and obese patients. Variation decreased with increasing BMI, but was extremely low in underweight subjects. Clear positive associations were seen with HbA1c levels above the 6.5% cutoff for geometric mean concentration and variation of hs-CRP while a diagnosis of type 2 diabetes was positively associated with the variation but not the geometric mean hs-CRP concentration.

Tables 6b and 6c show the associations between hs-CRP and disease history, life-style factors and medication intake. Family history of MI of at least one parent and the diagnosis of chronic bronchitis were associated with elevated hs-CRP concentrations. Hs-CRP showed less variation

in patients who reported angina pectoris, congestive heart failure (CHF) (Figure 5a), emphysema and at least one parent with a history of MI.

HDL cholesterol was inversely related to a geometric mean hs-CRP concentration with a higher variation in those patients who had an HDL cholesterol level above 35 mg/dl.

Patients reporting the intake of statins or other lipid-lowering drugs in general showed significantly lower geometric mean concentrations of hs-CRP and also less variation. For patients using ACE-inhibitors on the other hand, a higher variation was found while geometric mean concentrations seemed not to be associated with the intake of this medication (Figure 5b and Table 6c)

With very few exceptions, results for the variation model that included all variables at the same time did not differ much from the presented tables. Results showed the largest increases in variation for patients with HDL cholesterol concentrations above 35 mg/dl, older age, especially above 70, male gender, a log BNP level of $\geq$ 5.98 [pg/ml], and intake of anti-inflammatory medication (data not shown).

Table 6a: Association of time-invariant variables on the mean concentration and variation of hs-CRP. Variables of the base model

Variable		%-change from GM[a]	CI[b] lower	CI upper	p-value GM analysis	Proband group* / Reference group (ref) [% difference in within-patient variation]	p-value variation analysis	n
City	Athens	-29.54	-43.46	-12.19	0.002	12.4	0.0007#	108
	Augsburg	-25.95	-36.83	-13.19	0.0002	19.8		200
	Barcelona	9.80	-7.61	30.48	0.29	14.9		169
	Helsinki	-26.44	-37.27	-13.75	0.00016#	6.5		195
	Rome	-13.52	-27.74	3.50	0.11	4.1		134
	Stockholm	ref				ref		197
Sex	Male	-13.28	-23.78	-1.34	0.03	24.8	<0.0001#	788
	Female	ref				ref		215
Age [years]	< 50	28.07	6.26	54.35	0.009	25.5	<0.0001#	115
	50-59	ref				ref		271
	60-69	18.13	3.39	34.97	0.014	27.8		348
	≥ 70	28.26	10.45	48.95	0.001	37.2		269
BMI[c]	Linear: per increase in 5kg/m²	37.80	29.69	46.43	<0.0001#	--		999
	Obese (≥30)	86.02	59.97	116.31	<0.0001#	-45.8	<0.0001#	316
	Overweight (≥25<30)	33.76	16.51	53.57	<0.0001#	-19.9		483
	Normal (≥20<25)	ref				ref		189
	Underweight (<20)	-33.83	-59.49	8.10	0.099	-74.8		11
Number of MI	2 or more	13.10	-2.15	30.73	0.010	18.5	0.0027	150
	1	ref				ref		853
Smoking [packyears][d]	Linear: per increase in 25	16.31	9.65	23.38	<0.0001#	--		1002
	> 30.75	48.04	26.94	72.64	<0.0001#	1.1	0.082	228
	≤ 30.75	16.45	3.17	31.44	0.014	5.0		470
	Never smokers	ref				ref		304
HbA1c level[e]	High (≥6.5%)	26.24	7.23	48.61	0.005	20.7	0.0034	108
	Normal (<6.5%)	ref				ref		868
Log NT-pro BNP[f] **[pg/ml]**	Linear: per increase of 500 NT-pro BNP	38.43	20.61	58.89	<0.0001#			1003
	≥ 5.98	26.99	7.74	49.67	0.004	34.2	<0.0001#	245
	5.97 - 5.12	3.86	-10.94	21.11	0.63	0.03		252
	5.11 - 4.47	2.77	-11.32	19.09	0.72	-9.0		250
	< 4.47	ref				ref		256
Total Cholesterol level[g]	Linear: per increase in 40 mg/dl	15.53	9.60	21.79	<0.0001#	--		998
	High (>250mg/dl)	30.07	15.44	46.55	<0.0001#	-6.1	0.052	60
	At risk (200-250mg/dl)	23.85	-0.11	53.56	0.051	8.7		249
	Normal (<200mg/dl)	ref				ref		689

[a]GM: geometric mean; Geometric mean is the antilog of arithmetic mean of log-transformed variable, %change: antilog of effect estimate obtained from regression minus one multiplied by 100; [b]CI: Confidence Interval, [c]BMI: Body mass index, classification according to WHO (2000); [d]categories correspond interquartile ranges; [e]HbA1c: glycosylised haemoglobin; [f]NT-pro BNP: N-terminal proB-type natriuretic peptide, categories: quartiles; [g]measured at baseline # Statistically significant after adjusting for multiple testing ($\alpha = 0.00125$);

* Proband group refers to the respective category of the variable, e.g. Athens for city, the reference group is annotated as "ref", e.g. Stockholm for city

Table 6b: Association of disease history on the mean concentration and variation of hs-CRP. Base model + disease history

Variable		%-change from GM[a]	CI[b] lower	CI upper	p-value GM analysis	Proband group* / Reference group [% difference in within-patient variation]	p-value variation analysis	n
Type 2 Diabetes[c] excluding HbA1c from the model	Yes	4.22	-8.50	18.70	0.53	11.57	0.0052	198
	No	ref				ref		805
Angina pectoris[c]	Yes	2.54	-8.08	14.39	0.65	-11.0	<0.0001[#]	344
	No	ref				ref		658
Congestive heart failure[c]	Yes	2.83	-13.66	22.48	0.75	-24.9	<0.0001[#]	104
	No	ref				ref		899
Emphysema[c]	Yes	17.19	-16.64	64.75	0.36	-41.6	0.00024[#]	23
	No	ref				ref		980
Family history of MI[d]	≥ 1 parent	12.49	0.48	25.94	0.04	-20.5	<0.0001[#]	353
	No	ref				ref		547
Stroke[c]	Yes	-2.77	-21.78	20.86	0.80	1.6	0.094	62
	No	ref				ref		941
Hypertension[c]	Yes	-8.32	-17.18	1.48	0.093	6.3	0.061	511
	No	ref				ref		492
Chronic bronchitis[c]	Yes	36.47	10.97	67.81	0.003	-2.7	0.65	67
	No	ref				ref		936
Asthma[c]	Yes	16.81	-7.58	47.64	0.19	-9.5	0.15	47
	No	ref				ref		956

[a]GM: geometric mean, geometric mean is the antilog of arithmetic mean of log-transformed variable, %change: antilog of effect estimate obtained from regression minus one multiplied by 100; [b]CI: confidence interval; [c]ever physician diagnosed; [d]MI: myocardial infarction;
[#]statistically significant after adjusting for multiple testing ($\alpha = 0.00125$)
* Proband group refers to the group of patients with disease, e.g. Type 2 diabetes = yes, the reference group annotated as "ref" refers to subjects without the respective disease, e.g. Type 2 diabetes = no

Table 6c: Association of life-style factors and medication intake on the mean concentration and variation of hs-CRP. Base model + life-style variables and medication intake

Variable		%-change from GM[a]	CI[b] lower	CI upper	p-value GM analysis	Proband group* / Reference group (ref) [% difference in within-patient variation]	p-value variation analysis	n
Smoking status and ETS exposure excluding packyears of smoking from the model	Current regular smoker	23.68	-2.19	56.38	0.08	27.3	<0.0001#	72
	Occasional smoker	-27.94	-47.63	-0.86	0.04	-24.6		27
	No current smoker, constant ETS exposure	9.57	-6.31	28.14	0.25	-9.9		136
	No current smoker, no constant ETS exposure	ref				ref		767
Health status	Excellent/good	10.46	-1.13	23.39	0.08	6.5	0.067	592
	Moderate	ref				ref		342
	Bad/very bad	33.06	7.71	64.36	0.008	-11.2		68
Physical activity	Inactive	7.06	-7.16	23.46	0.35	7.0	0.0011#	219
	Partly/Irregularly active	3.09	-8.51	16.16	0.62	-13.1		280
	Regularly active/trained	ref				ref		504
HDL cholesterol[c] **[**mg/dl**]** adjusted for total cholesterol	Linear: per increase in 15 mg/dl	-8.15	-13.90	-2.03	0.0010#	--		998
	> 35	-16.78	-29.73	-1.45	0.033	36.0	<0.0001#	884
	≤ 35	ref				ref		114
Serum lipid reducers	Yes	-18.54	-29.98	-5.23	0.0079	-19.6	0.0015	858
	No	ref				ref		
Statins	Yes	-16.98	-28.28	-3.90	0.013	-18.4	0.00078#	841
	No	ref				ref		
ACE[d]**-inhibitors**	Yes	5.60	-7.59	20.67	0.42	18.3	0.00028#	606
	No	ref				ref		
Systemic anti-inflammatory medication	Yes	2.71	-8.97	15.88	0.66	6.6	0.14	234
	No	ref				ref		
Acetylsalicylic acid	Yes	-10.60	-23.55	4.56	0.16	-8.5	0.06	878
	No	ref				ref		
Diuretics	Yes	1.08	-10.66	14.36	0.86	4.0	0.03	277
	No	ref				ref		
Ca^{2+}-channel blockers	Yes	5.60	-7.59	20.67	0.42	4.1	0.04	184
	No	ref				ref		
Beta-blocker	Yes	1.21	-13.01	17.75	0.88	-11.6	0.005	845
	No	ref				ref		

[a]GM: geometric mean, geometric mean is the antilog of arithmetic mean of log-transformed variable, %change: antilog of effect estimate obtained from regression minus one multiplied by 100; [b]CI: confidence interval, [c]measured at baseline, [d]ACE: angiotensin converting enzyme; #Statistically significant after adjusting for multiple testing ($\alpha = 0.00125$); *Proband group refers to the respective category of the variable, e.g. excellent/good for health status; the reference group is annotated as "ref", e.g. moderate for health status

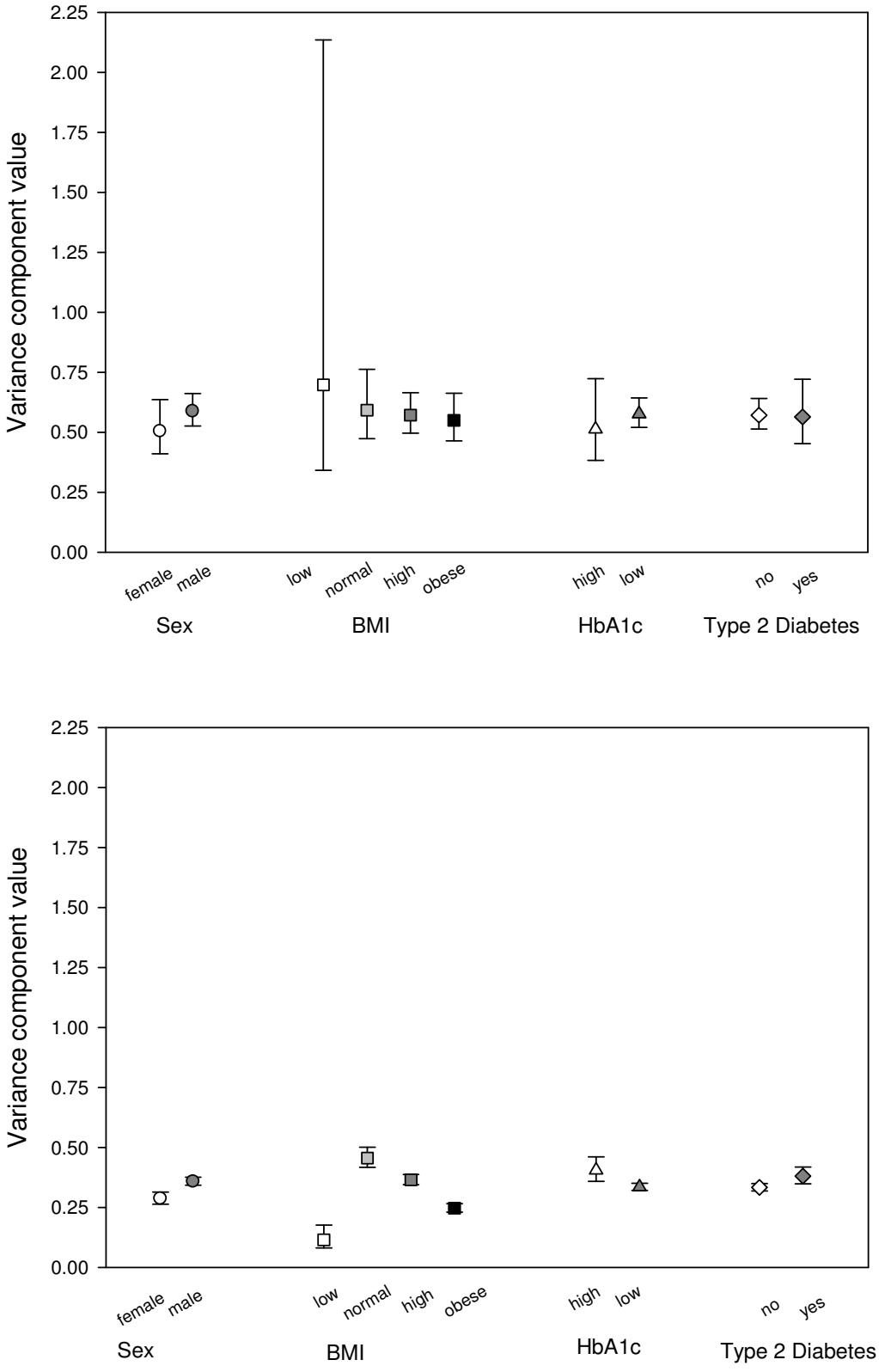

Figure 5a. Variation of mean hs-CRP between (above) and within subjects (below).
Individual hs-CRP measurements over time by patient characteristics (sex, body mass index (BMI), level of glycosylised haemoglobin (HbA1c) ≥ 6.5% and diagnosis of type 2 diabetes).

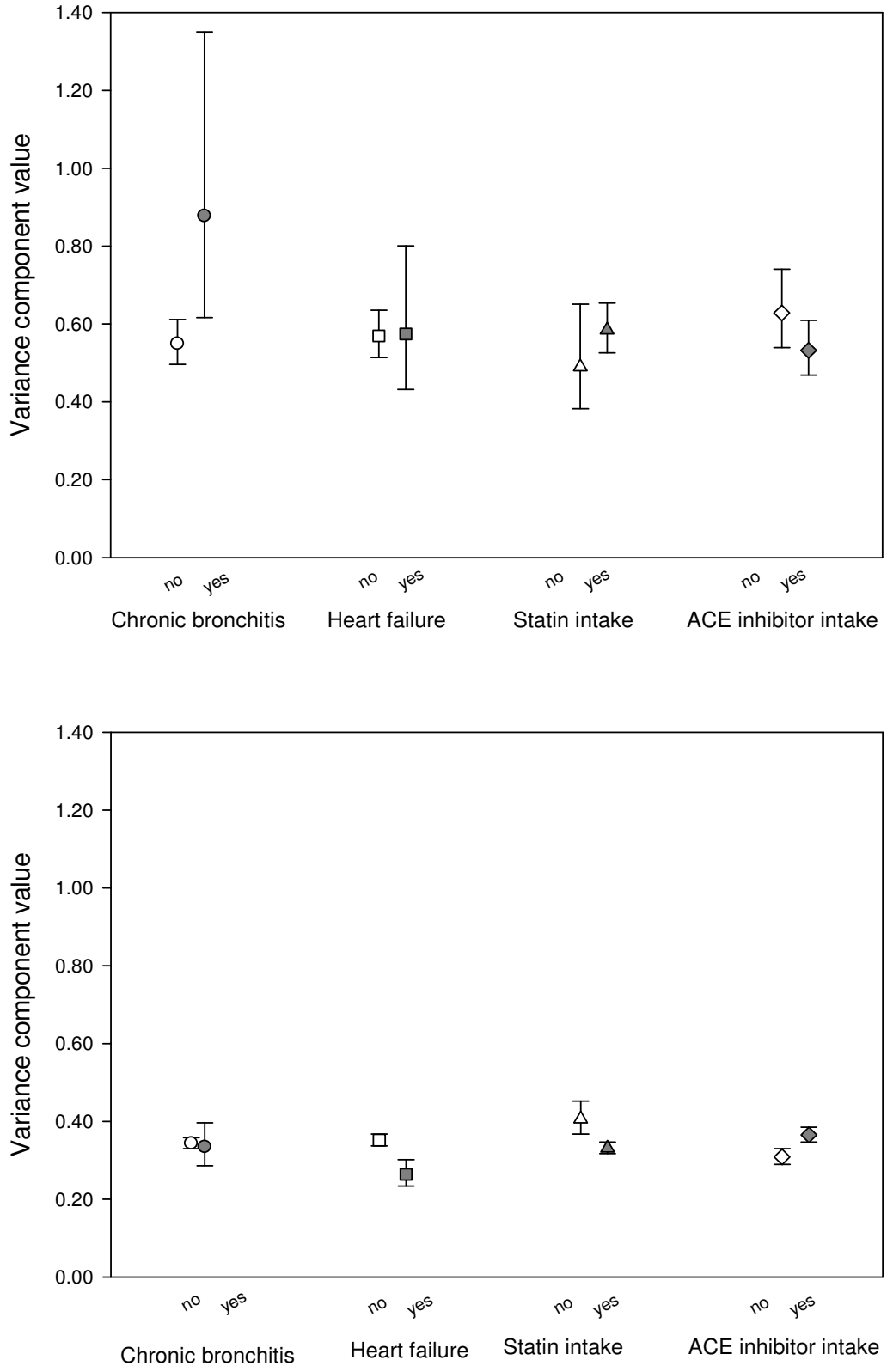

Figure 5b. Variation of mean hs-CRP between (above) and within subjects (below).
Individual hs-CRP measurements over time by patient diagnosis of chronic bronchitis or heart failure and intake of statins or angiotensin converting enzyme (ACE) inhibitors in 1,003 MI patients from the AIRGENE study.

As seen in Table 7, additional adjustment for associated medication intake did not change the results for co-morbidities. Table 8 summarises the results for different smoking-related variables. Twenty-five pack-years of smoking produced an increase of approximately 16% in the geometric mean of the hs-CRP concentration, and inclusion of smoking status in the model had little effect on this result. Including pack-years of smoking, however, removed the borderline effect for ex-smokers found in the model not including 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 hs-CRP concentrations, whereas occasional smokers seemed to have lower hs-CRP concentrations than nonsmokers not regularly exposed to ETS. The results were not statistically significant, however, especially when pack-years of smoking were included in the model. Also, the numbers of participants were low in several of the groups.

Results of the separate analyses for 770 men regarding cholesterol levels can be found in Table 9. Compared to the whole AIRGENE population, associations for total cholesterol (model 1a) and HDL cholesterol (model 2) were slightly stronger when only males were included in the analyses. Strong positive associations were seen for total cholesterol and LDL cholesterol but not for HDL cholesterol when parameters were added separately to the base model (models 1a to 1c). Adjustment for total cholesterol led to significantly negative associations for HDL cholesterol (model 2), which were only slightly weaker when additionally adjusted for LDL (model 4). No associations were found for triglycerides (models 1d and 5).

Table 7: Association of disease history on the mean concentration and variation of hs-CRP. Base model + base model additionally adjusted for medication intake.

Variable		%-change from GM[a]	CI[b] lower	CI upper	p-value GM analysis	Proband group*/ Reference group (ref) [% difference in within-patient variation]	p-value variation analysis
Angina pectoris[c]	Yes	2.54	-8.08	14.39	0.65	-11.0	<0.0001[#]
	No	ref				ref	
Angina pectoris[c] additionally adjusted for Ca^{2+} channel blocker	Yes	2.07	-8.56	13.93	0.72	-11.0	<0.0001[#]
	No	ref				ref	
Congestive heart failure[c]	Yes	2.83	-13.66	22.48	0.75	-24.9	<0.0001[#]
	No	ref				ref	
Congestive heart failure[c] additionally adjusted for ASA[d]	Yes	1.75	-14.61	21.25	0.85	-24.9	<0.0001[#]
	No	ref				ref	
Congestive heart failure[c] additionally adjusted for diuretics	Yes	2.61	-14.26	22.81	0.78	-24.9	<0.0001[#]
	No	ref				ref	
Congestive heart failure[c] additionally adjusted for ACE[e] inhibitors	Yes	6.11	-10.96	26.45	0.51	-24.9	<0.0001[#]
	No	ref				ref	
Emphysema[c]	Yes	17.19	-16.64	64.75	0.36	-41.6	0.00024[#]
	No	ref				ref	
Emphysema[c] additionally adjusted for systemic anti-inflammatory medication	Yes	16.61	-17.13	64.10	0.38	-41.6	0.00023[#]
	No	ref				ref	
Emphysema[c] additionally adjusted for ASA	Yes	16.17	-17.37	63.32	0.39	-41.6	0.00023[#]
	No	ref				ref	
Emphysema[c] additionally adjusted for diuretics	Yes	17.05	-16.79	64.65	0.37	-41.6	0.00024[#]
	No	ref				ref	
Emphysema[c] additionally adjusted for ACE inhibitors	Yes	19.05	-15.22	67.18	0.31	-41.6	0.00027[#]
	No	ref				ref	

[a]GM: geometric mean, geometric mean is the antilog of arithmetic mean of log-transformed variable, %change: antilog of effect estimate obtained from regression minus one multiplied by 100;
[b]CI: confidence interval, [c]ever physician diagnosed, [d]ASA: acetyl salicylic acid, [e]ACE: angiotensin converting enzyme; [#]Statistically significant after adjusting for multiple testing ($\alpha = 0.00125$)
* Proband group refers to the group of patients with disease, e.g. Angina pectoris = yes, the reference group annotated as "ref" refers to subjects without the respective disease, e.g. Angina pectoris = no

Table 8: Association of smoking and ETS exposure with the geometric mean level and variation of hs-CRP, adjusted for the variables of the base model

Variable		% change from GM[a]	CI[b] lower	CI upper	p-value GM analysis	Proband group* / Reference group (ref) [% difference in within-patient variation]	p-value variation analysis	n
Packyears of smoking excluding smoking status from the model	Linear: per increase of 25 packyears	16.31	9.65	23.38	<0.0001[a]	--	--	1002
Packyears of smoking including smoking status in the model	Linear: per increase of 25 packyears	14.88	7.59	22.67	<0.0001[a]	--	--	1002
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	99
	Ex smoker	19.67	5.97	35.16	0.004	-2.4		627
	Never Smoker	ref				ref		277
Smoking status including pack years of smoking in the model	Current smoker (regular/occasional)	4.59	-15.75	29.83	0.68	10.9	0.116	99
	Ex smoker	5.97	-7.32	21.16	0.40	-2.2		627
	Never Smoker	ref				ref		277
Smoking status and ETS exposure excluding packyears of smoking from the model	Current regular smoker	23.68	-2.19	56.38	0.08	27.3	<0.0001[b]	72
	Occasional smoker	-27.94	-47.63	-0.86	0.04	-24.6		27
	No current smoker, constant ETS exposure	9.57	-6.31	28.14	0.25	-9.9		136
	No current smoker, no constant ETS exposure	ref				ref		767
Smoking status and ETS exposure including packyears of smoking in the model	Current regular smoker	15.23	-8.79	45.59	0.23	26.8	<0.0001[b]	72
	Occasional smoker	-21.20	-42.66	8.29	0.14	-25.0		27
	No current smoker, constant ETS exposure	6.50	-8.81	24.38	0.43	-10.3		136
	No current smoker, no constant ETS exposure	ref				ref		767

[a]GM: geometric mean, geometric mean is the antilog of arithmetic mean of log-transformed variable, %change: antilog of effect estimate obtained from regression minus one multiplied by 100; [b]CI: Confidence interval;
*Proband group refers to the respective category of the variable, e.g. exsmoker for smoking status; the reference group is annotated as "ref", e.g. never smoker for smoking status

Table 9: Association of cholesterol and triglyceride level on hs-CRP concentration – 770* male post-MI patients (base model without adjustment for sex)

Variable	Total cholesterol per increase in 40mg/dl			HDL per increase in 15mg/dl			LDL per increase in 25mg/dl			Triglycerides per increase in 90mg/dl		
	% change from GM[a]	CI[b] lower	CI upper	% change from GM	CI lower	CI upper	% change from GM	CI lower	CI upper	% change from GM	CI lower	CI upper
Model 1 (a to d) Each parameter separately	20.92	13.57	28.73	-0.93	-8.56	7.35	16.23	10.11	22.68	4.95	-1.28	11.58
Model 2 Total cholesterol and HDL[c] without LDL[d] and triglycerides	24.24	16.27	32.76	-9.60	-16.80	-1.78	--	--	--	--	--	--
Model 3 Total cholesterol and LDL without HDL and triglycerides	14.63	3.77	26.62	--	--	--	6.09	-2.62	15.57	--	--	--
Model 4 Total cholesterol, HDL, LDL without triglycerides	20.05	7.75	33.76	-8.81	-16.29	-0.66	3.60	-5.13	13.15	--	--	--
Model 5 Total cholesterol and triglycerides without HDL and LDL	21.00	13.36	29.16	--	--	--	--	--	--	-0.27	-6.31	6.16

[a]GM: geometric mean, geometric mean is the antilog of arithmetic mean of log-transformed variable, %change: antilog of effect estimate obtained from regression minus one multiplied by 100;
[b]CI: confidence interval, [c]HDL: high density lipoprotein, [d]LDL: low density lipoprotein,
*only male patients with complete data on total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides were included

4.2 Life-style parameters and hs-CRP level

Life-style in the 24 hours preceding blood draw seemed to have no or a rather low influence on hs-CRP concentrations. Negative but not statistically significant associations were seen for alcohol and tea consumption and extreme stress or anger (Figure 6). However, the number of subjects that contributed to the effect was small, with only 260 patients reporting varying tea consumption and 500 reporting varying alcohol intake. While physical activity over the past 24 hours did not seem to be associated with geometric mean hs-CRP concentrations, a clear time course with an increase in hs-CRP was found if the patients were physically active between six and eleven hours before the blood withdrawal (Figure 7). For the other time-varying parameters, no such time-specific effects were seen.

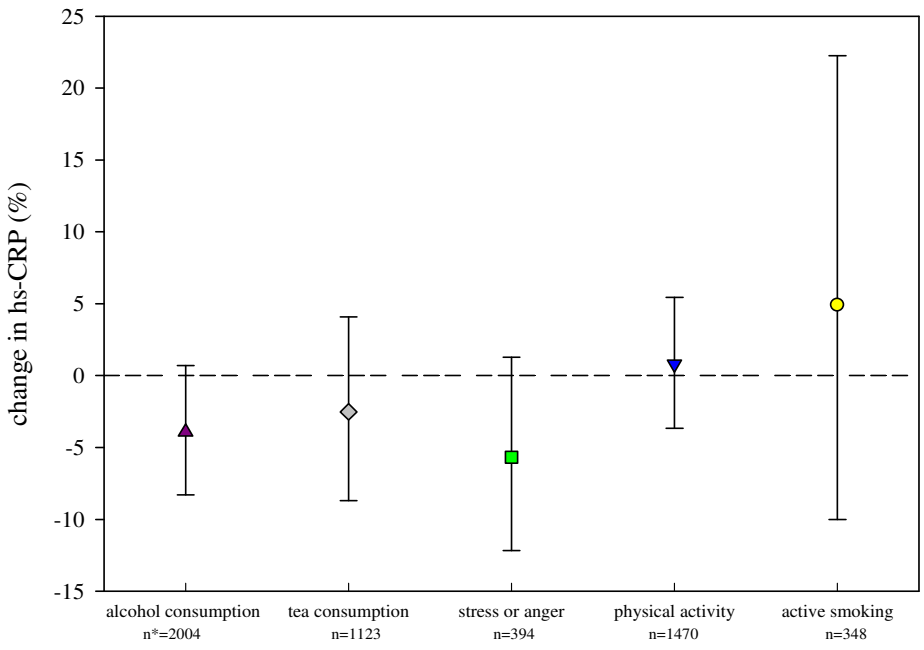

Figure 6. Associations between log hs-CRP and life-style parameters 24 hours before the blood withdrawal. *n: number of observations

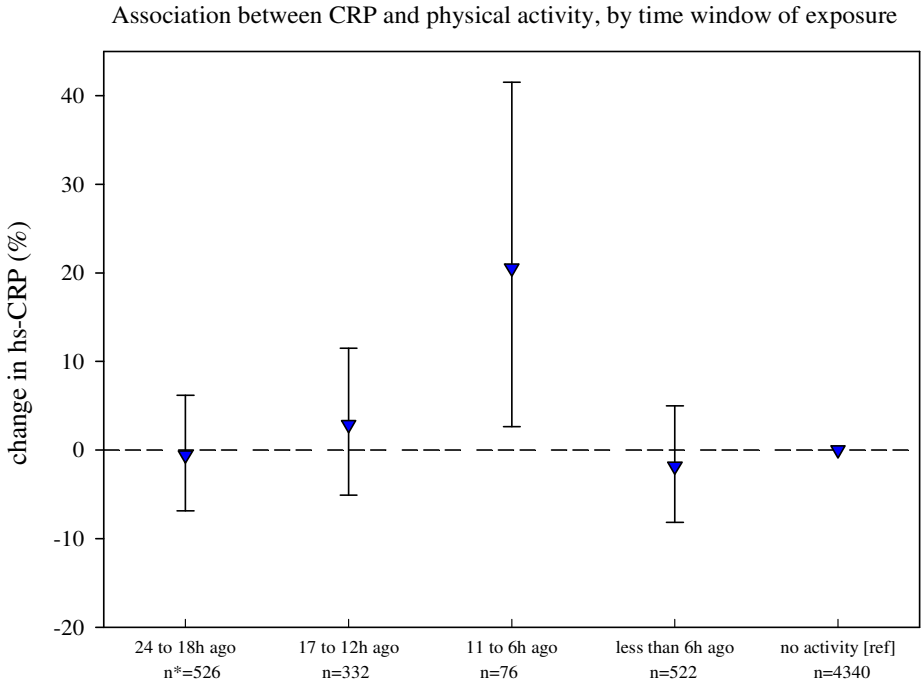

Figure 7. Associations between log hs-CRP and physical activity in 6 hour categories before the blood withdrawal. *n: number of observations, 14 missing values; ref: reference category

4.3 Air pollutants and hs-CRP concentration

Twenty four hour average concentrations of the pollutants and the description of the meteorological data for the six cities for the study period are given in Table 10. PNC and $PM_{2.5}$ were highest in the southern centres and lowest in Stockholm and Helsinki, while Augsburg showed intermediate levels (Figure 8) (Ruckerl et al., 2007), (Appendix III).

