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Environmental Effects and Gene-Environment Interactions: Air Pollution and Temperature Effects on Cardiovascular Risk Factors.

Thesis
Submitted for a Doctoral degree in Human Biology at the Faculty of Medicine, Ludwig-Maximilians-University, Munich, Germany

by
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from
Nördlingen
2011

With approval of the Medical Faculty
of the Ludwig-Maximilians-University of Munich

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Date of oral examination:	15.12.2011

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List of abbreviations

ACh	Acetylcholine
BMI	Body mass index
CHD	Coronary heart disease
ChT	Choline transporter
CHT1	Choline transporter gene
CRP	C-reactive protein
ECG	Electrocardiogram
HR	Heart rate
HRV	Heart rate variability
IGT	Impaired glucose tolerance
IL-6	Interleukin 6
NFE2L2	Nuclear factor (erythroid-derived 2)-like 2
PM	Particulate Matter
$PM_{2.5}$	PM with an aerodynamic diameter below 2.5µm
PM_{10}	PM with an aerodynamic diameter below 10µm
PNC	Particle number concentrations
RMSSD	Root mean square of successive differences
SDNN	Standard deviation of normal-to-normal intervals
SNP	Single nucleotide polymorphism
UFP	Ultrafine particles
vWf	Von Willebrand factor

1 Summary

Epidemiological studies have shown that elevated air pollution levels and day-to-day variations in air temperature are associated with increases in cardiovascular events such as arrhythmias, myocardial infarctions, and sudden cardiac death. Precursors of these events might be acute changes in heart rate, a reduced heart rate variability (HRV), and changes in the repolarization of the heart, such as QTc-prolongation as well as changes in T-wave amplitude. Furthermore, elevated levels of blood markers of inflammation and coagulation might also lead to the observed adverse cardiac health outcomes. There is already a large body of literature with regard to air pollution effects on HRV parameters and blood markers but the exact biological pathways are still unclear. Little is known about the association between temperature and HRV as well as blood markers. Moreover, potential mechanisms how air pollutants and temperature affect repolarization have received less attention. Researchers have reported that individuals with genetic predispositions or underlying diseases such as diabetes mellitus type 2 might be more susceptible to air pollution exposure. Therefore, more comprehensive investigations in these groups of individuals are necessary in order to gain a better insight in the biological mechanisms.

In the first publication of this thesis, I examined the effects of air temperature on markers of inflammation and coagulation in men with coronary or pulmonary disease. A temperature decrease was associated with changes in several blood biomarkers such as platelet counts, factor VII, fibrinogen, and C-reactive protein. However, the direction and timing of the relationship differed between patients with coronary and pulmonary disease. In a second publication, I observed a prolongation of the QT-interval in association with elevated levels of particulate matter (PM) in myocardial infarction survivors. This association was more pronounced in participants with at least one minor allele of the *NFE2L2* single nucleotide polymorphism (SNP) rs2364725 which is believed to be involved in the defense against oxidative stress. Furthermore, I detected immediate T-wave flattening and delayed increases in T-wave amplitude associated with elevated air pollution levels. The association between temperature and the T-wave amplitude was inversely U-shaped with highest values at 5°C. In a third study among participants with diabetes or impaired glucose tolerance (IGT), I detected reduced HRV, predominantly the standard deviation of normal-to-normal intervals, in association with increases in PM and ultrafine particles. These effects were more pronounced in participants with IGT. I also observed air pollution effect modifications by SNPs supposed to influence cardiac rhythm.

In conclusion, this thesis confirms and extends published results on short-

term air pollution effects on intermediate markers of cardiovascular system. Furthermore, it is among the first to examine air temperature effects on blood and ECG parameters. Certain medical conditions as well as certain genetic profiles seem to make some subpopulations more susceptible to environmental stressors. The observed changes in HRV and blood markers might partly explain the reported associations between environmental conditions and cardiovascular events.

2 Zusammenfassung

Epidemiologische Studien haben gezeigt, dass erhöhte Schadstoffkonzentrationen und Änderungen der Lufttemperatur mit sofortigen und verzögert auftretenden kardiovaskulären Ereignissen wie Arrhythmien, Herzinfarkten und plötzlichem Herztod zusammenhängen. Diesen Ereignissen gehen möglicherweise eine akute Änderung der Herzfrequenz, eine verminderte Herzratenvariabilität (HRV) und eine Veränderung des Repolarisationvorgangs des Herzens voraus wie etwa ein Verlängerung des QTc-Intervalls oder Änderungen der T-Wellen Amplitude. Darüberhinaus könnten auch erhöhte Entzündungs- und Gerinnungsmarker im Blut das Risiko für kardiovaskuläre Ereignisse erhöhen. Es wurden bereits eine Vielzahl von Studien zu Schadstoffeffekten auf HRV- und Blutparameter veröffentlicht. Allerdings sind die genauen biologischen Abläufe noch immer unklar. Es ist wenig über den Zusammenhang zwischen der Temperatur und HRV- und Blutparametern bekannt. Desweiteren wurden die potentiellen Mechanismen des Einflusses von Schadstoffen und Temperatur auf die Repolarisation bisher kaum untersucht. Studien haben außerdem gezeigt, dass Personen mit genetischen Prädispositionen oder mit bestimmten Grunderkrankungen, wie Diabetes mellitus Typ 2, vermutlich empfindlicher auf erhöhte Luftschadstoffkonzentrationen reagieren. Um einen besseren Einblick in die biologischen Mechanismen zu erhalten, sind folglich Studien mit Individuen, die möglicherweise suszeptibel auf Umwelteinflüssen reagieren, notwendig.
In dieser Arbeit habe ich den Effekt der Temperatur auf inflammatorische und koagulatorische Blutmarker bei Männern mit einer zugrunde liegender Herz- oder Lungenerkrankung untersucht. Ein Absinken der Temperatur zeigte eine Assoziation mit mehreren Blutmarkern unter anderem mit der Thrombozytenanzahl, Faktor VII, Fibrinogen und C-reaktivem Protein. Allerdings unterschieden sich die Richtung und der zeitliche Zusammenhang der Temperatureffekte zwischen herz- und lungenkranken Probanden. In einer zweiten Veröffentlichung beobachtete ich bei Herzinfarktüberlebenden eine Verlängerung des QT-Intervals im Zusammenhang mit erhöhten Feinstaubwerten. Diese Assoziation war bei Probanden mit mindestens einem seltenen Allel des *NFE2L2* Einzelnukleotid-Polymorphismus (SNP) rs2364725 stärker ausgeprägt. Man vermutet, dass das Gen *NFE2L2* an der Abwehr von oxidativem Stress beteiligt ist. Darüberhinaus beobachtete ich eine sofortige Erhöhung und eine verzögerte Abflachung der T-Wellen Amplitude in Assoziation mit erhöhter Schadstoffbelastung. Der Zusammenhang zwischen Temperatur und T-Wellen Amplitude war invers U-förmig mit den höchsten Werten bei 5°C. In einer dritten Studie mit Teilnehmern mit Diabetes oder gestörter Glukosetoleranz (IGT) fand ich eine verminderte HRV, insbeson-

dere für die Standardabweichung der NN- (normal to normal) Intervalle, im Zusammenhang mit einem Anstieg von Feinstaub und ultrafeinen Partikeln. Diese Effekte waren bei Personen mit IGT stärker ausgeprägt. Die Schadstoffeffekte wurden auch durch SNPs mit einem mutmaßlichen Einfluss auf den Herzrhythmus modifiziert.
Diese Arbeit bestätigt und erweitert die bereits veröffentlichten Studien zu Kurzzeiteffekten von Schadstoffen auf Parameter der kardiovaskulären Gesundheit. Darüberhinaus ist sie eine der ersten, die Temperatureffekte auf EKG und Blutmarker untersucht. Bestimmte Vorerkrankungen oder einige genetische Profile scheinen bestimmte Untergruppen der Bevölkerung empfindlicher gegenüber Umwelteinflüssen zu machen. Die von uns beobachteten Temperatur- und Schadstoffeffekte auf HRV- und Blutmarker erklären möglicherweise teilweise die bekannten Zusammenhänge zwischen Umweltfaktoren und kardiovaskulären adversen Ereignissen.

3 Introduction

3.1 Cardiovascular health

Cardiovascular disease is the leading cause of global mortality (WHO 2007). In 2004 worldwide 17.1 million people died from cardiovascular disease reflecting 29% of all global deaths. The most common reason for this disease is the development of atherosclerosis on the inner walls of the blood vessels which supply the heart or brain. Myocardial infarctions and strokes are acute events and are mainly caused by a thrombosis preventing blood flow within the heart or brain. Researchers worldwide explore risk factors for atherosclerosis and thrombosis in order to improve prevention and treatments.

3.1.1 Blood biomarkers and electrogardiogram parameters

Blood biomarkers
Blood markers of inflammation are assumed to be involved in the development of atherosclerosis leading to acute as well as chronic cardiovascular disease. C-reactive protein (CRP), a sensitive marker of the acute-phase response, is the most established inflammatory marker for the evaluation and prediction of cardiovascular health (Libby et al. 2002). CRP as well as interleukin 6 (IL-6), a pro-inflammatory cytokine which activates the release of CRP, are associated with the development of atherosclerosis. A link between inflammatory processes has been suggested to be mediated via an oxidative stress response and an imbalance of the autonomic nervous system (Brook et al. 2010). Blood markers of coagulation have also been identified as risk factors of cardiac health (Davi and Patrono 2007); for example platelets can adhere to the vessel wall and contribute to the development of atherosclerotic lesions, and in case of rupture they trigger the acute onset of arterial thrombosis. Von Willebrand factor (vWf) is a glycoprotein which mediates platelet adhesion in response to inflammatory stimuli and endothelial wall injury. Elevated levels of factor VII might indicate an activation of the clotting cascade. Fibrinogen is a precursor of fibrin which is responsible for thrombus formation. High levels of fibrinogen are a marker of systemic inflammation, while moderately elevated levels can indicate systemic activation of the clotting cascade.

Electrocardiogram parameters
Time domain and frequency domain parameters of heart rate variability (HRV) are putative markers of cardiac autonomic balance (Task Force 1996). Researchers reported that reduced HRV might be a precursor of cardiovascu-

lar problems, for instance myocardial infarctions and arrhythmia (Buccelletti et al. 2009, Lanza et al. 2006). Furthermore, changes in repolarization parameters such as a prolonged QT-interval and T-wave flattening are further indicators of adverse cardiac events (Ziegler et al. 2008, Lin et al. 2008).

3.1.2 Air pollution

During the last 20 years, researchers worldwide have reported increased numbers of myocardial infarctions (Bhaskaran et al. 2009a, Hsieh et al. 2010), hospital admissions or emergency room visits (Morris et al. 1995, Tsai et al. 2009, Zanobetti et al. 2000), and mortality due to cardiac problems (Dominici et al. 2005, Katsouyanni et al. 2001, Schwartz and Marcus 1990, Wong et al. 2008) on days with elevated air pollution levels. Especially particulate matter (PM) with aerodynamic diameters below 10µm (PM_{10}) and 2.5µm ($PM_{2.5}$), as well as ultrafine particles (UFP) with a size range of 0.01 to 0.1µm in diameter have been related to the reported adverse health effects. Although the effects were small, there is strong evidence of a causal relationship between elevated air pollution levels and cardiovascular health. Several biological pathways have been suggested which might explain the observed short-term air pollution effects on cardiovascular health (Brook et al. 2010, Mills et al. 2009, Pope and Dockery 2006):

- Inhaled particles deposit in the lung and might perturb the balance of the autonomic nervous system due to a stimulation of lung receptors or nerve endings in the human airways. This possibly leads to an activation of the sympathetic nervous system and to a withdrawal of the vagal tone. Accordingly, it has been shown that PM exposure is associated with an increase in heart rate (HR), a reduction in HRV, and changes in repolarization.

- Inhaled particles can induce inflammation, oxidative stress, and the excess of reactive oxygen species in the lung. On the one hand, inflammatory cytokines (e.g. IL-6), acute-phase reactants (e.g. CRP and fibrinogen), and vascular molecules (e.g. endothelins) are then released into the circulation possibly leading to a systemic inflammation. On the other hand, an imbalance of the autonomic nervous system and hence a decreased HRV might be the consequence.

- It has been shown in both animals and humans that UFP might even translocate into the circulation. It is assumed that these translocated particles induce oxidative stress and a local inflammation potentially destabilizing atherosclerotic plaques. Moreover, UFP could also have

direct effects on the vascular endothelium, plaques, and clotting possibly leading to an increased risk of ischemia or myocardial infarctions.

These suggested pathways might be activated at differing time points and they are assumed not to be exclusive but they rather overlap.
Overall, the observed air pollution effects on HRV and markers of inflammation and coagulation (Chahine et al. 2007, Rückerl et al. 2007, Schneider et al. 2010) could be precursors of the reported associations between air pollution and adverse cardiac events.
As it is hypothesized that ambient air pollution acts on the autonomic function via oxidative stress pathways (Brook et al. 2010) subjects with genetic predispositions related to these pathways might be especially susceptible. Accordingly, Chahine et al. (2007) and Schwartz et al. (2005) reported air pollution effects on HRV in individuals with a reduced defense against oxidative stress due to glutathione S-transferase M1 (*GSTM1*) deletion. Moreover, Park et al. (2006) observed an air pollution effect modification on HRV by a SNP in the hemochromatosis (HFE) gene which is supposed to be involved in iron uptake and oxidative stress.
Furthermore, it is assumed that individuals with diabetes, cardiac or pulmonary disease are susceptible subpopulations which react stronger to air pollution exposure than healthy individuals (Brook et al. 2010, Peel et al.2007, Zanobetti and Schwartz 2001). However, the knowledge of genetic or disease-related influences and mechanisms on susceptibility to air pollution is still very limited.