For hs-CRP, no associations between ambient air pollution and serum concentrations were observed for any of the analysed time lags (Table 11a). IL-6 and Fibrinogen on the other hand, which were examined in the same population in previous analyses (Ruckerl et al., 2007), (Appendix III), showed positive associations with levels of air pollution. In particular, an increase in IL-6 was seen when PNC were elevated 12–17 hours before the clinical visit and an increase in fibrinogen in association with a five day cumulative exposure to PM_{10} (Table 11b). Analyses of effect modification showed that for fibrinogen associations remained for the five day average exposure to PM_{10} for patients with elevated HbA1c levels (Figure 9).

Table 10. 24-hour average concentrations of the ambient air pollutants and meteorological parameters from 6 European cities during the AIRGENE study period

	Helsinki		Stockholm		Augsburg		Rome		Barcelona		Athens	
Study period[a]	*05.09.03 – 02.06.04*		*30.08.03 – 24.06.04*		*14.05.03 – 24.02.04*		*20.09.03 – 15.07.04*		*30.08.03 – 16.06.04*		*08.09.03 – 30.07.04*	
	Mean	95th	Mean	95th	Mean	95th	Mean	95th	Mean	95th	Mean	95th
PNC [1/cm³]	8534	15077	9748	17578	11876[b]	25135	35450[b]	69226	18133[b]	36526	20589[b]	47573
$PM_{2.5}$ [µg/m³]	8.2	19.4	8.8	19.1	17.4	29.3	24.5[b]	54.1	24.2[b]	62.7	23.0[b]	46.0
PM_{10} [µg/m³]	17.1	36.1	17.8	40.3	33.1	56.6	42.1	76.0	40.7[b]	88.7	38.5	64.6
CO [mg/m³]	0.31	0.46	0.29	0.43	0.58	1.00	1.40	2.47	0.59	0.92	1.48	3.23
NO_2 [µg/m³]	28.6	49.8	18.6	32.6	40.0	61.2	67.0	90.8	50.5	79.6	50.1	73.0
NO [µg/m³]	12.5	40.7	4.9	15.5	30.0	80.4	65.7	164.0	37.7	88.4	41.8	144.6
SO_2 [µg/m³]	4.2	10.1	1.9	4.9	3.0	5.7	4.1	9.2	4.7	9.6	10.3	23.2
O_3[c] [µg/m³]	46.8	89.0	60.6	96.9	54.4	115.3	45.3	99.6	28.2	76.5	59.8	100.2
Air temperature [°C]	3.1	14.7	4.7	15.1	10.2	25.1	13.4	23.9	15.2	23.2	17.6	29.3
Relative humidity [%]	76	91	82	94	69	92	80	95	67	86	67	84

[a]The study period started 5 days before the first measurement because air pollution concentrations up to 5 days before the blood withdrawals were considered.
[b]Data available on less than 95% of the days; [c]Average of 8 maximum hourly values within the past 24 hours

PNC: particle number concentration, $PM_{2.5}$: mass concentration of particles less than 2.5 µm in diameter; PM_{10}: mass concentration of particles less than 10 µm in diameter; CO: carbon monoxide; NO_2: nitrogen dioxide; NO: nitrogen monoxide; SO_2: sulphur dioxide; O_3: ozone.

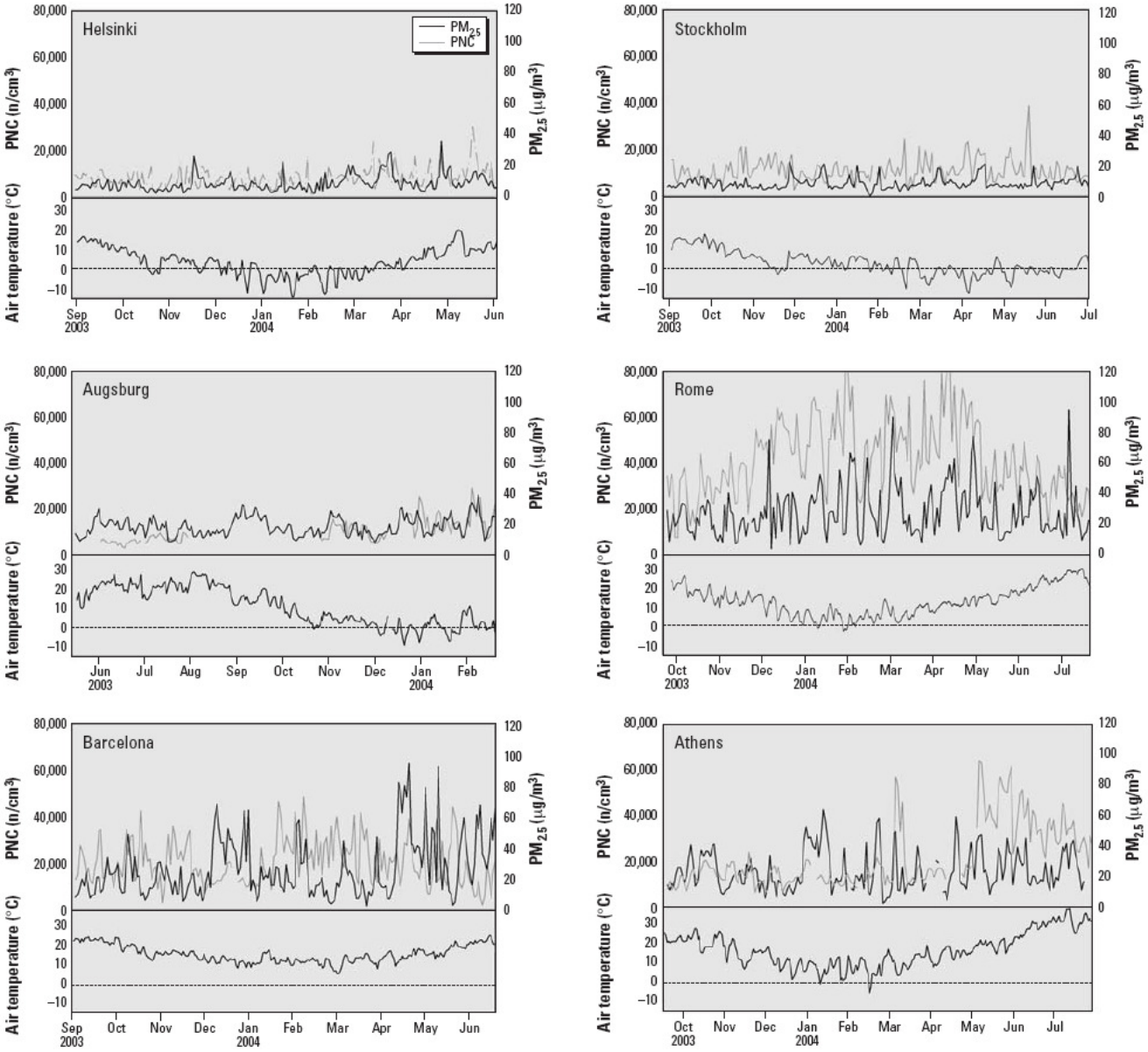

Figure 8. Time series of air pollution (PNC and $PM_{2.5}$) and air temperature in the six European cities of the AIRGENE study

Table 11a: Effects of particulate air pollutants on hs-CRP per increase in interquartile range (IQR) of air pollutant

			Hs-CRP[a] (all cities)			
Pollutant	**Time prior to blood withdrawal**	**IQR**	**%-change (GM[b])**	**95% CI[c]**		**p-value heterogeneity[d]**
				Lower	Upper	
PNC[e]	Lag 0	11852	1.33	-3.05	5.90	0.047
	Lag 1	11852	-1.52	-4.39	1.45	0.19
	Lag 2	11852	-1.63	-6.70	3.71	0.019
	5-d-aver.	11003	-0.08	-3.78	3.75	0.12
$PM_{2.5}$[f]	Lag 0	11.0	0.11	-1.95	2.21	0.71
	Lag 1	11.0	-0.06	-1.98	1.90	0.70
	Lag 2	11.0	0.11	-1.80	2.06	0.86
	5-d-aver.	8.6	-0.13	-2.15	1.92	0.94
PM_{10}[g]	Lag 0	17.4	-0.71	-2.75	1.37	0.16
	Lag 1	17.4	-0.63	-2.61	1.39	0.23
	Lag 2	17.4	-1.42	-4.23	1.47	0.086
	5-d-aver.	13.5	-1.35	-3.45	0.79	0.19

Table 11b: Effects of particulate air pollutants on IL-6 and Fibrinogen per increase in interquartile range (IQR) of air pollutant

			IL-6[h] (all cities)				**Fibrinogen (all cities except Athens)**			
Pollutant	**Time prior to blood withdrawal**	**IQR**	**%-change (GM[b])**	**95% CI**		**p-value hetero-geneity[c]**	**%-change (AM[i])**	**95% CI**		**p-value hetero-geneity**
				Lower	Upper			Lower	Upper	
PNC[e]	Lag 0	11852	1.88[†]	-0.16	3.97	0.72	0.40	-0.40	1.19	0.54
	Lag 1	11852	-0.67	-2.56	1.25	0.64	0.11	-0.69	0.91	0.12
	Lag 2	11852	-2.12[†]	-4.03	-0.17	0.055	0.09	-0.71	0.90	0.045
	5-d-aver.	11003	-0.93	-3.37	1.56	0.084	0.50	-2.20	3.20	0.009
$PM_{2.5}$[f]	Lag 0	11.0	0.46	-0.89	1.83	0.26	0.05	-0.48	0.58	0.36
	Lag 1	11.0	-0.39	-1.69	0.93	0.70	0.17	-0.35	0.69	0.55
	Lag 2	11.0	-0.23	-1.53	1.07	0.57	0.20	-0.32	0.71	0.26
	5-d-aver.	8.6	0.05	-1.37	1.50	0.66	0.38	-0.21	0.96	0.21
PM_{10}[g]	Lag 0	17.4	-0.34	-1.66	0.99	0.45	0.06	-0.43	0.55	0.53
	Lag 1	17.4	-0.69	-1.95	0.58	0.43	0.14	-0.35	0.63	0.83
	Lag 2	17.4	-1.59	-3.99	0.88	0.0030	0.24	-0.24	0.72	0.25
	5-d-aver.	13.5	-0.87	-2.28	0.55	0.15	0.60*	0.10	1.09	0.26

[a]CRP: c-reactive protein; [b]GM: geometric mean, geometric mean is the antilog of arithmetic mean of log-transformed variable, [c]CI: confidence interval, [d]heterogeneity determined with χ^2-test, α=10%; [e]PNC: particle number concentration; [f]$PM_{2.5}$: mass concentration of particles less than 2.5 µm in diameter; [g]PM_{10}: mass concentration of particles less than 10 µm in diameter, [h]IL-6: interleukin 6, [i]AM: arithmetic mean; *p<0.05; [†]p<0.1

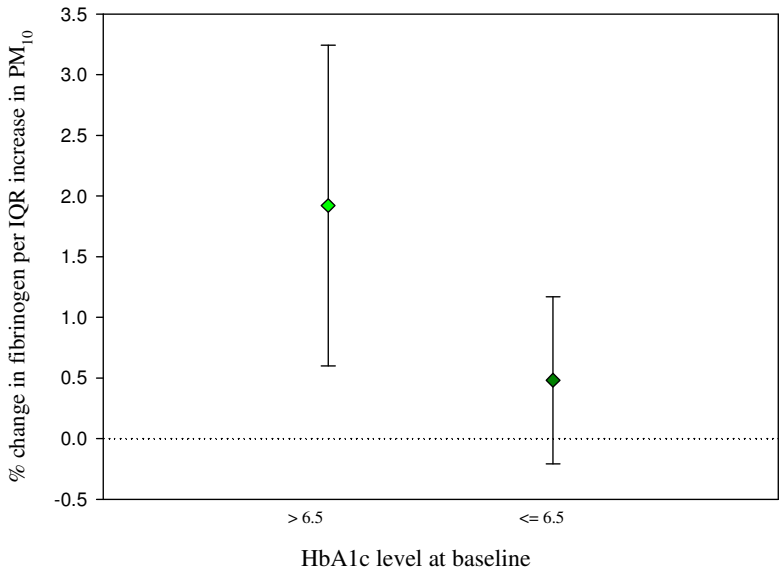

Figure 9. Effect modification of fibrinogen (five day average exposure) by glycosylised haemoglobin (HbA1c).

5 DISCUSSION

Data from the AIRGENE study, a large European multi-centre study on MI survivors, were used

1. a) to confirm known associations between time-invariant patient characteristics and hs-CRP concentrations,

   b) to extend the number of the characteristics published so far and

   c) to study the influence of the same parameters on the variation of hs-CRP, hypothesising that the diagnosis of certain diseases would be related to higher hs-CRP concentrations and a more pronounced variation.
2. to examine the associations between hs-CRP and life-style parameters in the 24 hours before the blood draw, hypothesising that the consumption of tea and alcohol would lead to lower hs-CRP concentrations, while extreme stress or anger, active smoking and physical activity would show a positive association and
3. to examine the association of hs-CRP concentrations and environmental parameters, especially particulate air pollutants, assuming a positive linear relationship.

Hs-CRP values measured in the AIRGENE population were on average slightly higher than in other studies. Subsamples of the three population based MONICA/KORA studies from Augsburg, Glasgow and Lille, for example, showed median concentrations of between 1.30 mg/L (Glasgow and Lille) and 1.38 mg/L (Augsburg) (Imhof et al., 2004), while the median hs-CRP concentration in the whole AIRGENE population was 1.61 mg/L. Similar concentrations to those of the MONICA/KORA populations were also seen in about 4,000 middle aged men and women of the Swedisch Malmö Diet and cancer study (Rosvall et al., 2007). The slightly higher concentrations in these data might in part be explained by the fact that the AIRGENE population consisted of MI survivors, who are likely to have higher hs-CRP concentrations due to their disease history. Ridker et al. (Ridker et al., 1998d), for example, reported median concentrations of 3.1 mg/L in participants who subsequently had recurrent MI, while their age and sex matched

healthy controls had a median concentration of 2.8 mg/L. Moreover, Geffken et al. (2001) reported from a study of approximately 6,000 elderly US citicens, that people without clinical cardiovascular disease had significantly lower mean hs-CRP concentrations compared to participants with clinical cardiovascular disease.

Considering the different regions of the AIRGENE study, the results of Imhof et al. (2004) for Augsburg are almost the same as for the AIRGENE Augsburg subsample. Also, the comparatively low levels for the Swedish and the relatively high concentrations for the Spanish subpopoulations in AIRGENE were seen in other studies (Garcia-Lorda et al., 2006; Rosvall et al., 2007).

Some studies report mean values, rather than the median; however, these data more or less reflect the picture found for the median concentrations. Mean hs-CRP concentrations for the whole AIRGENE population was 2.59 mg/L and therefore higher than mean concentration in two large studies in healthy voluteers (Geffken et al., 2001; Sung, 2006). Population based data from the Greek ATTICA study (Panagiotakos et al., 2004) were again lower than the mean concentrations of the Greek subpopulation of AIRGENE, as were data from a Finnish study (Saltevo et al., 2007).

As hs-CRP usually shows a skewed distribution, values are often log-transformed for analyses and the antilog of arithmetic mean, the geometric mean, is reported. A log-transformation reduces skewness in the data (Bland et al., 1996). The geometric mean was 1.39 mg/L in the AIRGENE population. A comparison of geometric means from the MONICA studies in Augsburg, Glasgow and Lille with the geometric mean of the AIRGENE population showed that the geometric mean of MONICA Augsburg was lower than that of the whole AIRGENE population, while those of Glasgow and Lille were higher. Again, geometric means of patients with chronic cardiovascular disease were higher than for those without (Danesh et al., 2004; Hoffmeister et al., 2001; Tuomisto et al., 2006).

5.1 Determinants of hs-CRP concentration and variation

Results of the AIRGENE data mostly confirmed previously published associations between time-invariant patient characteristics and hs-CRP. In contrast to the initial hypothesis, the diagnosis of any of the diseases recorded in this study seemed not to be related to higher hs-CRP levels, with the exception of chronic bronchitis. Moreover, the variation of hs-CRP was significantly lower in patients with the studied co-morbidities, the only exceptions being the diagnosis of diabetes and a baseline level of HbA1c of at least 6.5%.

A variety of studies have examined influences on the level of hs-CRP and most of the results are in line with the results from the AIRGENE dataset, such as for elevated BMI and obesity (Garcia-Lorda et al., 2006; Greenfield et al., 2004; Sung, 2006; Thorand et al., 2006; Verdaet et al., 2004), for smokers compared to non-smokers (Frohlich et al., 2003; Garcia-Lorda et al., 2006; Geffken et al., 2001; Verdaet et al., 2004) and for subjects with low HDL-cholesterol levels (Garcia-Lorda et al., 2006; Greenfield et al., 2004).

While some investigators did not report any sex differences (Garcia-Lorda et al., 2006), others found lower concentrations in men consistent with the results from AIRGENE (Bowden et al., 2006; Frohlich et al., 2003; Hutchinson et al., 2000; Imhof et al., 2004). Hutchinson et al. (2000) hypothesised that the sex difference might be due to oestrogen intake in women and a study on diabetic women showed significantly higher hs-CRP concentrations in those receiving HRT (Bowden et al., 2006). In the AIRGENE data, intake of HRT showed a slightly positive but non-significant association with hs-CRP (data not shown), consistent with this hypothesis.

As for the influence of age on hs-CRP concentrations, some authors found a positive linear relationship (Hutchinson et al., 2000) while a lack of association has also been reported (Greenfield et al., 2004). It appears that a U-shaped function, as seen in these data, has not been reported before; this could be due the way the relationships were modelled and/or to the fact that the AIRGENE data were based on MI survivors, while most studies have been conducted in the general population.

A higher variation in diabetic patients compared to non-diabetics was seen, however geometric mean hs-CRP concentrations did not differ significantly, in contrast to results of other authors (Pradhan et al., 2001). Subjects with elevated HbA1c (≥ 6.5%), an indicator of glucose control, however, showed higher hs-CRP concentrations and higher variation even in this comparatively homogeneous population of MI survivors. In this dataset, high levels of HbA1c seem to reflect uncontrolled rather than undiagnosed diabetes as 89% of those with HbA1c values above 6.5% reported a diagnosis of diabetes. Only half of the AIRGENE population diagnosed with diabetes on the other hand met the HbA1c criterion of 6.5%. This might indicate that metabolically stable diabetic patients are at less risk compared to unstable diabetics.

No publication seems to deal with the association between hs-CRP and N-terminal proB-type natriuretic peptide (Nt-pro BNP), an indicator for left ventricular dysfunction. However, in patients with chronic heart failure a worse New York Heart Association classification was associated with higher concentrations of hs-CRP and Nt-pro BNP (Windram et al., 2007). Moreover, a study in patients with non-ST elevation acute coronary syndromes showed that Troponin T, NT pro-BNP and hs-CRP were predictors of risk for major events such as death, new acute coronary syndrome, revascularization and heart failure in a multivariate analysis. The authors followed that a multimarker approach based on Troponin T, hs-CRP and NT-proBNP provides a more accurate prognosis regarding acute coronary syndrome, with a worse outcome for those with two or three elevated biomarkers (Tello-Montoliu et al., 2007). These studies indicate, that hs-CRP and Nt-pro BNP have a positive association, as found in the AIRGENE population.

None of the so far published works examines the within-patient variation of hs-CRP concentrations among different subgroups such as males and females or patients having certain medical conditions. Interestingly, the analysed data showed that an increase in geometric mean hs-CRP concentrations and a higher variation were not necessarily related. Subjects that reported angina pectoris, CHF or emphysema showed a lower variation compared to participants who did not report any of these disorders. These findings remained statistically significant even after

adjustment for multiple testing and associated medication intake. Emphysema is often caused by smoking (Petty, 2002) and in the AIRGENE population more than 80% of the emphysema patients were past or current smokers. As emphysema and early stage CHF do not necessarily include an inflammatory component, it is also conceivable that the lower variation of hs-CRP in these patients is merely a marker for a different mechanism, such as an underlying genetic component. Studies on twins have shown that there is a substantial genetic contribution to baseline hs-CRP concentrations (Pepys et al., 2003) and in genetical analyses of the Airgene dataset minor alleles of several variants in selected candidate genes such as *CRP*, *Fibrinogen*, *IL-6* and *IL-10* were found to be significantly associated with intra-individual variation of hs-CRP concentrations (Kolz et al., 2007).

Several studies, also in agreement with the data presented here, have shown that statin therapy reduces circulating hs-CRP (Albert et al., 2001a; Albert et al., 2001b; Libby et al., 2002; Ridker et al., 2001; Ridker et al., 2008; Rosenson et al., 2003). Clinical trials indicate that the proven efficacy of various compounds in primary and secondary prevention of coronary heart disease, such as statins and aspirin, may, at least in part, be mediated by modifying the consequences of the inflammatory response on vascular risk. In particular, additional non-lipid, anti-atherothrombotic and anti-inflammatory effects have been suggested for some statins and the clinical benefit in several studies occurred too early to be explained simply by an impact on the atherosclerotic process (Rosenson et al., 1998). In addition to a change in the lipid profile, especially on the LDL cholesterol level, and an improvement in endothelial function, some statins reduce inflammatory cells within the plaque. This leads to increased plaque stability as fewer enzymes are produced which degrade the extra-cellular matrix and weaken the plaque. Moreover, statins improve the haemostatic balance due to a reduction in platelet aggregation and various coagulation factors such as tissue factor (Rosenson et al., 1998; Welty et al., 1997). Data from the Cholesterol and Recurrent Events (CARE) trial (Ridker et al., 1998d) showed that the relative risk of recurrent coronary events was highest in the group with consistent evidence of inflammation, indicated by hs-CRP and SAA levels above the 90th percentile, which was assigned

to placebo, while the lowest risk was found in the group of patients without signs of inflammation who were additionally on statins. The results also demonstrated that statin intake in the group of patients with high hs-CRP and SAA levels reduced the risk of a recurrent coronary event by 54% to the level of those on placebo but without inflammatory activity. Moreover, the JUPITER (Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin) trial showed for the first time in a prospective study a beneficial effect of statin intake on cardiovascular endpoints in addition to reduced CRP and LDL cholesterol levels in people with elevated hs-CRP but LDL cholesterol below current treatment levels (Ridker et al., 2008). Further evidence has been found in studies indicating a decrease of IL-6 during treatment with some statins (Koenig, 1999; Rosenson et al., 1999). A reduction in IL-6 might then lead to a reduced production of CRP.

In addition, CRP lowering effects have been seen with ASA (Ikonomidis et al., 1999), and ACE inhibitors (Soriano et al., 2007) however, in the AIRGENE data, associations were not significant. An increase in CRP in association with HRT has been reported (Cushman et al., 1999; Ridker et al., 2000) and could be confirmed in this dataset.

Cholesterol, which is transported in the form of lipoproteins, plays an important role in the development of atherosclerosis. LDL cholesterol serves as the principal carrier, and it provides an exogenous source of cholesterol and other cellular nutrients to hepatic and extra hepatic cells through LDL receptor-mediated uptake. Early stages of arterial lipoprotein modification, marked by generation of bioactive products of lipid peroxidation, can occur with little change in cellular receptor recognition. The uptake of these modified products facilitates the intracellular accumulation of lipoprotein-derived cholesterol and cholesteryl esters, characteristic of arterial foam cell formation, one of the first steps in the development of atherosclerotic lesions. HDL cholesterol, on the other hand, is a component of reverse cholesterol transport, which takes cholesterol back from extrahepatic tissues to the liver (Hajjar, 1997). Statin therapy has been shown to be associated with a regression of coronary artery disease, when LDL is substantially reduced and HDL is increased. This might indicate that statin benefits are derived from both

reductions in atherogenic lipoprotein levels and increases in HDL (Nicholls et al., 2007). Data of 770 males of the AIRGENE population showed strong positive associations with CRP for total cholesterol and LDL cholesterol but no association for HDL cholesterol when parameters were added separately to the base model. HDL and total cholesterol had opposing associations with a stronger effect of total cholesterol on hs-CRP. Therefore, the negative association between HDL and hs-CRP only became apparent after adjusting the model for total cholesterol. Adjustment for LDL, on the other hand, weakened the association for total cholesterol and hs-CRP, while LDL cholesterol only showed significantly positive associations without adjustment for total cholesterol. In previous studies, HDL was negatively associated with hs-CRP concentrations while neither LDL nor total cholesterol showed significant associations, however, the methods used in these studies were slightly different to the one used here (Fredrikson et al., 2004; Garcia-Lorda et al., 2006; Ryu et al., 2005).

Whether different factors affect each other and, if so, how, remains speculative. It is possible that a combination of variables amplifies the variation although it is also conceivable that certain combinations of factors may reduce variation. Additionally, factors that are associated with a high variation could just be indicators for a different mechanism. For example, the increase in variation that was seen in our data in association with medication intake might be a direct effect of the medication itself. However, it is more likely that the high variation is due to the underlying disease which led to the prescription of the drug.

5.2 Short-term influences on hs-CRP

Regarding time-varying life-style parameters, results point in the expected direction for alcohol and tea consumption and active smoking, however they were not significant, possibly due to a lack of patients with a variation in those endpoints.

In respect to tea and alcohol consumption, no publication seems to have addressed its effects within the 24 hours post-consumption. A slight decrease in hs-CRP in association with tea and alcohol intake was found; however the number of subjects that contribute to the effect was small, with only 260 patients reporting varying tea consumption and 500 reporting varying alcohol

intake. Several authors have shown a hs-CRP lowering effect of regular black tea (De Bacquer et al., 2006; Steptoe et al., 2007) and of moderate alcohol consumption (Imhof et al., 2004).

Several studies have demonstrated that regular moderate to high exercise leads to a decrease in hs-CRP concentrations (Elosua et al., 2005; Pitsavos et al., 2007) although results are conflicting (Fontana et al., 2007), and some authors assign the detected negative association to a lower BMI in those who exercise rather than to a direct effect of physical activity on inflammatory markers (Elosua et al., 2005; Verdaet et al., 2004). Short term influences, however, have rarely been looked at. Research in sports medicine has examined the effect of extremely strenuous activities on inflammatory markers, among them hs-CRP, and studies report a positive association (Kim et al., 2007; Margeli et al., 2005). However, the reported studies were conducted in people whose activities, even for professional athletes, must be considered extreme, such as a 200 km footrace. A short immediate increase in hs-CRP six to eleven hours after physical activity was found in this population of MI survivors that quickly returns to baseline concentration. In contrast to these findings, a study in post-menopausal women, who conducted light to moderate physical activity, did not report any increase in hs-CRP one hour or 24 hours after exercise compared to baseline concentrations (Davis et al., 2007). Also, hs-CRP concentrations measured immediately and 48 hours after a seven km hill race did not differ from baseline (Simpson et al., 2005). These conflicting results might be explained by differing level of exercise. It is also possible that they are due to different time frames used in the studies. Work on the time course of hs-CRP concentrations after surgical procedures showed a rapid increase starting six to eight hours after an operation, with the highest peak around 48 hours which returns to baseline between 72 and 144 hours post intervention (Colley et al., 1983).

5.3 Response to environmental factors

It is still unclear why some subjects develop cardiovascular diseases or suffer from an MI due to certain triggers while others do not. Heavy physical exertion (Albert et al., 2000; Mittleman et al., 1993), extreme anger (Mittleman et al., 1995) and cocaine or marijuana use (Mittleman et al., 1999; Mittleman et al., 2001) have been reported as causes for an acute MI. Also, environmental stimuli such as second hand tobacco smoke (Barnoya et al., 2005) noise (Babisch, 2006), meteorology (Medina-Ramon et al., 2006; Medina-Ramon et al., 2007; Morabito et al., 2005; Sarna et al., 1977) and air pollution (Lanki et al., 2006; Peters et al., 2001a) 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 level of inflammatory markers, and/or a higher variation in inflammation might represent one possible explanation.

A higher variation was seen in patients with elevated HbA1c and self reported type 2 diabetes. It is plausible, but speculative, that these subgroups also show a stronger reaction to environmental factors, e.g. a more pronounced inflammatory response.

5.3.1 Air pollution and hs-CRP

The current analyses are focused on the association between hs-CRP and air pollution as environmental parameter. Hs-CRP has been one of the first acute phase reactants to be examined in association with air pollution in literature. Increased concentrations have been shown during an air pollution episode in Germany in healthy men (Peters et al., 2001b) and for ambient PM_{10} levels currently present in Europe (Seaton et al., 1999). Additionally, in a panel of coronary heart disease patients, an increase in hs-CRP above the 90^{th} percentile was found in association with ambient particles with a lag of two days (Ruckerl et al. 2006) (Appendix IV). The AIRGENE dataset did not show any associations between the measured air pollutants such as PNC, $PM_{2.5}$, PM_{10} or gaseous pollutants and hs-CRP; however IL-6 and fibrinogen concentrations were elevated in association with increased ambient particles (Ruckerl et al., 2007) (Appendix III). A possible explanation for the lack of association between hs-CRP and air pollution in these data

might be the widespread intake of statins in the AIRGENE population. It has been shown that statins reduce CRP through inhibition of its hepatic synthesis (Arnaud et al., 2005). IL-6, which is produced upstream to the production of CRP in the liver, is not affected by this compound. Also, fibrinogen has been implicated to be reduced by fibrates but not statins (Rosenson et al., 2001).

This study, in line with many others (Albert et al., 2001a; Dubowsky et al., 2006; Ridker et al., 2001; Rosenson et al., 2003), showed a clear negative association between statin intake and hs-CRP concentrations (Peters et al., 2007). It can be hypothesised that the intake of statins attenuates the impact of environmental parameters and might therefore, in addition to recommended guidelines, be beneficial in certain particularly susceptible subgroups to avoid adverse cardiovascular effects of environmental stimuli. Zeka et al. (2006) reported evidence for a greater effect of black carbon on inflammatory markers among non-users of statins. However, more research in this area is clearly needed.

5.3.2 Possible biological mechanisms

The exact mechanisms linking the inhalation of ambient air particles to an acute exacerbation of cardiovascular disease are not completely understood (Brook et al., 2004). Increased concentrations of hs-CRP are known to predict cardiovascular events in healthy subjects (Pepys et al., 2003) and alveolar inflammation induced by particles may either directly or via oxidative stress lead to systemic inflammation with increased levels of blood coagulability, progression of atherosclerosis, and destabilization or even rupture of vulnerable plaques, resulting in acute ischemic events (Brook et al., 2004; Peters et al., 1997; Pope et al., 2004; Seaton et al., 1995; Seaton et al., 1999). Hs-CRP, even at lower than medically relevant concentrations, can be considered as a sensitive marker reflecting systemic inflammation caused by particles.

In this dataset, in addition to a higher mean concentration in hs-CRP a higher variation in certain subgroups was found (Ruckerl et al., 2007 Appendix II) which might be one possible link for the reported associations between air pollution and adverse cardiovascular outcomes. Persistently elevated concentrations as well as acute changes in concentrations of inflammatory markers have

been associated with an increased risk of cardiovascular events in cohort studies (Koenig et al., 1999; Ridker et al., 1997).

MI patients were selected for this study, as it has been shown that individuals with certain diseases, such as diabetes and MI, have an enhanced susceptibility for air pollution related conditions, possibly due to a disease induced increased inflammatory burden (Bateson et al., 2004; Brook et al., 2008; Goldberg et al., 2001; Zanobetti et al., 2001). In this dataset, mean hs-CRP concentrations were not significantly higher in diabetic patients, but a higher variation was seen compared to non-diabetics. Furthermore, subjects with increased HbA1c (≥6.5%) showed higher hs-CRP concentrations and higher variation.

Recent analyses demonstrate that diabetics seem to react especially strongly to environmental stimuli such as air pollution. O'Neill et al. (2007) report a positive association between air pollution and markers of inflammation in a panel of diabetic patients. Dubowsky et al. (2006) found that patients with diabetes, obese and especially individuals with metabolic syndrome showed stronger associations between air pollution and hs-CRP than subjects without any of these conditions. Results were similar for IL-6 (Dubowsky et al., 2006). However, the number of individuals was very limited in their analyses. In the AIRGENE dataset, analyses of effect modification showed that for fibrinogen associations were slightly higher and almost significant for the exposure to PM_{10} for patients with elevated HbA1c levels compared to patients with HbA1c levels below 6.5%.

The biological mechanisms that apply to the association between air pollution and atherogenesis could also promote the development of diabetes. Insulin resistance, the main biological pathway that causes type 2 diabetes, is triggered by oxidative stress and proinflammatory mediators at the cellular and transduction level. Likewise, autonomic nervous system imbalance and impaired endothelial function can also lead to blunted insulin action. Air pollution is capable of causing such physiological responses (Brook et al., 2008). However, these hypotheses are still highly

speculative and more research on parameters that make a person especially prone to cardiovascular disease is needed.

Hypothesised mechanisms also differ regarding different size fractions of ambient particles. PM_{10} is suggested to affect the upper bronchi and therefore lead to an inflammation in the lung, whereas the smaller particles potentially transfer into the blood and start a systemic inflammatory response. According to Geiser (2002), UFP are rapidly translocated into the blood. It is therefore possible that the delay that has been observed in a previous study on hs-CRP and air pollution (Ruckerl et al., 2006) is due to the time needed to initiate the acute-phase response after a fast UFP translocation.

5.4 Strengths and limitations of the study design

The chosen study design, using repeated measurements per subject collected over a period of several months, has some unique features making it especially suitable for these analyses.

Firstly, several repeated measurements per person lead to a more stable estimate of the average hs-CRP concentration for the evaluation of the patient characteristics that affect hs-CRP concentrations, compared to a single measurement. Although great care was taken in excluding blood samples that might have been influenced by a current cold or minor operation at the time of blood withdrawal, it is impossible to account for all possible parameters. The statitistical power, on the other hand, is weaker than it would be for the same amount of independent measurements.

Variation in hs-CRP can only be determined if the dataset contains repeated measurements. For the analysis of time-variant life-style parameters, the advantage of the repeated measurement design lies in the fact that time invariant parameters are automatically controlled for. Possible confounders that do not or only slightly change in a period of months, such as sex, age or BMI and even unknown factors are accounted for by the study design as each person serves as his or her own control. A drawback is, however, that only those patients who report differing exposure over the course of the study add to the statistical power.

Very similar to the analyses of time-variant parameters, the analysis of air pollutants and hs-CRP uses the advantages of the repeated measurements design. While the time invariant parameters remain approximately the same, the exposure varies on different examination days. In contrast to the aforementioned analyses, subjects have little influence over the level of air pollution to which they are exposed.

It has to be considered that the examined population consists of MI survivors, who differ from a healthy or otherwise diseased population. Therefore, the results of these analyses might not be generalisable to other population subgroups. Regarding the public health impact, however, MI survivors certainly need special attention as despite efficacious interventions to treat acute MI (1998a; 1998b; 2002; Freemantle et al., 1999; Latini et al., 1995) and despite declining secular trends over the last 25 years, these patients are still at increased risk for recurrent ischemic events and CHF. The occurrence of unstable angina and recurrent MI as well as of CHF is particularly high during the first half year after the index MI and decreases constantly during the following three to five years (Hellermann et al., 2003; Jokhadar et al., 2004; McGovern et al., 2001; Rosamond et al., 1998). Therefore, it is very well conceivable that during this vulnerable period, triggers such as stress, certain weather conditions or noise (Ising et al., 2004; Mittleman et al., 1995; Willich et al., 2006) and also exposure to traffic (Peters et al., 2004) might provoke the onset of a new cardiac event.

6 CONCLUSION

This work confirms and extends published results on the association between patient characteristics and intake of medication and hs-CRP levels in a panel of male and female MI survivors. None of the short-term parameters measured in this study seemed to influence hs-CRP concentrations. Moreover, in the AIRGENE dataset, ambient air pollution was not associated with hs-CRP concentrations, possibly due to a widespread intake of statins in the examined subjects.

In addition, this study is the first to measure within-patient variation in hs-CRP concentration in a large study population. It was not possible to trace variation back to one single source in these data. Males, elderly individuals, smokers, and patients with increased HbA1c concentrations had greater intra-individual variation in repeated measurements of hs-CRP. Of these subgroups, elderly, smokers and patients with elevated HbA1c also showed a higher mean concentration compared to the reference group. For males, patients with a normal BMI, subjects without history of MI and hypertensives, data revealed lower mean concentrations but higher variation. This result might indicate that in patients with manifest cardiovascular disease, in particular after MI, several hs-CRP measurements may be necessary to adequately characterise their risk, especially in defined subgroups.

The study only included MI survivors, so the conclusions are limited to this subgroup. MI survivors are at increased risk for recurrent ischemic events, primarily in the first six months after the MI, but also years later. They might therefore be especially vulnerable to environmental stimuli that can trigger acute events. However, whether the variation seen in subgroups of the study participants makes these patients additionally susceptible to adverse environmental variables needs further investigation. It would be interesting to see if the variation in hs-CRP found in MI survivors differs from the variation in healthy populations or patients with other diseases, such as diabetes. Like patients with cardiovascular disease, diabetics showed higher hs-CRP concentrations compared to a healthy population. However, nothing is known about the variation of hs-CRP in these patients.

The effect of medication, especially statins, could not be examined in detail in this study, as almost 90% of the participants report an intake of statins. The lack of detectable associations for air pollution and hs-CRP, on the other hand may be attributed to exactly this widespread intake of statins in our population, which might suggest a protective effect against environmental, proinflammatory stimuli. However, to resolve the issue of whether a prophylactic intake of statins may protect certain subgroups from adverse environmental parameters, further research is needed.

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Appendix I

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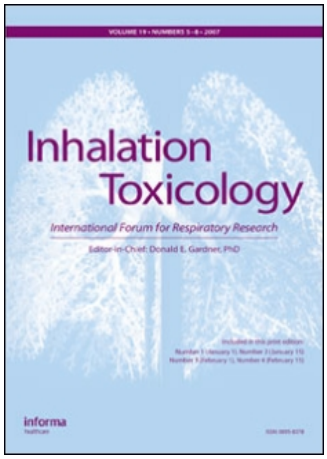

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Air Pollution and Inflammatory Response in Myocardial Infarction Survivors: Gene–Environment Interactions in a High-Risk Group

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Ambient air pollution has been associated with an increased risk of hospital admission and mortality in potentially susceptible subpopulations, including myocardial infarction (MI) survivors. The multicenter epidemiological study described in this report was set up to study the role of air pollution in eliciting inflammation in MI survivors in six European cities, Helsinki, Stockholm, Augsburg, Rome, Barcelona, and Athens. Outcomes of interest are plasma concentrations of the proinflammatory cytokine interleukin 6 (IL-6) and the acute-phase proteins C-reactive protein (CRP) and fibrinogen. In addition, the study was designed to assess the role of candidate gene polymorphisms hypothesized to lead to a modification of the short-term effects of ambient air pollution. In total, 1003 MI survivors were recruited and assessed with at least 2 repeated clinic visits without any signs of infections. In total, 5813 blood samples were collected, equivalent to an average of 5.8 repeated clinic visits per subject (97% of the scheduled 6 repeated visits). Subjects across the six cities varied with respect to risk factor profiles. Most of the subjects were nonsmokers, but light smokers were included in Rome, Barcelona, and Athens. Substantial inter- and intraindividual variability was observed for IL-6 and CRP. The study will permit assessing the role of cardiovascular disease risk factors, including ambient air pollution and genetic polymorphisms in candidate genes, in determining the inter- and the intraindividual variability in plasma IL-6, CRP, and fibrinogen concentrations in MI survivors.

Epidemiological research has indicated that ambient air pollution is associated with an increase in mortality and morbidity of respiratory and cardiovascular diseases (Katsouyanni et al., 2001; Le Tertre et al., 2002; Zanobetti et al., 2003). Particulate matter (PM) appears to be the air pollutant most consistently associated with adverse health outcomes. The inhalable fraction of ambient aerosols, measured as PM_{10} or $PM_{2.5}$ (particles with an aerodynamic diameter less than 10 μm or less than 2.5 μm), is considered to be responsible for most of the adverse health effects. The number of ultrafine particles (0.01 to 0.1 μm) in ambient air is hypothesized to be of particular concern (Wichmann & Peters, 2000).

Coronary heart disease (CHD) is a common chronic health condition in Western societies (Tunstall-Pedoe et al., 2000). Two main pathological processes, namely atherosclerosis and thrombosis, lead to an acute coronary syndrome (ACS) such as unstable angina, non-ST-elevation myocardial infarction (NSTEMI), or ST-elevation myocardial infarction (STEMI) (Naghavi et al., 2003a, 2003b). The typical atherosclerotic lesion is a fibro-lipid plaque composed of a pool of lipids covered with a connective tissue cap (Ross, 1999). Although the plaque narrows the coronary arteries, ACS only occurs when a plaque erodes, fissures, or ruptures and a thrombus is formed that occludes the arteries, partially or totally, and impedes blood flow. There is a strong link between inflammation and CHD since factors involved in inflammation and infection seem to play a pro-atherogenic role and inflammation has been identified as a potent risk factor for the ACS (Ross, 1999). Other risk factors such as cigarette smoking, diabetes, or high body mass index (BMI) have also been found to be associated with low-grade systemic inflammation (Woods et al., 2000), providing a further link between inflammation and ACS. On the other hand, factors alleviating systemic inflammation, such as moderate exercise or weight loss, reduce the risk of acute coronary events.

Acute-phase proteins, like C-reactive protein (CRP) or fibrinogen, have been identified as biomarkers for inflammatory processes and are important determinants of plaque rupture (Ridker et al., 2004). CRP may also exert direct pro-atherogenic effects by various mechanisms (Pasceri et al., 2000). This classical acute-phase protein has now emerged as a reliable predictive marker for cardiac events in patients with CHD and in healthy individuals. Recently, its application in clinical practice has been recommended by the American Heart Association and the Centers for Disease Control for subjects being at intermediate risk of CHD (Pearson et al., 2003).

Ambient particulate matter has been associated with systemic responses, including increases in CRP and fibrinogen in healthy individuals in cross-sectional settings (Peters et al., 2001; Schwartz, 2001; Pekkanen et al., 2000). Additionally, in longitudinal studies changes in ambient particulate air pollution were associated significantly with changes in the CRP level (Seaton et al., 1999; Ruckerl et al., 2006).

The multicenter epidemiological study described in this report was set up to study the role of air pollution in eliciting inflammation in MI survivors in six European cities (Figure 1). Outcomes of interest are plasma concentrations of the proinflammatory cytokine interleukin 6 (IL-6) and the acute-phase proteins CRP and fibrinogen. IL-6 is thought to play a major role in mediating stimuli from activated macrophages for example by smoking (Woods et al., 2000). IL-6 can stimulate the synthesis of acute-phase proteins, such as CRP and fibrinogen (Figure 2).

FIG. 1. Location of the study centers in Europe.

Fibrinogen was also considered a risk factor for its contribution to the formation of blood clots. In addition, the study was designed to assess the role of candidate gene polymorphisms hypothesized to lead to a modification of the short-term effects of ambient air pollution.

METHODS

Study Design

A multicenter longitudinal study of MI survivors was performed in six European cities—Athens (Greece, 3.1 million inhabitants), Augsburg (Germany; 0.5 million inhabitants), Barcelona (Spain; 1.5 million inhabitants), Helsinki (Finland; 0.5 million inhabitants), Rome (Italy; 2.7 million inhabitants), and Stockholm (Sweden; 1.0 million inhabitants)—chosen to include a variety of geographical conditions and air pollution levels. At each location, the goal was to recruit 200 post-MI patients resulting in a study population of 1200 MI survivors. In each subject 6 repeated clinical examinations were scheduled: one every 4 weeks. Therefore, in total 7200 clinical examinations were anticipated.

Study Population

Candidates for the study were identified in population registries of patients with MI [Augsburg (Lowel et al., 2005), Barcelona, Stockholm] or in administrative databases of hospital admissions (Athens, Helsinki, Rome). They were contacted either directly or through their hospitals, depending on the national ethical requirements.

MI was defined based on the recommendation by the European Society of Cardiology/American College of Cardiology Committee (2000). Inclusion criteria were (a) survival of a MI between 3 mo and 6 yr before entry into the study, corresponding to a MI in the years between 1997 and 2003, and (b) age between 35 and 80 yr. Exclusion criteria were: (a) a myocardial infarction and/or interventional procedure (PTCA, bypass surgery) less than 3 mo before the beginning of the study; (b) not resident in the study area; (c) an extended period of absence from the study area planned during the study period; (d) any major illness preventing patients from complying with the study protocol; (e) chronic inflammatory diseases and/or anti-inflammatory medication modifying the biomarkers considered in the study; and (f) only one or no valid blood sample available per patient. In addition, if a patient had had a cold/flu, urinary-tract infection, gastrointestinal infection, respiratory infection, or a surgery or a major dental procedure in the 3 days before the clinical visit, then the samples collected at that visit were excluded.

Preferably, currently nonsmoking MI survivors were recruited. Ex-smokers had to have quit 3 months before the start of the study to be considered as nonsmokers. But light current smokers were accepted in some centers.

Field Study

All partners received approval of the study protocol by their local human subjects committees. Informed consent was obtained from all patients at the first clinical visit after a detailed description of the study protocol.

Clinical Characterization at Baseline

The health status of each patient was assessed at the first visit at the local study center. The cohorts were characterized with respect to their cardiovascular risk factor profile. This part was crucial in order to describe similarities and differences of the cohorts recruited in the six locations.

The protocol included a history of CHD and other comorbidities. A baseline questionnaire assessed regular exercise, smoking history, environmental tobacco smoke exposures, socioeconomic status, and alcohol intake. All medication taken was recorded, including brand name, dose, and intake pattern. Clinical measurements included a blood pressure measurement, the determination of the BMI, and a resting 12-lead electrocardiogram (ECG). A blood serum sample was drawn to determine serum lipids including total cholesterol, high-density lipoprotein (HDL) cholesterol, and glycosylated haemoglobin (HbA1c). Ethylenediamine tetraacetic acid (EDTA)–plasma samples were collected to assess CRP, fibrinogen, IL-6, and N-terminal proB-type natriuretic peptide (NT-proBNP, only at baseline). For DNA analyses a single blood sample was collected in a 9-ml EDTA tube for each participant at the first visit and stored at –80°C until samples were shipped on dry ice for DNA isolation to the laboratory at the GSF-National Research Center for Environment and Health in Neuherberg, Germany.

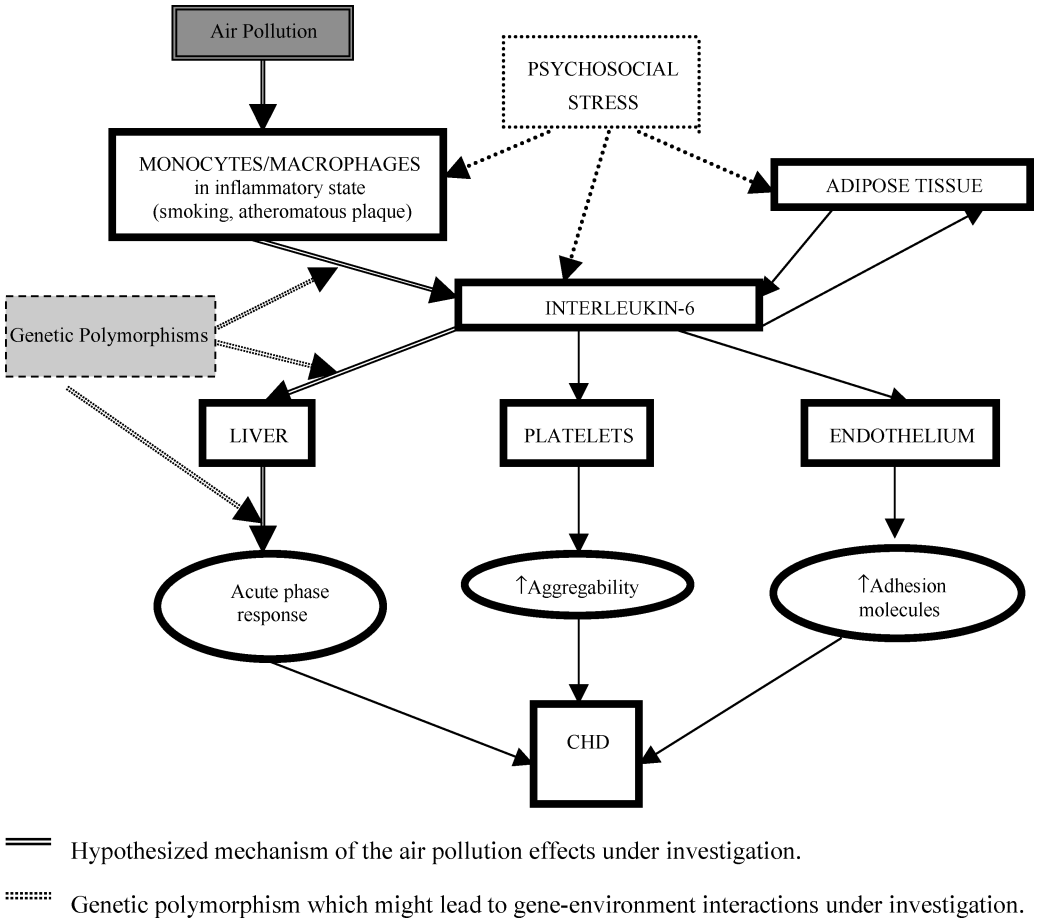

FIG. 2. Role of inflammatory pathways in the development and exacerbation of coronary heart disease (adapted after (Woods et al., 2000)).

Laboratory results outside the normal range and alarming ECG or blood pressure measurements were reported to the patient through the local study center with the advice to see her or his health care provider for repeated analysis and potential treatment.

Repeated Clinical Visits

Each clinical visit was to be scheduled on the same day and at the same time of the week to minimize the impact of circadian variation and the impact of the day of the week. If the patient was unable to comply with this criterion, another day of the week was selected with an appointment at the same hour (plus/minus 1 h). If patients suffered from acute infections such as a cold or influenza during the 3 days before the scheduled visit, examinations were postponed or blood samples were excluded from analyses.

At each clinical visit, an EDTA–plasma sample was collected for inflammatory marker determination (CRP, fibrinogen, and IL-6) and a short questionnaire was administered. Information on time-varying factors such as smoking or the time of the last meal was collected in a short questionnaire. A 7-day recall on medication intake was obtained. Venous blood samples were taken in EDTA tubes according to standardized procedures. Samples were cooled down and stored at 4°C until further processing, which was within 4 h after blood withdrawal. To obtain plasma samples, EDTA–blood was centrifuged at 4°C in a precooled centrifuge for 20 min at 2500 × g. The plasma was then collected with a pipette and aliquots for analysis were prepared. Plasma aliquots were stored in each study centre at –80°C until they were shipped on dry ice to the central laboratory in Ulm, Germany. Blood samples were analyzed by means of a commercial enzyme linked immunosorbent assay (ELISA) for IL-6 (quantitative high sensitive IL-6 Immunoassay, RD Systems GmbH, Wiesbaden, Germany), immunonephelometry for fibrinogen and high sensitivity CRP (Dade Behring Marburg GmbH, Marburg, Germany). Blood samples from Athens did not pass the quality assurance assessments for fibrinogen determinations and therefore no fibrinogen data are available for this city.

In addition, most of the patients volunteered to keep a diary during the course of the study on cardiovascular and respiratory symptoms, overall health status, smoking behavior, physical activity, and times spent outdoors, in traffic, or in rooms where other people smoked.

Air Pollution and Meteorological Data

Air pollution data from fixed monitoring sites representing urban background concentrations were collected for each city according to standard procedures already employed in several European studies of air pollution (Katsouyanni et al., 1995). Measurement of ambient concentrations of air pollutants concurrent with the clinical examinations was used to characterize the population average exposures. Air pollution concentrations include the traditional air pollutants such as gaseous pollutants and particulate matter. In addition, particle number concentrations (PNC) measurements were included by leveraging and extending previous measurements (Aalto et al., 2005).

Hourly means of the gaseous air pollutants (CO, SO_2, O_3, NO_x, NO_2) and of particles (PM_{10}, $PM_{2.5}$) and meteorological variables (air temperature, relative humidity, air pressure) were obtained through the city-specific air monitoring networks and the meteorological services. If data was recorded locally at smaller units, at least 50% of the data for 1 h needed to be present in order for the hourly value to be considered useable. For valid mean values over 8 or 24 h, at least 75% of the observations needed to be present. All pollutant data were calculated for individual average exposures, thus taking the time of blood withdrawal into account.

Genotyping

Genotyping of single-nucleotide polymorphisms (SNPs) and one deletion/insertion variant was performed in selected candidate genes involved in the regulation of inflammatory responses, which may potentially modify the susceptibility of individuals to environmental exposures. To get the maximal information of the genes, we have analyzed several DNA variants per gene. We selected all haplotype tagging SNPs known at the time, possibly functional SNPs as well as DNA variants showing an association in other studies with CVD or related phenotypes.

Genotyping analyses were carried out by using the MassARRAY system (Sequenom, San Diego, CA) according to Weidinger et al. (2005). Briefly, genomic DNAs were amplified by polymerase chain reaction (PCR) using HotStarTaq DNA polymerase (Qiagen, Hilden, Germany). Genotyping assays were carried out using 5 ng genomic DNA. PCR primers were used at 167 nM final concentrations for a PCR volume of 6 μl. The PCR conditions were a hot start at 95°C for 15 min, followed by denaturing at 95°C for 30 s, annealing at 56°C for 30 s, extension at 72°C for 1 min for 45 cycles, and finally incubation at 72°C for 10 min. PCR products were first treated with shrimp alkaline phosphatase (SAP; Amersham, Freiburg, Germany) for 20 min at 37°C to remove excess dNTPs and afterward for 10 min at 85°C to inactivate SAP. ThermoSequenase (Amersham) was used for the base extension reactions. Extension primers were used at a final concentration of 5.4 μM in 10-μl reactions. The PCR conditions were a hot start at 95°C for 15 min, followed by denaturing at 95°C for 30 s, annealing at 56°C for 30 s, extension at 72°C for 1 min for 45 cycles, and finally incubation at 72°C for 10 min. All reactions (PCR amplification, base extension) were carried out in a Tetrad PCR thermal cycler (MJ Research). The final base extension products were treated with SpectroCLEAN resin (Sequenom) to remove salts in the reaction buffer. This step was carried out with a Multimek 96 channel autopipette (Beckman Coulter), and 16 μl of resin/water suspension was added into each base extension reaction, making the total volume 26μl. After rapid centrifugation (2000 rpm, 3 min) in an Eppendorf centrifuge 5810, 10 nl of reaction solution was dispensed onto a 384 format SpectroCHIP (Sequenom) prespotted with a matrix of 3-hydroxypicolinic acid by using a SpectroPoint nanodispenser (Sequenom). A modified Bruker Biflex matrix-assisted laser desorption ionization–time-of-flight mass spectrometer (Sequenom) was used for data acquisitions from the SpectroCHIP. Genotyping calls were made in real time with MassArray RT software (Sequenom).

Quality Assurance Measures for the Field Study

A study manual was developed describing the methods of the study, including standardized operating procedures (SOPs) for specific parts of the field study. The SOPs were developed and approved by all centers. Questionnaires were translated into the different languages by the local partners. A 2-day training session in Augsburg instructed the investigators from the centers on the implementation of the SOPs in the field. Before the field phase, the personnel who conducted the examinations were trained locally on the basis of the study manual.

The progress of the field study, including patient recruitment, training of the study personnel, and the number of clinical visits, was continuously monitored. At each study center a site visit was conducted by a scientist of the coordinating partner at the beginning of the field phase. During this site visit, the study components were assessed based on a questionnaire, deviations were discussed with the local investigators, and procedures were altered if necessary. The process was documented in a quality assurance report available to all project partners.

RESULTS

Recruitment of Cohort

In total, 1003 MI survivors were recruited, who fulfilled the inclusion and none of the exclusion criteria and had at least two valid, repeated blood samples taken (Table 1). These were 84% of the targeted 1200 patients. Fifty-eight patients had to be excluded because they did not fulfil the inclusion and exclusion criteria (a–e). Based on the questionnaire data at the clinical visits, we excluded 255 of 6068 collected blood samples. Blood samples were excluded when patients had acute respiratory infections or reported surgical procedures in the 3 days before the clinic visit, since these could have severely altered the concentrations of the inflammatory markers. As a result, 69 patients who had less than 2 valid blood samples remaining were excluded. Overall, 5813 plasma samples for inflammatory marker determination were available, which represented 96.6% of the scheduled six blood samples within 1003 patients. The average number of repeated visits per study subject ranged from 4.5 in

TABLE 1
Study description

City	Study period	Patients recruited for baseline examinations	Patients fulfilling the inclusion and exclusion (a–e) criteria	Patients with ≥2 usable blood samples (exclusion criterion f)	Excluded blood samples	Usable blood samples	Percent of scheduled[a] blood samples	Blood samples: mean per patient
Helsinki	10.09.03–02.06.04	212	202	195	7	1155	99%	5.9
Stockholm	03.09.03–24.06.04	207	201	197	4	1168	99%	5.9
Augsburg	19.05.03–24.02.04	213	207	200	7	1144	95%	5.7
Rome	25.09.03–15.07.04	163	149	134	115[c]	741	92%	5.5
Barcelona	03.09.03–16.06.04	183	180	169	11	1119	110%	6.6
Athens	13.09.03–30.07.04[b]	151	132	108	111[c]	486	75%	4.5
Total	19.05.03–30.07.04	1129	1071	1003	255	5813	97%	5.8

[a]Six visits were scheduled for all patients included (column 5); however, some patients in Barcelona, Rome, and Stockholm had 7 or 8 visits.
[b]Two additional visits on 11/03/05 and 17/03/05.
[c]Blood withdrawals were conducted in Rome and Athens even though patients had recorded infections to ensure compliance.

Athens to 6.6 in Barcelona. The study period was 9 to 11 mo in all study centers starting earliest on May 19, 2003, in Augsburg and ending latest on July 30, 2004, in Athens, with 2 additional patient visits in spring 2005.

Baseline Characteristics of the Cohort

Table 2 presents the age and sex distribution of the study participants. More women participated in the Nordic centers Stockholm and Helsinki compared to the other centers. Partic-

TABLE 2
Baseline characteristics of 1003 myocardial infarction survivors from 6 European cities: Disease history

Parameter	Helsinki $N = 195$	Stockholm $N = 197$	Augsburg $N = 200$	Rome $N = 134$	Barcelona $N = 169$	Athens $N = 108$	p Value
Sex = male (%)	68.7	70.6	82.0	86.6	83.4	87.0	<.0001[1]
Age (yrs)[a]	64.6 (45–78)	64.0 (38–76)	61.9 (39–76)	62.7 (39–79)	62.1 (37–81)	54.7 (38–75)	<.0001[4]
Myocardial infarction							
First MI (%)	81.5	85.8	87.5	87.3	86.4	80.6	.37[1]
Last MI to study (yrs)[a]	2.7 (0.6–5.8)	2.3 (0.6–3.9)	2.1 (0.5–3.4)	2.7 (0.4–6.0)	2.1 (0.4–5.9)	2.4 (0.5–5.5)	.0015[2]
MI in family history (%)							overall <.0001[1]
Yes (mother and/or father)	47.7	44.7	30.5	29.1	20.7	34.3	<.0001[1]
No	41.0	44.2	58.0	64.2	70.4	54.6	<.0001[1]
Information incomplete	11.3	11.2	11.5	6.7	8.9	11.1	.7010[1]
Self-reported history (%)[b]							
Angina pectoris	39.0	47.7	21.0	27.6	29.6	41.7	<.0001[1]
Arrhythmia	31.3	20.8	24.0	23.1	13.0	21.3	.0029[1]
Congestive heart failure	14.9	16.2	13.0	6.0	1.8	5.6	<.0001[1]
Hypertension	51.3	49.7	51.0	55.2	46.2	54.6	.73[1]
Diabetes	21.0	18.3	17.5	17.2	23.7	21.3	.63[1]
Any respiratory disease	7.2	6.6	10.5	22.4	13.6	6.5	<.0001[1]
Hay fever	10.3	14.2	10.0	11.9	4.1	0	<.0001[3]
Chronic renal disease	3.6	2.0	5.0	5.2	9.5	1.9	.019[3]
Arthrosis	18.5	21.8	17.5	34.3	30.2	1.9	<.0001[3]

Note. Numbers in p value column represent: [1]chi-square test, [2]median test, [3]Fisher's exact test, and [4]ANOVA.
[a]Mean (range).
[b]Ever doctor diagnosed.

ipants had a similar age range; however, a higher proportion of young men were recruited in Athens. The proportion of patients with first MI was similar across centers, but the mean time since the last MI was shortest in Augsburg and Barcelona and longest in Rome and Helsinki. Family history of MI was more frequent in the Nordic countries. A history of angina pectoris was more frequent in Athens and Stockholm. Diabetic patients were distributed equally among study centers. A higher proportion of patients in Rome reported a history of respiratory disease, but frequency of symptoms at baseline and the use of medication to treat respiratory diseases were not more frequent in Rome. Therefore, the self-reported history may reflect the emphasis on respiratory diseases at Columbus hospital, where the patients had their baseline visit.