3.1.3 Air temperature

In recent years, the influence of weather conditions, such as air temperature, on population health has received greater attention. Especially the effects of heat waves (Kosatsky 2005, Ostro et al. 2009) but also the effects of changes in moderate temperature on cardiac health have been examined (Bhaskaran et al. 2009b). Authors have reported an U- or J-shaped association between air temperature and myocardial infarctions or mortality (Bhaskaran et al. 2009b, Baccini et al. 2008). That is, cold as well as hot temperatures increase the number of cardiovascular disease events with lowest effects on days with a mean temperature between 15°C and 25°C depending on the geographical region. These observed findings might be explained by the following underlying mechanisms:

- Cold temperatures lead to a stimulation of cold receptors in the skin and to a constriction of skin vessels in order to reduce heat loss. An increase

in blood pressure and HR might be the consequence (Alperovitch et al. 2009, Keatinge et al. 1984, Näyhä 2005). It has also been suggested that cold temperatures trigger blood clotting in participants exposed to cold air or water (De Lorenzo et al. 1999, Keatinge et al. 1984). Authors reported a seasonal variation of inflammatory markers such as CRP (Sung 2006) and IL-6 (Kanikowska et al. 2009) with higher levels during winter compared to spring and autumn. Furthermore, it has been shown that short-term decreases in air temperature lead to enhanced levels of CRP, IL-6, and fibrinogen (Schneider et al. 2008).

- In hot weather, the skin vessels enlarge and sweating as well as cardiac work increases. The risk of thrombosis is enhanced because of an increased blood viscosity (Keatinge et al. 1986, Näyhä 2005). Furthermore, increases in HR have been observed in participants exposed to heat under controlled conditions (Keatinge et al. 1986). Kanikowska et al. (2009) also observed higher levels of IL-6 during summer compared to spring and autumn.

It has been shown that the elderly, women, and individuals with diabetes are particularly vulnerable to temperature extremes in association with mortality (Analitis et al. 2008, Medina-Ramon et al. 2006, Stafoggia et al. 2006). However, the association between temperature and electrocardiogram (ECG) parameters as well as blood markers of inflammation and coagulation have been rarely investigated. It is assumed that climate change might lead to both increases in average ambient temperature and unexpected drops in temperature due to higher temperature variability. Therefore, further research on temperature effects on cardiovascular risk markers is necessary.

3.2 Specific aims and results

The main objectives of this thesis were to investigate:

1. The association between air temperature and blood markers of inflammation and coagulation in men with coronary or pulmonary disease.

2. Air pollution and temperature effects on repolarization parameters and potential air pollution effect modifications by single nucleotide polymorphisms (SNPs) involved in detoxification pathways in myocardial infarction survivors.

3. Modifications of air pollution effects on HR and HRV by SNPs with an influence on cardiac rhythm among participants with type 2 diabetes or impaired glucose tolerance.

For all manuscripts, I developed the specific focus of the research question, performed the statistical analyses and interpreted the results. I wrote the first complete draft of the manuscripts and finalized them based on the co-authors comments. I revised the manuscripts based on the reviewers' comments after discussions with my supervisors and wrote the responses to the reviewers.

The analyses of this work are based on three different panel studies conducted in Erfurt (first publication) and Augsburg (second and third manuscript), Germany. In each study for each participant clinical examinations were performed repeatedly over a period of several months. Therefore, each participant acts as his/her own control while the exposures to environmental factors changed over the time course of the study. Data on health status as well as disease and smoking history were gathered at a baseline visit and were constant over time. Other time varying potential confounders such as medication intake was updated at each study visit. For a more detailed description of the study populations of the first and second study see Rückerl et al. 2006, Hildebrandt et al. 2009, and Peters et al. 2007. The associations between air temperature or air pollution and health outcomes were analyzed using additive mixed models with random participant effects. An appropriate covariance structure was chosen in order to account for dependencies between repeated measurements. All models were adjusted for meteorological variables and long-term time trend.

In the first publication, I investigated the association between air temperature and blood markers in potentially susceptible non-smoking individuals with coronary heart disease (CHD, 57 participants with 578 blood withdrawals) or with a history of doctor-diagnosed impaired lung function and/or chronic pulmonary disease (38 participants with 381 blood withdrawals). In contrast to the initial hypothesis, the two panels did not show similar responses to temperature drops. A 10°C decrease in the 5-day-average of temperature before the blood withdrawal led to an increase in platelet counts (%-change from the mean: 3.0%; 95%-confidence interval: [0.6%;5.5%]) and fibrinogen (5.5% [1.3%; 9.7%]), and to no change in CRP in patients with pulmonary disease. Opposed to this, the same decrease in the 5-day average temperature resulted in a decrease in platelet counts (-3.6% [-6.9%;-0.4%]), fibrinogen (-5.2% [-11.0%;0.7%]), and CRP (-16.1% [-26.8%;-3.8%]) in individuals with CHD. Diabetic individuals of the CHD panel seemed to be a susceptible subpopulation as they showed a strong 2-day delayed increase in factor VII (12.1%[5.8%;18.9%]) associated with temperature drops whereas no effect was observed for patients with a pulmonary disease (comprising only two individuals with diabetes) and non-diabetic CHD individuals. The rea-

son for differing temperature effects in patients with pulmonary or coronary heart disease might be the complex interplay of blood markers and unknown underlying mechanisms influencing the response to air temperature. The panels also differed in disease status and medication. Furthermore, CHD patients were older and suffered more often from comorbidities than patients with pulmonary disease. The combination of all these factors possibly affected the susceptibility to temperature changes.

In the second publication, I analyzed air pollution and temperature effects on repolarization parameters and HR. I also investigated potential air pollution effect modifications by SNPs supposed to be involved in detoxification pathways. Overall, 67 myocardial infarction survivors transmitted 1745 16sec-ECGs via telephone using a portable ECG device (Philips Viapac). Only obese participants with a body mass index (BMI)$\geq$ 30kg/m^2 or participants with an intake of beta-adrenergic receptor blockers showed an immediate increase in HR in association with elevated $PM_{2.5}$ levels. HR did not change in patients with a BMI<30kg/m^2 or without beta-adrenergic receptor blockers. A one-day lagged prolongation of the Bazett-corrected QT-interval (QTc) was observed with $PM_{2.5}$, PM_{10}, and coarse particles in all participants, e.g. increases in $PM_{2.5}$ led to a 0.5% [0.0;1.0%] QTc-prolongation. This association was more pronounced in patients with one (0.6%[0.1;1.0%]) or two (1.2%[0.4;2.1%]) minor alleles of the nuclear factor (erythroid-derived 2)-like 2 (*NFE2L2*) SNP rs2364725, whereas participants with no minor allele did not react to $PM_{2.5}$ increases. The *NFE2L2* gene is believed to be involved in the defense against oxidative stress (Goldring et al. 2004). It can only be speculated that the defense is more activated in patients with common alleles, whereas patients with at least one minor allele are more susceptible to PM. In contrast, increases in particle number concentrations (PNC), a proxy for UFP, led to a 4-day lagged QTc-shortening in participants with at least one minor allele of rs2364725. Different effects of PM and PNC might reflect different biological pathways activated by different particle properties. T-wave amplitude, another marker for the repolarization process, was positively associated with air pollution levels 0-23h and 24-47h before ECG transmission and inversely associated with levels more than 48h prior. Furthermore, I found an inverse U-shaped association between temperature and T-wave amplitude with highest values at 5°C. Immediate and lagged decreases in the T-wave amplitude by 5-9% in association with a temperature decline of 5°C on days with average temperatures below 5°C (cold effects) and with a 5°C increase in temperature on days above 5°C were observed. Changes in T-wave amplitude and QT-interval can be precursors of sudden cardiac death or arrhythmia. In general, the observed air pollution and

temperature effects on HR and repolarization parameters indicate potential triggers of adverse cardiac health outcomes.

In the third study I investigated very acute effects on an hourly basis of ambient air pollutants on HR and HRV parameters in 61 individuals with type 2 diabetes mellitus or impaired glucose tolerance (IGT). The HRV parameters of interest were the standard deviation of normal-to-normal intervals (SDNN) and the root mean square of successive differences (RMSSD). About 130 SNPs involved in cardiac rhythm which have already been identified in the literature were used as potential modifiers of the air pollution effects. In order to reduce the number of performed tests, regression trees for longitudinal data (Sela and Simonoff 2010) were used to single out SNPs with an influence on repeated measurements of ECG parameters. Only these influential SNPs were then used as potential effect modifiers. With this method I was able to select more than 130 SNPs from published genome-wide association studies and did not have to restrict the analysis to only a few candidate SNPs. The analysis of the 207 ECG recordings comprising 1153 1h-intervals revealed concurrent and lagged decreases in SDNN by about 2-5% in association with elevated levels of PM, UFP, black carbon, and sulfate. The stronges effect modification was for SNP rs333229. Only participants with at least one minor allele showed a reduction in SDNN. This SNP is located in the 3'untranslated region of the choline transporter gene (*CHT1*) (Neumann et al. 2005). *CHT1* encodes the high-affinity choline transporter (ChT) which carries choline into acetylcholine (ACh)-synthesizing neurons. ACh is a neurotransmitter of the sympathetic and parasympathetic system. Therefore, variations in *CHT1* may account for variations in ACh neurotransmission which might lead to a modulation of HR and HRV. However, it can only be speculated that changes in ChT affect the response to air pollution. An association between increases in PM_{10} and $PM_{2.5}$ and a concurrent reduction in RMSSD (-5.3%; [-9.3;-1.1%] and -7.2%[-12.2;-1.8%], respectively) was also observed. Furthermore, RMSSD changed by -3.8%[-7.1;-0.5%] and -5.2%[-9.8;-0.4%] in association with elevated black carbon levels with a lag of 1h and 6h, respectively. Elevated air pollution levels led to a concurrent reduction in RMSSD only in individuals with at least one minor allele of rs2096767, respectively. In contrast, people with no or one minor allele of rs2745967 exhibited an immediate decrease in RMSSD in association with air pollutants. I observed no air pollution effects on HR. In general, air pollution effects on SDNN and RMSSD seemed to be more pronounced in individuals with IGT than with diabetes. By selecting subjects with IGT, I intended to study the impact of air pollutants in subjects with an enhanced risk for type 2 diabetes mellitus but who were not as heavily treated by beta-adrenergic receptor

blockers, statins, or anti-diabetic medications as individuals with manifest diabetes. These medications may obliterate the effects of particle exposures. As in this study 81% of participants with diabetes took beta-blockers, statins, or anti-diabetic medication it might not have been possible to detect ambient air pollution effects on ECG-parameters for these individuals.

3.3 Conclusion

This work confirms and extends published results on short-term effects of environmental stimuli on markers of cardiovascular health. Susceptibility to environmental conditions seems to depend among others on disease status and medication intake. Accordingly, I observed differences in the direction and timing of temperature effects on blood markers of inflammation and coagulation between patients with CHD and pulmonary disease. Furthermore, HR increased in association with elevated air pollution levels only in myocardial infarction survivors without intake of beta-adrenergic receptor blockers. Moreover, air pollution effects on HRV parameters were only detected in participants with IGT but not in more heavily medicated individuals with diabetes. In general, I investigated environmental effects only in participants with underlying chronic diseases; therefore the results cannot be generalized to the whole population. However, analyzing particularly susceptible patients might show stronger reactions to environmental conditions and give better insights into possible mechanistic pathways. Moreover, as the prevalence of diabetes is increasing worldwide, it is important to conduct further investigations especially in this subpopulation.
Not only disease status but also genetic predispositions might have an influence on susceptibility to environmental conditions. Genes involved in detoxification pathways or with an influence on cardiac rhythm are particularly interesting. Correspondingly, I found a QTc-prolongation and a reduced HRV (SDNN) only in participants with at least one minor allele of SNPs involved in detoxification (*NFE2L2*) or in cardiac rhythm (*CHT*), respectively.
Overall, in this thesis I observed changes in HR, HRV, repolarization, and blood markers of inflammation and coagulation in association with changes in air temperature and air pollution. These changes may contribute to the observed associations between environmental conditions and cardiovascular morbidity and mortality. Although the detected associations between air pollution and ECG and blood markers are only small and might be considered subclinical, they can give ideas about possible underlying biological pathways. Moreover, even small acute changes in blood and ECG parameters may lead to atherosclerotic progression and chronic effects.

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4 Air temperature and inflammatory and coagulation responses in men with coronary or pulmonary disease during the winter season

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Journal: Occupational and Environmental Medicine
Volume: 67
Pages: 408 - 416
Year: 2010

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Air temperature and inflammatory and coagulation responses in men with coronary or pulmonary disease during the winter season

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The Focus Network Nanoparticles and Health (NanoHealth) coordinates and focuses all Helmholtz Zentrum München research on health effects and the characterisation of aerosols. It comprises research projects in the Institutes of Ecological Chemistry, Epidemiology, Inhalation Biology, Radiation Protection, and Toxicology at Helmholtz Zentrum München.

Accepted 19 October 2009

ABSTRACT

Background and Objective Air temperature changes are associated with increased cardiovascular and respiratory risk, but the roles of inflammatory and coagulation markers are not well understood. We investigated the associations between temperature and several blood markers in patients with coronary heart disease (CHD) and pulmonary disease (PD).

Methods Two studies were conducted in Erfurt, Germany, over two successive winters. 578 and 381 repeated blood measurements were collected from 57 CHD and 38 PD patients, respectively. Data on patient characteristics and disease history were gathered at baseline. Meteorological data were collected from existing networks. Associations were analysed using additive mixed models with random patient effects. Effect modification by diabetes status was investigated only in CHD patients, as only two PD patients had diabetes.

Results Mean daily air temperature varied between −13°C and 16°C in both study periods. A 10°C decrease in the 5-day temperature average before blood withdrawal led to an increase in platelet counts (% change from the mean: 3.0%, 95% CI 0.6% to 5.5%) and fibrinogen (5.5%, 1.3% to 9.7%), no change in C-reactive protein in PD patients, and a decrease in C-reactive protein in CHD patients. A 2-day delayed increase in factor VII associated with temperature decrease was seen in CHD patients (4.9%; 0.7% to 9.2%), while PD patients showed no effect. 'Effects in CHD patients without diabetes' into 'Effects on factor VII in CHD patients without diabetes'.