Regarding BMI, 70 to 80% of all participants were overweight or obese (Table 3). The proportion of obese subjects ranged between 22% and 37% in the different cities. The best scores with respect to total cholesterol and HDL to total cholesterol ratio were observed in Barcelona and Stockholm, while the lipid profiles were most disadvantageous in Athens. In addition, Stockholm showed the lowest prevalence of HbA1c equal or greater than 6.5%.

Only in Helsinki, Stockholm, and Augsburg was it possible to avoid recruiting current regular smokers and still reach the recruitment goals in a reasonable time frame (Table 4). Also, a history of smoking was more prevalent in the southern European centers among current nonsmokers, particularly Athens and Barcelona. Self-assessed health status ranged between "good" and "average," but was best in Athens. Patients from Athens and Barcelona reported the highest rates of inactivity, and the highest proportion of physically active subjects came from Augsburg. Low education was substantially rarer in Stockholm than in other centers. However, one has to note that the education was categorized separately by each center, so that differences might also be attributable to the differences in definitions. Most of the participants were already retired at the time of the study, with the highest proportion of working subjects in Athens. The lowest alcohol consumption was seen among participants in Athens, while the highest number of heavy drinkers was observed in Augsburg. The majority of the patients were treated with beta-blockers, ACE inhibitors, and lipid-lowering drugs, as well as antithrombotic therapy with aspirin to prevent recurrent myocardial infarctions (Table 5). Treatment was less vigorous in Athens.

Repeated Measurements of Inflammatory Markers

The highest CRP levels were observed in Barcelona, while the lowest were observed in Helsinki (Table 6). While IL-6 and fibrinogen were also high in Barcelona, the other cities were more comparable for these markers. Nevertheless, substantial between-subject and within-subject variability was observed for all three blood markers in all cities (Figure 3). CRP and IL-6 displayed more skewed distributions than fibrinogen. The proportion of subjects whose CRP levels were always above 3 mg/L varied between cities (6% in Athens, 14% Barcelona, 10% Rome, 7% Augsburg, 7% Helsinki, and 11% Stockholm). Similarly, the proportion of subjects whose CRP concentrations varied between below and above 3 mg/L differed between centers (40% in Athens, 52% Barcelona, 38% Rome, 39% Augsburg, 32% Helsinki, and 38% Stockholm).

To check the reliability of the laboratory tests, blind duplicates were sampled. During the follow-up examinations, an additional EDTA- or citrate-monovette was scheduled to be filled from the same butterfly or needle from every 30th patient. These samples were assigned a special identification number but otherwise were to be treated like all other samples. Results for the original and the duplicate blood sample were compared in up to 35 samples. Overall, the coefficient of variation (CV) between duplicate samples was smallest for the CRP measurements and largest for IL-6 (Table 6). The lowest duplicate sample CV was seen in Augsburg and the highest in Rome.

Genotyping

Based on literature research altogether 13 genes with 111 different SNPs were selected. The candidate genes were C-reactive protein (*CRP*), interleukin 6 (*IL6*), fibrinogen alpha, beta, and gamma (*FGA*, *FGB,* and *FGG*), interleukin 10 (*IL10*), interleukin 18 (*IL18*), toll-like receptor 4 (*TLR4*), tumor necrosis factor alpha (*TNFa*), lymphotoxin alpha (*LTA*), and the nuclear factor kappa-B family (*NFkB1*, *RELA,* and *NFkB1A*). For *NFkB1* and *IL-10* some of the SNPs were not in the Hardy–Weinberg equilibrium (HWE); therefore, some more SNPs were analyzed that were in linkage disequilibrium (LD) with the originally problematic SNPs. For *NFkB1* also a deletion (NFkBdel) was analyzed. For *CRP* a tri-allelic SNP was included as well.

Altogether 134 SNPs were genotyped. For 11 SNPs no assay could be established or the assay did not provide valid results (rs2227439, rs2066864, rs1554286, rs1800896, rs6703630, rs360723, rs980455, rs1609993, rs4648050, rs2233411, rs10782383). Three SNPs turned out to be monomorphic in the investigated study population (rs2069830, rs6051, rs2066870) and six had an allele frequency of less than 1% (rs2069860, rs2070034, rs2070033, rs3093544, rs5744263, rs5030710). All together, 114 SNPs were taken into the statistical analyses. The average success rate was 99.1%.

For quality control, a sex determination was performed for all samples by amplification of a partial sequence of the amelogenin gene (*AMELX*). In addition, sex determination was performed with validated genotyping assays as a second independent method. Samples showing inconsistencies were excluded from further analysis.

Negative controls were included in all assays. To control for reproducibility of genotyping data 30% of randomly selected samples were genotyped in duplicate. The discrepancy rate was 0.18%. Each SNP was tested for departures from HWE by means of a chi-square test or Fisher's exact test depending on allele frequency. Seventeen SNPs showed departures from HWE (rs2070011, rs10494879,

TABLE 3
Baseline characteristics of 1003 myocardial infarction survivors from 6 European cities: Measured CHD risk factors

Parameter	Helsinki $N = 195$	Stockholm $N = 197$	Augsburg $N = 200$	Rome $N = 134$	Barcelona $N = 169$	Athens $N = 108$	p Value
Body mass index[a]	28.6 (19.1–48.9)	27.6 (17.5–43.2)	28.7 (19.1–48.4)	27.7 (19.0–39.4)	28.8 (19.3–43.5)	28.8 (20.8–46.3)	.0039[4]
Body mass index class (%)[b]							Overall .0833[3]
Underweight	1.1	2.0	1.5	1.5	1.2	0.1	0.72[3]
Normal weight	19.5	25.4	14.5	20.9	16.6	15.7	0.10[1]
Overweight	45.6	50.3	48.5	51.5	45.6	47.2	0.89[1]
Obese	32.8	22.3	35.5	26.1	36.7	37.0	.010[1]
Systolic blood pressure (mm Hg)[a]	139.9 (93–209)	137.6 (97–196)	128.4 (84–198)	134.7 (95–188)	129.5 (81–196)	136.1 (100–190)	<.0001[4]
Diastolic blood pressure (mm Hg)[a]	79.5 (52–112)	80.4 (53–112)	78.1 (47–112)	77.6 (54–114)	77.4 (45–126)	82.3 (6–122)	.0010[4]
WHO/ISI blood pressure categories (%)[c]							Overall <.0001[3]
Optimal/normal/high normal	49.7	54.3	73.5	56.7	69.2	53.7	<.0001[1]
Mild/moderate hypertensive	0.0	42.6	23.0	41.0	27.8	39.8	<.0001[1]
Severe hypertensive	8.7	3.0	3.0	2.2	2.9	3.7	.0431[3]
Blood biomarkers determined at local laboratories							
Total cholesterol (mg/dl)[a]	182.2 (91.1–291.9)	173.4 (96.7–324.7)	181.0 (107.0–316.0)	190.6 (12.0–321.0)	193.2 (119.0–390.0)	195.4 (92.0–293.0)	<.0001[4]
HDL cholesterol (mg/dl)[a]	54.0 (22.0–119.3)	53.7 (30.9–116.0)	47.9 (24.0–98.0)	43.7 (25.0–87.0)	52.7 (28.0–105.0)	46.1 (24.0–87.0)	<.0001[4]
High ratio total cholesterol/HDL cholesterol (%)[d]	11.8	6.6	16.0	28.4	7.7	34.3	<.0001[1]
HbA1c (%)[a]	5.9 (4.7–9.2)	5.0 (3.8–9.9)	5.6 (4.7–9.8)	5.4 (2.8–8.7)	5.1 (3.8–9.8)	5.8 (3.7–10.5)	<.0001[4]
HbA1c $\geq$ 6.5% (%)[e]	15.9	6.6	9.5	8.2	10.7	14.8	.010[1]

Note. Numbers in p value column indicate: [1]chi-square test, [2]median test, [3]Fisher's exact test, and [4]ANOVA.

[a]Mean and range in parentheses.

[b]Guidelines of German Society for Nutrition (DGE).

[c]1999 WHO/ISH Guidelines for the management of hypertension: "optimal/normal/high normal" systolic blood pressure (SBP) <140 mm Hg or diastolic blood pressure (DBP) <90 mm Hg; "mild/moderate hypertensive" 140 mm Hg $\leq$ SBP <180 mm Hg or 90 mm Hg $\leq$ DBP < 110 mm Hg; "severe hypertensive" 180 mm Hg $\leq$ SBP or 110 mm Hg $\leq$ DBP. The higher category applies when a patient's systolic pressure and diastolic blood pressure fall into different categories.

[d]"High" $\geq$5 mg/dl.

[e]Indication for diabetes: HbA1c $\geq$ 6.5%.

rs3024496, rs6676671, rs3024491, rs1800890, rs2069827, rs230521, rs3774956, rs3774964, rs1801, rs1020759, rs230498, rs11722146, rs3091257, rs1799724, rs28362491). They were kept in the data set because this study is investigating a patient population.

Air Pollution

Concentration of ambient air pollutants and weather parameters are presented in Table 7. Concentrations of gaseous as well as particulate air pollutants were higher in the southern European countries than in the Nordic countries.

TABLE 4
Baseline characteristics of 1003 myocardial infarction survivors from 6 European cities: CHD risk factors from questionnaire

Parameter	Helsinki $N = 195$	Stockholm $N = 197$	Augsburg $N = 200$	Rome $N = 134$	Barcelona $N = 169$	Athens $N = 108$	p Value
Smoking status (%)							<.0001[3]
Never smoker	39.5	29.9	31.0	25.4	14.2	10.2	<.0001[1]
Ex or occasional smoker	59.0	69.5	69.0	65.7	72.8	51.9	.0019[1]
Current smoker	1.5	0.5	0	9.0	13.0	38.0	<.0001[3]
Packyears (cigarettes only)[a]	9.1 (0–65.0)	12.2 (0–73.8)	15.1 (0–205.2)	21.8 (0–171.8)	28.1 (0–192.3)	35.6 (0–174.0)	<.0001[2]
Physical activity (%)[b]							Overall <.0001[1]
Inactive	18.5	7.6	4.0	28.4	42.0	47.2	<.0001[1]
Partly active	19.0	17.3	13.0	14.2	5.9	10.2	.0052[1]
Unregularly active	17.9	22.8	10.5	14.2	8.3	8.3	.0002[1]
Regularly active	33.3	40.1	42.5	38.8	33.7	30.6	0.19[1]
Trained	11.3	12.2	30.0	4.5	10.1	3.7	<.0001[1]
Low education (%)[c]	26.2	10.7	24.0	23.1	35.5	28.7	<.0001[1]
Employment (%)							Overall .0005[1]
Full-time work	24.1	25.9	28.5	34.3	30.2	45.4	.0027[1]
Part-time work	6.2	14.7	6.5	7.5	7.7	4.6	.011[1]
No work	69.2	59.4	65.0	58.2	62.1	50.0	.020[1]
Alcohol intake (%)[d]							Overall <.0001[1]
None	14.4	7.1	14.5	19.4	17.2	36.1	<.0001[1]
Moderate	76.9	80.7	65.0	67.9	69.2	53.7	<.0001[1]
Heavy	8.7	12.2	19.5	12.7	13.6	10.2	.037[1]
Health status (%)[e]							
Bad or very bad health	5.1	5.0	9.0	7.4	8.2	5.5	<.0001[3]

Note. Numbers in p value column indicate: [1]chi-square test, [2]median test, [3]Fisher's exact test.

[a]Mean and range in parentheses.

[b]Swiss Health Survey 2002.

[c]Patients were divided into pre- and postwar education according to their age and then center-specific cut points of education years (sum of school years/professional training/college and university) were applied.

[d]"None" 0 g/day, "moderate" <20 g/day for women and <40 g/day for men, "heavy" ≥20 g/day for women and ≥40 g/day for men.

[e]Scale 1 ("excellent") to 5 ("very bad").

Nevertheless, sulfur dioxide concentrations were low in all settings.

DISCUSSION

A group of MI survivors was recruited in six European cities and characterized at baseline. Although recruitment was a major challenge to all centers, 94% of the proposed 1200 MI survivors were recruited and 84% could be included for analyses on the repeated blood markers. Within patients included in the study, follow-up was excellent as 97% of the scheduled blood samples could be collected.

At the outset, the study attempted to recruit nonsmoking MI survivors to control for additional exposures to particles by active smoking and sidestream smoke. However, it only partly succeeded in restricting recruitment to this group. This may reflect the prevalence of smoking in MI survivors in southern Europe. Based on these characteristics and the highly demanding protocol, the study participants cannot be considered a random sample of the population of MI survivors in the six cities.

The majority of patients were treated with lipid-lowering agents, which stabilize atherosclerotic lesions and reduce subsequent risk in MI survivors (Koenig, 2005). Statin use has been implicated to reduce plasma CRP concentrations (Kathiresan et al., 2006). In addition, patients were also taking systemic anti-inflammatory medication for the treatment of other diseases such as for example respiratory disease. A considerable proportion of subjects still had high blood pressure in a standardized setting, indicating that insufficient treatment of hypertension is still prevalent even in this group of high-risk patients (Antikainen et al., 2006).

TABLE 5
Baseline characteristics of 1003 myocardial infarction survivors from 6 European cities: Treatment

Parameter	Helsinki $N = 195$	Stockholm $N = 197$	Augsburg $N = 200$	Rome $N = 134$	Barcelona $N = 169$	Athens $N = 108$	p Value
Beta blocker (%)	95	91	92	75	75	64	<.001[1]
ACE inhibitors (%)	50	51	72	81	59	52	<.001[1]
Calcium channel blockers (%)	14	22	15	26	21	29	.0055[1]
Diuretics (%)	23	25	44	33	23	10	<.001[1]
Lipid-lowering medication (%)	85	89	90	83	86	74	.0039[1]
Antithrombotic medication (%)	97	97	99	95	98	93	.058[2]
Other systemic anti-inflammatory medication (%)	29.2	7.1	22.0	12.7	13.6	2.8	<.0001[1]
Hormone replacement therapy (women) (%)	8.2	4.6	1.5	0.0	0.0	0.0	<.0001[2]

Note. Numbers in p value column indicate: p value from [1]chi-squared test; [2] p value from Fisher's exact test.

Interindividual variation with respect to the inflammatory markers was quite high in all six study centers. Also, a substantial proportion of the subjects had CRP concentrations above 3 mg/L, which is considered to be associated with a high risk for ACS (Koenig et al., 2004). Intraindividual variability for CRP and IL-6 was high within repeated visits, even in a city that indicated very low variability in blind duplicated samples, such as Augsburg. Fibrinogen

TABLE 6
Repeated measurements of inflammatory markers based on 5813 blood samples collected from 1003 myocardial infarction survivors from 6 European cities

	Measured samples						Quality control	
City	Blood samples	Number of patients	Mean	Median	Mean CV[a] (%)	Median CV[a] (%)	Number of duplicate samples	Mean CV in duplicate samples
CRP (mg/L)								
Helsinki	1155	195	1.98	1.37	53.6	35.2	35	1.7
Stockholm	1168	197	2.86	1.59	52.0	42.6	10	4.1
Augsburg	1144	200	2.26	1.40	56.8	31.3	14	2.1
Rome	741	134	2.56	1.62	52.3	36.1	27	7.3
Barcelona	1119	169	3.52	2.17	59.3	35.9	16	1.5
Athens	486	108	2.52	1.49	55.9	36.7	0	—
Fibrinogen (g/L)								
Helsinki	1155	195	3.76	3.69	9.5	8.3	35	6.0
Stockholm	1168	197	3.53	3.40	11.1	10.0	10	3.4
Augsburg	1144	200	3.34	3.27	9.0	7.4	14	2.5
Rome	741	134	3.24	3.12	14.5	14.0	27	11.2
Barcelona	1119	169	3.99	4.00	11.9	11.6	16	3.9
Athens	—	—	—	—	—	—	—	—
IL-6 (pg/mL)								
Helsinki	1155	195	3.16	2.32	41.7	28.4	35	13.6
Stockholm	1168	197	2.67	2.07	37.5	38.2	10	23.4
Augsburg	1144	200	2.60	2.17	43.6	29.1	14	3.2
Rome	741	134	3.18	2.33	39.6	30.8	27	21.9
Barcelona	1119	169	3.58	3.01	48.4	29.0	16	6.8
Athens	486	108	3.19	2.53	49.4	30.2	0	—

[a]Coefficients of variation (CV) were calculated for the repeated measurements of the individuals.

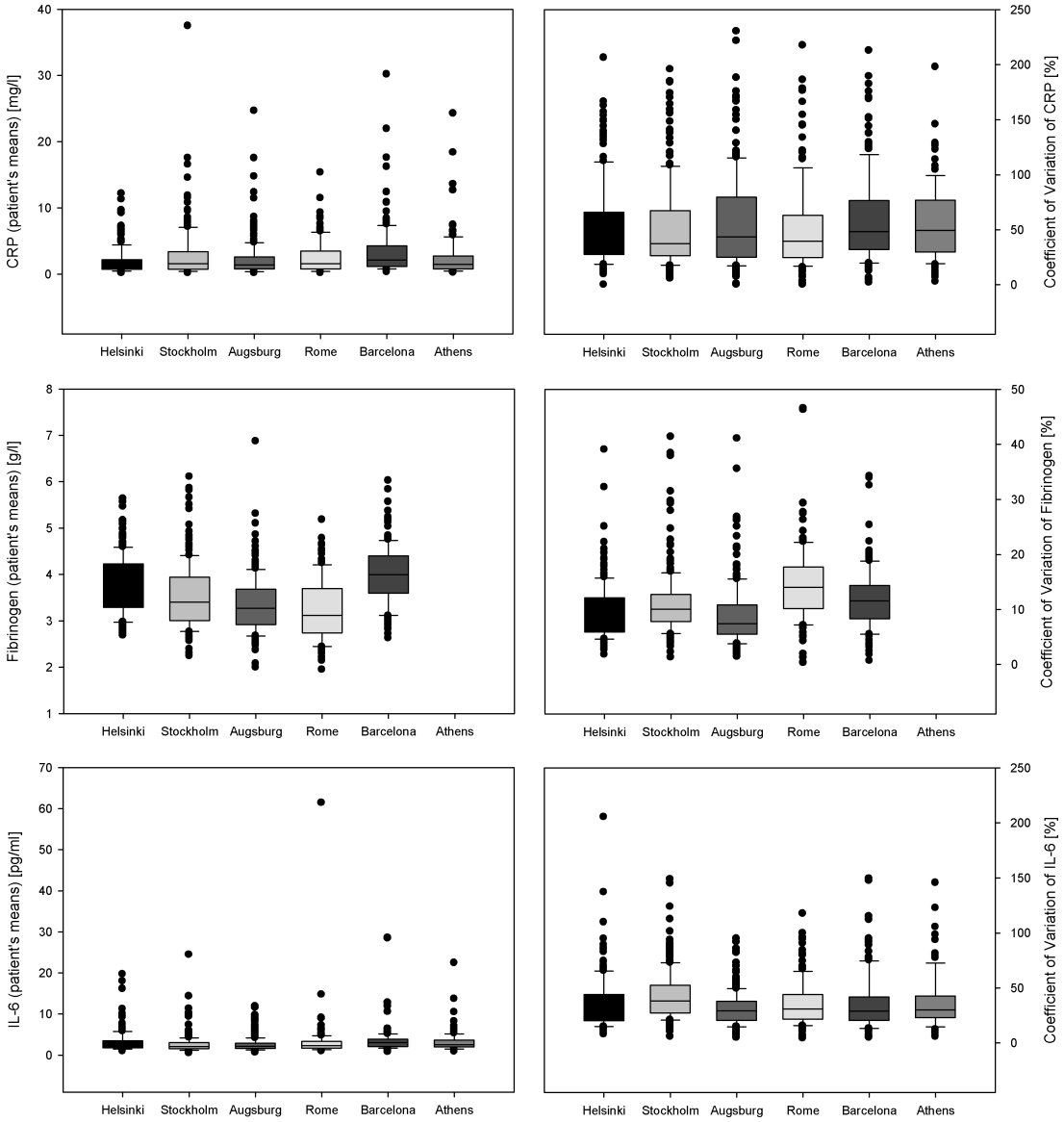

FIG. 3. Distribution of mean CRP, fibrinogen and IL-6 concentrations and person-specific coefficients of variation by city.

concentrations showed less interindividual as well as intra-individual variability.

Strengths and Limitations

The study is based on a common protocol and SOPs applied in six different European cities. Site visits were conducted to ensure uniform procedures in all centers. The analyses of the inflammatory markers were done in one central laboratory and blinded duplicate samples monitored the variability of procedures within centers.

The study was designed to assess the impact of ambient air pollution in a large cohort of MI survivors and gene–environment interactions. It applies a repeated measurement design for assessing the impact of environmental time-varying factors on inflammatory markers in a potentially susceptible subgroup. The inflammatory markers assessed have a half-life of 3 days or less. Therefore, the repeated measurements taken once every 4 wk can be regarded as being uncorrelated over time, but more similar within individuals than between individuals.

Air pollution in all selected cities originates mostly from motor vehicle traffic; the local weather conditions, population density and mobility, and pollution control strategies also contribute to the variability in the concentration of the pollutants and their seasonal patterns. In some cities, long-range transport plays an additional role in determining short-term variation in air pollution concentrations (Vallius et al., 2005). The study was conducted mostly in wintertime, when day-to-day variation is

TABLE 7
24-Hour average concentrations of the ambient air pollution and weather parameters during the AIRGENE study period from 6 European cities

	Helsinki, 05.09.03–02.06.04		Stockholm, 29.08.03–24.06.04		Augsburg, 14.05.03–24.02.04		Rome, 20.09.03–15.07.04		Barcelona, 29.08.03–16.06.04		Athens, 08.09.03–30.07.04	
Study period[a]	Mean	95th	Mean	95th	Mean	95th	Mean	95th	Mean	95th	Mean	95th
Particle number concentrations (PNC) ($1/cm^3$)	8534	15,077	9748	17,578	11,876[b]	25,135	35,450[b]	69,226	18,133[b]	36,526	20,589[b]	47,573
$PM_{2.5}$($\mu g/m^3$)	8.2	19.4	8.8	19.1	17.4	29.3	24.5[b]	54.1	24.2[b]	62.7	23.0[b]	46.0
PM_{10} ($\mu g/m^3$)	17.1	36.1	17.8	40.3	33.1	56.6	42.1	76.0	40.7[b]	88.7	38.5	64.6
CO (mg/m^3)	0.31	0.46	0.29	0.43	0.58	1.00	1.40	2.47	0.59	0.92	1.48	3.23
NO_2 ($\mu g/m^3$)	28.6	49.8	18.6	32.6	40.0	61.2	67.0	90.8	50.5	79.6	50.1	73.0
NO ($\mu g/m^3$)	12.5	40.7	4.9	15.5	30.0	80.4	65.7	164.0	37.7	88.4	41.8	144.6
SO_2($\mu g/m^3$)	4.2	10.1	1.9	4.9	3.0	5.7	4.1	9.2	4.7	9.6	10.3	23.2
O_3 (8-h average) ($\mu g/m^3$)	46.8	89.0	60.6	96.9	54.4	115.3	45.3	99.6	28.2	76.5	59.8	100.2
Air temperature (°C)	3.1	14.7	4.7	15.1	10.2	25.1	13.4	23.9	15.2	23.2	17.6	29.3
Relative humidity (%)	76	91	82	94	69	92	80	95	67	86	67	84

[a]The study period started 5 days before the first measurement because a priori air pollution concentrations up to 5 days before the blood withdrawals were considered.

[b]Data on less than 95% of the days available.

highest in European cities for all primary combustion-related pollutants.

Adverse health effects of ambient air pollution on the general population of the six cities have been documented; daily concentrations of particulate matter and gases have been linked to mortality (Katsouyanni et al., 1997, 2001; Touloumi et al., 1994; Michelozzi et al., 1998) and to hospital admissions for respiratory diseases (Anderson et al., 1997; Spix et al., 1998; Sunyer et al., 1997; Ponka & Virtanen, 1994). Associations between daily levels of NO_2, CO, SO_2, PM_{10}, and particle number concentrations and hospital admissions for CHD, arrhythmias, and congestive heart failure (CHF) have been found in Augsburg, Barcelona, Helsinki, Rome, and Stockholm, separately and combined (Michelozzi et al., 1999; D'Ippoliti et al., 2003; Forastiere et al., 2005; von Klot et al., 2005; Sahu et al., 2006; Sunyer et al., 2003). Data collected as part of the WHO-MONICA project in Augsburg have previously been used to study the association between air pollution and markers of inflammation which are predictors of cardiovascular disease (Peters et al., 1997, 2000, 2001). In Helsinki, short-time variations in the concentration of ultrafine particles have been shown to be associated with declines in lung function among asthmatic adults (Penttinen et al., 2001) and increases in the risk of ischemia during moderate exercise in patients with CHD within the ULTRA project (Pekkanen et al., 2002). By examining the effects of air pollutant concentrations on inflammatory markers, this study explores possible pathways that may help explain the aforementioned observed effects on health outcomes (Brook et al., 2004; Schulz et al., 2005; Seaton et al., 1995).

So far, only small studies were conducted to assess the association between ambient air pollution and inflammatory markers (Ruckerl et al., 2006; Seaton et al., 1999; Riediker et al., 2004). For example, Rückerl and colleagues studied 57 patients with coronary artery disease collecting a total of 579 blood samples and showed associations between PM_{10} as well ultrafine particles and inflammatory markers (Ruckerl et al., 2006). In Helsinki, Stockholm, Augsburg, and Barcelona, each substudy increased the number of subjects at least threefold and doubled the number of blood samples, while Rome and Athens might have not substantially increased power compared to earlier studies (Ruckerl et al., 2006; Seaton et al., 1999; Riediker et al., 2004). This large study has been designed to address the variability of responses to elevated air pollution concentrations across Europe and to detect potential susceptible subgroups based on their genetic susceptibility for an aggravated inflammatory response to external stimuli. In an ad hoc power calculation submitted with the proposal, it was estimated that the study had a power of 0.80 to detect an association between air pollution and inflammatory markers as previously reported with a p value of .05 in subgroups based on SNPs with a frequency of 10% to 15%.

Selection of the study group is a critical point when setting up a panel study. We chose MI survivors to study a susceptible subgroup of the population (von Klot et al., 2005). However, current treatment specifically with lipid-lowering medication may counteract the hypothesized air pollution effects. This fact may weaken our study, but at the same time it would be an important fact that may have implications for risk assessments. Also,

we aimed at including nonsmokers to minimize confounding by acute smoking. However, that proved to be impossible in southern Europe, where MI survivors do not quit smoking as frequently as in northern Europe. Therefore, the analyses will need to address the long-term as well as short-term impact of active smoking. In addition, visits of patients were excluded due to acute diseases that impact inflammation during the course of the study, such as acute respiratory infections as well as an acute hepatitis.

The study design makes possible the assessment not only of short-term adverse health effects of air pollution and the potential role of genetic polymorphisms in modifying these effects, but also of the roles of cardiovascular disease risk factors, including genetic polymorphisms in candidate genes, in determining inter- and intraindividual variability in inflammatory marker concentrations. However, cross-sectional between-city comparisons will be problematic as the participants are not a random sample of the cities' myocardial infarction survivors. The differences between the cities as documented in the baseline description in this article reflect a combination of differences in risk factor profiles between northern and southern Europe and of differences based on self-selection and different sampling strategies. In the future, a follow-up of this patient cohort could permit an evaluation of the role of variation in inflammatory marker concentrations as a risk factor in MI survivors. Previously published studies have only measured inflammatory marker concentrations once or twice and have assessed their role in predicting future recurrent CHD events. However, the data collected as part of this study will be useful in the assessment of repeated measurements as predictors of future events. In addition, the study might be able to assess the long-term risk associated with ambient air pollution if further data on the continuing exposure at the place of residence is collected.

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APPENDIX: THE AIRGENE STUDY GROUP

The AIRGENE study group comprises the following partners:

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Fredrik Nyberg, employed by AstraZeneca, is also Lecturer in Epidemiology at Karolinska Institute. AstraZeneca did not contribute any direct financing to this study.

Appendix II

Regina Rückerl, Annette Peters, Natalie Khuseyinova, Mariarita Andreani, Wolfgang Koenig, Christa Meisinger, Konstantina Dimakopoulou, Jordi Sunyer, Timo Lanki, Fredrik Nyberg, Alexandra Schneider:
Determinants of the acute phase protein CRP in MI survivors: The role of co-morbidities and environmental factors.

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

Regina Rückerl,[1] Annette Peters,[1,2] Natalie Khuseyinova,[3] Mariarita Andreani,[4] Wolfgang Koenig,[3*] Christa Meisinger,[1,5] Konstantina Dimakopoulou,[6] Jordi Sunyer,[7,8,9,10] Timo Lanki,[11] Fredrik Nyberg,[12,13] and Alexandra Schneider[1]

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-

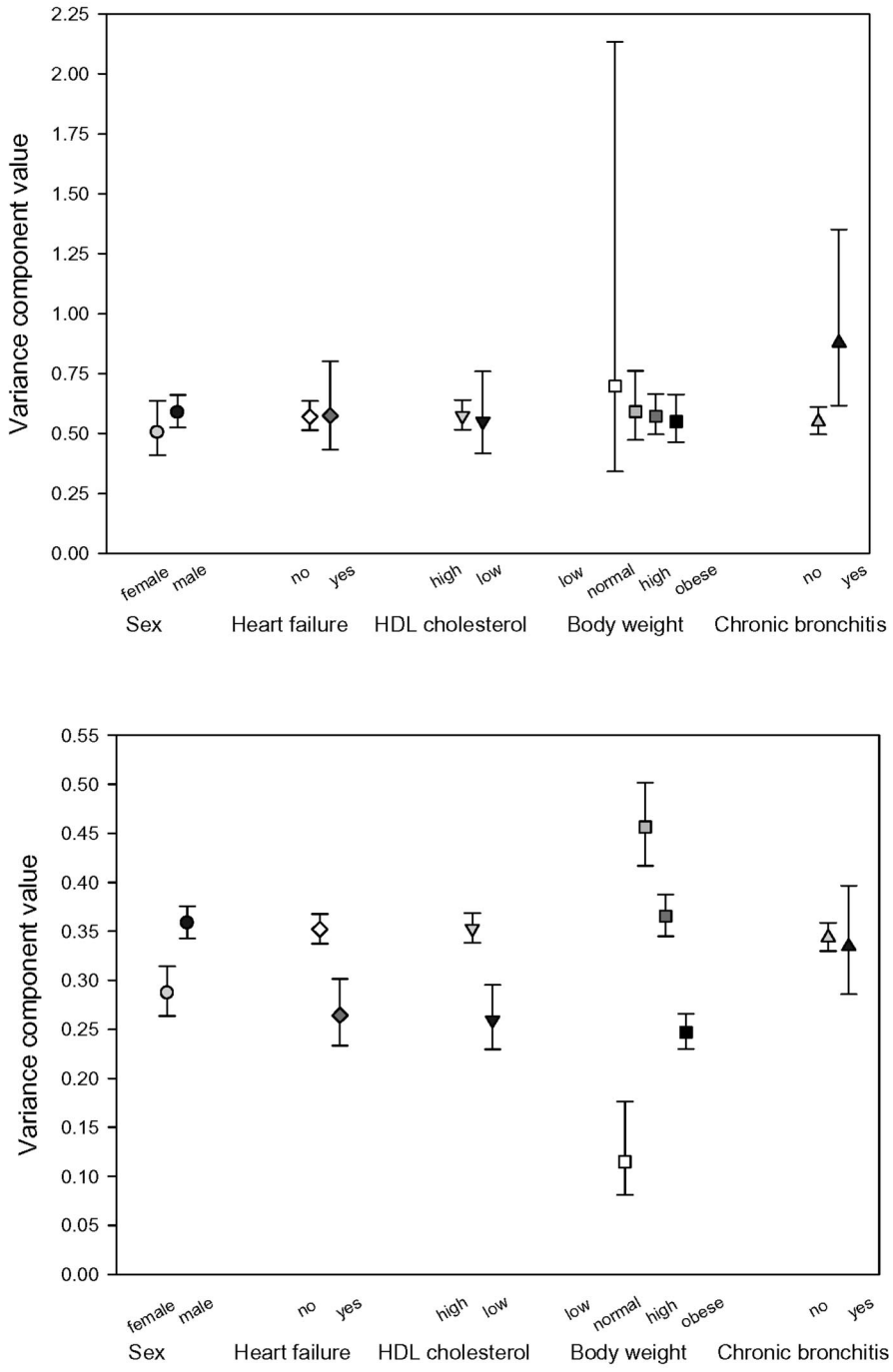

Fig. 1. Variation in mean hsCRP concentration between (upper panel) and within (lower panel) individuals for separate hsCRP measurements made over time.

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.

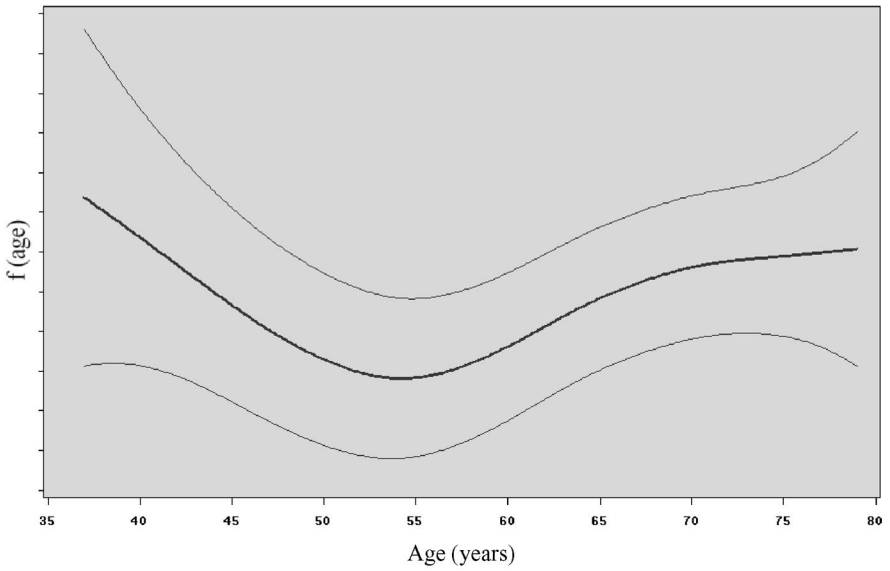

Fig. 2. Smooth spline of log hsCRP concentration [f(age)] on patient age in 1003 MI patients from the AIRGENE study, after adjustment for all of the variables in the base model.

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

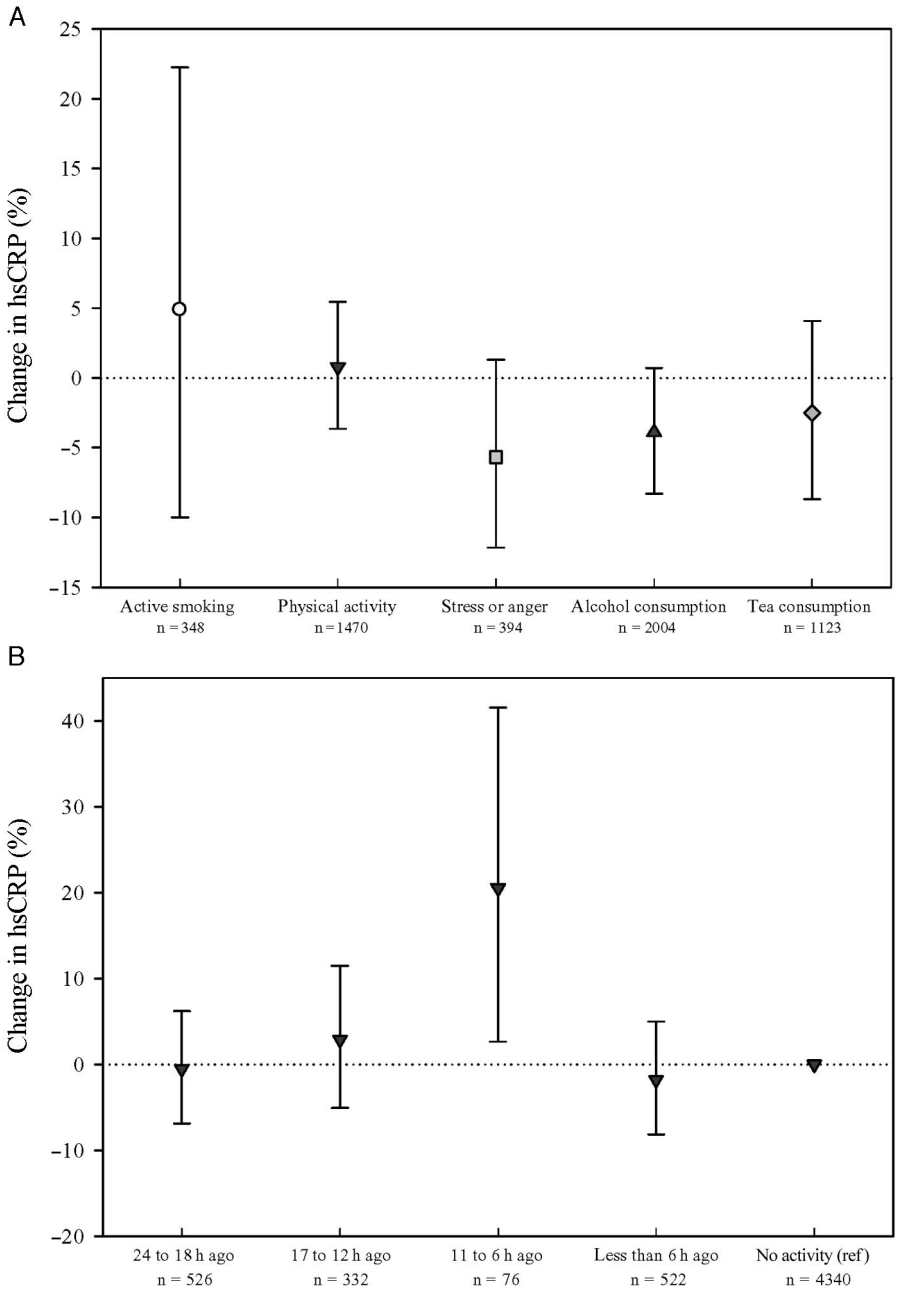

Fig. 3. Association between log hsCRP concentration and various variables (5813 visits in 1003 AIRGENE patients).
(A), Associations between log hsCRP concentration and lifestyle factors 24 h before blood draw. (B), Associations between log hsCRP concentration and physical activity in 6-h intervals before 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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Appendix III

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Air Pollution and Inflammation (Interleukin-6, C-Reactive Protein, Fibrinogen) in Myocardial Infarction Survivors

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Air Pollution and Inflammation (Interleukin-6, C-Reactive Protein, Fibrinogen) in Myocardial Infarction Survivors

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Background: Numerous studies have found that ambient air pollution has been associated with cardiovascular disease exacerbation.

Objectives: Given previous findings, we hypothesized that particulate air pollution might induce systemic inflammation in myocardial infarction (MI) survivors, contributing to an increased vulnerability to elevated concentrations of ambient particles.

Methods: A prospective longitudinal study of 1,003 MI survivors was performed in six European cities between May 2003 and July 2004. We compared repeated measurements of interleukin 6 (IL-6), fibrinogen, and C-reactive protein (CRP) with concurrent levels of air pollution. We collected hourly data on particle number concentrations (PNC), mass concentrations of particulate matter (PM) < 10 μm (PM_{10}) and < 2.5 μm ($PM_{2.5}$), gaseous pollutants, and meteorologic data at central monitoring sites in each city. City-specific confounder models were built for each blood marker separately, adjusting for meteorology and time-varying and time-invariant covariates. Data were analyzed with mixed-effects models.

Results: Pooled results show an increase in IL-6 when concentrations of PNC were elevated 12–17 hr before blood withdrawal [percent change of geometric mean, 2.7; 95% confidence interval (CI), 1.0–4.6]. Five day cumulative exposure to PM_{10} was associated with increased fibrinogen concentrations (percent change of arithmetic mean, 0.6; 95% CI, 0.1–1.1). Results remained stable for smokers, diabetics, and patients with heart failure. No consistent associations were found for CRP.

Conclusions: Results indicate an immediate response to PNC on the IL-6 level, possibly leading to the production of acute-phase proteins, as seen in increased fibrinogen levels. This might provide a link between air pollution and adverse cardiac events.

Key words: air pollution, C-reactive protein, CRP, epidemiology, fibrinogen, IL-6, inflammation, myocardial infarction, ultrafine particles. *Environ Health Perspect* 115:1072–1080 (2007). doi:10.1289/ehp.10021 available via *http://dx.doi.org/* [Online 18 April 2007]

Ambient air pollution has been associated with cardiovascular mortality (Forastiere et al. 2005; Peters et al. 2000; Schwartz and Dockery 1992) and hospital admissions for various cardiovascular diseases (Burnett et al. 1997; Schwartz 1999). Also, an elevated risk for acute myocardial infarction (MI) (Lanki et al. 2006; Peters et al. 2001a) and cardiorespiratory symptoms (de Hartog et al. 2003) has been reported in relation to air pollution. Some studies have suggested that patients with preexisting coronary heart disease (CHD) (Goldberger et al. 2001) might be a particularly susceptible population.

The exact mechanisms linking the inhalation of ambient air particles to an acute exacerbation of cardiovascular disease are not completely understood (Brook et al. 2004). Alveolar inflammation induced by particles may either directly or via oxidative stress lead to systemic inflammation with increased levels of blood coagulability, progression of atherosclerosis, and destabilization or even rupture of vulnerable plaques, resulting in acute ischemic events (Brook et al. 2004; Seaton et al. 1995).

So far, studies using repeated measures to assess the association between ambient air particles and inflammatory markers have had controversial results. In addition, they have been conducted only on a small scale, with samples sizes ranging from 9 to 112 (Riediker et al. 2004; Ruckerl et al. 2006; Seaton et al. 1999). In larger studies, however, associations have been based on single blood measurements (Zeka et al. 2006), and the examined populations have encompassed healthy and diseased subjects, covering a variety of diseases. All these differences might explain the conflicting results.

For interleukin 6 (IL-6), hypothesized to play a central role in the triggering of the inflammatory process (Woods et al. 2000), associations with high levels of particulate matter (PM) < 10 μm in aerodynamic diameter (PM_{10}) have been shown (van Eeden et al. 2001), although a study in elderly subjects in the United Kingdom (Seaton et al. 1999) did not reveal significant associations with ambient PM_{10}. The present study was designed to address the responses of IL-6, fibrinogen, and C-reactive protein (CRP) to elevated air pollution levels in a large cohort of MI survivors across Europe. We were particularly interested in MI survivors because they are especially prone to a progression of atherosclerosis and adverse cardiovascular events.

Materials and Methods

Study population. A prospective longitudinal study of post-MI patients was performed in six European cities—Athens (Greece), Augsburg [Germany, KORA (Cooperative Health Research in the Augsburg Region) (Lowel et al. 2005)], Barcelona (Spain), Helsinki (Finland), Rome (Italy), and Stockholm (Sweden)—chosen to include a

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variety of geographic conditions and air pollution characteristics [see Appendix 1 for participants; see Supplemental Material (http://www.ehponline.org/docs/2007/10021/suppl.pdf) for data]. The study design is described in detail elsewhere (Peters et al., in press). In brief, we 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 with MI or interventional procedures < 3 months before the beginning of the study or with chronic recurring inflammatory diseases such as Crohn's disease were not included.

Preferably, current nonsmokers were recruited. All partners approved the study protocol at their 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.

Clinical measurements. Patients were invited to participate in six to eight clinical visits between May 2003 and July 2004. The visits were scheduled every 4–6 weeks on the same weekday and at the same time of the day to minimize the impact of weekly and circadian variation. At the first visit, a baseline questionnaire was administered regarding health status, medication intake, and smoking history. Blood pressure and body mass index (BMI) were measured and a blood serum sample was drawn to assess baseline serum lipids, glycosylized hemoglobin (HbA1c; an indicator of diabetic status) and N-terminal proB-type natriuretic peptide (NT-proBNP; an indicator for left ventricular dysfunction).

At each clinical visit a 7-day recall on medication intake was obtained. Venous ethylenediamine tetraacetic acid (EDTA)–plasma samples were collected for the determination of the inflammatory markers. Samples were cooled and stored at 4°C until further processing within a maximum of 4 hr. The EDTA-blood was centrifuged at 4°C in a precooled centrifuge for 20 min at 2,500 × *g*. 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 by means of a commercial enzyme-linked immunosorbent assay (ELISA) for IL-6 (quantitative high sensitive IL-6 immunoassay; RD Systems GmbH, Wiesbaden, Germany) and immunonephelometry for fibrinogen and high-sensitivity CRP (Dade Behring Marburg GmbH, Marburg, Germany). Because CRP and fibrinogen concentrations were measured by a fully automated assay, only single measurements were available, except for results above and below the detection limit, which were double-checked. Within- and between-patient variability for a number of blood samples that were tested as quality assurance measures are described elsewhere (Peters et al., in press).

Air pollution and meteorologic data. Air pollution data from fixed monitoring sites representing urban background concentrations were collected for each city according to standard procedures already employed in several European studies of air pollution (Aalto et al. 2005; Katsouyanni et al. 1996). We obtained hourly means of particles [black smoke (BS), black carbon (BC), mass concentration of PM_{10}, and mass concentration of particles < 2.5 μm in diameter ($PM_{2.5}$)], gaseous air pollutants (carbon monoxide, sulfur dioxide, ozone, nitric oxide, nitrogen dioxide) and meteorologic variables (air temperature, relative humidity, barometric pressure, dew point temperature) through city-specific air monitoring networks and meteorologic services. If data were recorded locally at smaller units, at least 50% of the data for 1 hr needed to be present for the hourly value to be considered useable. For valid 8- or 24-hr mean values, at least 75% of the observations needed to be present. Particle number concentration (PNC) measurements as indicator for ultrafine particles were performed using condensation particle counters (CPC; 3022A; TSI, Shoreview, MN, USA) in all centers.

Missing data on the aggregate level were replaced using a formula adapted from the APHEA (Air Pollution and Health—A European Approach) method (Katsouyanni et al. 1996) [see Supplemental Material (http://www.ehponline.org/docs/2007/10021/suppl.pdf)]. We calculated apparent temperature by using the formula of Steadman (1984) and Kalkstein and Valimont (1986).

We used moving averages of ambient concentrations of air pollutants and meteorogic variables to characterize the exposures by calculating the individual 24-hr average exposure for each person immediately preceding the clinical visit (lag 0) up to 4 days (lag 1–lag 4). In addition, we calculated the mean of lags 0–4 for the air pollution data and the mean of lags 0 and 1, the mean of lags 2 and 3, the mean of lags 0–3, and the mean of lags 0–4 for the meteorologic variables, if at least half of the relevant lags were available.

Statistical analyses. **Analytical strategy.** Given previous findings, we hypothesized that particulate air pollution induces systemic inflammation. Specifically, we assumed that IL-6 would increase in association with increased levels of ambient particle concentrations of the preceding or same day, because immediate effects on IL-6 have been shown before (van Eeden et al. 2001), and the cytokine has a very short half life (2–6 hr) (Riches et al. 1992).

We also hypothesized that an acute-phase response involving *de novo* synthesis of proteins in the liver would require an induction time of 1–2 days. This would translate to an increase in fibrinogen concentrations with elevated particle concentrations of the previous 5 days [half-life 2–3 days (Thomas 1998)] and an increase in CRP in association with ambient particle concentrations 2–3 days before blood withdrawal [half-life 19 hr (Koenig et al. 2003)]. Similar results have been shown in previous studies (Ruckerl et al. 2006; Seaton et al. 1999).

Statistical model. We analyzed data using mixed-effects models with random patient effects accounting for repeated measures. Because the half-lives of the markers were 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 were used to allow for nonparametric exposure–response functions (Greven et al. 2006). IL-6 and CRP needed to be log-transformed to fulfill the model assumption of residual normality.

City-specific confounder models without air pollutants were built for each blood marker separately. In addition to potential time-varying confounders, we included time-invariant patient characteristics associated with the mean levels of inflammatory markers to permit the assumption of a normally distributed random patient intercept.

In a first step, time-invariant factors were selected for all cities combined. In the second step, for each city a more parsimonious model was selected out of the formerly chosen variables [see Supplemental Material, Table 1 (http://www.ehponline.org/docs/2007/10021/suppl.pdf)]. With this strategy, we adjusted for variables that influenced the mean levels of the respective blood markers in the single cities, such as age, sex, and BMI. These variables varied among the cities, possibly reflecting underlying differences in the populations across Europe as well as chance influences. To ensure sufficient adjustment for season and meteorology, long-term time trend and apparent temperature were forced into all models. Additionally, relative humidity, time of day, and day of the week were included if this adjustment improved the model fit. We considered lag 0, the mean of lags 0 and 1, the mean of lags 2 and 3, and the mean of lag 0–3 for the weather variables; for fibrinogen, we additionally assessed the mean of lags 0–4. P-splines were used to model continuous covariables and were compared with linear terms and polynomials of degrees 2 and 3. All decisions on goodness-of-fit were based on Akaike's Information Criterion (AIC) (Akaike 1973). Only after this

adjustment did we examine mean changes of the inflammatory markers in association with air pollution. Single air pollutants were added and effects estimated linearly. After the city-specific data analyses, we assessed heterogeneity between centers (Normand 1999). We combined city-specific effect estimates using meta-analysis methodology (Van Houwelingen et al. 2002). Additionally, we checked whether active smoking, levels of NT-proBNP > 80 pg/mL (de Lemos et al. 2003), and HbA1c > 6.5%, respectively, modified the effects of air pollution on blood parameters.

Data were analyzed using the statistical package SAS version 9.1 (SAS Institute Inc., Cary, NC, USA). Effect estimates are presented as percent change of geometric mean of the blood marker level (IL-6, CRP) and change of the arithmetic mean level (fibrinogen, percent of overall mean) together with 95% confidence intervals (CIs) based on an increase in air pollution concentrations from the first to the third quartile [interquartile range (IQR)].

Sensitivity analyses. We performed sensitivity analyses to explore the robustness of the models by using a more parsimonious and an extended model. Also, indicator variables for season and for potential inflammation due to diseases or surgery shortly before the blood withdrawal were added to the model.

Results

Study population. Baseline characteristics of the study population are given in Table 1. In total, 1,003 MI survivors who had at least two valid repeated blood samples were taken into the analyses. These were 84% of the targeted 1,200 patients.

Blood parameters. Of 6,068 collected blood samples, 255 had to be excluded due to acute infections or surgical procedures 3 days before the clinic visit, because they could have severely altered concentrations of inflammatory

Table 1. Baseline characteristics of 1,003 MI survivors from six European cities.

Characteristic	Helsinki (*n* = 195)	Stockholm (*n* = 197)	Augsburg (*n* = 200)	Rome (*n* = 134)	Barcelona (*n* = 169)	Athens (*n* = 108)	*p*-Value
Percent male	68.7	70.6	82.0	86.6	83.4	87.0	< 0.0001*
Age [mean years (range)]	64.6 (45–78)	64.0 (38–76)	61.9 (39–76)	62.7 (39–79)	62.1 (37–81)	54.7 (38–75)	< 0.0001**
BMI [mean (range)]	28.6 (19.1–48.9)	27.6 (17.5–43.2)	28.7 (19.1–48.4)	27.7 (19.0–39.4)	28.8 (19.3–43.5)	28.8 (20.8–46.3)	0.0039**
First MI (%)	81.5	85.8	87.5	87.3	86.4	80.6	0.37*
Self-reported history (%)[a]							
Angina pectoris	39.0	47.7	21.0	27.6	29.6	41.7	< 0.0001*
Arrhythmia	31.3	20.8	24.0	23.1	13.0	21.3	0.0029*
Congestive heart failure	14.9	16.2	13.0	6.0	1.8	5.6	< 0.0001*
Hypertension	51.3	49.7	51.0	55.2	46.2	54.6	0.73*
Diabetes	21.0	18.3	17.5	17.2	23.7	21.3	0.63*
Chronic renal disease	3.6	2.0	5.0	5.2	9.5	1.9	0.019#
Asthma	5.1	5.6	4.5	6.7	4.7	0.0	0.0946#
Any respiratory disease	7.2	6.6	10.5	22.4	13.6	6.5	< 0.0001*
Indication of COPD[b]	29.2	30.6	20.5	15.7	27.8	13.9	0.007*
Total cholesterol (mg/dL)[c] (range)	182.2 (91.1–291.9)	173.4 (96.7–324.7)	181.0 (107.0–316.0)	190.6 (120.0–321.0)	193.2 (119.0–390.0)	195.4 (92.0–293.0)	< 0.0001**
HDL cholesterol (mg/dL)[c] (range)	54.0 (22.0–119.3)	53.7 (30.9–116.0)	47.9 (24.0–98.0)	43.7 (25.0–87.0)	52.7 (28.0–105.0)	46.1 (24.0–87.0)	< 0.0001**
HbA1c [% (range)][c]	5.9 (4.7–9.2)	5.0 (3.8–9.9)	5.6 (4.7–9.8)	5.4 (2.8–8.7)	5.1 (3.8–9.8)	5.8 (3.7–10.5)	< 0.0001##
Statins (%)	83	88	89	79	85	73	0.0001*
Lipid-lowering medication (%)	85	89	90	83	86	74	0.0039*
Antithrombotic medication (%)	97	97	99	95	98	93	0.058†
No. of blood samples	1,155	1,168	1,144	741	1,119	486[d]	
IL-6 [mean (pg/mL)]	3.16	2.67	2.60	3.18	3.58	3.19	
GM (range)[e]	2.46 (0.92–19.7)	2.02 (0.48–24.4)	2.16 (0.61–11.8)	2.32 (0.95–61.4)	2.85 (0.76–28.51)	2.52 (0.84–22.40)	
Fibrinogen [mean (g/L)]	3.76	3.53	3.34	3.24	3.99	—	
GM (range)[e]	3.69 (2.68–5.63)	3.44 (2.24–6.11)	3.27 (2.00–6.87)	3.14 (1.94–5.18)	3.91 (2.62–6.02)	—	
CRP [mean (mg/L)][f]	1.98	2.86	2.26	2.56	3.52	2.52	
GM (range)[f]	1.18 (0.16–12.15)	1.42 (0.16–37.44)	1.18 (0.16–24.65)	1.40 (0.16–15.33)	2.03 (0.33–30.16)	1.32 (0.23–24.25)	

COPD, chronic obstructive pulmonary disease.
[a]Ever physician diagnosed. [b]Evaluated using a questionnaire on symptoms.[c]Blood biomarkers determined at local laboratories. [d]For fibrinogen N = 0. [e]Geometric mean of patients' geometric mean of repeated measurements. [f]Values of CRP < 0.16 could not be measured and were set to 0.16. *p*-Values determined with *chi-square test, **ANOVA, #Fisher´s exact test, ##Median-test, †Kruskal-Wallis test.

Table 2. Twenty-four-hour average concentrations of the ambient air pollution concentrations and meteorologic parameters from six European cities during the AIRGENE study period.[a]

Pollutant	Helsinki 5 Sep 03–2 Jun 04 Mean (95th)	Stockholm 30 Aug 03–24 Jun 04 Mean (95th)	Augsburg 14 May 03–24 Feb 04 Mean (95th)	Rome 20 Sep 03–15 Jul 04 Mean (95th)	Barcelona 30 Aug 03–16 Jun 04 Mean (95th)	Athens 8 Sep 03–30 Jul 04 Mean (95th)
PNC (1/cm^3)	8,534 (15,077)	9,748 (17,578)	11,876[b] (25,135)	35,450[b] (69,226)	18,133[b] (36,526)	20,589[b] (47,573)
$PM_{2.5}$ (μg/m^3)	8.2 (19.4)	8.8 (19.1)	17.4 (29.3)	24.5[b] (54.1)	24.2[b] (62.7)	23.0[b] (46.0)
PM_{10} (μg/m^3)	17.1 (36.1)	17.8 (40.3)	33.1 (56.6)	42.1 (76.0)	40.7[b] (88.7)	38.5 (64.6)
CO (mg/m^3)	0.31 (0.46)	0.29 (0.43)	0.58 (1.00)	1.40 (2.47)	0.59 (0.92)	1.48 (3.23)
NO_2 (μg/m^3)	28.6 (49.8)	18.6 (32.6)	40.0 (61.2)	67.0 (90.8)	50.5 (79.6)	50.1 (73.0)
NO (μg/m^3)	12.5 (40.7)	4.9 (15.5)	30.0 (80.4)	65.7 (164.0)	37.7 (88.4)	41.8 (144.6)
SO_2 (μg/m^3)	4.2 (10.1)	1.9 (4.9)	3.0 (5.7)	4.1 (9.2)	4.7 (9.6)	10.3 (23.2)
O_3 [8-hr average ([μg/m^3)]	46.8 (89.0)	60.6 (96.9)	54.4 (115.3)	45.3 (99.6)	28.2 (76.5)	59.8 (100.2)
Air temperature (°C)	3.1 (14.7)	4.7 (15.1)	10.2 (25.1)	13.4 (23.9)	15.2 (23.2)	17.6 (29.3)
Relative humidity (%)	76 (91)	82 (94)	69 (92)	80 (95)	67 (86)	67 (84)

95th, 95th percentile.
[a]The study period started 5 days before the first measurement because *a priori* air pollution concentrations up to 5 days before the blood withdrawals were considered. [b]Data available on < 95% of the days.

markers. Overall, 5,813 plasma samples remained. For Athens, fibrinogen levels could not be assessed. IL-6, fibrinogen, and CRP showed a moderate correlation, with the Spearman correlation coefficient ranging from 0.41 to 0.51 for all single measurements and from 0.49 to 0.55 for the mean values per patient, with the data of all cities combined. The single cities showed similar correlation coefficients, Barcelona being the only exception, with a low correlation between fibrinogen and IL-6 ($r = 0.22$ and 0.25, respectively).

Air pollutants. The 24-hr average concentrations of the pollutants and meteorologic data are given in Table 2. PNC and $PM_{2.5}$ were highest in the southern cities and lowest in Stockholm and Helsinki, whereas Augsburg showed intermediate levels (Figure 1).

Regression results. The pooled results for the regression of the three blood markers are summarized in Table 3. IL-6 showed borderline significant increases in association with PNC and NO_2 with lag 0, one of the two *a priori* specified lags (Figure 2). Because IL-6 showed positive associations for lag 0, we analyzed the 24 hr of air pollution exposure before the blood withdrawal in more detail. PNC results indicate a time response with a slight increase 6–11 hr after an exposure, a clear increase with 12–17 hr after an exposure, and a drop back to the level of 0–5 hr thereafter (Figure 3). Results for 6–11 as well as 12–17 hr for all single cities show clear positive associations, except for Helsinki and Athens (6–11 hr) and Helsinki and Stockholm (12–17 hr).

Fibrinogen was associated with an increase for the 5-day-average exposure of PM_{10}. Other pollutants also indicate an increase for the 5-day-averages, but CIs were wide. In addition to the effect for the cumulative exposure, we found an increase for fibrinogen with lag 3 for $PM_{2.5}$ and PM_{10} (Figure 3). For lag 3, results of the single cities show clear positive associations with $PM_{2.5}$ for all cities except for Augsburg, where no association was seen. For PM_{10} and lag 3, results are heterogeneous. Except for

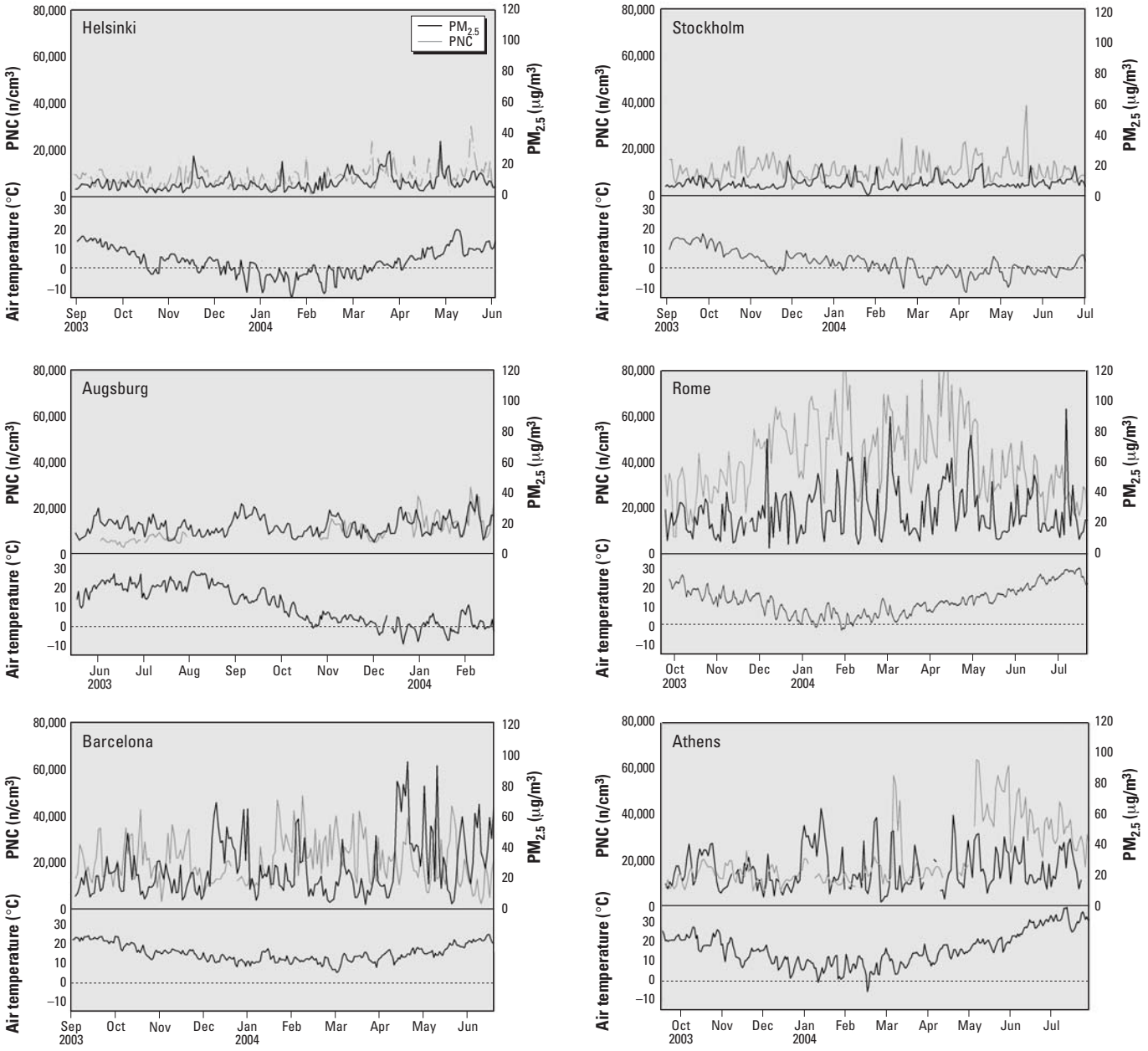

Figure 1. Time series of air pollution (PNC and $PM_{2.5}$) and air temperature in the six European cities of the AIRGENE study.