Conclusions This study suggests that temperature decrease is associated with change in several blood parameters. The complex interplay of blood markers at low temperature may contribute to the observed association between cold and cardiovascular mortality and morbidity.

What this paper adds

- Air temperature changes have been associated with increased cardiovascular and respiratory risk for mortality and morbidity, but the roles of inflammatory and coagulation markers and adhesion molecules in these relationships are not well understood.
- We observed significant changes in adhesion molecules and in inflammatory and coagulation blood markers.
- We found some conflicting associations in patients with coronary heart disease and pulmonary disease.
- The complex interplay of blood markers at low temperature may contribute to the observed association between temperature decrease and cardiovascular mortality and morbidity.
- Susceptible subgroups should take preventive/precautionary measures when the temperature drops.

INTRODUCTION

Several studies have shown that changes in air temperature are associated with hospital admissions[1 2] and mortality[3–5] due to cardiovascular events. Elderly people and those with diabetes or chronic obstructive pulmonary disease (COPD) seem to be susceptible to extreme weather conditions.[6–8] However, the underlying mechanisms of the relationship between temperature and cardiovascular morbidity remain poorly understood.

Earlier studies on the controlled exposure of participants to cold air or water revealed an increase in the levels of red blood cells (RBC), platelets, factor VII (FVII) and fibrinogen and a decline in von Willebrand factor (vWf).[9–11] A Taiwanese study[12] found higher mean fibrinogen and FVII concentrations on days with an ambient temperature equal to or below 20°C compared to days with an ambient temperature above 20°C. Seasonal variations in C-reactive protein (CRP) levels were observed in healthy Koreans with higher values during winter and spring than in summer.[13] The association between temperature and repeated fibrinogen, CRP and interleukin-6 (IL-6) measurements was assessed in a multi-centre European study of survivors of myocardial infarction.[14] A decrease in temperature 3 days before the measurement was associated with an increase in fibrinogen, CRP and IL-6 levels seemed to be related to average temperature changes in the previous 5 days.

It is hypothesised that a wide range of blood markers are involved in the development of atherosclerosis leading to chronic as well as acute cardiovascular disease. In patients with coronary or pulmonary disease, increased levels of inflammatory or coagulation markers might predict future cardiovascular events.[15 16] Therefore,

elevated blood markers due to decreased temperature might indicate cardiovascular health. The objective of our study was to assess the influence of temperature on repeated measurements of cellular blood components, adhesion molecules and coagulation as well as inflammatory markers as indicators for cardiovascular health in susceptible subgroups. We investigated the direction and temporal delay of the temperature–blood marker response and whether this differs in subjects with pulmonary and cardiovascular disease. Our hypothesis was that there would be a similar response to temperature stimuli in both groups. Abnormalities in the haemostatic system and low-grade systemic inflammation were found in people with diabetes.[17 18] Nakaji *et al*[19] observed an increase in diabetes-related mortality during the winter season in Japan; thus, we were also interested in the possible effect modifications of diabetes status on the relationship between temperature and blood markers.

METHODS

Study populations

As part of the University of Rochester Particulate Matter Center investigations, two prospective panel studies were conducted in Erfurt, Germany. The first study was carried out between 15 October 2000 and 27 April 2001 with a group consisting of non-smoking men with coronary heart disease (CHD). Male participants with a history of doctor-diagnosed impaired lung function and/or chronic pulmonary disease took part in the second study between 15 October 2001 and 6 May 2002. All participants signed a written consent form and the study protocol was approved by the Ethics Commission of the Bavarian Chamber of Physicians ('Bayerische Landesaerztekammer'). Patients taking anti-coagulants (except for acetylsalicylic acid) and those with an implanted cardiac pacemaker, a recent (<3 months previously) myocardial infarction, bypass surgery, coronary angioplasty or diabetes mellitus type I were excluded from both groups. Additionally, participants in the pulmonary disease (PD) group with pneumoconiosis (silicosis or asbestosis), more than three hospitalisations for COPD in the previous year, or continuing oxygen therapy and those who had taken antibiotics more than four times during preceding 6 months or winter half-year were excluded.

Clinical measurements

For each participant up to 12 clinical examinations were scheduled every two weeks on the same week day and at the same time of day. At the first visit, a baseline questionnaire was administered regarding health status, pulmonary and cardiac symptoms, medication and smoking history. Each visit included a short interview and ethylenediaminetetraacetic acid (EDTA) and citrate plasma samples were drawn (Becton Dickinson, Franklin Lakes, NJ). Samples were centrifuged and aliquots were immediately stored at −20°C until analysis. All blood specimens were analysed using an Abbott Cell-Dyn 1800 cell counter (Abbott, Wiesbaden, Germany). Intercellular adhesion molecule 1 (ICAM-1), endothelial-leukocyte adhesion molecule (E-selectin) (R&D Systems, Wiesbaden, Germany), prothrombin fragment 1+2 (Dade Behring, Marburg, Germany) and soluble CD40 ligand (sCD40L) were measured by means of a commercial enzyme-linked immunosorbent assay (ELISA). sCD40L measurements were only available for the CHD group. D-dimer and vWf were analysed using an immunoturbidimetric method and FVII by clotting time (Diagnostica Stago, Asnieres-sur-Seine, France). Fibrinogen, CRP (high-sensitivity assay) and serum amyloid A (SAA) were analysed by immunonephelometry (Dade Behring).

Meteorological data

Hourly data on temperature, relative humidity and barometric pressure were collected from existing networks. For each person and visit, the individual 24 h average of each meteorological variable preceding the visit (0–23 h before blood withdrawal) for up to 4 days (24–47 h, 48–71 h, 72–95 h, 96–119 h) before the examination were calculated if more than two thirds of the hourly measurements were available for this period. Additionally, we calculated the mean of these 5 days (5-day average).

Statistical analysis

Analyses were performed with SAS (v 9.1) in both groups separately. The medians of patient-specific correlation coefficients between all blood markers were calculated. In order to compare the blood parameters between the groups, the two datasets were combined and mixed models with random patient effects were conducted including a dummy variable for the group effect. We assumed a compound symmetry structure for the covariance matrix, as the half-lives of the markers were much shorter than the intervals between the visits. To compare the meteorological variables between the groups, a regression model with a first-order autoregressive covariance structure was used to account for the dependencies between the repeated measurements. The p value of the group effect indicates whether the blood markers and meteorological variables differ significantly between the groups. The patient characteristics of both groups were compared using a t test for metric variables and a χ^2 test for categorical variables. In case of less than five observations in one category, Fisher's exact test was used.

Regarding the estimation of temperature effects, the data of each group were analysed separately using additive mixed models with random patient effects and a compound symmetry covariance structure. In both groups, prothrombin fragment 1+2, CRP, SAA and D-dimer and, only in the CHD group, sCD40L and FVII were log-transformed in order to produce normally distributed residuals. Penalised splines (P-splines) were used to allow for non-linear exposure–response functions and for non-linear confounder adjustment (S Greven, H Küchenhoff, A Peters, personal communication, 2008).

Confounder models were built separately for each blood parameter. Model selection was carried out by minimising Akaike's information criterion. Continuous confounders were included linearly or smoothly as P-splines depending on the Akaike's information criterion value. Long-term time trend and relative humidity with the same lag as the temperature term were forced into the model. Barometric pressure with the same lag as the analysed temperature lag and day of the week were only included in case of model fit improvement. Two variables with questionnaire information indicating an airway infection or antibiotic intake 2 weeks before blood withdrawal were forced into the model to account for possible inflammation unrelated to a change in temperature. In addition, for the PD group, a variable for corticosteroid intake was also taken into account. Observations with obviously large residuals in the confounder model were excluded.

After completion of the confounder models, the effects of temperature lags were estimated linearly as well as smoothly as P-splines. The linearly estimated temperature effects are presented as per cent change in the outcome mean per 10°C decrease in temperature together with 95% CIs. We chose to

present the effects of a decrease in temperature because both groups were mainly studied in the winter.

Effect modification

We considered body mass index (BMI) (≤30 kg/m^2 vs >30 kg/m^2), hypertension (yes vs no), age (≤60 years vs >60 years) and statins (intake vs no intake) as potential effect modifiers. The interaction with diabetes type II (yes vs no) was only analysed for the CHD group as only two patients in the PD group had diabetes. Additionally, interactions with smoking status (current smokers vs non-smokers) and β2-agonists (intake vs no intake) were analysed for PD patients.

Sensitivity analyses

To check the robustness of our models we performed several sensitivity analyses. Temperature effects were calculated including observations with extremes in the residuals to avoid exclusion of observations possibly sensitive to temperature changes and to test the sensitivity of the results to our main model. Also, patients with pulmonary disease (COPD, chronic bronchitis) were excluded from the CHD group, and patients with coronary disease (CHD, angina pectoris and previous myocardial infarction) from the PD group. Additionally, we separately added particulate matter with a diameter below 10 μm (PM_{10}) or ultrafine particles (UFP) with the same lag as temperature, as well as with the most influential lag, to the original confounder model. Polynomial distributed lag (PDL) models were calculated, which include all temperature lags simultaneously. In order to avoid multicollinearity, we used an Almon distributed lag model,[20] which forces estimates to a polynomial shape. The sum of the lagged temperature effects resulting from a PDL model were compared with the effect of the 5-day temperature average.

RESULTS

Study populations

Of the initial 61 CHD patients, one refused to participate, two were excluded due to disease causing changes in blood markers (leukaemia, lymphoma) and one was excluded because of constantly elevated levels of leukocytes and erythrocytes, indicating an unknown underlying disease. The CHD group therefore comprised 57 men. Fifty five patients participated in 12 scheduled visits, while two attended nine and eight examinations, respectively. If patients reported an acute infection and/or surgery during the 2 weeks before the examination or if nurses saw signs of acute infection, the blood samples of the respective visits were excluded (46 blood samples from 19 patients). As not all patients were able to give the scheduled amount of blood at each visit, 578 (85%) blood samples remained for analysis.

The data of 38 of 42 male PD participants were used for analyses: three participants with a single measurement and one patient with a cancer diagnosis were excluded. In total, 438 (96%) of the targeted 456 visits were carried out. Blood withdrawal failed for technical reasons on 10 occasions and 47 blood samples were excluded because of fever the week before withdrawal or a diagnosed airway infection on the day of the visit. Thus, 381 (87% of 438 blood withdrawals) valid blood samples were available for analysis. The majority of patients had chronic bronchitis and/or asthma with normal pulmonary function tests measured as forced expiratory volume in 1 s (FEV_1)/forced vital capacity ratios above 70%. Two patients had mild, seven moderate (50%≤$FEV_1/FEV_{1predicted}$ <80%) and five severe (30%≤ $FEV_1/FEV_{1predicted}$ <50%) COPD.

The PD and CHD groups are described in table 1. CHD patients were significantly older than PD patients: the age of CHD and PD patients ranged from 51 to 76 years and from 35 and 78 years, respectively. Significantly more patients with chronic bronchitis were observed among CHD patients with diabetes than without diabetes. Overall, the PD group was healthier as the participants tended to be less obese and it included only two patients with diabetes, while the CHD group included 13 patients with diabetes.

Clinical measurements

Levels of blood parameters are summarised in table 2. Except for E-selectin, vWf, D-dimer and SAA, PD patients tended to have higher values than CHD patients. Only platelets, prothrombin fragment 1+2 and ICAM-1 showed significant differences between the groups. CHD patients with diabetes had higher E-selectin and ICAM-1 levels compared to CHD patients without diabetes. CRP and SAA showed a moderate correlation of r=0.7 (CHD) and r=0.6 (PD) in both groups. None of the remaining blood markers were strongly correlated ($|r|<0.4$) in either group.

Meteorological data

Meteorological data were measured on 198 and 204 days for the CHD and PD groups, respectively (see online supplemental table 1). The mean temperature during both study periods was 4°C (SD 5°C). Mean relative humidity was 84% (SD 9%) and 83% (SD 12%) and mean barometric pressure was 973 hPa (SD 10 hPa) and 980 hPa (SD 11 hPa) for the CHD and PD groups, respectively. Only barometric pressure showed a significant difference between the groups. The meteorological variables exhibited only low correlation ($|r|\leq 0.3$). Online figure 1 compares the time series of temperature in both groups.