Augsburg, all cities present positive associations, with Helsinki being the highest. Associations for $PM_{2.5}$ and PM_{10} for the 5-day-average exposures were positive in all cities.

Analyses of effect modification showed that for fibrinogen associations remained for the 5-day average exposure to PM_{10} for nonsmokers and patients with elevated NT-proBNP and HbA1c levels (Figure 4). Results for the single cities revealed clear positive associations for patients with elevated HbA1c levels in Helsinki and Barcelona, and small positive associations in Augsburg and Rome, whereas no association was found for Stockholm. Helsinki and Barcelona showed clear increases in fibrinogen levels with increased PM_{10} for patients with high NT-proBNP levels, whereas for the other cities only small increases were found. Active smokers were present only in Rome and Barcelona, and interactions with smoking thus were calculated only for these two cities. The combined results are driven mainly by the results from Barcelona, which indicate a strong positive association for nonsmokers. For CRP, no associations between ambient air pollution and serum concentrations were observed for either the *a priori* hypothesized time span or other lags.

Sensitivity analyses. We performed sensitivity analyses for all blood markers, using selected air pollutants and the *a priori* specified lags (Table 4). For IL-6 and PNC, additional confounders in the model led to a clear positive result, whereas all other models, including the chosen model, were more conservative. For fibrinogen, overall results remained clearly positive and stable with PM_{10}. With PNC a strong yet not significant association was found for the model without time-independent covariates. For CRP, results did not change in dependence on the model.

Discussion

We measured IL-6, fibrinogen, and CRP, three blood markers that indicate an inflammatory response, in MI survivors in six European cities. Pooled results show an increase in IL-6 when concentrations of PNC were elevated 12–17 hr before the clinical visit. Cumulative exposure to PM_{10} was associated with an increase in fibrinogen. No

Table 3. Effects of air pollution on blood biomarkers per increase in IQR of air pollutant (pooled effect estimates).

		IL-6 (all cities)			Fibrinogen (all cities except Athens)			CRP (all cities)		
Pollutant, IQR	Time before blood withdrawal	% change (GM)	95% CI	*p*-Value heterogeneity	% change (AM)	95% CI	*p*-Value heterogeneity	% change (GM)	95% CI	*p*-Value heterogeneity
PNC[a]										
11852	Lag 0	1.88**	−0.16 to 3.97	0.72	0.40	−0.40 to 1.19	0.54	1.33	−3.05 to 5.90	0.047
11852	Lag 1	−0.67	−2.56 to 1.25	0.64	0.11	−0.69 to 0.91	0.12	−1.52	−4.39 to 1.45	0.19
11852	Lag 2	−2.12**	−4.03 to −0.17	0.055	0.09	−0.71 to 0.90	0.045	−1.63	−6.70 to 3.71	0.019
11003	5-day average	−0.93	−3.37 to 1.56	0.084	0.50	−2.20 to 3.20	0.009	−0.08	−3.78 to 3.75	0.12
$PM_{2.5}$[b]										
11.0	Lag 0	0.46	−0.89 to 1.83	0.26	0.05	−0.48 to 0.58	0.36	0.11	−1.95 to 2.21	0.71
11.0	Lag 1	−0.39	−1.69 to 0.93	0.70	0.17	−0.35 to 0.69	0.55	−0.06	−1.98 to 1.90	0.70
11.0	Lag 2	−0.23	−1.53 to 1.07	0.57	0.20	−0.32 to 0.71	0.26	0.11	−1.80 to 2.06	0.86
8.6	5-day average	0.05	−1.37 to 1.50	0.66	0.38	−0.21 to 0.96	0.21	−0.13	−2.15 to 1.92	0.94
PM_{10}[c]										
17.4	Lag 0	−0.34	−1.66 to 0.99	0.45	0.06	−0.43 to 0.55	0.53	−0.71	−2.75 to 1.37	0.16
17.4	Lag 1	−0.69	−1.95 to 0.58	0.43	0.14	−0.35 to 0.63	0.83	−0.63	−2.61 to 1.39	0.23
17.4	Lag 2	−1.59	−3.99 to 0.88	0.0030	0.24	−0.24 to 0.72	0.25	−1.42	−4.23 to 1.47	0.086
13.5	5-day average	−0.87	−2.28 to 0.55	0.15	0.60*	0.10 to 1.09	0.26	−1.35	−3.45 to 0.79	0.19
CO										
0.34	Lag 0	0.57	−0.63 to 1.79	0.95	0.24	−0.45 to 0.92	0.11	−0.01	−1.72 to 1.73	0.18
0.34	Lag 1	0.44	−0.79 to 1.68	0.72	0.32	−0.35 to 1.00	0.38	−1.51	−3.30 to 0.32	0.19
0.34	Lag 2	−2.36	−4.82 to 0.17	0.0054	−0.44	−1.11 to 0.23	0.078	−2.35	−6.84 to 2.36	0.0025
0.31	5-day average	−0.28	−2.53 to 2.02	0.067	0.12	−0.81 to 1.05	0.062	−0.85	−5.37 to 3.90	0.051
NO_2										
15.9	Lag 0	1.31**	−0.24 to 2.89	0.97	0.05	−0.50 to 0.60	0.84	0.41	−1.93 to 2.81	0.68
15.9	Lag 1	0.93	−0.55 to 2.43	0.78	0.04	−0.49 to 0.57	0.64	1.15	−1.18 to 3.54	0.86
15.9	Lag 2	−1.38	−4.35 to 1.68	0.00024	0.05	−0.71 to 0.80	0.056	−0.28	−4.05 to 3.63	0.0081
10.1	5-day average	−0.19	−3.08 to 2.78	0.0014	0.24	−0.45 to 0.93	0.057	1.40	−0.92 to 3.79	0.19

Abbreviations: AM, arithmetic mean; GM, geometric mean. *A priori* specified lags: IL-6: lag 0 and lag 1; fibrinogen: 5-day average; CRP: lag 2.
[a]IQR (24-hr, 5-day average), 11852.39408, 11002.9686 n/cm^3. [b]IQR (24-hr, 5-day average), 10.99720847, 8.59343322 µg/m^3. [c]IQR (24-hr, 5-day average), 17.36794382, 13.5380001 µg/m^3.
*$p < 0.05$; **$p < 0.1$.

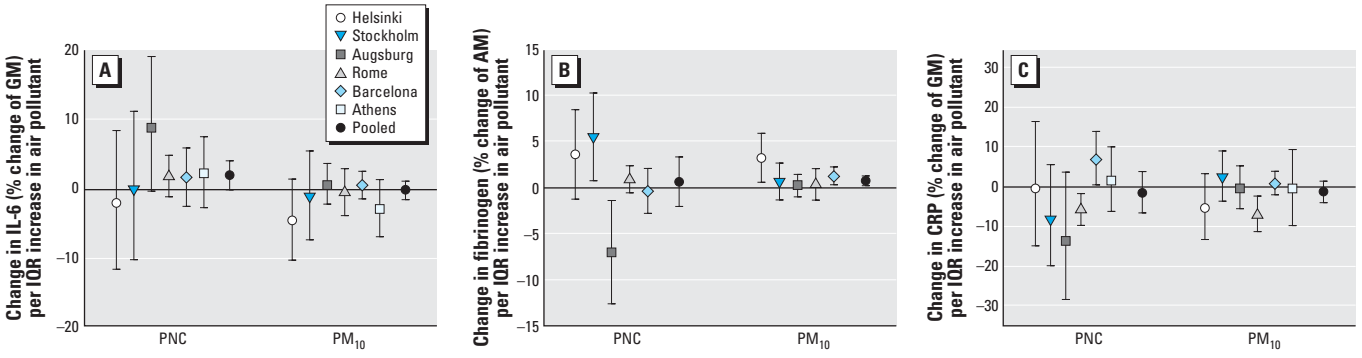

Figure 2. Association between PNC and PM_{10} and blood markers for the single cities for the *a priori* specified lags. (*A*) IL-6. (*B*) Fibrinogen. (*C*) CRP. Abbreviations: AM, arithmetic mean; GM, geometric mean.

consistent associations could be detected for CRP.

Air pollution seems to affect susceptible subgroups (Goldberg et al. 2001, 2006; Katsouyanni et al. 2001; Pope et al. 2002). We therefore examined MI survivors, a subgroup with an increased risk for readmission to the hospital (von Klot et al. 2005), in six European cities, covering a wide range of gaseous and particulate air pollutants. There is a strong link between inflammation and CHD because factors involved in inflammation and infection seem to play a pro-atherogenic role, and inflammation has been identified as a potent risk factor for acute ischemic syndromes (Ross 1999). Other risk factors such as cigarette smoking (Danesh et al. 1999: Fröhlich et al. 2003), diabetes (Thorand et al. 2007), or high BMI (Danesh et al. 1999: Thorand et al. 2006) have also been found to be associated with low-grade systemic inflammation, providing a further link between inflammation and acute coronary events.

Previous studies have shown an association between air pollution and blood markers of inflammation and coagulation. We examined IL-6 because of its role in the inflammatory cascade (Woods et al. 2000). IL-6 is produced by different cells in the body, including lymphocytes, monocytes, and endothelial cells. It is thought to play a major role in mediating stimuli from activated macrophages—for example, by smoking. IL-6 is the key cytokine that stimulates the synthesis of all major acute phase proteins, including CRP and fibrinogen (Woods et al. 2000). The latter factor induces an increase in blood viscosity and promotes thrombus formation (Koenig 2003).

In a study in the United Kingdom (Seaton et al. 1999), no significant associations were seen for a 3-day cumulative exposure to ambient PM_{10} and IL-6 levels. At high pollution levels, however, such as in road tunnels or during forest fires, positive associations have been observed (Hilt et al. 2002; van Eeden et al. 2001). Our results indicate an increase in IL-6 within 12 hr after exposure, a requisite first step in the stimulation of the *de novo* synthesis of acute-phase proteins in the liver, triggered by ambient particles.

Fibrinogen, an acute-phase protein, also plays a crucial role in the coagulation cascade. Studies regarding its association with air pollution are inconclusive. It has been shown to increase in association with high levels of ambient particles such as in an air pollution episode (Peters et al. 1997) or in controlled human exposure studies (Ghio et al. 2000). Also, positive associations, such as for $PM_{10,}$ have been reported at levels comparable to those measured in the present study (Pekkanen et al. 2000; Schwartz 2001). However, also null associations (Pope et al. 2004; Ruckerl et al. 2006) and even decreases in fibrinogen concentration in association with air pollutants have been reported (Khandoga et al. 2004; Seaton et al. 1999). Our study indicates an increase in fibrinogen for lag 3 and the 5-day cumulative exposure for PM_{10}.

CRP, a well-known biomarker of systemic inflammation, has been one of the first acute-phase reactants to be examined in association with air pollution in several studies. Increased concentrations have been shown during an air pollution episode in Germany in healthy men, 45–64 years of age (Peters et al. 2001b) as well as for ambient PM_{10} levels currently present in Europe (Seaton et al. 1999). Additionally, in a panel of CHD patients, an increase in CRP above the 90th percentile was found in association with ambient particles (Ruckerl et al. 2006). Similar analyses did not reveal any effects in our data, which might be attributed to differences in the two panels. The AIRGENE panel consisted of both males and females and was on average slightly younger, but had more severe diseases than the subjects studied previously. On the other hand, the average CRP levels were lower in the AIRGENE panel.

Overall, these studies suggest associations between inflammation and ambient air pollution concentrations, especially particles, although the effects between studies differ for individual data. To date, the reason for the heterogeneity is largely unknown. Different pollution mixtures, underlying medical conditions, treatments or diets with high antioxidant levels might be possible explanations.

We observed immediate associations between PNC and IL-6 and cumulative effects between PM_{10} and fibrinogen. This is a surprising finding, which might be attributed to chance, because PM_{10} and PNC were not highly correlated in most cities. It is, however, also possible that their mode of action is different. Ultrafine particles or attached substances might translocate quickly into the bloodstream (Geiser 2002) and lead to the observed changes in IL-6 without having a direct impact on the lung. $PM_{10,}$ on the other hand, might only exert an indirect systemic impact by provoking an inflammatory response in the lung that eventually causes oxidative stress, leading to the observed delayed increase in fibrinogen. However, these explanations are highly speculative. Further, PNC and PM_{10} differ not only by size but also by composition and redox activity (Cho et al. 2005), but the implications for the mechanisms are difficult to judge. When the city-specific results are examined, the immediate association between IL-6 and PNC was strongest in Augsburg, whereas the association between PM_{10} and fibrinogen was strongest in Helsinki for the 5-day-average exposure. Because these city-specific estimates were not heterogeneous, this may reflect the expected variation between independent studies. It also might point to differences in measurement error with respect to population average exposures characterized by central monitoring sites.

One possible explanation for the lack of associations between air pollutants and CRP in our data could be the high prevalence of

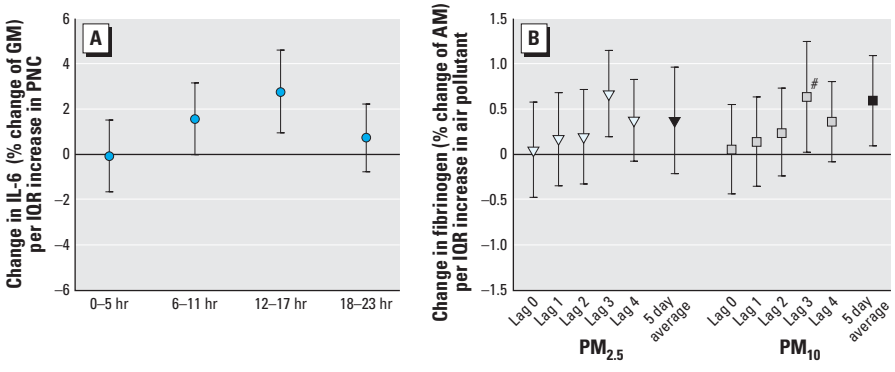

Figure 3. Pooled effects of PNC on IL-6 (*A*) and of $PM_{2.5}$ and PM_{10} (*B*) on fibrinogen, different lags. Abbreviations: AM, arithmetic mean; GM, geometric mean. Error bars indicate 95% CIs.
[#]Heterogeneity between the cities present.

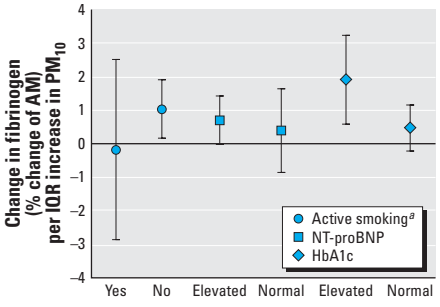

Figure 4. Effects of PM_{10} on fibrinogen (5-day average) as modified by active smoking, levels of NT-proBNP and HbA1c as indicators of left ventricular dysfunction and diabetes, respectively. AM, arithmetic mean. Error bars indicate 95% CIs.
[a]Active smokers were only present in Rome and Barcelona; interaction thus calculated only for these two cities.

lipid-lowering drugs intake, particularly statins, which have been shown to reduce CRP through inhibition of its hepatic synthesis (Arnaud et al. 2005). Studies have shown that long-term therapy with a statin significantly lowers plasma levels of CRP (Ridker et al. 2001; Sandhu et al. 2005). IL-6, which is produced upstream to the production of CRP in the liver, is not affected by this compound. Also, fibrinogen has been implicated to be reduced by fibrates but not statins (Rosenson et al. 2001). Because the majority of our patients reported an intake of statins, subgroup analyses did not seem reasonable.

Increased concentrations of CRP are known to predict cardiovascular events in healthy subjects (Danesh et al. 2000). Also, elevated levels of IL-6 have been found to be associated with total mortality (Harris et al. 1999) and with risk of future fatal and nonfatal MI (Ridker et al. 2000). Whether the short-term increases in IL-6 and fibrinogen observed in this study actually lead to an increased risk for an acute coronary syndrome, however, remains to be shown. A long-term follow-up study examining cardiovascular end points might help to answer the question whether subjects with elevated levels of inflammatory proteins in response to environmental stimuli have an increased risk of acute ischemic syndromes.

Strengths and limitations. The study is based on a common protocol and standard operating procedures applied in six European cities. Site visits were conducted to ensure uniform procedures. The analyses of the inflammatory markers were done in one central laboratory, and blinded duplicate samples were measured for quality assurance.

Some of the measured biomarkers (e.g., CRP) are affected by health-related events such as acute infection or surgery (Thomas

Table 4. Sensitivity analyses: results for different models on selected outcomes.

	IL-6 (lag 0)			Fibrinogen (mean of 5-day average)			CRP (lag 2)		
Pollutant, model	Estimate (% change GM)	95% CI	Heterogeneity (*p*-value)	Estimate (% change AM)	95% CI	Heterogeneity (*p*-value)	Estimate (% change GM)	95% CI	Heterogeneity (*p*-value)
PNC									
Main model	1.88	–0.16 to 3.97	0.72	0.5	–2.20 to 3.20	0.009	–1.63	–6.70 to 3.71	0.02
Additional covariates[a]	2.16*	0.08 to 4.29	0.85	0.26	–2.96 to 3.48	0.004	–1.64	–7.02 to 4.05	0.01
No time-independent covariates	1.16	–0.85 to 3.21	0.46	2.77	–0.15 to 5.69	0.001	–1.92	–6.93 to 3.36	0.02
Including risk of potential inflammation 4–7 days before blood withdrawal	1.91	–0.14 to 3.99	0.70	0.48	–2.37 to 3.32	0.01	–1.74	–6.78 to 3.57	0.02
Including seasonal interaction (season = winter)	1.97	–0.11 to 4.09	0.61	0.35	–0.80 to 1.51	0.02	–1.18	–6.59 to 4.55	0.01
PM_{10}									
Main model	–0.34	–1.66 to 0.99	0.45	0.60*	0.10 to 1.09	0.26	–1.42	–4.23 to 1.47	0.086
Additional covariates[a]	–0.16	–1.48 to 1.17	0.53	0.81*	0.17 to 1.45	0.19	–1.08	–3.05 to 0.92	0.11
No time-independent covariates	–0.64	–1.94 to 0.69	0.44	0.36	–0.77 to 1.48	0.02	–2.04	–5.05 to 1.07	0.04
Including risk of potential inflammation 4–7 days before blood withdrawal	–0.35	–1.66 to 0.99	0.44	0.75	0.12 to 1.38	0.34	–1.43	–4.21 to 1.43	0.09
Including seasonal interaction (season = winter)	–0.15	–1.71 to 1.42	0.40	0.78	–0.02 to 1.57	0.50	–1.33	–3.61 to 1.00	0.55

Estimates for CRP and IL-6 are expressed as percent change in expected geometric mean (GM); estimates for fibrinogen are expressed as absolute change in expected mean, expressed as percent of overall arithmetic mean (AM).
[a]Additional covariates included time-independent variables present in at least two cities, hour of blood withdrawal, and relative humidity. *$p < 0.05$.

Appendix 1: The AIRGENE study group comprises the following partners:

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2. ***University of Ulm, Department of Cardiology (Germany):*** W. Koenig, N. Khuseyinova, G. Trischler.
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7. ***University of Athens Medical School, Department of Hygiene and Epidemiology (Athens, Greece):*** K. Katsouyanni, A. Chalamandaris, K. Dimakopoulou, D. Panagiotakos; Department of Cardiology (Athens, Greece): C. Stefanadis, C. Pitsavos, C. Antoniades, C. Chrysohoou, J. Mitropoulos.
8. ***University of Helsinki, Department of Physical Sciences (Helsinki, Finland):*** M. Kulmala, P. Aalto, P. Paatero.

2000). We therefore carefully excluded blood samples that might have been strongly influenced by other sources than air pollution before the statistical analyses. Moreover, thorough confounder adjustment was done to rule out the possibility that the detected associations resulted from meteorologic influences or seasonal differences, and repeated measures decreased the chance of confounding by time-independent variables, because each person served as his or her own control.

Even though we included city-specific patient characteristics to account for differences in the panels, the city-specific estimates of the air pollution effects still varied. However, for those results indicating an association between air pollution and inflammation, these variations did not exceed the expected random variation. But it is also quite possible that the air pollution mixture, socioeconomic factors, or genetic background are responsible for these modifications. Indeed, we did observe effect modification for patients with elevated HbA1c and NT-proBNP.

We observed only small changes in the acute-phase response that are not on the scale of a bacterial infection (Thomas 2000) or surgery and presumably do not have any direct clinical relevance. Other factors, such as 10 pack-years of smoking, in comparison, led to increases of 2.7% (95% CI, 1.2–4.3) for IL-6, 1.2% (95% CI, 0.7–1.7) for fibrinogen, and 5.5% (95% CI, 3.0–8.0) for CRP in our data. A higher BMI of 5 kg/m^2 was associated with significantly higher levels of IL-6 (16%), fibrinogen (3.8%), and CRP (38%). Smoking and overweight may be of concern in subpopulations, whereas air pollution usually affects whole populations and there is generally no voluntary component to the risk. Based on a publication by Cesari et al. (2003), we estimated that the increase in IL-6 we found in association with PNC might lead to a 0.7% (95% CI, –0.06 to 1.5) increased risk of CHD in elderly people without baseline cardiovascular risk. Despite the high prevalence of statin intake, our data still indicate an inflammatory response in association with air pollution. We therefore hypothesize that ambient air pollution might increase plaque vulnerability by these subclinical inflammatory responses.

Conclusion

Our results indicate an immediate response of IL-6 to ambient air pollution, which might lead to the synthesis of acute-phase proteins, as indicated by increased fibrinogen levels. The lack of detectable associations for CRP may be attributed to a widespread intake of statins in our population, which might suggest a protective effect against environmental, proinflammatory stimuli—an intriguing phenomenon that deserves further study.

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Appendix IV

Regina Rückerl, Angela Ibald-Mulli, Wolfgang Koenig, Alexandra Schneider, Gabriele Woelke, Josef Cyrys, Joachim Heinrich, Victor Marder, Mark Frampton, H. Erich Wichmann, and Annette Peters:
Air Pollution and Markers of Inflammation and Coagulation in Patients with Coronary Heart Disease

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Air Pollution and Markers of Inflammation and Coagulation in Patients with Coronary Heart Disease

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Rationale: **Ambient air pollution has been shown to be associated with cardiovascular morbidity and mortality.**
Objectives: **A prospective panel study was conducted to study the early physiologic reactions characterized by blood biomarkers of inflammation, endothelial dysfunction, and coagulation in response to daily changes in air pollution in Erfurt, Germany.**
Methods: **Blood parameters were repeatedly measured in 57 male patients with coronary heart disease during the winter of 2000/2001. Fixed-effects linear and logistic regression models were applied, adjusting for trend, weekday, and meteorologic parameters.**
Measurements: **Hourly data on ultrafine particles (UFPs; number concentration of particles from 0.01 to 0.1 μm), mass concentration of particles less than 10 (PM_{10}) and 2.5 μm in diameter, elemental and organic carbon, gaseous pollutants, and meteorologic data were collected at central monitoring sites.**
Main Results: **Increased levels of C-reactive protein above the 90th percentile were observed for an increase in air pollution concentrations of one interquartile range. The effect was strongest for accumulation mode particles, with a delay of 2 d (odds ratio [OR], 3.2; confidence interval [CI], 1.7, 6.0). Results were consistent for UFPs and PM_{10}, which also showed a 2-d delayed response (OR, 2.3; CI, 1.3, 3.8; and OR, 2.2; CI, 1.2, 3.8, respectively). However, not all of the blood markers of endothelial dysfunction and coagulation increased consistently in association with air pollutants.**
Conclusion: **These results suggest that inflammation as well as parts of the coagulation pathway may contribute to the association between particulate air pollution and coronary events.**

Keywords: acute-phase reaction; air pollution; blood coagulation; cardiovascular diseases; C-reactive protein

Increasing evidence suggests that ambient air pollution may adversely affect the cardiovascular system. It has been shown that ambient air pollution leads to increased cardiovascular mortality (1–6), and studies found associations between ambient air pollution and hospital admissions for various cardiovascular diseases, including congestive heart failure (7–9). Also, an increased risk for acute myocardial infarction (MI) (10) and cardiorespiratory symptoms (11) has been reported in association with particulate air pollution.

The exact mechanisms linking the inhalation of ambient air particles to an acute exacerbation of cardiovascular disease are not completely understood (12). Seaton and coworkers (13) hypothesized that inhaled particles would lead to alveolar inflammation, which increases the level of blood coagulability, thus leading to an increased risk of ischemic events in susceptible individuals. DeMeo and colleagues (14) found reduced oxygen saturation in association with particulate matter of less than 2.5 μm in diameter ($PM_{2.5}$). Pope and colleagues (15), who linked long-term exposure to particulate air pollution to various causes of mortality, found a strong and robust association between $PM_{2.5}$ and cardiovascular disease mortality. They concluded that exposure to particulate air pollution and cardiopulmonary mortality risk is linked by accelerated pulmonary and systemic inflammation. Moreover, Peters and coworkers (16) demonstrated increased levels of plasma viscosity during an air pollution episode in central Europe, compared with less polluted days. Increased plasma concentrations of C-reactive protein (CRP), the classic acute-phase protein, were also shown during the 1985 air pollution episode (17).

There is a strong link between inflammation and coronary heart disease (CHD) because factors involved in inflammation and infection seem to play a proatherogenic role and inflammation has been identified as a potent risk factor for acute coronary syndrome. Systemic inflammation could result in destabilization or even rupture of vulnerable atheromatous plaques, leading to acute ischemic events.

Most of the cited studies have been conducted in the general population or in elderly healthy subjects. This study looks at a susceptible subgroup to provide insight into the ways in which air pollution might precipitate death in persons with underlying heart disease, based on the hypothesis that particulate air pollution can alter cardiovascular function.

Repeated measurements of markers of an early inflammatory response, cell recruitment and coagulation, were compared with concurrent levels of air pollution. Our primary hypothesis was that CRP, a well-known marker for inflammation, would increase in association with a rise in levels of air pollution. Moreover, we analyzed various other markers of inflammation (serum amyloid A [SAA]), cell adhesion (E-selectin, von Willebrand factor antigen [vWf], intercellular adhesion molecule 1 [ICAM-1]), and coagulation (fibrinogen, factor VII [FVII], prothrombin fragment 1+2, D-dimer) on a more explorative basis hypothesizing that the levels of these blood markers would also go up in association with higher levels of air pollution, as seen in Figure 1. Some results have been previously presented in form of an abstract (18).

METHODS

Study Design

As part of the University of Rochester Particulate Matter Center, a prospective panel study was conducted between October 15, 2000, and April 27, 2001, in Erfurt, Germany. The panel consisted of male patients with CHD who were scheduled for 12 subsequent clinical visits. Each

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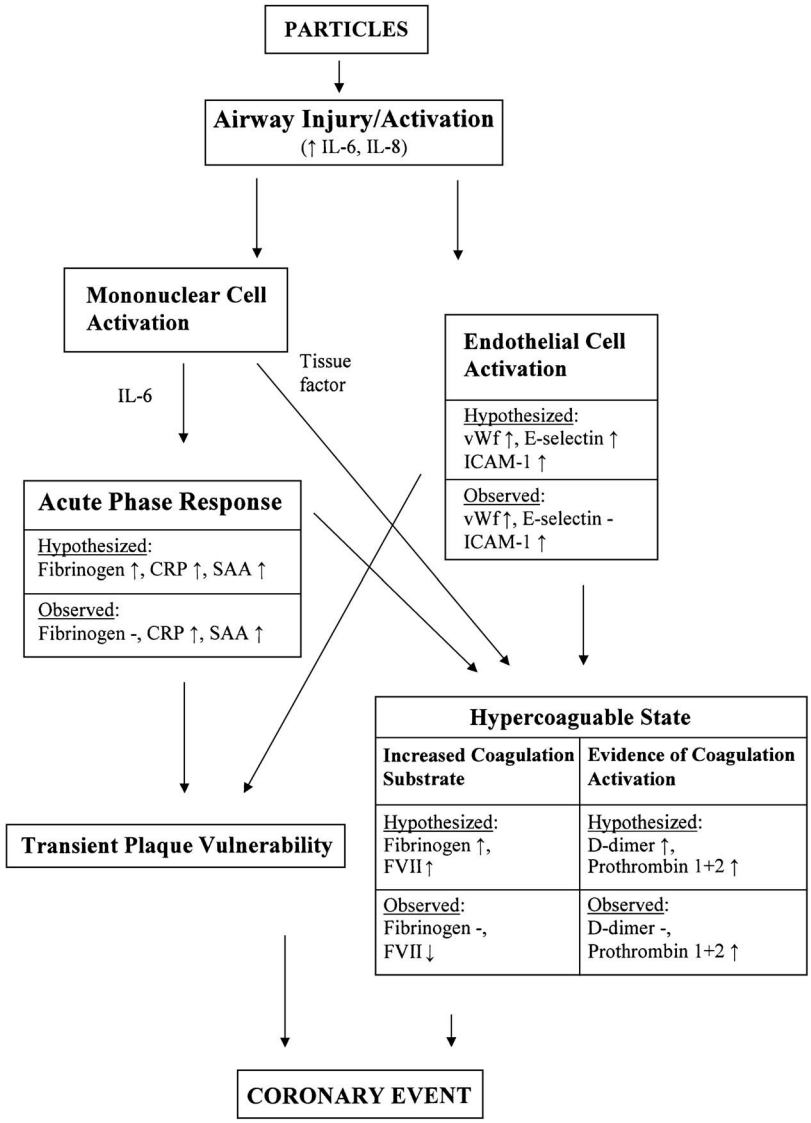

Figure 1. Conceptual model showing hypothesized as well as observed effects of the cellular events linking particulate exposure to cardiopulmonary events. CRP = C-reactive protein; FVII = factor VII; ICAM-1 = intercellular adhesion molecule 1; IL = interleukin; SAA = serum amyloid A; vWf = von Willebrand factor.

clinical visit included a short interview and the withdrawal of a blood sample. At the first visit, a baseline questionnaire was administered regarding health status, pulmonary and cardiac symptoms, medication intake, and smoking history.