Effects of air temperature

There was no evidence in either group for a deviation in the linearity of the relationship between temperature and blood markers (data not shown). Figures 1 and 2 show the immediate, lagged and cumulative effects of a 10°C decrease in temperature. All figures are presented with the same scaling except for RBC which exhibits very small temperature effects. The association between temperature and RBC was quite similar for both groups, but with an immediate significant effect in CHD patients (0.7%, 0.0% to 1.4%). In contrast, CHD patients reacted to a drop in temperature, particularly the 5-day average, with decreased (−3.6%, −6.9 to −0.4%) platelet levels, whereas PD patients responded with increased (3.0%, 0.6% to 5.5%) platelet levels. A decline in temperature was associated with an increase in sCD40L in CHD patients, with the 5-day temperature average showing the largest effect (18.5%, 5.1% to 33.5%). Only in PD patients was a decline in all temperature lags associated with increased E-selectin levels. A drop in temperature for the 5-day average led to a 4.6% (0.2% to 9.1%) increase in ICAM-1 in CHD patients. A −6.5% (−12.7% to −0.2%) reduction in vWf 24–47 h before blood withdrawal was detected in PD patients. We observed large differences between the groups for the relationship between temperature and FVII. While PD patients showed an immediate decrease in FVII (−5.6%, −10.4 to −0.7%), CHD patients exhibited an inverse association of roughly the same size with a delay of 2 days (4.9%, 0.7% to 9.2%). We found only in PD patients an association between temperature and prothrombin fragment 1+2 with a delay of 1–3 days with the largest effect for the 5-day average (25.9%, 7.6% to 47.3%). No clear effects were detected for fibrinogen. A

Table 1 Baseline characteristics of the PD and CHD groups

	CHD group (n=57)				PD group (n=38)	
	All mean (SD) or n(%)	No diabetes mean (SD) or n(%)	Diabetes, N (%)	p Value (no diabetes vs diabetes)	mean (SD) or n(%)	p Value (PD vs CHD)
Age (years), mean (SD)	66.3 (6.0)	66.0 (6.2)	67.3 (5.7)	0.50*	53.8 (12.3)	<0.001*
BMI‡ (kg/m^2), mean (SD)	28.1 (3.4)	28.2 (3.4)	27.6 (3.5)	0.57*	25.5 (3.7)	0.06*
Age (years)						
≤60	8 (14)	7 (16)	1 (8)	0.67‡	17 (45)	<0.001†
>60	49 (86)	37 (84)	12 (92)		21 (55)	
BMI (kg/m^2)						
≤30	42 (74)	31 (70)	11 (85)	0.48‡	33 (87)	0.12†
>30	15 (26)	13 (30)	2 (15)		5 (13)	
Smoking						
Never smoker	15 (26)	14 (32)	1 (8)	0.15‡	8 (21)	0.003‡
Ex-smoker	42 (74)	30 (68)	12 (92)		23 (61)	
Current smoker	0 (0)	0 (0)	0 (0)		7 (18)	
History of						
Coronary heart disease	57 (100)	44 (100)	13 (100)	–	11 (29)	<0.001‡
Angina pectoris	39 (68)	29 (67)	10 (77)	0.73‡	15 (40)	0.004†
Myocardial infarction	43 (57)	33 (75)	10 (77)	0.89†	8 (21)	<0.001†
Bypass surgery/balloon dilatation	49 (68)	37 (84)	12 (92)	0.67‡	4 (11)	<0.001‡
COPD§ (mild to severe)	5 (9)	5 (11)	0 (0)	0.58‡	14 (37)	<0.001†
Chronic bronchitis	2 (4)	0 (0)	2 (15)	0.05‡	29 (76)	<0.001‡
Emphysema	0 (0)	0 (0)	0 (0)	–	5 (13)	0.009††
Bronchial asthma	0 (0)	0 (0)	0 (0)	–	20 (53)	<0.001††
Hypertension	40 (70)	32 (73)	8 (62)	0.44†	14 (37)	0.001†
Diabetes	13 (23)	0 (0)	13 (100)	–	2 (5)	0.02‡
Medication use						
Acetylsalicylic acid	53 (93)	42 (96)	11 (85)	0.22‡	11 (29)	<0.001‡
Statins	28 (49)	22 (50)	6 (46)	0.81†	7 (18)	0.002†
β2-Agonists	4 (7)	3 (7)	1 (8)	1.00†	21 (56)	<0.001‡
Theophylline	6 (11)	5 (11)	1 (8)	1.00†	17 (45)	<0.001†
Glucocorticosteroids	4 (7)	3 (7)	1 (8)	1.00†	18 (47)	<0.001‡

*t test;
†χ^2 test;
‡Fisher's exact test.
BMI, body mass index; CHD, coronary heart disease; COPD, chronic obstructive pulmonary disease; PD, pulmonary disease.

decrease in temperature 96–119 h before blood withdrawal and in the 5-day average led to a borderline significant reduction of about 5% in CHD patients, whereas PD patients showed elevated fibrinogen levels for a temperature decrease 24–47 h (5.0%, 1.2% to 8.7%), 48–71 h (5.2%, 2.0% to 8.4%) and 72–95 h (3.4%, 0.5% to 6.4%) before blood withdrawal and for the 5-day temperature average (5.5%, 1.3% to 9.7%). We detected only in CHD patients influential associations between a temperature decrease and CRP. Nearly all temperature lags showed a statistically significant or borderline decrease in CRP, with the largest influence for the 5-day temperature average (−16.1%, −26.8% to −3.8%). In both groups we found no influence of temperature on WBC, D-dimer or SAA (data not shown).

Effect modification

Only CHD patients with diabetes exhibited a decrease in platelets in association with a decline in temperature (figure 3). Participants with and without diabetes showed elevated and decreased levels of E-selectin and vWf, respectively, due to a decrease in temperature, which were not significant. We observed the largest interaction effects between temperature and diabetes on FVII. Patients with diabetes showed strongly elevated FVII values for a decrease in temperature for almost all lags, while there were no effects for patients without diabetes. No significant interactions by diabetes were detected in association with the remaining blood markers.

Temperature effects on platelets were more pronounced in CHD patients without statin intake, whereas CHD patients taking statins showed larger effects on E-selectin (data not shown). In PD patients we observed only temperature effects on prothrombin fragment 1+2 in patients with statin intake. Throughout all 24 h lags, these participants exhibited significantly elevated levels due to a drop in temperature, whereas the effect of patients who did not take statins was smaller and mostly not significant. In both groups, no significant interactions of temperature with age, BMI or hypertension were detected. Smoking status and intake of β2-agonists did not modify the temperature–blood marker response in PD patients.

Sensitivity analyses

In both groups the temperature effects remained robust after adjustment for air pollution. Only in CHD patients were temperature effects on RBC, ICAM-1 and vWf more pronounced after adjusting for PM_{10} with the same lag as temperature. The exclusion of PD patients from the CHD group and of patients with coronary disease from the PD group did not change our

Table 2 Blood marker levels for PD and CHD patients

	CHD group (N=57)							PD group (N=38)		
	All		No diabetes		Diabetes		p Value* (no diabetes vs diabetes)			
	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)		n	Mean (SD)	p Value (PD vs CHD)
WBC (×1000/μl)	576	6.7 (1.6)	447	6.6 (1.6)	129	7.2 (1.6)	0.15	379	7.0 (1.9)	0.36
RBC (×1000/μl)	575	4.6 (0.4)	447	4.6 (0.3)	128	4.5 (0.4)	0.33	379	4.6 (0.3)	0.66
Platelets (×1000/μl)	572	184.4 (64.1)	445	180.0 (43.4)	127	199.8 (108.0)	0.39	380	255.9 (62.9)	<0.001
sCD40L (pg/ml)	538	5256 (4480)	423	5327 (4751)	115	4993 (3299)	0.79	–	– (–)	–
E-selectin (ng/ml)	571	54.1 (26.3)	446	49.4 (19.8)	125	70.7 (37.5)	0.03	376	50.0 (20.7)	0.60
ICAM-1 (ng/ml)	572	271.2 (72.1)	446	259.1 (58.1)	126	314.2 (96.7)	0.01	377	300.7 (97.6)	0.02
vWF (% activity)	544	134.3 (57.3)	423	131.4 (53.4)	121	144.6 (68.5)	0.28	349	126.4 (43.4)	0.41
FVII (% activity)	535	120.7 (33.0)	418	119.7 (33.3)	117	124.1 (31.6)	0.78	345	130.2 (32.8)	0.06
Prothrombin fragment 1+2 (nmol/l)	541	1.5 (1.8)	423	1.5 (1.6)	118	1.7 (2.5)	0.18	345	2.2 (1.0)	<0.001
Fibrinogen (g/l)	573	2.9 (0.7)	444	2.9 (0.7)	129	2.9 (0.7)	0.98	374	3.0 (0.7)	0.41
D-dimer (μg/ml)	546	0.7 (1.1)	425	0.6 (0.5)	121	1.1 (2.1)	0.22	347	0.4 (0.6)	0.14
CRP (mg/l)	575	3.4 (4.1)	446	3.1 (3.3)	129	4.6 (5.8)	0.08	379	3.5 (5.5)	0.77
SAA (mg/l)	575	5.1 (8.7)	446	4.8 (8.9)	129	6.0 (7.9)	0.23	378	4.6 (8.4)	0.64

*p Value of fixed group effect in mixed effects model.
CHD, coronary heart disease; CRP, C-reactive protein; E-selectin, endothelial-leukocyte adhesion molecule; FVII, factor VII; ICAM-1, intercellular adhesion molecule 1; PD, pulmonary disease; RBC, red blood cell count; SAA, serum amyloid A; sCD40L, soluble CD40 ligand; vWF, von Willebrand-factor antigen; WBC, white blood cell count.

findings. The sums of the lagged temperature effects resulting from PDL analyses were comparable to the effects of 5-day temperature average on all blood markers in both groups (data not shown).

DISCUSSION

Summary

We investigated the cardiovascular effects of temperature in two susceptible subgroups. We observed opposite air temperature effects between the groups for platelets, E-selectin, prothrombin fragment 1+2, FVII and fibrinogen. In association with a 10°C decline in temperature, PD patients showed an increase in blood parameters except for FVII, while CHD patients showed constant or decreased blood marker levels. Inverse immediate and lagged effects were found on sCD40L, which was measured in the CHD group only. Immediate decreased FVII levels in association with a 10°C decrease in the 24 h temperature average were detected in PD participants, whereas CHD patients showed increased FVII levels with a delay of 48–71 h. Patients with CHD and diabetes showed in particular a strong association between temperature and platelets as well as FVII, whereas no or opposite effects were observed in both PD and CHD participants without diabetes, respectively. The results indicate that decreases in temperature might lead to changes in coagulation markers and adhesion molecules, suggesting a biological mechanism for the observed temperature-related variation in cardiovascular event frequency.

Air temperature and blood parameters

RBC tended to increase as temperature decreased. These findings support other studies which showed elevated RBC after cooling.[9 11] Elwood *et al*[21] found an absolute increase in platelets of $16\times10^3/\mu l$ in association with a 16°C decrease in temperature which is comparable with the $10\times10^3/\mu l$ platelet increase in PD patients for the same temperature decline found in our analysis. Increased platelet counts were also observed during mild surface cooling,[11] probably as a consequence of cold-induced sympathetic activity. Contrary to our findings in PD participants, we observed a positive association in CHD patients. Considering the significantly increased sCD40L levels in CHD participants, the decrease in platelets could be the consequence of platelet aggregation due to activation of platelets.[22] Unfortunately, no sCD40L data were available for the PD group to allow us further investigate this hypothesis. De Lorenzo *et al*[10] showed that vWf, a glycoprotein which mediates platelet adhesion, decreased in healthy participants due to cold adaptation following whole body water immersion. In our results, reduced vWf levels in association with a drop in temperature were seen in both groups. However, the effects were mostly not significant.

FVII activates the clotting cascade and Woodhouse *et al*[23] observed enhanced FVII clotting activity values in the elderly during winter. However, one should only cautiously refer to studies comparing winter-time and summer-time levels of blood markers, as the differences may be due to differences in influenza occurrence rather than temperature changes. Yeh *et al*[12] found higher mean FVII levels in Taiwanese subjects on days with a temperature equal to or below 20°C in comparison to days exceeding 20°C. Although temperature ranges between our and the Taiwanese study differed, we also detected cold effects on FVII in CHD patients. Opposite effects were observed in PD participants. Several studies[21 23 24] observed enhanced fibrinogen levels in the elderly during winter. To our knowledge there was only one study which assessed the influence of temperature on repeated measurements of fibrinogen. Schneider *et al*[14] observed increased fibrinogen levels in association with a decrease in temperature in survivors of myocardial infarction. A 10°C drop in temperature 72–95 h before blood withdrawal led to a 1.32% (0.2% to 2.4%) increase in fibrinogen. We also found lagged temperature effects in PD patients, but our effects were larger and significant for several lags. However, in CHD patients a delayed decrease in fibrinogen was found. This is in accordance with the report by Frohlich *et al*[25] who reported peak fibrinogen levels in April and lower levels in winter. Fibrinogen is a precursor of fibrin which is responsible for thrombus formation. High levels of fibrinogen are a marker of systemic inflammation, while low levels can indicate systemic activation of the clotting cascade. Schneider *et al*[14] detected elevated values of CRP in relation to a decline in temperature. However, we found no effect on CRP in PD patients and a decrease in CRP for almost all lags in CHD patients. The findings on seasonal changes of CRP are controversial. While Sung[13] reported seasonal variation in CRP levels with a peak in winter,

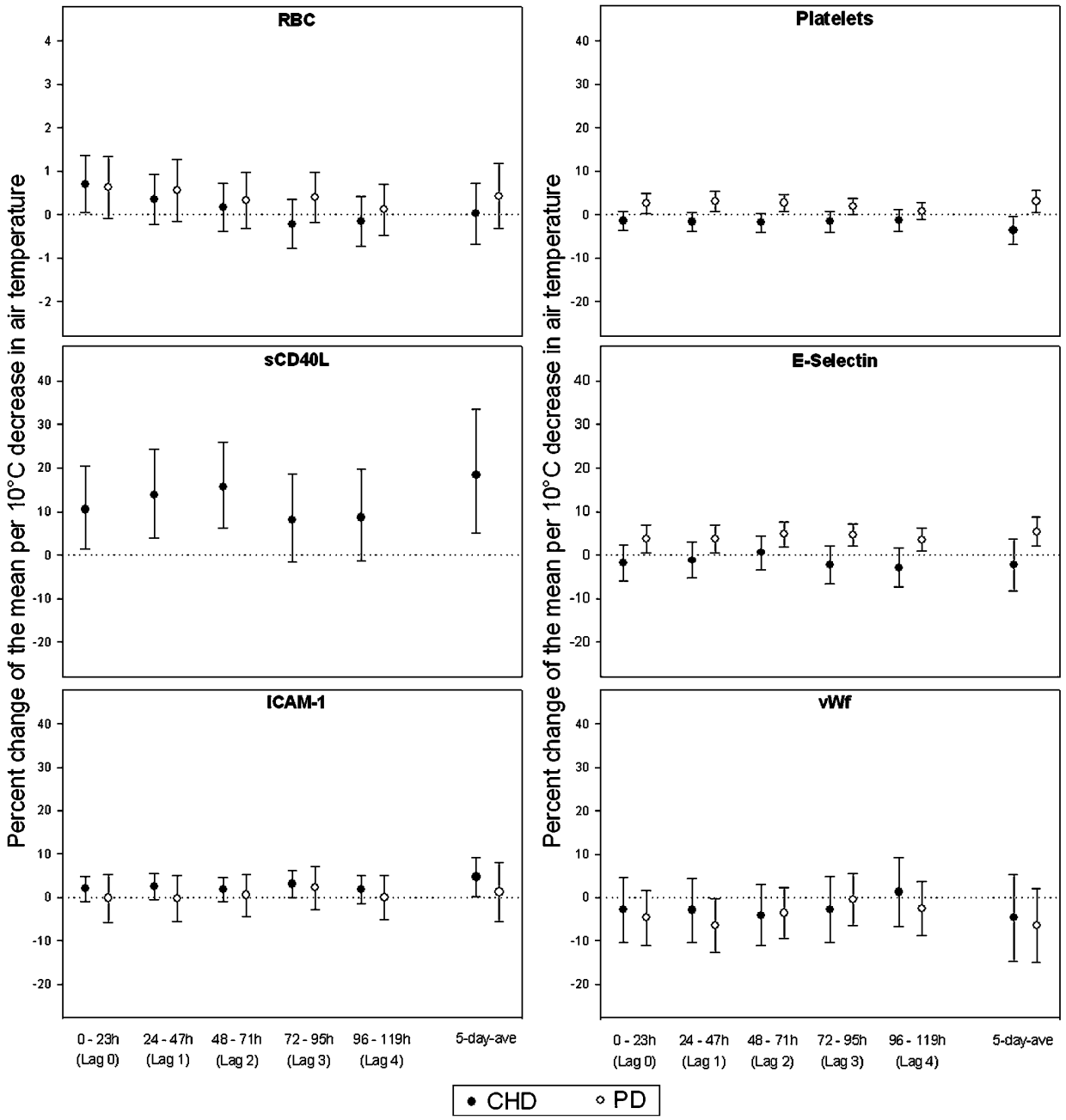

Figure 1 Per cent change per 10°C decrease in temperature in mean levels of red blood cells (RBC), platelets, soluble CD40 ligand (sCD40L), E-selectin, intercellular adhesion molecule 1 (ICAM-1) and von Willebrand-factor antigen (vWF). CHD, coronary heart disease; PD, pulmonary disease.

Horan *et al*[26] and Frohlich *et al*[27] found no seasonal variation. CRP is the most established inflammatory marker used to evaluate and predict cardiovascular health.[15] However, we mainly observed changes in coagulation markers which might also be relevant in the development of acute coronary syndromes and in thrombus formation.[28]

Air temperature and blood parameters in patients with diabetes

We detected interaction effects between diabetic status and temperature on platelets, E-selectin, vWf and FVII in CHD patients. The inverse influence of a drop in temperature on FVII in all CHD patients might be based on the strong inverse temperature effect in people with diabetes. CHD patients without diabetes showed non-significant effects comparable to the estimates for the PD group, which included only two patients with diabetes. While smaller or opposite effects were detected in patients without diabetes, a decline in temperature also led to decreased platelets and slightly increased E-selectin and vWf levels in patients with diabetes. The haemostatic system in individuals with diabetes exhibits various abnormalities[17] such as endothelial

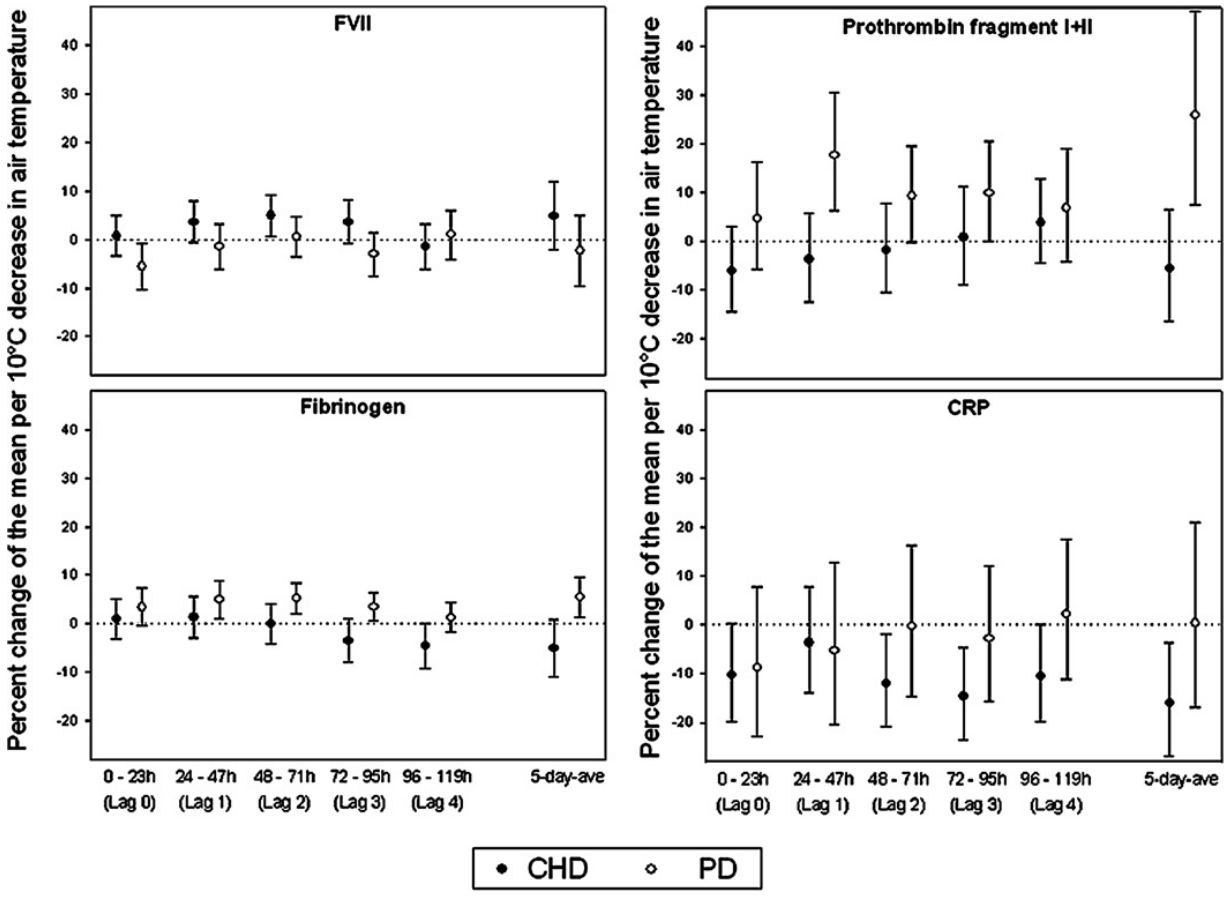

Figure 2 Per cent change per 10°C decrease in temperature in mean levels of factor VII (FVII), prothrombin fragment 1+2, fibrinogen and C-reactive protein (CRP). CHD, coronary heart disease; PD, pulmonary disease.

damage and elevated coagulation markers. The decrease in platelets in association with a temperature drop might be due to enhanced vWf levels which promote platelet adhesion to the damaged endothelial tissue and subsequent platelet activation.[29] Our analyses revealed that patients with diabetes seem to be a susceptible subpopulation which reacted more strongly to cold temperature. This finding needs further investigation as Schwartz[6] and Medina-Ramon and Schwartz[30] observed in case-only analyses increased hospital admissions and deaths in patients with diabetes on extremely hot days but no effects of extreme cold. As the study periods of our groups were limited to the cold season we expected no heat effects.

In summary, contrary to our initial hypothesis, the relationship between temperature and most blood markers differed between the groups or clear temperature effects were detected only in one group. CHD patients in our study were older and more often had hypertension and diabetes than PD patients. Medication also varied because of different disease patterns between the groups. The combination of all these factors possibly affected blood markers and susceptibility to temperature. Thus, differences in the effect of temperature between the groups could not be fully explained by a single patient characteristic. It is possible that underlying, but currently unknown, mechanisms influencing the association between temperature and blood markers might differ between the two susceptible subgroups. However, it is also possible that our results are only due to chance.

Air temperature and cardiovascular events

As several studies[5 31] observed increases in cardiovascular events due to a decline in temperature, our analyses might identify intermediate steps linking temperature changes to cardiovascular health. The influence of temperature on cardiovascular events has also been described by U-, V- or J-shaped functions.[2 32–34] In this context, it is important to note that the clinical examinations of the CHD and PD patients took place from October to April or May, respectively. The maximum 24 h averages in temperature during both study periods never exceeded 16°C. We assume that our results reflect the left part of a U- or V-shaped temperature—blood marker response as it has been shown that cardiovascular mortality is lowest on days with a mean temperature between 15°C and 20°C.[34]

Strengths and limitations

We were able to analyse intra-individual variation as patients with PD or CHD participated in up to 12 consecutive blood measurements. Further strengths are the non-linear confounder adjustment and the information on patient characteristics allowing us to perform subgroup analyses. However, the findings are limited in that the measured blood markers are easily influenced by infections. CRP is particularly sensitive to infectious disease and can increase a thousand-fold within a short time.[35] We therefore excluded the blood samples of participants with an acute infection during their visit and we additionally

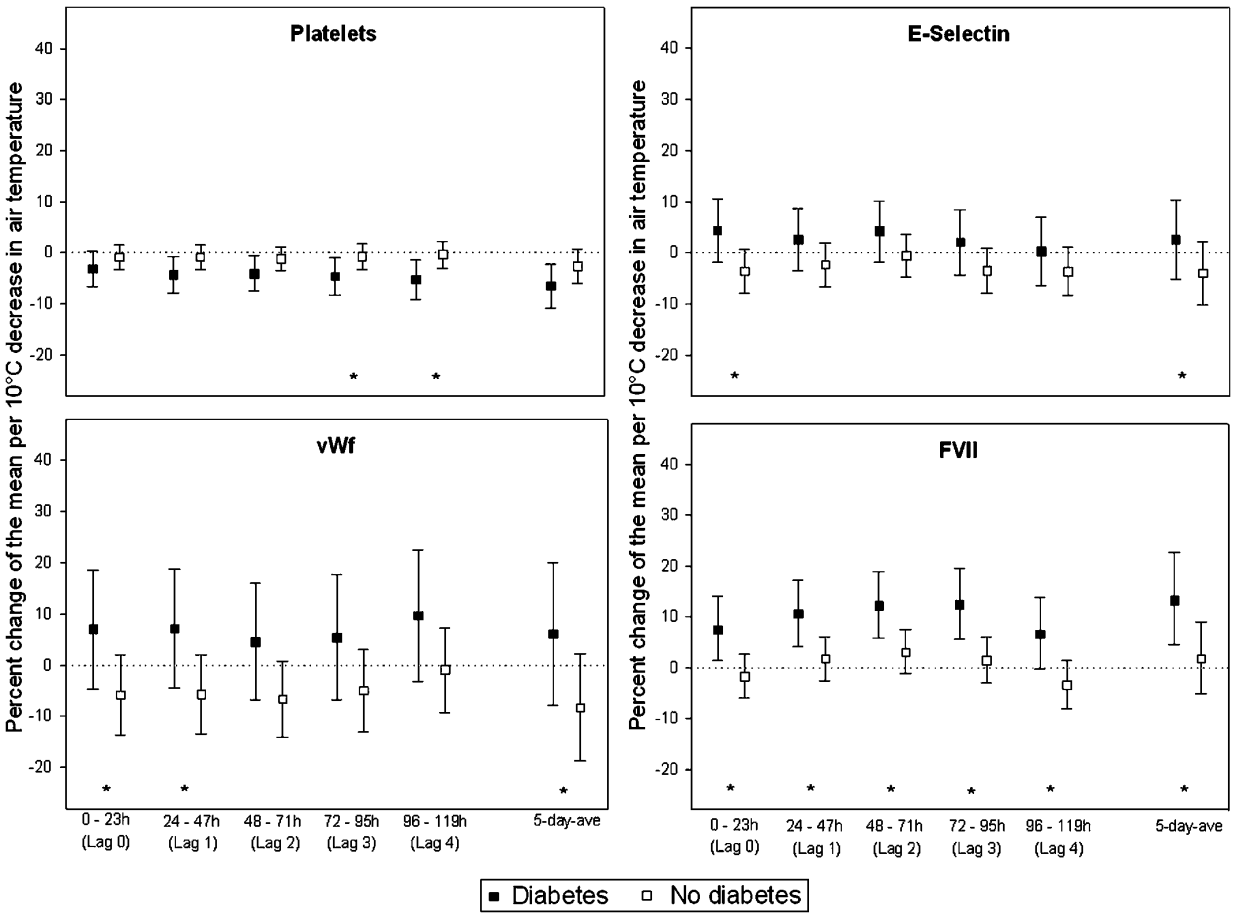

Figure 3 Per cent change per 10°C decrease in temperature in mean levels of blood markers for coronary heart disease patients with and without diabetes. *Interaction is significant.

adjusted for intake of antibiotics and airway infections during the 2 weeks prior to the examination. It can therefore be assumed that the presented effects result from a temperature decrease and not from an infectious disease. It has also been shown that air pollutants might be associated with blood markers.[36–42] It is still unclear whether air pollutants confound or modify the temperature–blood marker response. However, adjustment for air pollution hardly changed our results.

There is no standard technique to determine sCD40L and it has been shown that sCD40L levels may be influenced by the method employed. However, in our study all samples were analysed at the same time and under the same conditions (for further details, see Rückerl *et al*[22]). Moreover, the change in the sCD40L level within one patient was used for analysis, rather than the absolute level, and therefore the assay itself cannot have influenced the results.

One limitation is that we investigated the influence of temperature on blood parameters in men with severe disease on different medication. Hence, they are a highly selected group which might show differing reactions to temperature changes compared to women or healthy subjects, leading to reduced generalisability of our results. On the other hand, analysing vulnerable groups might provide more mechanistic insights and provide ideas for instituting new precautions. A further limitation is that only outdoor temperature was measured although people usually spend a lot of time indoors, especially in winter. However, as numerous studies have shown that the risk for cardiovascular events increases during the cold season,[5 34] we assume that short exposure to outdoor temperature can also influence blood markers.