Sixty-one nonsmoking men, aged 50 yr or older, with physician-diagnosed CHD were recruited through a local cardiologist. Patients with pacemakers, recent (< 3 mo ago) MI, bypass surgery, or balloon dilatation were not included because the inflammatory processes involved in such a procedure might not yet have subsided. Persons with type 1 diabetes or on anticoagulation therapy (except for antiplatelet agents) were also not included. A written, informed consent was obtained from all subjects. The study protocol was approved by the German Ethics Committee of the "Bayerische Landesärztekammer" in Munich, Germany. All methods used in the study were conducted according to standard operating procedures and were tested beforehand in a 2-wk pilot study.

Air Pollution Monitoring

Concentrations of different ambient air pollutants were measured at one fixed monitoring site in the city center representing urban background levels. The measurement site was put up especially for carrying out epidemiologic studies (19, 20) and all measurements were conducted according to the standard operating procedures developed within the framework of previous studies (21–23).

Continuous ultrafine particle (UFP) counts (0.01–0.1 μm), accumulation mode particle (AP) counts (0.1–1.0 μm), and fine-particle mass ($PM_{2.5}$) were measured with the mobile aerosol spectrometer (MAS). The MAS, described previously (24, 25), consists of two different, commercially available instruments covering different size ranges. Particles in the size range from 0.01 to 0.5 μm were measured using a differential mobility particle sizer (TSI, Aachen, Germany). Particles in the size range from 0.1 to 2.5 μm were classified by an optical laser aerosol spectrometer (PMS, Leonberg, Germany).

PM_{10} (particulate matter < 10 μm in diameter) data were collected by the tapered element oscillating microbalance method (TEOM 1400a; Rupprecht and Patashnik, Albany, NY) and continuous data on elemental (EC) and organic carbon (OC) were measured with an ambient carbon monitor (ACM 5400; Rupprecht and Patashnick). Data on meteorologic variables for this period as well as concentrations of gaseous air pollutants were collected from existing networks. Missing values of the ambient UFPs between January 20 and February 13 were imputed by a linear regression model based on parallel measurements with a condensation particle counter and a scanning mobility particle sizer. The R squares for the regression model was 0.96. Also, between December 2000 and May 2001, approximately 15% of the $PM_{2.5}$ measurements by MAS were lost. These missing values were replaced by corrected data based on parallel measurements with TEOM-PM_{10} and Harvard Impactor-$PM_{2.5}$. (Air Diagnostic and Engineering, Inc., Naples, ME).

For each person and visit, the individual 24-h average of pollutants immediately preceding the clinical visit (lag 0) up to Day 5 (lag 1–4) and 5-d running means before the examination were calculated if more than two-thirds of the hourly measurements were available for this period.

Clinical Measurements

The clinical visits were scheduled on the same weekday (Monday to Friday) and time (8:00 A.M. to 5:00 P.M.) for each patient once every 2 wk.

At each visit, ethylenediaminetetraacetic acid and citrate plasma samples were drawn (Becton Dickinson, Franklin Lakes, NJ). Samples were centrifuged and aliquots were immediately stored at −20°C until analysis. CRP (high-sensitivity assay), SAA, and fibrinogen were analyzed by immunonephelometry (Dade Behring, Marburg, Germany). ICAM-1, E-selectin (R&D Systems, Wiesbaden, Germany), and prothrombin fragment 1+2 (Dade Behring) were measured by means of a commercial ELISA. D-dimer and vWf were analyzed using an immunoturbidimetric method and FVII by clotting time measurement (Diagnostica Stago, Asnieres-sur-Seine, France).

Study Subjects

Fifty-seven of 61 patients were included in the analyses. One patient refused to participate, and three patients had to be excluded for the following reasons: two were diagnosed with leukemia or lymphoma and one patient had constantly elevated levels of white and red blood cells, indicating an unknown hematologic disorder. Fifty-five patients participated in 12, one patient participated in nine, and one patient participated in eight scheduled visits (99% completeness). Blood samples of patients reporting an acute infection and/or surgery during the 2 wk before the examination were excluded from the analysis (46 blood samples [7%] of 19 different patients). Also, 18 blood samples (3%) in 15 patients showing implausibly low fibrinogen values (< 1.0 g/L) on nephelometry were excluded from the analysis. Finally, not all patients were able to give the scheduled amount of blood at each visit. Therefore, between 544 blood samples (87%) and 581 blood samples (92%) remained for analysis, depending on the marker.

Statistical Analyses

Continuous concentrations of the blood markers were analyzed using linear regression models. Also, values above the 90th percentile were assessed using logistic regression models (17). Generalized additive models, including pollutant and confounder variables, were used for fixed-effects regression with individual intercepts for each patient. Long-term time trend, an indicator variable for each subject, weekday of the visit, and the meteorologic parameters air temperature, relative humidity, and barometric pressure, each with lag 0 to lag 3, were considered as potential confounders. Because the half-life of most markers is only a couple of hours and the visits took place in 2-wk intervals, it was assumed that no autocorrelation was present in the patient data, and no adjustment for autocorrelation was made.

Prothrombin fragment 1+2, FVII, SAA, CRP, and E-selectin were log-transformed before analysis because their residuals remained skewed after multivariate modeling.

Model building was done for each blood parameter separately without an air pollution variable. To explore the shape of the association between confounders and blood markers, nonparametric smooth functions on the basis of locally weighted least squares were applied for all confounders. Model fit was rated on the basis of the Akaike information criterion (AIC). In the final model, nonparametric smooth functions were replaced by appropriate polynomials (degree 2 or 3) or natural splines based on lowest AIC. After the model fit was completed, dose–response functions of the confounders were checked visually and in case implausible shapes were observed, degrees of freedom were decreased. Each pollutant was added separately to the final model.

Data were analyzed using the statistical package SAS version 8.2 (SAS Institute, Inc., Cary, NC) and S-Plus version 6.0 (Mathsoft Engineering and Education, Inc., Cambridge, MA).

Logistic regression models were used to determine whether the effect was limited to the upper tail of the distribution. Confounder adjustment was done in the same way as described for the linear regression models; however, more parsimonious models were used. Sensitivity analyses were done to explore the influences of the different confounder models.

RESULTS

Patient Characteristics

Patient characteristics are summarized in Table 1. The study population comprised 57 nonsmoking men, aged 51 to 76 yr. Approximately 84% of them were retired. Except for one person, all patients had stopped smoking at least 1 yr before the examinations.

Air Pollutants

The distributions of the 24-h average concentrations of the particulate and gaseous pollutants as well as meteorologic data are given in Table 2.

PM_{10}, $PM_{2.5}$, and AP were highly correlated (r = 0.90–0.91), whereas UFPs were only moderately correlated with PM_{10} and $PM_{2.5}$ (r = 0.57 and 0.41, respectively). $PM_{2.5}$ showed a moderate negative correlation with air temperature (r = −0.5; Figure 2). EC and OC showed high correlation (r = 0.96) and were also highly correlated with all other particle fractions (r = 0.63–0.90). Also, CO and NO_2 were highly correlated (r = 0.82), whereas the correlation for UFPs with NO_2 was slightly lower than with CO (r = 0.75 and 0.82, respectively).

Blood Parameters

Levels of blood parameters are summarized in Table 3. Parameters of the acute-phase response, SAA and CRP, were correlated (r = 0.53), as were the adhesion molecules ICAM-1 and E-selectin (r = 0.53). However, no significant correlation was seen between markers of an acute-phase response and adhesion molecules (r = 0.08 to 0.31). SAA and CRP also showed a moderate correlation with fibrinogen (r = 0.44 and 0.34, respectively).

TABLE 1. CHARACTERISTICS OF THE STUDY POPULATION, 57 MEN WITH HISTORY OF CORONARY HEART DISEASE IN ERFURT, GERMANY, WINTER 2000/2001

	Mean (*SD*)
Age, yr	66 (6.0)
BMI, kg/m^2	28 (3.4)
	No. (%)
History of	
Coronary heart disease	57 (100)
Angina pectoris	40 (68)
Myocardial infarction	43 (75)
Bypass surgery/balloon dilatation	49 (86)
Stroke	3 (5)
Diabetes mellitus	13 (23)
Hypertension	40 (70)
Chronic bronchitis	2 (4)
COPD	5 (9)
Hay fever	2 (4)
Chronic kidney disease	6 (11)
Smoking	
Never smoker	15 (26)
Ever smoker	42 (74)
Medication use	
Platelet aggregation inhibitors	
Acetylsalicylic acid	52 (91)
Thienopyridines	3 (5)
Antihyperlipidemic medication	29 (51)

Definition of abbreviations: BMI = body mass index; COPD = chronic obstructive pulmonary disease.

TABLE 2. SUMMARY STATISTICS OF DAILY CONCENTRATIONS (24-h AVERAGE) OF AIR POLLUTANTS AND METEOROLOGIC VARIABLES IN ERFURT, GERMANY, BETWEEN OCTOBER 12, 2000, AND APRIL 27, 2001 (INCLUDING IMPUTED VALUES)

Variable	No.	Mean (± *SD*)	Min.	First Quartile	Median	Third Quartile	Max.	IQR	IQR (*5-d average*)
UFPs,* n/cm³	196	12,602 (± 6,455)	2,542	7,326	11,444	17,332	34,294	10,005	6,821
AP, n/cm³	167	1,593 (± 1,034)	328	821	1,238	2,120	4,908	1,299	1,127
$PM_{2.5}$,† μg/m³	197	20.0 (± 15.0)	2.6	9.7	14.9	26.1	83.7	16.4	12.2
PM_{10}, μg/m³	154	20.0 (± 13.0)	5.4	10.8	15.6	26.0	74.5	15.2	12.8
Organic carbon, μg/m³	126	1.5 (± 0.6)	0.3	1.1	1.4	1.8	3.4	0.7	0.5
Elemental carbon, μg/m³	126	2.6 (± 2.4)	0.2	1.0	1.8	3.2	12.4	2.3	1.8
NO_2, μg/m³	198	34.3 (± 11.4)	8.0	25.3	34.0	42.5	68.4	17.2	9.3
CO, mg/m³	198	0.52 (± 0.29)	0.11	0.33	0.44	0.60	1.93	0.27	0.22
Temperature, °C‡	198	4.1 (± 4.8)	−10.4	0.5	4.4	7.9	13.2	7.4	5.9
Barometric pressure, hPa	198	973.4 (± 9.7)	949.5	966.3	972.9	980.0	995.7	13.6	10.8
Relative humidity,§ %	198	83.5 (± 8.8)	55.8	78.9	84.3	88.8	100.0	10.0	9.1

Definition of abbreviations: AP = accumulation mode particles (particles with a size range of 0.1 to 1.0 μm); IQR = interquartile range; $PM_{2.5}$ = mass concentration of particles less than 2.5 μm in diameter; PM_{10} = mass concentration of particles less than 10 μm in diameter; UFPs = ultrafine particles (number concentration of particles with a size range of 0.01 to 0.1 μm in diameter).

* For UFPs, 13% of the hourly measurements were imputed.

† For $PM_{2.5}$, 15% of the hourly measurements were imputed.

‡ For temperature, 0.5% of the hourly measurements were imputed.

§ For relative humidity, 0.5% of the hourly measurements were imputed.

Regression Results

Results for the regression of different blood markers are summarized in Table 4 (logistic regression) and Table 5 (linear regression). Effect estimates are presented together with 95% confidence intervals (95% CI) based on an increase in air pollution concentration from the first to the third quartile (interquartile range).

Inflammation and adhesion. For CRP, the odds of observing concentrations above the 90th percentile were consistently increased in association with PM_{10} and UFPs (Figure 3) as well as AP, NO_2, and CO for lag 2. The highest odds ratio (OR) was seen with AP, whereas EC and OC showed no significant results.

The OR for observing high ICAM-1 levels increased, especially for lag 1 and 2. This pattern was seen for PM_{10} (Figure 3), AP, EC and OC, and CO. For ICAM-1, a decrease with lag 0 was also found for most pollutants. Results for SAA indicate an increase in association with particulate air pollution (e.g., with UFP concentrations); however, results are not as strong and consistent as for CRP (Figure 3). Linear regression analyses looking at the continuous distribution did not reveal significant results for CRP, ICAM-1, and SAA. Also, E-selectin did not show any association with ambient air pollution (Figure 3).

Linear regression analyses of vWf (Table 5) revealed statistically significant positive associations for most pollutants with lag 0 and for the 5-d average exposure (Figure 3). For $PM_{2.5}$ and AP, the

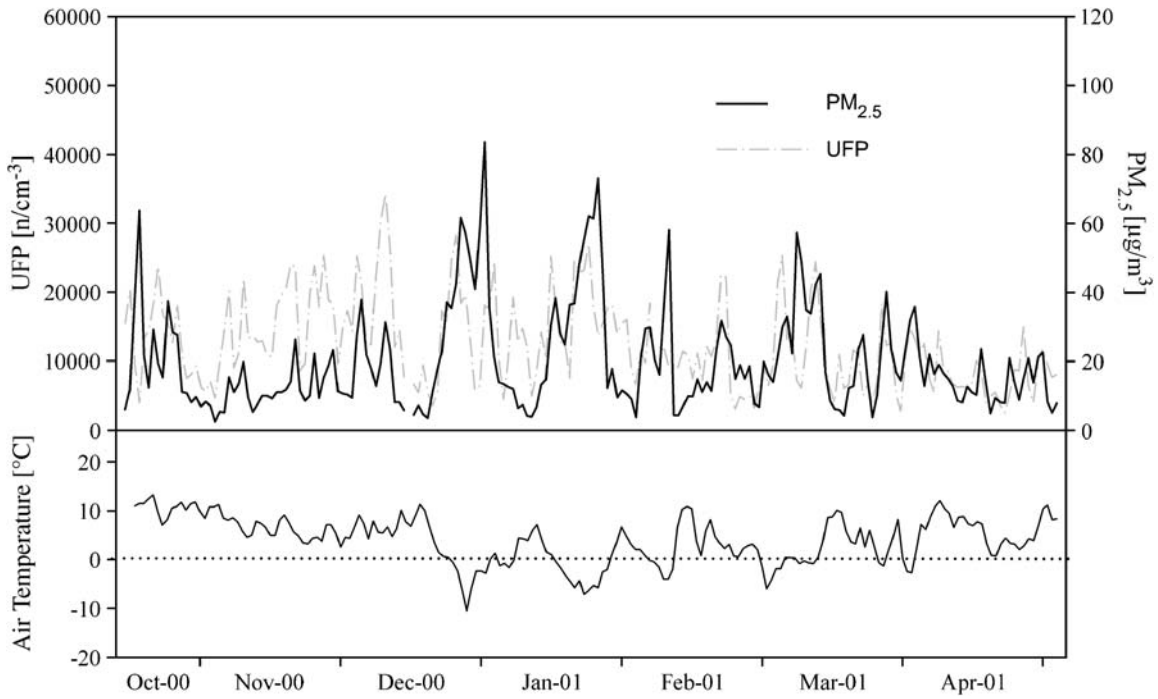

Figure 2. Time series of number concentrations of particles sized 0.01 to 0.1 μm (ultrafine particles [UFP]) and mass concentrations of particles less than 2.5 μm in diameter ($PM_{2.5}$) together with air temperature in Erfurt, Germany, between October 2000 and April 2001.

TABLE 3. BIOMARKERS FOR 57 MALE PATIENTS WITH CORONARY HEART DISEASE IN ERFURT, GERMANY, WINTER 2000/2001

Parameter	No.	Mean (*SD*)	Min.	Median	90th Percentile	Max.
CRP, mg/L	579	3.7 (6.5)	0.2	2.0	8.5	93.2
SAA, mg/L	579	6.9 (35.1)	0.80	3.0	8.5	761
Factor VII, % activity	543	124 (61)	42	118	158	839
vWf, % activity	549	135 (59)	21	134	209	393
Fibrinogen, g/L	577	2.9 (0.70)	1.24	2.90	3.81	5.73
Prothrombin fragments 1+2, nmol/L	543	1.5 (1.8)	0.7	1.24	1.7	19.6
D-dimer, μg/ml	549	0.7 (1.1)	0.2	0.42	1.3	10.0
ICAM-1, ng/ml	578	272 (75.7)	50.0	263	362	717
E-selectin, ng/ml	578	59.2 (122)	1.7	49.2	85.4	2,922

Definition of abbreviations: CRP = C-reactive protein; ICAM-1 = intercellular adhesion molecule 1; SAA = serum amyloid A; vWf = von Willebrand factor antigen.

effect was limited to the 5-d average exposure. Associations for the 1-d lag were found to be even stronger than for lag 0; however, this was not consistent throughout all pollutants.

Blood coagulation. In linear regression, a consistent decrease in the mean of percentage of activity was found with FVII for almost all pollutants for the 5-d average exposure, indicating a cumulative effect (Figure 3). With the exception of $PM_{2.5}$ and NO_2, this decrease was also consistently found for lag 2.

Logistic regression results for FVII were in agreement with the results of the linear regression.

For prothrombin fragment 1+2, the logistic regression revealed constant increases of the OR, with lag 4 showing a consistent pattern in all measured pollutants (data not shown). Fibrinogen only revealed very few significant effects, which might be due to chance. Analyses of D-dimer revealed a null result in linear as well as in logistic models (Figure 3).

Sensitivity analyses. Thorough sensitivity analyses were conducted for the logistic regression models comparing different models with varying number of confounder variables.

For CRP, adding temperature, relative humidity, and air pressure resulted in higher AIC values. In these models, the results for the two-lagged effect of UFPs and AP were confirmed; however, these had generally wider confidence intervals. For prothrombin fragment 1+2, the AIC was reduced by adding air pressure to the model. However, estimates were up to twofold higher and results for the AP destabilized. Therefore, the more conservative and more stable model was used. Throughout all models, stable results were found for lag 2 with the UFPs and with lag 4 for AP, PM_{10}, $PM_{2.5}$, EC, and OC. The results for ICAM-1 also remained stable throughout all models. Moreover, we conducted sensitivity analyses comparing the results for those patients who were on lipid-lowering drugs, primarily statins, with those who were not. Results for the linear regression show that the effects were mainly driven by the patients who were not on lipid-lowering medication. The effects were larger than the overall effects but had wide confidence intervals due to reduced power. Stratified analyses for CRP showed stronger effects in the patients taking statins.

We compared the results of a random-effects model with those of the fixed-effects model for the linear regression, showing consistent effect estimates (FVII, AP, 5-d average exposure: OR, −4.3; 95% CI, −8.1, −0.5). Some associations were found to have a nonlinear exposure response function as marked in Tables 4 and 5. Nonlinearity weakens the evidence for a strong influence of these pollutants; however, for the CRP, all associations were linear (Table 4).

DISCUSSION

Summary

Our findings suggest increases in CRP and ICAM-1 in association with ambient air particles. For these markers, the effects were limited to the higher values of the parameters, showing an increase in the odds of observing high levels of the respective parameters with elevated levels of air pollution. CRP rose with a delay of 2 d for all measured pollutants except for EC and OC. For ICAM-1, a 1- and 2-d delayed increase was associated with most pollutants.

Mean concentrations of vWf were shifted toward higher values revealing the strongest effect for the 5-d average exposure to almost all pollutants. Moreover, a rise in prothrombin fragment 1+2 in association with all pollutants was seen, which was consistent for lag 4. For FVII, clear and consistent negative associations were observed.

UFPs, NO_2, and CO were measured simultaneously and therefore allow a rough estimate on how well the two gases can be used as surrogates for the exposure to combustion-derived particles. Our results show a high correlation among these three pollutants, and also the estimates for the blood markers show comparable results, especially in logistic regressions. Similar conclusions have been drawn by Cyrys and colleagues (26), who found UFPs, NO_2, and CO to be strongly correlated and to reflect motor vehicle traffic.

Possible Mechanisms

This study was based on the following mechanistic hypotheses (Figure 1): Airway injury or activation of blood cells, such as monocytes, caused by particles deposited in the alveoli leads to a release of proinflammatory cytokines interleukin (IL)-6 and IL-8. Increased production of IL-6 and IL-8 activates mononuclear as well as endothelial cells, initiating the hepatic synthesis of acute-phase proteins, such as CRP and SAA, and an upregulation of the expression of adhesion molecules as markers of endothelial dysfunction. An enhanced acute-phase response as well as endothelial cell activation increase procoagulant activity, indicated by a rise in coagulation proteins or evidence of activation of the clotting cascade (27). These changes in blood parameters, together with plaque instability, may ultimately lead to a coronary event in susceptible patients.

Inflammatory Pathway

Regarding CRP, Seaton and coworkers (28) observed a positive association between exposure to ambient PM_{10} and CRP concentrations in elderly subjects in the United Kingdom. For CRP, our findings are in accordance with those of Seaton and colleagues

TABLE 4. EFFECTS OF AIR POLLUTION ON BLOOD PARAMETERS PRESENTED AS OR FOR AN INCREASE IN THE BLOOD MARKER ABOVE THE 90th PERCENTILE (LOGISTIC REGRESSION) PER INCREASE IN INTERQUARTILE RANGE AIR POLLUTANT IN 57 MEN WITH CORONARY HEART DISEASE IN ERFURT, GERMANY, WINTER 2000/2001

	Time Period before Blood Withdrawal	CRP		ICAM-1	
		OR	95% CI	OR	95% CI
PM_{10}	0 to 23 h	1.2	0.8, 1.9	1.3	0.9, 1.8
	24 to 47 h	2.0*	1.1, 3.6	3.1*‡	2.0, 4.8
	48 to 71 h	2.2*	1.2, 3.8	3.4*	2.2, 5.2
	5-d-mean	2.0*	1.2, 3.7	3.4*	2.2, 5.3
$PM_{2.5}$	0 to 23 h	1.1	0.7, 1.8	0.7*‡	0.4, 0.9
	24 to 47 h	1.5†	0.9, 2.5	1.3	0.8, 1.8
	48 to 71 h	1.2	0.8, 1.9	1.8*	1.2, 2.7
	5-d-mean	1.4	0.9, 2.3	1.1	0.8, 1.5
AP	0 to 23 h	0.7	0.5, 1.2	0.6*‡	0.4, 0.9
	24 to 47 h	1.5	0.9, 2.6	1.8*	1.2, 2.8
	48 to 71 h	3.2*	1.7, 6.0	1.6†	1.0, 2.5
	5-d mean	1.5	0.8, 3.0	0.9	0.6, 1.5
UFPs	0 to 23 h	0.7	0.5, 1.2	0.6*	0.4, 1.0
	24 to 47 h	1.2	0.7, 2.0	1.0	0.6, 1.6
	48 to 71 h	2.3*	1.3, 3.8	1.3‡	0.8, 2.2
	5-d mean	1.5	0.9, 2.6	0.8	0.5, 1.2
EC	0 to 23 h	1.2	0.7, 2.0	1.0‡	0.7, 1.6
	24 to 47 h	1.3	0.7, 2.4	2.6*‡	1.7, 3.8
	48 to 71 h	1.6	0.9, 2.7	4.0*	2.5, 6.1
	5-d mean	1.2	0.7, 2.1	2.2*‡	1.4, 3.3
OC	0 to 23 h	1.2	0.7, 1.9	0.9‡	0.6, 1.3
	24 to 47 h	1.3	0.8, 2.1	2.0*‡	1.3, 3.2
	48 to 71 h	1.4	0.8, 2.4	3.0*	1.8, 4.8
	5-d mean	1.2	0.7, 1.8	1.3‡	0.8, 2.0
NO_2	0 to 23 h	1.1	0.7, 1.8	0.6†	0.4, 1.0
	24 to 47 h	1.4	0.9, 2.3	1.0‡	0.7, 1.6
	48 to 71 h	2.0*	1.2, 3.3	1.0‡	0.6, 1.5
	5-d mean	1.4†	1.0, 2.0	0.8‡	0.6, 1.1
CO	0 to 23 h	0.9	0.7, 1.2	0.8*	0.6, 1.0
	24 to 47 h	1.0	0.7, 1.5	1.5*	1.2, 1.9
	48 to 71 h	1.5*	1.1, 2.1	1.7*‡	1.3, 2.3
	5-d mean	1.1	0.8, 1.6	1.2‡	1.0, 1.6

Definition of abbreviations: AP = accumulation mode particles (particles with a size range of 0.1 to 1.0 μm); CI = confidence interval; CRP = C-reactive protein; EC = elemental carbon; ICAM-1 = intercellular adhesion molecule 1; OC = organic carbon; OR = odds ratio; $PM_{2.5}$ = mass concentration of particles less than 2.5 μm in diameter; PM_{10} = mass concentration of particles less than 10 μm in diameter; UFPs = ultrafine particles (number concentration of particles with a size range of 0.01 to 0.1 μm).

* $p < 0.05$.

† $p < 0.1$.

‡ Nonlinearity observed in the model.

Final models: CRP, adjusted for relative humidity lag 4, ns (df = 2) + temperature lag 3, ns (df = 2) + trend (linear) + ID; ICAM-1, adjusted for temperature lag 0, ns (df = 4) + trend (linear) + ID.

and are also consistent with those previously reported from Augsburg, Germany (17). Increased concentrations of CRP are known to predict cardiovascular events in healthy subjects (29). Also, elevated levels of IL-6 have been found to be associated with total mortality (30) and with risk of future fatal and nonfatal MI (31). PM_{10} is suggested to affect the upper bronchi and therefore lead to an inflammation in the lung, whereas the smaller particles potentially transfer into the blood and start a systemic inflammatory response. Our data indicate a systemic delayed response to air pollution. According to Geiser (32), particles are rapidly translocated into the blood. It is therefore possible that the delay we observed is due to the time needed to initiate the acute-phase response after a rapid UFP translocation. With a half-life of 19 h (33), CRP is down-regulated rapidly and therefore does not show any elevation 3 to 4 d after an increase in air pollution.

Endothelial Dysfunction

Adhesion molecules, such as ICAM-1, mediate the contact between circulating leukocytes and endothelial cells. ICAM-1 induces a tight binding of leukocytes to the endothelium. In this way, leukocytes can leave the blood stream and enter the subendothelial space (34–37). ICAM-1 has been shown to predict acute coronary events as well as angina pectoris in a prospective cohort of apparently healthy men (38). Also, an increase in ICAM-1 in association with diesel exhaust was shown in bronchial biopsies in a panel of 15 human volunteers (39). Moreover, particles collected in Provo, Utah (40), enhanced the expression of ICAM-1 in primary cultures of human epithelium. Our data are in accordance with the literature indicating an up-regulation in ICAM-1 expression primarily with lag 1 and 2.

In addition, vWf may serve as a marker of endothelial dysfunction. In healthy mice, increased vWf expression on hepatic endothelium was detected after application of UFPs (41). vWf reflects endothelial cell release and probably vascular reactivity. Vascular reactivity could be secondary to inflammation, and because vWf can mediate platelet adhesion to damaged endothelium, this could be a predictor of coronary events (34, 42).

Coagulation Pathway

In contrast to our initial hypothesis, the various clotting factor levels showed no consistent pattern in association with air pollution.

FVII, one of the key enzymes of the extrinsic system of the coagulation cascade, is activated by tissue factor. Complexes of tissue factor with factor VIIa are central to the activation of factor X and to the formation of thrombin, which mediates the conversion of fibrinogen to fibrin (43). Results for FVII in the literature are inconsistent (28, 44). In our study, FVII activity decreased significantly in association with most pollutants.

Regarding fibrinogen levels, we did not find any consistent results in association with air pollution. Controlled human exposure studies (45, 46) as well as epidemiologic studies (47, 48) demonstrated positive associations between fibrinogen or plasma viscosity and air pollution. However, decreases in fibrinogen levels also have been reported and the significance of these results is unknown (28, 41).

Prothrombin fragment 1+2 is cleaved from prothrombin when it is activated to thrombin by factor Xa, thereby representing a marker of activation of the coagulation pathway (34, 49, 50). Our data indicate an increased concentration in association with ambient air pollutants. This significant increase indicates that an early step of blood coagulation has been activated. However, this activation was not associated with increased formation of fibrin, as would be detected by elevated D-dimer levels. The elevated levels of prothrombin fragment 1+2 are an important finding that shows that air pollution not only induces inflammation but also coagulation.

The large number of blood markers measured in this study revealed inconsistencies that were already observed in previous studies (13). One possible explanation is that various particle fractions or components differ in their effects. CRP, for example, did not show any association with EC and OC, whereas other blood markers showed quite strong effects. Moreover, diverse time patterns in the reaction to air pollution, due to the differing biological mechanisms, are conceivable and were also seen in the data.

While the results for inflammation and air pollution seem consistent, inhomogeneity exists in terms of coagulation markers. Our data strongly indicate that the pathway that links airway

TABLE 5. EFFECTS OF AIR POLLUTION ON BLOOD PARAMETERS PRESENTED AS PERCENTAGE OF CHANGE FROM THE MEAN/GEOMETRIC MEAN IN THE BLOOD MARKER (LINEAR REGRESSION) PER INCREASE IN INTERQUARTILE RANGE AIR POLLUTANT IN 57 MALE PATIENTS WITH CORONARY HEART DISEASE IN ERFURT, GERMANY, WINTER 2000/2001

		vWf		FVII	
	Time Period before Blood Withdrawal	% Change (*mean*)	95% CI	% Change (*GM*)[§]	95% CI
PM_{10}	0 to 23 h	4.0	−0.6, 8.5	−6.6*	−10.4, −2.5
	24 to 47 h	6.0*	0.6, 11.5	−8.4*†	−12.3, −4.3
	48 to 71 h	1.1†	−4.9, 7.0	−5.9*	−9.6, −2.0
	5-d mean	6.1	−0.6, 12.8	−8.0*	−12.4, −3.4
$PM_{2.5}$	0 to 23 h	3.9	−0.3, 8.1	−2.5	−6.2, 1.4
	24 to 47 h	3.1	−1.6, 7.8	−2.8	−6.1, 0.6
	48 to 71 h	3.6†	−1.1, 8.3	−2.3	−5.0, 0.6
	5-d mean	5.6*	0.5, 10.8	−3.5*	−6.4, −0.4
AP	0 to 23 h	4.8*†	0.2, 9.3	0.0	−2.9, 3.0
	24 to 47 h	5.9*†	0.4, 11.5	−2.9‡	−6.1, 0.4
	48 to 71 h	7.0*	0.7, 13.4	−3.6*	−6.8, −0.3
	5-d mean	13.5*	6.3, 20.6	−4.1*	−7.9, −0.3
UFPs	0 to 23 h	3.4	−2.2, 9.0	1.3†	−2.3, 5.1
	24 to 47 h	3.5	−2.7, 9.7	−6.6*	−10.6, −2.4
	48 to 71 h	3.1	−3.4, 9.6	−6.4*	−10.4, −2.3
	5-d mean	7.8*	1.4, 14.3	−6.6*	−10.7, −2.3
EC	0 to 23 h	5.0*	0.0, 10.1	−5.7*	−10.5, −0.7
	24 to 47 h	7.6*	1.4, 13.7	−6.9*†	−11.2, −2.3
	48 to 71 h	1.1†	−5.2, 7.4	−4.2‡	−8.4, 0.2
	5-d mean	5.7†‡	−0.5, 12.0	−6.0*	−10.5, −1.2
OC	0 to 23 h	5.5*	0.2, 10.8	−6.1*	−10.6, −1.4
	24 to 47 h	8.0*	2.1, 13.9	−7.2*	−11.4, −2.8
	48 to 71 h	3.5†	−2.6, 9.6	−3.8	−8.2, 0.9
	5-d mean	7.4*†	2.0, 12.8	−5.6*	−9.8, −1.1
NO_2	0 to 23 h	5.7*	0.7, 10.7	−3.0	−6.7, 0.9
	24 to 47 h	4.1	−1.2, 9.4	−2.0†	−5.5, 1.7
	48 to 71 h	3.9	−1.5, 9.4	−1.5†	−4.8, 1.9
	5-d mean	5.7*	1.6, 9.8	−2.8‡	−5.5, 0.0
CO	0 to 23 h	4.4*	1.4, 7.5	−1.4	−3.8, 1.1
	24 to 47 h	2.7†	−0.8, 6.1	−2.6*†	−4.8, −0.3
	48 to 71 h	2.0†	−1.7, 5.8	−2.8*	−5.1, −0.4
	5-d mean	4.9*	1.0, 8.8	−3.0*	−5.5, −0.4

Definition of abbreviations: EC = elemental carbon; FVII = factor VII; OC = organic carbon; $PM_{2.5}$ = mass concentration of particles less than 2.5 μm in diameter; PM_{10} = mass concentration of particles less than 10 μm in diameter; UFPs = ultrafine particles (number concentration of particles with a size range of 0.01 to 0.1 μm); vWf = von Willebrand factor antigen.

* $p < 0.05$.

† Nonlinearity observed in the model.

‡ $p < 0.1$.

§ Percentage of change per interquartile range increase in air pollution.

Final models: vWf: air pressure, lag 1, ns (df = 2) + relative humidity, lag 1, ns (df = 4) + air temperature, lag 3, ns (df = 5) + trend, ns (df = 3)+ ID + weekday; factor VII: air pressure, lag 3, ns (df = 4) + relative humidity, lag 0, ns (df = 5) + air temperature, lag 1, ns (df = 3) + trend, ns (df = 4) + ID + weekday.

injury from air pollution and coronary events may include increased expression of adhesion molecules and a proinflammatory response. Furthermore, the coagulation system (prothrombin fragment 1+2) is activated, although not sufficiently enough to cause increased fibrin formation, as would have been reflected by elevated D-dimer levels. We do not have an explanation for the decreased concentrations of fibrinogen and FVII, but, whatever the cause, they may act to protect against clinical events secondary to coronary thrombosis. Our study represents measurements of background levels of blood markers and does not reflect changes that might relate to acute clinical events.

Strengths and Limitations

A strength of this study is the achievement of 99% of all scheduled visits. Moreover, a wide range of markers, reflecting different pathways, were measured within the same setting. All of the measured biomarkers may increase in response to a number of unspecific stimuli, such as infectious diseases or surgery. CRP is particularly sensitive and can increase a thousandfold within a short time in response to such triggers (34). Therefore, we excluded all blood samples that potentially might have been affected by sources other than air pollution. All patients were asked about infections and surgery in the 2 wk before the blood withdrawal. Reasons for physician visits as well as hospital admissions were recorded, and study nurses documented signs of an acute respiratory infection in the patient during regular clinic visits. None of the samples that revealed especially high or low levels of the respective biomarker were excluded unless a reason was known.

We used logistic regression analyses in addition to linear regression because Peters and colleagues (16) had suggested that effects of air pollution may be seen on a small number of subjects with high values of a particular marker with less effect on the mean level. Also, some parameters showed a few extreme outliers, which strongly influenced the regression results.

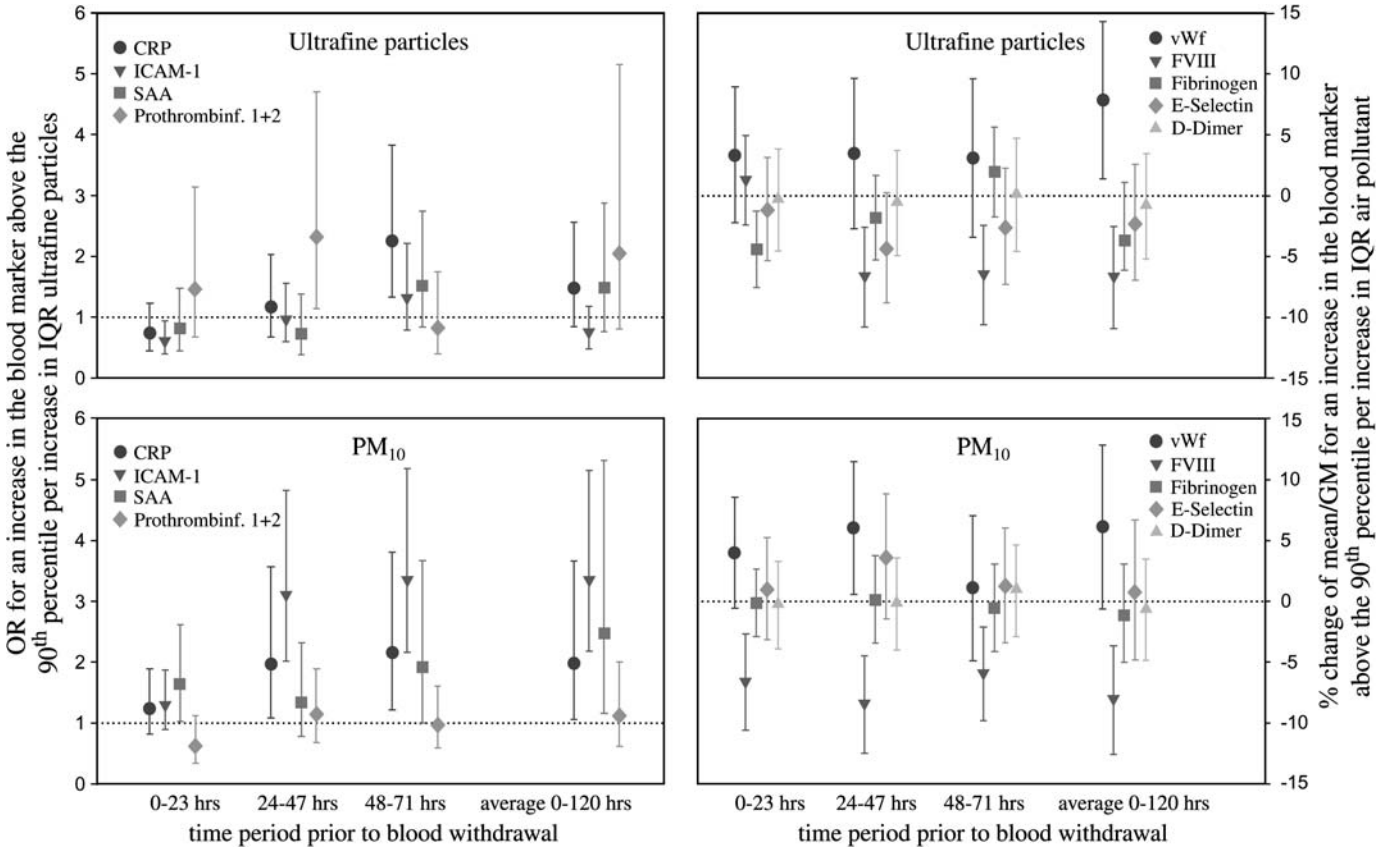

Figure 3. Effects of UFPs and PM_{10} on blood markers of inflammation, endothelial dysfunction, and coagulation; lag 0 to lag 2 and 5-d average exposure. Fifty-seven male patients with coronary heart disease in Erfurt, Germany, winter 2000/2001. GM = geometric mean; IQR = interquartile range; OR = odds ratio.

Although the fixed-effect models were adjusted for individual time-invariant factors, by design no adjustment for time-dependent individual-level variables was possible.

A variety of pollutants were used for the analyses, because different pollutants may point toward different properties of the aerosol, and also represent different sources of air pollution. However, by testing multiple blood parameters and a set of air pollutants, the possibility that some effects might have occurred by chance cannot be excluded. Because the air pollution parameters are closely correlated, we considered especially consistent patterns in the data as actual effects. Moreover, thorough confounder adjustment for meteorologic variables was done to rule out the possibility that the detected associations resulted from meteorologic influences or seasonal differences.

Only one central measurement site was used for the collection of ambient air pollution. However, the spatial representativeness of this site has been analyzed in detail previously by Cyrys and colleagues (51), who measured sulfate and PM_{10} levels simultaneously at three additional monitoring sites in the Erfurt area. The relatively high intersite correlation between the monitoring stations (0.69–0.98) indicates that regional episodes of sulfates and PM_{10} in Erfurt can be identified using one fixed monitoring site and that our site is generally representative for the urban background level of air pollution within Erfurt. Erfurt is a small city with one air mass confined by a mountain ridge on three sides and high rises on the fourth side. Because most of the participants of our panel were already retired, we assume that they spent the greater part of their day within the vicinity of their residence within the city of Erfurt.

Many studies have demonstrated that individual exposures to PM are poorly correlated spatially with ambient concentrations (52). Some longitudinal exposure assessment studies of PM and specific PM components with repeated measures have found higher correlations between personal exposures and ambient concentrations. Janssen and coworkers (53) showed, for example, that ambient, indoor, and personal concentrations of $PM_{2.5}$ were highly correlated in two European cities.

However, the correlation many epidemiologists are interested in is not that between total personal exposure and outdoor concentrations but the correlation between that component of personal exposure that can be attributed to outdoor particles and the outdoor concentrations. Ebelt and colleagues (54) demonstrated that ambient concentrations and the contribution of ambient particles to personal PM exposure were highly correlated, with a Pearson correlation coefficient of 0.81 for $PM_{2.5}$, of 0.71 for PM_{10} and of 0.73 for the coarse fraction ($PM_{10} - PM_{2.5}$). Moreover, they show that ambient concentrations and exposure to nonambient $PM_{2.5}$ are independent, which is an important assumption in epidemiologic studies that use ambient concentration as a surrogate for personal exposure. They conclude that their results give support to the use of ambient monitoring data in time series analyses. Cyrys and coworkers (55), who compared the relationship of indoor and outdoor levels of fine-particulate mass, particle number concentrations, and black smoke, concluded that ambient concentrations of $PM_{2.5}$ and black smoke can be used as good approximations of indoor concentrations.

A limitation to the study is that the examined panel consisted of male patients only, with a history of CHD, who were all taking

cardiac medication. Therefore, they represent a highly selected group and the study results might not be generalizable to other population groups, such as females with CHD or healthy subjects.

A differentiation between chronic and acute effects of higher levels of blood markers in the patients is not possible with this study design. Because of the short observation time, it is not clear whether these changes can lead to an onset or exacerbation of the disease. We observed short-term changes in various blood parameters; however, the implications for patients remain speculative. On the other hand, changes in blood markers due to air pollution have recently been observed not only in patients with CHD but also in young and healthy persons (56).

Conclusions

The study adds to the evidence that elevated levels of ambient air pollution may cause systemic inflammatory and coagulation responses. These changes in blood markers could represent additional risk factors, which, in susceptible individuals, such as patients with CHD, could increase the likelihood of serious arterial vascular thrombotic events on exposure to high levels of air pollutants.

Conflict of Interest Statement: None of the authors have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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Appendix V

AIRGENE Questionnaires

BASELINE QUESTIONNAIRE

AIRGENE

ID Number:

(To be completed via interview)

Examiner please note that fields marked with ✘ refer to inclusion or exclusion criteria; other questions are for baseline characterization!

---

1. Interviewer ID _____

2. ID-Number: _____

3. Date of birth: |___|___|.|___|___|.|___|___|
   d d m m y y

4. Sex: ❑ Male ❑ Female

5. Marital Status:

   married ❑ single ❑ divorced ❑ widowed ❑

6. Ethnicity **(INT: please do not read this question out loud except when you are unsure)**

   caucasian ❑ black ❑ asian ❑ mixed ❑

7. Education

   7.1. School years (including primary school) |___|___| years

   7.2. Professional Training |___|___| years

   7.3 College/University |___|___| years

Inclusion criteria

8. INT: if known, do not read out loud

✘ Did a doctor diagnose a myocardial infarction? ❑ Yes ❑ No

(if 'No' subject has to be excluded)

if 'Yes':

8.1 How many infarctions have you had? |___|

8.2 When did you have your first infarction? |___|___|.|___|___|.|___|___|
d d m m y y

8.3 ✘ When did you have your last infarction? |___|___|.|___|___|.|___|___|
d d m m y y

(if less than 6 months ago or more than 5 ½ years ago subject has to be excluded)

8.4. ✘ Age at onset of last MI |___|___|

(if less than 35 years or more 74 years subject has to be excluded)

9. Did your mother have a myocardial infarction? ❑ Yes ❑ No ❑ don´t know

9.1 If 'Yes' at what age? (*first infarction*)?

at |___|___| years of age
❑ don´t know

10. Did your father have a myocardial infarction? ❑ Yes ❑ No ❑ don´t know

10.1 If 'Yes': at what age (*first infarction*)?

at |___|___| years of age
❑ don´t know

11. ✘ Have you had recent (< 6 months) coronary by-pass surgery or a balloon dilatation (PTCA)?
(if 'Yes' subject has to be excluded) ❑ Yes ❑ No

12. ✘ Have you smoked during the past 3 months? ❑ Yes ❑ No
(preferably current non-smokers only)

if 'Yes':

12.1 had you less than 1 cigarette, cigar or pipe of tobacco a day? ❑ Yes, less
❑ No, one or more

13. ✘ Do you suffer from any of the following diseases which might prohibit participation in the study:
(if 'Yes' subject has to be excluded)

13.1 Crohns disease ❑ Yes ❑ No

13.2 Ulcerative colitis ❑ Yes ❑ No

13.3 Rheumatoid arthritis ❑ Yes ❑ No

13.4 Hemophilia ❑ Yes ❑ No

13.5 HIV ❑ Yes ❑ No

14. ✘ Do you plan to leave your home for months during the study period?
(if 'Yes' subject has to be excluded) ❑ Yes ❑ No

15. ✘ Are you able to come to the study center once every four weeks for another 5 visits?
(if 'No' subject has to be excluded) ❑ Yes ❑ No

16. ✘ At each visit a blood sample will be taken. Do you agree to have your blood taken?
(if 'No' subject has to be excluded) ❑ Yes ❑ No

Disease History

17. Did a doctor ever diagnose one or more of the following diseases?
Please ask if you don´t understand one of the following:

17.1	Angina Pectoris	❑ Yes ❑ No
17.2	Arrhythmias	❑ Yes ❑ No
17.3	Congestive heart failure	❑ Yes ❑ No
17.4	Other heart problems	❑ Yes ❑ No
17.5	Diabetes	❑ Yes ❑ No

If 'yes':

17.5.1 Type of diabetes

Type I ❑
Type II ❑
don't know ❑

17.5.2

Year of diagnosis |___|___|___|___|
or
Age at diagnosis |___|___|

17.6	Hypertension	❑ Yes ❑ No
17.7	Stroke	❑ Yes ❑ No
17.8	Asthma	❑ Yes ❑ No
17.9	Chronic bronchitis	❑ Yes ❑ No
17.10	Emphysema	❑ Yes ❑ No
17.11	Hay fever	❑ Yes ❑ No
17.12	Chronic renal diseases	❑ Yes ❑ No
17.13	Arthrosis	❑ Yes ❑ No
17.14	Other chronic diseases	❑ Yes ❑ No

Specify: _____

18.1 Have you had wheezing or whistling in your chest at any time in the last ***12 months***? ❑ Yes ❑ No

if 'Yes'**:**

18.1.1 Have you had this wheezing or whistling when you did ***not*** have a cold? ❑ Yes ❑ No

18.2 Do you ***usually*** cough during the day, or at night, in the winter? ❑ Yes ❑ No

if 'Yes'**:**

18.2.1 Do you cough like this on most days for as much as three months each year? ❑ Yes ❑ No

18.3 Do you ***usually*** bring up any phlegm from your chest during the day, or at night, in the winter? ❑ Yes ❑ No

if 'Yes'*:*

18.3.1 Do you bring up phlegm like this on most days for as much as three months each year ? ❑ Yes ❑ No

---

Health status and CCS functional class

19. Do you have a cardiac pacemaker? ❑ Yes ❑ No

20. In your opinion, your health status compared to your age group is in general:

❑ excellent

❑ good

❑ moderate

❑ bad

❑ very bad

21. **Which of the following daily activities can you manage without angina pectoris symptoms?**

(Only one answer possible)

21.1 *Vigorous activities*, such as lifting heavy objects, shoveling snow or participating in strenuous sports such as skiing, ball games or running ❑

21.2 *Moderate activities,* such as walking, climbing stairs rapidly or after meals, moving a table, pushing a vacuum cleaner, bowling, or playing golf ❑

21.3 *Ordinary activities*, such as walking one or two blocks, or climbing 1 flight of stairs at a normal pace under normal conditions ❑

21.4 *No physical activity* without discomfort or angina symptoms even at rest ❑

Physical activity

The following questions refer to physical activity, separate for light physical activity and vigorous physical activity.

22. Light physical activities are activities that make you breathe hard like brisk walking, hiking, dancing, light gardening, heavy housework or other light physical activities.

22.1 On average, on how many days per week do you engage in such activities (0 to 7 days)? |___|

22.2 On average, how long do you engage in such activities on those days? _____min

22.3 How many years or months have you been doing these activities?

|___|___| years |___|___| months

23. Vigorous physical activities are activities that make you sweat that you perform for at least 20 min (e.g. jogging, aerobics, swimming, fast cycling, ball games, heavy gardening)
On average, how many days a week do you pursue vigorous physical activities? |___|

Work and living conditions

24. Are you currently working? ❑ Yes ❑ No
INT: yes also if currently away from work because of illness

24.1 if 'Yes', do you work:

full time ❑

part time ❑

24.2 Are you exposed to fumes, gases or dust at work? ❑ Yes ❑ No

if 'Yes', specify:_____

24.3 Do people smoke regularly in a room where you work? ❑ Yes ❑ No

25. How old is the house you live in **|___|___|** or
when was it built? |___|___|___|___|

26. Is your home/apartment situated close to a busy road, that is, at most 50m away from it? ❑ Yes ❑ No
(busy road = at least 10.000 vehicles per day)
☞ *(please provide examples)*
if 'No': go to question 28

if 'Yes':

27. Does your home/apartment have one or more windows that face a busy canyon road? ❑ Yes ❑ No

(busy canyon road = at least 10.000 vehicles per day with houses on both sides of the street for at least 100m; the width of the space between the houses is less than 3 times the average height of the houses
☞(please provide example)

if 'Yes':

27.1 How many floors does the house you live in have? |___|___| floors
INT: Including ground floor (basement=0, ground floor=1, one storey up = 2 etc.)

27.2 On which floor is your apartment? Floor |___|___|
INT: Including ground floor (basement=0, ground floor=1, one storey up = 2 etc.)

28. How much are you annoyed by the traffic noise at home if the windows are kept open?
(please circle a number from 0-10 on the scale)
☞(please provide a scale and read off the corresponding number) |___|

29. How much are you annoyed by air pollution at home, i.e., smell of traffic and industry, when you keep the window open?
(please circle a number from 0-10 on the scale)
☞(please provide a scale and read off the corresponding number) |___|

30. During the summer months, do you usually sleep with the windows open? ❑ Yes ❑ No

31. During the winter months, do you usually sleep with the windows open? ❑ Yes ❑ No

32. Does your home have central heating? ❑ Yes ❑ No

32.1 If 'No', which of the following fuels do you use regularly for heating?

(more than one answer possible)

coal, wood ❑
gas ❑
electricity ❑
oil ❑

33. Do you regularly use a gas range or gas oven for cooking? ❑ Yes ❑ No

34. Do you have air conditioning? ❑ Yes ❑ No

35. Did you drink any alcoholic beverages in the past 12 months? ❑ Yes ❑ No

if 'No': go to question 38

if 'Yes':

36. How much beer, wine and liquor did you drink during the last weekend? (Friday evening, Saturday and Sunday)

beer or cider (accurate to 0,5 l) |___|___|,|___| liters

light beer (accurate to 0,5 l) |___|___|,|___| liters

alcohol free beer (accurate to 0,5 l) |___|___|,|___| liters

wine /sparkling wine (accurate to 0,2 l) |___|___|,|___| liters

liquor |___|___| glasses à 0,02 l

37. How much beer, wine and liquor did you drink at the last working day? If this day is a Friday please relate to the day before (Thursday)

beer or cider (accurate to 0,5 l) |___|___|,|___| liters

light beer (accurate to 0,5 l) |___|___|,|___| liters

alcohol free beer (accurate to 0,5 l) |___|___|,|___| liters

wine /sparkling wine (accurate to 0,2 l) |___|___|,|___| liters

liquor |___|___| glasses à 0,02 l

38. Does anyone, not including yourself, smoke regularly inside your home? ❑ Yes ❑ No

If 'Yes':

38.1 How many people, not including yourself, in your household smoke? |___|___|

39. Have you ever smoked cigarettes for as long as a year?

❑ No

❑ Yes, < 1 cig per day (occasional smoker)

❑ Yes, >= 1 cig per day

If 'No' or '< 1 cig per day': Please go to question 41

If 'Yes':

39.1 How old were you when you started smoking cigarettes? |___|___| years

39.2 How many years did you smoke 1 to 10 cigarettes per day? |___|___| years

39.3 How many years did you smoke 11 to 20 cigarettes per day? |___|___| years

39.4 How many years did you smoke 21 to 30 cigarettes per day? |___|___| years

39.5 How many years did you smoke more than 30 cigarettes per day? |___|___| years

if more than 0 years:
39.5.1 How many cigarettes per day did you smoke on average at that time? |___|___|

39.6. Did you stop smoking cigarettes at any time and then start again? ❑ Yes ❑ No

if 'Yes':
39.6.1 How many years in total did make such cigarette smoking breaks? |___|___| years

40. Have you now stopped smoking cigarettes? ❑ Yes ❑ No

40.1 if 'Yes', When did you give up smoking cigarettes? Year |___|___|___|___|
or
Age |___|___| years old

40.2 if 'No': How many cigarettes do you currently smoke per day on average? |___|___|

INT: please check that the whole cigarette smoking history is covered.
(Calculation: 40.1 (or age today if still smoking cigarettes) minus 39.1 (age at start) minus 39.6.1 (years of cigarette breaks) should equal 39.2 plus 39.3 plus 39.4 plus 39.5)
If there are problems, try to ask the patient again

41. Have you ever smoked cigars/cigarillos/pipes for as long as a year? ❑ Yes ❑ No

If 'No':

Please go to the Food Frequency Questionnaire.

if 'Yes':

41.1 How old were you when you started smoking cigars/cigarillos/pipes? |___|___| years

41.2 How much did you smoke on average during your years of smoking cigarillos, cigars or pipe?

Number of cigarillos per day |___|___|

Number of cigars per day |___|___|

Pipes per week |___|___|___|

41.3 Did you stop smoking cigars/cigarillos/pipe at any time and then start again? ❑ Yes ❑ No

if 'Yes':

41.3.1 How many years in total did you make such smoking breaks? |___|___| years

42. Have you now stopped smoking cigars/cigarillos/pipe? ❑ Yes ❑ No

42.1 if 'Yes': When did you give up smoking cigars/cigarillos/pipe? Year |___|___|___|___| or Age |___|___| years old

42.2 if 'No': How much do you currently smoke on average?

Number of cigarillos per day |___|___|

Number of cigars per day |___|___|

Pipes per week |___|___|___|

Please go to the Food Frequency Questionnaire

Evaluation for participation

⚠ *For Nurses and Doctors only, please do **not** read to participant!*

43. ✘ Is the patient physically and mentally able to participate? ❑ Yes ❑ No

44. ✘ Is there any other indication to exclude the patient from the study? ❑ Yes ❑ No

If 'Yes', specify:_____

45. Patient read and signed the consent form and got a copy? ❑ Yes ❑ No

46. Date |___|___|.|___|___|.|___|___|
d d m m y y

Notes/Comments:_____

_____

ID-No.: |__|__|__|__|__|__|

Short Questionnaire AIRGENE **Date:** |___|___|.|___|___|.|___|___|
d d m m y y

DOCTOR VISITS AND SYMPTOMS

The examinations of the AIRGENE study can only be carried out, if there are no conditions inducing inflammation. Please answer the following questions regarding symptoms and doctor's visits:

1. Did you have a cold or flu in the past 3 days? ❑ Yes ❑ No
2. Did you have an acute urinary tract infection during the past 3 days? ❑ Yes ❑ No
3. Did you have an acute gastro-intestinal infection during the past 3 days? ❑ Yes ❑ No
4. Did you have an acute respiratory infection during the past 3 days? ❑ Yes ❑ No

---

⚠ *Examiner only, please do not read out loud:*

5. Do you see any sign of a respiratory infection? ❑ Yes ❑ No

---

6. Did you have a surgery during the past 3 days? ❑ Yes ❑ No
7. Did you have a severe dental intervention? ❑ Yes ❑ No

⚠ ***If any of the above questions is answered with yes, the examination has to be postponed. Please make a new appointment with the patient.***

8. Did you visit a doctor/dentist or were you admitted to a hospital for any acute illness during the past week? ❑ Yes ❑ No

8.1 if Yes, for what reason:

- Angina Pectoris? ❑
- Urinary tract infection? ❑
- Gastro-intestinal infection ❑
- Respiratory condition? ❑
- Other condition ❑ (specify):_____

LIVING CONDITIONS/NUTRITION

9.How did you get here?

❑ walked ❑ cycled ❑ other

10. Did you smoke during the past 24 hours? ❑ Yes ❑ No

if Yes:

10.1 How many cigarettes/cigars/cigarillos/pipes did you smoke? |___|___|

10.2 When was your last cigarette/cigar/cigarillo/pipe |___|___|:|___|___|
h h m m

11. Were you in rooms where people smoked during the last 24 hours? ❑ Yes ❑ No

if Yes:

11.1 How long did you spend there? (1h accuracy) |___|___| hours

❑ Less than 1 hour

11.2 When did you leave |___|___|:|___|___|
h h m m

12. When did you last have something to eat (including snacks) |___|___|:|___|___|
INT: Time the last meal/snack was finished h h m m

13. Did you drink any black or green tea in the past 24 hours? ❑ Yes ❑ No

13.1 if yes: At what time was the last tea? |___|___|:|___|___|
h h m m

14. Did you drink any alcoholic beverages in the past 24 hours? ❑ Yes ❑ No

14.1 if yes: At what time was the last drink? |___|___|:|___|___|
h h m m

15. Did you exert yourself in the past 24 hours?
(exertion = physical activities that made you breathe hard like brisk walking, hiking, dancing, light gardening, heavy housework or other physical activities) ❑ Yes ❑ No

if yes:

15.1 How long was the exertion? |___|___|___| minutes

15.2 When was the end of the exertion? |___|___|:|___|___|
h h m m

16. Did you feel extreme anger or stress during the past 24 hours? ❑ Yes ❑ No

MEDICATION

17. Have there been any changes in your medication since your last visit? ❑ Yes ❑ No

17.1if Yes:

Have you stopped taking a medication? ❑ Yes ❑ No

⚠ *Please enter the date of last intake into the medication sheet.*

17.2 Did you take or have you started taking a new medication? ❑ Yes ❑ No

⚠ *Please check the diary and update the medication sheet.*

18 Has the dosage of your medication changed since the last visit (more or less tablets, different concentration or ingredient? ❑ Yes ❑ No

⚠ *Please enter the date of last intake into the medication sheet and enter the medication with the new dosage in the medication sheet, using a new number.*

19 Which medication did you take during the past 7 days?

Med No.	**Full name of medication**	**daily**	**on demand**	**other schedule**	**specify**

⚠ Please make sure that all medication reported here is also documented in the medication sheet!

Medication sheet

ID-No.: |___|___|___|___|

Do you take any medication? Yes No

Sheet Nr: |___|

Med. No.	**Full name of medication**	**ATC-Code** *(7 digits)*	**Concentration of active ingredient**	**Total dose** (number of pills etc. per day) (if not taken daily, use /: 1/week for 1 pill a week)	**On demand**	**Start (Date)** dd.mm.yy (only if during study)	**> 7 days before start of study**	**End (Date)** dd.mm.yy (only if during study)	**Continued at end of study**
						|__|__|.|__|__|.|__|__|		|__|__|.|__|__|.|__|__|	
						|__|__|.|__|__|.|__|__|		|__|__|.|__|__|.|__|__|	
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						|__|__|.|__|__|.|__|__|		|__|__|.|__|__|.|__|__|	

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PERSONAL CONTRIBUTION

As part of the AIRGENE team I worked on the development of the questionnaires and standard operation procedures used in the AIRGENE study. I was also involved in training the study nurses and responsible for the quality assurance of the study.

I took part in drafting the analyses concept, analysed parts of the data and drafted the two first author manuscripts. I critically reviewed the manuscripts in which I am listed as co-author.

Hiermit erkläre ich, Regina Rückerl, dass ich die vorliegende Dissertation selbständig angefertigt habe. Ich habe mich außer der angegebenen keiner weiteren Hilfsmittel bedient und alle Erkenntnisse, die aus dem Schrifttum ganz oder annähernd übernommen sind, als solche kenntlich gemacht und nach ihrer Herkuft unter Bezeichnung der Fundstelle einzeln nachgewiesen. Ich habe bisher noch keinen Promotionsversuch unternommen, und die vorliegende Dissertation wurde nicht in gleicher oder ähnlicher Form bei einer anderen Stelle zur Erlangung eines akademischen Grades eingereicht.

München, den 09.09.2009

(Regina Rückerl)

PUBLICATIONS

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