CONCLUSION

The present study suggests that a decrease in temperature is associated with changes in several blood parameters. The direction and timing of the relationship differed between CHD and PD patients. The complex interplay of blood markers at low temperature may contribute to the observed association between cold and cardiovascular mortality and morbidity.

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Funding This study received funding from the U.S. Environmental Protection Agency through STAR grants R 827354 and RD832415 to the University of Rochester.

Competing interests None.

Ethics approval This study was conducted with the approval of the Ethics Commission of the Bavarian Chamber of Physicians ('Bayerische Landesaerztekammer').

Provenance and peer review Not commissioned; externally peer reviewed.

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Supplemental Material, Table 1. Description of meteorological variables in the CHD and PD panel.

	Panel	**N**	**Mean**	**SD**	**Min**	**25%**	**Median**	**75%**	**Max**	**IQR**
Air temperature	CHD	198	4.1	4.8	-10.5	0.7	4.4	7.8	13.2	7.1
(°C)	PD	204	4.1	5.4	-12.8	0.2	4.7	8.4	15.7	8.1
Barom. pressure	CHD	198	973.2	9.8	949.8	966.2	972.9	979.9	995.6	13.7
(hPa)	PD	204	979.7	10.6	952.8	972.6	979.7	987.1	1002.3	14.5
Rel. humidity	CHD	198	83.5	8.9	56.5	78.5	84.5	89.1	100	10.6
(%)	PD	204	82	11.7	48.8	75	84.3	90.6	100	15.6

CHD: Coronary heart disease, PD: Pulmonary disease, SD: Standard deviation, IQR: Interquartile range

5 Altered cardiac repolarization in association with air pollution and air temperature in myocardial infarction survivors

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Journal: Environmental Health Perspectives
Volume: 118 (12)
Pages: 1755 - 1761
Year: 2010

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Altered Cardiac Repolarization in Association with Air Pollution and Air Temperature among Myocardial Infarction Survivors

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Background: Epidemiological studies have shown that ambient particulate matter (PM) and changes in air temperature are associated with increased cardiopulmonary events.

Objective: We hypothesized that patients with previous myocardial infarction (MI) experience changes in heart rate (HR) and repolarization parameters, such as Bazett-corrected QT interval (QTc), and T-wave amplitude (Tamp), in association with increases in air pollution and temperature changes.

Methods: Between May 2003 and February 2004, 67 MI survivors from the Augsburg KORA-MI registry repeatedly sent 16 sec electrocardiograms (ECGs) with a personal transmitter (Viapac) via telephone to the Philips Monitoring Center, where ECG parameters were immediately analyzed. Meteorological data and air pollutants were acquired from fixed monitoring sites on an hourly basis. Additive mixed models were used for analysis. Effect modification by patient characteristics was investigated.

Results: The analysis of the 1,745 ECGs revealed an increased HR associated with interquartile range (IQR) increases in PM levels among participants not using beta-adrenergic receptor blockers and among those with body mass index ≥ 30 kg/m^2. We observed a 24- to 47-hr lagged QTc prolongation [0.5% change (95% confidence interval, 0.0–1.0%)] in association with IQR increases in levels of PM ≤ 2.5 μm in aerodynamic diameter, especially in patients with one [0.6% (0.1–1.0%)] or two [1.2% (0.4–2.1%)] minor alleles of the nuclear factor (erythroid-derived 2)-like 2 (*NFE2L2*) single-nucleotide polymorphism rs2364725. Positive immediate (0–23 hr) and inverse delayed (48–71 hr up to 96–119 hr) associations were evident between PM and Tamp. We detected an inverse U-shaped association between temperature and Tamp, with a maximum Tamp at 5°C.

Conclusions: Increased air pollution levels and temperature changes may lead to changes in HR and repolarization parameters that may be precursors of cardiac problems.

Key words: air pollution, air temperature, epidemiology, myocardial infarction, panel study, repolarization. *Environ Health Perspect* 118:1755–1761 (2010). doi:10.1289/ehp.1001995 [Online 15 September 2010]

Numerous studies have shown that elevated ambient air pollutants and changes in air temperature are associated with increases in hospital admissions and mortality due to cardiovascular events (Analitis et al. 2008; Pope and Dockery 2006). The effects of air pollution on heart rate (HR) and heart rate variability (HRV) have been studied more extensively since the initial publications by Pope et al. (1999), Peters et al. (1999), and Gold et al. (2000). For example, researchers reported a reduced HRV in susceptible participants such as senior adults (Luttmann-Gibson et al. 2006) and patients with coronary artery disease (CAD) (Timonen et al. 2006). Increased levels of air pollution have been shown to enhance the risk for ST-segment depression (Pekkanen et al. 2002) and arrhythmia (Berger et al. 2006). It is hypothesized that the observed associations between HR and HRV and air pollutants are a consequence of the activation of the autonomic nervous system or a direct affection of the electric system of the heart (Pope and Dockery 2006). Little is known about the influence of temperature on HR and HRV. Drops in temperature may activate the sympathetic nervous system via stimulation of cold receptors in the skin, which may result in increased catecholamine levels. The consequences are vasoconstriction and increased blood pressure (Alpérovitch et al. 2009; Pääkkönen and Leppaluoto 2002).

Potential mechanisms of the influence of air pollutants and temperature on repolarization have received less attention. Some researchers have hypothesized that a prolonged QT interval and T-wave abnormalities might trigger the onset of arrhythmias (Roden 2008) and increase the risk for coronary deaths (Greenland et al. 2003). Only a few studies have investigated the relationship between elevated levels of particulate matter (PM) air pollution and repolarization thus far (Ghelfi et al. 2008; Henneberger et al. 2005; Zareba et al. 2009), and little is known about the temperature influence on these parameters.

The main objective of our study was to evaluate the influence of air pollutants and air temperature on repeated measurements of HR and repolarization parameters, such as Bazett-corrected QT interval (QTc) (Bednar et al. 2001) and T-wave amplitude (Tamp).

Because specific single-nucleotide polymorphisms (SNPs) have been reported to modulate the QT interval (Pfeufer et al. 2009), we examined modifications of the association between air pollution and electrocardiogram (ECG) parameters by SNPs involved in detoxification pathways.

Materials and Methods

Study population. A panel study of nonsmoking myocardial infarction (MI) survivors was conducted in Augsburg, Germany, between 30 May 2003 and 1 February 2004, as a substudy of the AIRGENE (Air Pollution and Inflammatory Response in Myocardial Infarction Survivors: Gene-Environment Interaction in a High Risk Group) study. Only persons who had survived an MI between 3 months and 6 years before entry into the study were included. All methods used in the study center were conducted according to common standard operating procedures (Peters et al. 2007). All participants gave written informed consent, and the study protocol was approved by the German ethics commission (Bayerische Landesaerztekammer). During a clinical

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Supplemental Material is available online (doi:10.1289/ehp.1001995 via http://dx.doi.org/).

The AIRGENE study was funded as part of the European Union's 5th Framework Programme, key action 4: "Environment and Health," contract QLRT-2002-02236. This research has been funded wholly or in part by the U.S. Environmental Protection Agency through Science to Achieve Results grants RD827354 and RD832415 to the University of Rochester. This article has not been subjected to the agency's required peer and policy review and therefore does not necessarily reflect the views of the agency, and no official endorsement should be inferred.

The authors declare they have no actual or potential competing financial interests.

Received 26 January 2010; accepted 15 September 2010.

examination, a baseline questionnaire (Peters et al. 2007) was administered regarding health status, self-report of current medication intake, and smoking history; blood pressure and body mass index (BMI) also were measured. A more detailed description of the patient recruitment and the study panel can be found elsewhere (Peters et al. 2007).

Clinical measurements. At the beginning of the study, the participants were asked whether they wanted to transmit an ECG only in case of cardiac symptoms or daily at the same time during the whole study period. Participants were invited to call the Philips Monitoring Center in Düsseldorf, Germany, at any time and transmit an ECG via telephone (land line or mobile). For this purpose, a participant had to fix a 12-lead personal ECG transmitter (Philips Viapac, Philips Telemedicine and Healthcare Services, Dusseldorf, Germany; size ~ 13 × 7 × 5 cm; weight, 35 g) with a belt on the chest, which placed the electrodes automatically in the correct position (Mampuya 2002; Mischke et al. 2005). The medical staff of the monitoring center questioned the individuals about cardiac symptoms and whether they had been in traffic or had been exposed to great physical strain or anger 2 hr before the transmission. The staff, which comprised cardiologists and trained medical professionals, immediately evaluated the ECG manually.

Each participant repeatedly transmitted 16 sec ECGs to the monitoring center. If an individual sent two ECGs within 30 min, we excluded the first measurement assuming that the participant thought that the first transmission was incorrect and hence transmitted a second one. If there were several transmissions per day and per patient, only the first one (in case of no second transmission within 30 min) was included in the analysis. ECG measurements with a pacemaker rhythm, frequent ectopic ventricular beats, or atrial fibrillation were excluded. One individual with an internal defibrillator was kept in the study because he had a normal heart rhythm without conduction abnormalities during ECG transmissions.

The outcomes of interest were HR, QTc, Tamp, PQ interval, ST-segment changes, and ventricular and supraventricular ectopic beats. RR-, QT-, and PQ-interval durations were measured manually in lead II. We chose lead II for QT measurements because in clinical conditions this lead is considered representative of the overall electrical forces of the heart. For Tamp, we used ECG leads I, II, and V1–V6, and the median value from those eight original leads was taken for each cardiac cycle. For ST-segment leads LII (inferior wall), V2 (anterior wall), and V5 (lateral wall) were used, respectively, and the ST segment was the median value over each 16-sec period.

Genotyping. DNA was extracted from ethylenediaminetetraacetic acid anticoagulated blood using a salting out procedure. In our analysis, we used 12 SNPs located in four genes involved in detoxification pathways. The SNPs were rs10183914, rs1806649, rs1962142, and rs2364725 in the nuclear factor (erythroid-derived 2)-like 2 (*NFE2L2*) gene; rs1048942 and rs2606345 in the cytochrome P450 family 1 member A1 (*CYP1A1*) gene; rs1799945 and rs1800562 in the hemochromatosis (*HFE*) gene; rs1695, rs6591256, and rs4891 in the glutathione *S*-transferase pi (*GSTP1*) gene; and a deletion in glutathione *S*-transferase mu 1 (*GSTM1*). SNPs with a minor allele frequency (MAF) < 5% were excluded. Each SNP was tested for deviations from Hardy-Weinberg equilibrium (HWE). For the analyses, SNPs were coded linearly, counting the number of minor alleles. For frequencies of rare homozygote genotypes < 5%, the hetero- and minor homozygote genotypes were combined into one group.

Air pollution and meteorological data. Air pollution data from fixed monitoring sites representing urban background concentrations were collected according to standard procedures (Rückerl et al. 2007). Hourly means of carbon monoxide (CO), nitrogen dioxide (NO_2), and PM with an aerodynamic diameter ≤ 10 μm or 2.5 μm (PM_{10}, $PM_{2.5}$) were available. Particle number concentrations (PNC) were obtained as a proxy for ultrafine particles (UFP) with an aerodynamic diameter of 0.01–0.1 μm. Additionally, we computed coarse particles ($PM_{10-2.5}$) as the difference between PM_{10} and $PM_{2.5}$. Meteorological variables (air temperature, relative humidity, barometric pressure) were obtained through a monitoring system operated by the Bavarian Environment Agency (Bayerisches Landesamt für Umwelt).

For each person and ECG transmission, we determined average exposures for 0–23 hr before ECG transmission and for up to four 24-hr periods before (24–47, 48–71, 72–95, 96–119 hr) if more than two-thirds of the hourly air pollution or meteorological measurements were available for a given period. Additionally, we calculated mean exposures during the 5 days (120 hr) before ECG transmissions. Missing data on the aggregate level were replaced using a formula adapted from the APHEA (Air Pollution and Health: A European Approach) method (Berglind et al. 2009).

Statistical analysis. The longitudinal data were analyzed with the SAS statistical package (version 9.1; SAS Institute Inc., Cary, NC, USA) using additive mixed models with a random patient effect. To account for the dependencies between the inordinate repeated measurements, we assumed a spatial covariance structure. The elements of this covariance matrix decline as the elapsed time between two measurements increases.

For analysis of air pollution effects, we identified confounders for each ECG parameter separately. Potential confounders were long-term time trend, day of the week, temperature, relative humidity, and barometric pressure. The possible lags for meteorology were defined as 0–23, 24–47, 48–71, and 72–95 hr before ECG transmission. The confounders were modeled linearly or as penalized splines (P-splines) to allow for nonlinear relationships. The lag and shape that minimized the Akaike information criterion (AIC) was selected. If a confounder was included as a P-spline, we checked whether a polynomial led to a smaller AIC. Barometric pressure and day of the week were selected only if model fit was improved. For the analysis of temperature effects, only trend and relative humidity with a corresponding lag to the analyzed temperature lag were included as confounders.

After assessing the confounder model, single air pollution or temperature lags were added, and the effects were estimated linearly.

SNP selection. The influence of the selected SNPs on the mean or variability of the ECG parameters was estimated separately. In order to assess the association between an SNP and variability, mixed models with two different covariance structures were calculated. As described above, the first model used a spatial covariance matrix with blocks identical for each participant, but the second model included a matrix with three different blocks identical only for patients with the same genotype. If the likelihood ratio test revealed a significant difference between the two models, we assumed that the variability of the ECG parameter differed between the genetic groups. For SNP selection, the alpha-level was corrected for the number of independent tests following a modified Bonferroni procedure (Li and Ji 2005).

Effect modification. Interaction variables were added to the model in order to estimate the air pollution or temperature effects of the corresponding subgroups. For air pollution, separately included interaction variables were age (age < 60 vs. ≥ 60 years), BMI (< 30 vs. ≥ 30 kg/m^2), beta-adrenergic receptor blockers (intake vs. no intake), sex (women vs. men), season (summer April–September vs. winter October–March), smoking status (ex-smoker vs. never-smoker), being in traffic 2 hr before the ECG transmission (yes vs. no), and SNPs with a significant influence on the mean or variability of at least one ECG parameter. For temperature, we assessed interactions with age, BMI, intake of beta blockers, sex, and season. To investigate possible interaction effects between exposure variables, we calculated smooth interaction functions of air pollution and temperature using R version 2.5.0 (R Foundation for Statistical Computing 2008). For this purpose, we used the exposure lags that showed the strongest associations

with the ECG parameters in the performed main analyses that included exposure variables separately as fixed effects in mixed models.

Sensitivity analyses. In order to check the robustness of our models, we performed several sensitivity analyses. For air pollution and temperature effect estimation, we excluded ECGs with ectopic beats or ECGs transmitted when participants indicated they were having cardiac problems, respectively. In a further sensitivity analysis, individuals with QRS intervals > 120 msec in at least two ECGs were excluded. Furthermore, we performed an alternative confounder model including patient characteristics such as blood pressure, BMI, sex, smoking status, or physical activity if this minimized the AIC. Afterward, the meteorological confounders were selected as described above. Because PNC was not measured for several weeks during the study period, we additionally estimated the association between PM or gaseous pollutants and ECG parameters only on days with existing PNC measurements.

For temperature effect estimation we added barometric pressure, PM_{10}, or $PM_{2.5}$ separately to the original confounder model. Barometric pressure was included with the same lag as the analyzed temperature lag. PM was included either with the same lag as temperature or with the lag exhibiting the largest association with the outcome in the air pollution analysis. The shape (linear, smooth, polynomial) of barometric pressure and air pollutants that minimized AIC was chosen, respectively. Additionally, temperature effects were estimated as P-splines.

Table 1. Description of the study population of 67 participants with at least one MI.

Clinical characteristic	Mean ± SD or total (%)
Age (years)	59.3 ± 8.5
BMI (kg/m^2)	29.0 ± 4.3
Systolic blood pressure (mmHg)	127.7 ± 19.9
Diastolic blood pressure (mmHg)	77.7 ± 10.5
Sex (men)	58 (87)
BMI (kg/m^2)	
< 30	42 (63)
≥ 30	25 (37)
ECG transmission time period	
Winter (October–March)	791 (45)
Summer (April–September)	954 (55)
Type 2 diabetes mellitus	9 (13)
First MI	59 (87)
Angina pectoris	14 (21)
Arrhythmias	15 (22)
Congestive heart failure	6 (9)
Hypertension	28 (42)
Occupational status	
Full-time or part-time employment	29 (43)
Exposed to toxic gases, dust, or fumes during work	15 (22)
Smoking	
Never-smoker	14 (21)
Ex-smoker[a]	53 (79)
Current medication intake	
Beta blockers	62 (93)
Angiotensin-converting enzyme inhibitors	48 (72)
Calcium-channel blockers	8 (12)
Nitrates	9 (13)
Statins	59 (88)
Diuretics	22 (32)
Acetylsalicylic acid	61 (91)
Other antithrombotics	7 (10)

All patient characteristics were determined with a questionnaire during a clinical examination.
[a]Had stopped smoking at least 3 months before start of the study.

Results

Study population. A total of 75 individuals participated in the substudy and received a Viapac system from Philips. Two patients were ineligible because they had not had a previous MI according to the official criteria; we excluded five patients because they did not transmit any ECG or transmitted only one ECG to the monitoring center. We excluded individual ECG measurements for the following reasons: abnormal pacemaker rhythm or ventricular trigeminus (51 ECGs from 3 patients), atrial fibrillation (37 ECGs from 5 patients), transmission while not in Augsburg (14 ECGs from 10 patients) or from an unknown location (2 ECGs from 2 patients), second ECG transmitted within 30 min (3 ECGs from 3 patients), and second ECG transmission within 1 day but not within 30 min of another transmission (14 ECGs from 9 patients). We excluded one patient because only one eligible ECG was available after we excluded a second ECG due to atrial fibrillation. Therefore, 1,745 ECGs from 67 patients were available for the analyses. Only 3 of 21 persons who agreed to transmit ECGs on a daily basis did so; 46 persons declared intention to transmit ECGs only in case of cardiac problems. However, in both groups only 16 ECGs (2%) were sent due to heart trouble, respectively. The participants transmitted on average 26 ECGs (range, 3–178 ECGs) and experienced an MI on average 2.2 years (range, 0.6–3.5 years) before study entry. The age of the participants ranged from 40 to 76 years. In Table 1, we provide further clinical patient characteristics.

Clinical measurements. Table 2 describes the ECG parameters analyzed. HR, QTc, Tamp, and PQ interval were not correlated according to the median of patient-specific Spearman correlation coefficients (data not shown). We did not evaluate ST-segment changes because they were available for only 72 ECGs (4%). We did not analyze ectopic beats because only 76 ECGs (4%) from 19 participants and 31 ECGs (2%) from 12 participants exhibited ventricular and supraventricular ectopic beats, respectively.

Air pollution and meteorological data. During the study period the 24-hr averages ± SD were 33.4 ± 13.2 μg/m^3 for PM_{10}, 15.8 ± 7.7 μg/m^3 for $PM_{10–2.5}$, 17.7 ± 6.2 μg/m^3 for $PM_{2.5}$, 11,809 ± 6,253/cm^3 for PNC, 40.7 ± 10.8 μg/m^3 for NO_2, 0.6 ± 0.2 mg/m^3 for CO, 10.8 ± 9.9°C for air temperature, 69.1 ± 14.5% for relative humidity, and 1018.2 ± 6.9 hPa for barometric pressure [for additional information, see Supplemental Material, Table 1 (doi:10.1289/ehp.1001995)]. Spearman correlation coefficients among the three PM measurements and between CO and PNC, $PM_{10–2.5}$ and NO_2, and temperature and relative humidity revealed moderate or strong correlations ($|r| > 0.5$). The remaining air pollutants and meteorological covariates were not correlated. Supplemental Material, Figure 1 (doi:10.1289/ehp.1001995), depicts daily 24-hr averages of $PM_{2.5}$, PNC, and temperature during the study period. About 29% of the PNC measurements and ≤ 2% of all other air pollution and meteorological variables were missing due to a device failure.

Estimated effects of air pollution. Table 3 shows the percent changes in arithmetic mean values of the ECG parameters [with 95% confidence intervals (CIs)] per interquartile range (IQR; difference between the third and first quartile) increase in PM and PNC. IQR increases in PM levels were associated with significant increases in QTc 24–47 hr later, and borderline significant increases in QTc were associated with 48- to 71-hr lag and 5-day average PM_{10} and $PM_{2.5}$ levels. IQR increases in CO concentrations were also associated with QTc prolongation [0.4% (95% CI, 0.1–0.7%)] 24–74 hr later (data not shown). In general, Tamp was positively associated with air pollution levels 0–23 hr and 24–47 hr before ECG transmission and inversely associated with levels more than 48 hr before, with a significant positive association with $PM_{2.5}$ 0–23 hr before and a significant inverse association with $PM_{10–2.5}$ 96–119 hr before transmission (Table 3). In addition, Tamp was positively associated with NO_2 0–23 hr [3.0% (0.2–5.7%)] and 24–47 hr [3.1% (0.1–6.1%)] before transmission. We observed no significant associations between air pollutants and HR (Table 3) or PQ interval (data not shown).

Table 2. Description of ECG parameters.

ECG parameter	*n*	Mean ± SD	Minimum	25%	Median	75%	Maximum	IQR
HR (beats/min)	1,745	71.5 ± 11.6	40	63	71	79	114	16
QTc (msec)	1,744	400.6 ± 32.6	270	380	400	420	560	40
Tamp (μV)	1,743	213.4 ± 154.8	–200	100	200	300	700	200
PQ interval (msec)	1,743	164.1 ± 27.8	110	140	160	180	290	40

25% and 75% are 25th and 75th percentiles, respectively.

Estimated effects of air temperature. HR, QTc, and PQ interval were not associated with temperature changes (data not shown), but we observed an inverse U-shaped association between temperature and Tamp with highest Tamp at 5°C (Figure 1A). To obtain separate temperature effect estimates for warmer and colder days, we modeled temperature as a linear variable and added an interaction term between temperature and a variable indicating mean temperature above or below 5°C. For all lags we observed a 5–9% decrease in Tamp associated with a temperature decline of 5°C on days with average temperatures < 5°C (cold effects) and with a 5°C increase in temperature on days > 5°C (heat effects; Figure 1B).

Effect modification. HR significantly increased in association with an IQR increase in $PM_{2.5}$ 0–23 hr before ECG transmission in individuals with BMI ≥ 30 kg/m^2 [1.8% (0.4–3.1%)] and in individuals not using beta blocker medication [2.4% (0.4–4.5%); Figure 2]. These effect modifications were significant (p-value < 5%) for BMI but only borderline significant for intake of beta blockers. However, only five participants with 200 ECGs did not take beta blockers. No significant interactions between PM and BMI or intake of beta blockers were evident for QTc. Tamp was significantly increased in association with an IQR increase in $PM_{2.5}$ 0–23 hr before ECG transmission in participants with a BMI < 30 kg/m^2 and in patients taking beta blocker medications. However, the interaction effects were not significant. Additionally, we observed a positive association between Tamp and an IQR increase in CO 24–47 hr before ECG transmission among participants with BMI < 30 kg/m^2 [2.7% (0.5–4.8%)] and inverse associations with CO among those with BMI ≥ 30 kg/m^2 [–3.7% (7.4–0.0%)].

Table 3. Percent change of the outcome mean per IQR increase in pollutant.

	Estimate (95% CI)			
Hr before transmission	PM_{10}[a] (µg/m^3)	$PM_{10-2.5}$[b] (µg/m^3)	$PM_{2.5}$[c] (µg/m^3)	PNC[d] (/cm^3)
HR				
0–23 hr	0.4 (–0.3 to 1.2)	0.2 (–0.5 to 1.0)	0.5 (–0.2 to 1.2)	–0.4 (–1.2 to 0.5)
24–47 hr	0.5 (–0.3 to 1.3)	0.5 (–0.2 to 1.3)	0.5 (–0.2 to 1.1)	–0.6 (–1.4 to 0.3)
48–71 hr	0.2 (–0.6 to 0.9)	0.1 (–0.6 to 0.9)	0.1 (–0.6 to 0.8)	–0.7 (–1.5 to 0.1)
72–95 hr	0.3 (–0.5 to 1.1)	0.3 (–0.5 to 1.0)	0.2 (–0.5 to 1.0)	–0.4 (–1.2 to 0.5)
96–119 hr	–0.1 (–0.9 to 0.7)	0.1 (–0.7 to 0.9)	–0.2 (–0.9 to 0.6)	–0.5 (–1.5 to 0.4)
5-day average	0.3 (–0.3 to 1.0)	0.4 (–0.3 to 1.1)	0.3 (–0.4 to 1.0)	–1.2 (–2.6 to 0.1)*
QTc				
0–23 hr	0.2 (–0.4 to 0.7)	0.0 (–0.5 to 0.5)	0.3 (–0.2 to 0.8)	0.0 (–0.6 to 0.6)
24–47 hr	0.7 (0.2 to 1.2)**	0.8 (0.3 to 1.3)**	0.5 (0.0 to 1.0)**	0.5 (–0.1 to 1.0)
48–71 hr	0.4 (–0.1 to 0.9)*	0.4 (–0.1 to 0.9)	0.4 (0.0 to 0.9)*	0.2 (–0.4 to 0.8)
72–95 hr	0.3 (–0.2 to 0.8)	0.3 (–0.2 to 0.8)	0.3 (–0.2 to 0.8)	0.1 (–0.5 to 0.7)
96–119 hr	0.3 (–0.2 to 0.8)	0.3 (–0.3 to 0.8)	0.3 (–0.2 to 0.8)	–0.6 (–1.3 to 0.1)
5-day average	0.4 (0.0 to 0.8)*	0.4 (0.0 to 0.8)*	0.4 (0.0 to 0.9)*	0.2 (–0.8 to 1.1)
Tamp				
0–23 hr	3.3 (0.0 to 6.5)*	2.9 (–0.3 to 6.0)*	3.3 (0.2 to 6.3)**	1.7 (–1.7 to 5.1)
24–47 hr	3.0 (–0.4 to 6.4)*	2.5 (–0.8 to 5.8)	2.8 (–0.3 to 5.9)*	2.8 (–0.6 to 6.1)
48–71 hr	–0.8 (–4.1 to 2.4)	–0.8 (–4.0 to 2.3)	–0.5 (–3.5 to 2.5)	–2.4 (–6.1 to 1.3)
72–95 hr	–1.8 (–5.1 to 1.4)	–2.1 (–5.4 to 1.1)	–1.3 (–4.2 to 1.6)	–3.0 (–6.7 to 0.7)
96–119 hr	–2.3 (–5.5 to 0.9)	–3.2 (–6.4 to –0.1)**	–0.9 (–3.8 to 2.0)	–0.8 (–4.6 to 2.9)
5-day average	0.1 (–2.8 to 3.0)	–0.5 (–3.4 to 2.5)	0.8 (–2.3 to 4.0)	–0.7 (–7.0 to 5.6)

[a]IQR 24-hr average, 19.5 µg/m^3; IQR 5-day average, 11.9 µg/m^3. [b]IQR 24-hr average, 10.9 µg/m^3; IQR 5-day average, 6.5 µg/m^3. [c]IQR 24-hr average, 8.4 µg/m^3; IQR 5-day average, 6.3 µg/m^3. [d]IQR 24-hr average, 7,481/cm^3; IQR 5-day average, 6,974/cm^3. *p < 0.1. **p < 0.05.

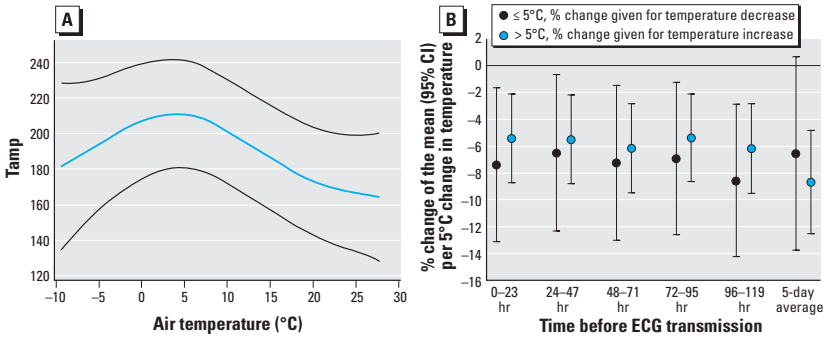

Figure 1. P-spline of air temperature in association with Tamp (*A*) and mean change in Tamp (with 95% CIs) associated with a 5°C increase or decrease in air temperature on days with average air temperature above or below 5°C, respectively (*B*). Both A and B are adjusted for long-term time trend and relative humidity.

We did not evaluate associations with rs18005862 in *HFE* and rs1048943 in *CYP1A1* because MAFs were < 5%. Although rs10183914 in the *NFE2L2* gene was not in HWE, we did not exclude it because our study comprises a highly selected group of MI survivors and not a random population sample. We corrected the global significance level of 5% for testing associations between SNPs and mean levels or variability of ECG parameters for the 8 independent tests resulting in an adjusted alpha-level of 0.05/(8 × 2) = 0.003125. No SNP was significantly associated with mean levels of ECG parameters (data not shown), but some were significantly associated with variability in ECG parameters [see Supplemental Material, Table 2 (doi:10.1289/ehp.1001995)].

We detected interaction effects between particulate air pollutants and genotypes only for rs2364725 in the *NFE2L2* gene on QTc (Figure 3). Nineteen participants with 363 ECGs were homozygous carriers of the minor allele (G), 28 participants with 885 ECGs were heterozygous, and 20 participants with 497 ECGs were homozygous carriers of the major allele (T). IQR increases in PM 24–47 hr before ECG transmission were associated with a prolonged QTc of about 0.5–1.5% only in patients with at least one minor allele. We also observed a similar pattern for PM exposures 0–23 hr before ECG transmission (data not shown). An IQR increase in PNC exposure 96–119 hr before transmission was inversely associated with QTc in patients with one or two minor alleles [–0.8% (–1.5 to –0.1%) and –2.2% (–3.4 to –1.0%), respectively] but not in other patients [0.6% (–0.4 to 1.6%)].

We detected no other significant air pollution effect modifications with other variables. Temperature effects were not modified by any variable (data not shown).

Sensitivity analyses. After excluding 10 patients having at least two ECGs with QRS intervals > 120 msec the air pollution associations were in general slightly more pronounced (data not shown). We observed an immediate and 24- to 47-hr lagged increase in HR of about 1% in association with PM. None of the other sensitivity analyses resulted in any notable changes in associations between air pollutants or temperature and the outcomes. We found no evidence for a deviation of linearity of temperature effects on HR or QTc.

Discussion

Our analyses showed no main effects of air pollutants on HR overall, but we observed significant positive associations between PM 0–23 hr before and HR among participants with BMI ≥ 30 kg/m^2 and among those not using beta blocker medications. We observed

a prolonged QTc interval in association with increases in PM levels 24–47 hr before ECG transmission, with stronger associations among participants with one or two minor alleles of the *NFE2L2* SNP rs2364725. However, patients with at least one minor allele showed shortened QTc in association with an increase in PNC 96–119 hr before. Tamp decreased in association with both cold and warm temperatures, with maximum Tamp around 5°C.

Air pollutants and ECG parameters. Several authors have reported inverse associations between air pollutants and HRV in the elderly (Luttmann-Gibson et al. 2006; Schwartz et al. 2005). It is hypothesized that air pollutants may activate the sympathetic nervous system directly or indirectly, which possibly leads to an increased HR and reduced HRV. UFP might even translocate into the systemic circulation and affect the electric system of the heart directly (Pope and Dockery 2006). It has been shown that patients not using beta blocker medications exhibit a stronger reduction in HRV in association with PM exposure compared with patients using beta blockers (de Hartog et al. 2009). We observed an increased HR with exposure to air pollutants only among our patients with 200 ECGs not using beta blockers. Beta blockers constrain the activation of the sympathetic tone; thus, participants using beta blockers might be less susceptible to activation of the sympathetic nervous system by air pollutants. Because of the small number of participants not taking beta blockers and even if patient characteristics did not differ significantly between individuals with and without beta blocker intake, it is still possible that the observed differences in the estimated air pollution effects are related to something other than medication intake. Consistent with our findings, Chen et al. (2007) reported stronger positive associations between $PM_{2.5}$ and HR in individuals with BMI ≥ 30 kg/m^2.

Henneberger et al. (2005) detected an immediate positive association of 24-hr averages of organic carbon with QTc. Furthermore, Liao et al. (2010) reported immediate and delayed QTc prolongations associated with elevated 30-min averages of $PM_{2.5}$. However Lux and Pope (2009) observed no association between $PM_{2.5}$ and repolarization parameters in elderly participants. In our analysis we found lagged associations between PM and QTc. Associations were more pronounced among participants with one or two minor alleles of the *NFE2L2* SNP rs2364725. The *NFE2L2* gene is believed to be involved in the defense against oxidative stress (Goldring et al. 2004). We can only speculate that the defense is more activated in patients with common alleles, whereas patients with at least one minor allele are more susceptible to PM. In contrast, we observed inverse associations between QTc and PNC, a proxy for UFP, in accordance with two chamber studies (Samet et al. 2009; Zareba et al. 2009) that reported QTc shortening in healthy nonsmoking subjects who were exposed to UFP. Different effects of PM and PNC might reflect different biological pathways activated by different particle properties. Tamp indicates the repolarization of the ventricles and Henneberger et al. (2005) reported a 7.3% decrease in Tamp in association with an increase in UFP 0–23 hr before ECG measuring. A subsequent analysis of Yue et al. (2007) suggested that this association was driven by traffic-related UFP specifically. We observed a 4-day delayed decrease and an immediate elevation of Tamp in relation with all PM parameters which cannot be explained by a single patient characteristic such as BMI. It can only be speculated that a combination of medication intake and disease history may be involved in the susceptibility to air pollution. A study by Schneider et al. (2010) also observed opposed variations in Tamp responses depending on the considered $PM_{2.5}$ lag. In general, changes in repolarization might be the result of changes in the ion channel function or a direct effect of the autonomic nervous system on the ventricular myocardium (Ghelfi et al. 2008; Henneberger et al. 2005; Zareba et al. 2009). However, the understanding of the complex biologic pathways is still very limited.

It has been shown that T-wave alternans (TWA) is a reliable predictor for sudden cardiac death (Stein et al. 2010). Zanobetti et al. (2009) observed an association between black carbon and TWA in CAD patients. Prolonged QTc are a risk factor for cardiac arrhythmia (Roden 2008) and cardiovascular mortality (Ziegler et al. 2008). Our results suggest that elevated levels of air pollutants might trigger changes in Tamp and QTc and therefore might predispose to additional cardiovascular problems in individuals who had already experienced an MI.

Temperature and ECG parameters. Yamamoto et al. (2007) found increased HR and decreased HRV after exposing six healthy Japanese to a heated condition (37°C) in a chamber study. Bruce-Low et al. (2006) observed similar results among participants with ECG measurements before and during exposure to heat in a sauna. In our analyses, HR did not appear to be altered by temperature changes, but during the study period 24-hr averages of temperature never exceeded 28°C, which is similar to the temperature

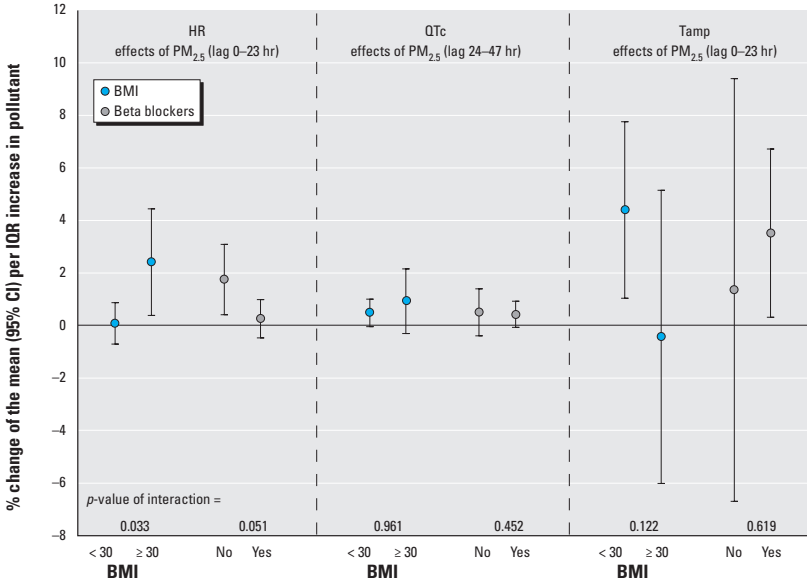

Figure 2. Subgroup-specific associations of IQR increases in $PM_{2.5}$ with ECG parameters (adjusted for long-term time trend and meteorology; $PM_{2.5}$ IQR, 8.4 µg/m^3).

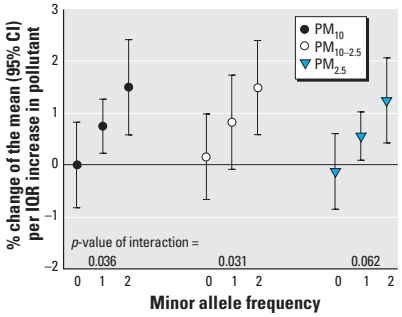

Figure 3. Associations between IQR increases in PM and QTc (lag, 24–47 hr) according to minor allele frequencies of *NFE2L2* rs2364725 (T, wild-type allele; G, minor allele; adjusted for long-term time trend and meteorology; IQRs: PM_{10}, 19.5 µg/m^3; $PM_{10-2.5}$, 10.9 µg/m^3; $PM_{2.5}$, 8.4 µg/m^3).

conditions before heat exposure in the previously described studies.

Several studies reported a U- or J-shaped influence of apparent temperature on cardiorespiratory mortality; the lowest mortality was observed for temperatures between 15 and 25°C (Baccini et al. 2008; McMichael et al. 2008). However, Baccini et al. (2008) conducted their study only in the summer, whereas our study took place during almost 1 year with low temperatures during the winter. A study conducted by McMichael et al. (2008) also included Asian and South American cities with high mean temperatures.

Lin et al. (2008) reported an increased risk of cardiovascular events during a follow-up of 30 days among patients with T-wave flattening at the time of the emergency department visit. In our study Tamp decreased with temperature increases as well as decreases. Wolf et al. (2009) observed an inverse relation between temperature and MI occurrence. Therefore, temperature might act as a trigger for T-wave flattening in susceptible individuals, leading to an enhancement of already existing cardiovascular problems.

Because core temperature should not be affected by small changes in ambient temperature, we can only speculate that our observed associations are probably affected by changes in the autonomic nervous system or by loading effects on the heart possibly mediated by cutaneous blood flow regulation or neural input from temperature sensors in the skin.

Strengths and limitations. A strength of this study is the ability to analyze intraindividual variation because patients transmitted ECG parameters on several occasions. Further strengths are the nonlinear confounder adjustment and the detailed information on patient characteristics and SNPs allowing us to perform several subgroup analyses. Because our estimated effects remained stable in several sensitivity analyses, our results seem to be quite robust. Cardiac arrhythmias alter the interpretation of the ECG parameters; therefore, to have a homogeneous ECG data set, we excluded ECGs with pacemaker rhythms, ventricular trigeminus, or atrial fibrillation. One limitation is that we measured only outdoor exposure, whereas in general people spend a lot of time indoors. However, a study of Cyrys et al. (2004) revealed that ambient concentrations of $PM_{2.5}$ and black smoke can be used as good approximation of indoor concentrations. However, personal measurements of air pollutants should be taken into account in future studies if possible. Because several studies (Analitis et al. 2008; Zanobetti and Schwartz 2008) have shown that changes in temperature are associated with increases in cardiovascular mortality, we assumed that also a short exposure to outdoor temperature possibly influences ECG parameters. A variety of exposure and outcome variables have been used for the analyses, so we cannot exclude that some associations occurred only by chance. A further limitation is that our panel comprised a highly selected group of MI survivors who were taking a variety of medications and might have different reactions to air pollution and temperature compared with healthy people. Thus, generalizability of our results is uncertain. On the other hand, analyzing vulnerable patients might give better insight into possible mechanistic pathways

Conclusion

Our results indicate that IQR increases in air pollutants were associated with an increase in mean HR among MI patients with BMI ≥ 30 kg/m^3 and among those not using beta blockers. We observed QTc prolongation in association with an IQR increase in PM in patients with at least one minor allele of a *NFE2L2* SNP. We detected inconsistent associations between air pollution and Tamp, and nonlinear associations between ambient temperature and Tamp. Overall, we observed changes in HR and repolarization parameters associated with air pollutant exposures and temperature changes that are possible precursors for additional cardiovascular problems in individuals who had already experienced an MI.

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Supplemental Material, Table 1. Description of daily concentration of air pollutants and meteorological variables between May 2003 and February 2004 (absolute correlations coefficients larger than 0.5 are highlighted in grey).

	N	Mean (±SD)	Min.	25%	Median	75%	Max.	IQR	IQR_5	Spearman correlation coefficient							
										PM_{10}	$PM_{10-2.5}$	$PM_{2.5}$	PNC	NO_2	CO	Temp.	Rel. hum.
PM_{10} [µg/m³]	247	33.4 (±13.2)	7.1	23.0	33.0	42.4	70.9	19.5	11.9	1							
$PM_{10-2.5}$ [µg/m³]	243	15.8 (± 7.7)	-0.8	9.9	15.9	20.8	34.7	10.9	6.6	0.96	1						
$PM_{2.5}$ [µg/m³]	244	17.7 (±6.2)	6.2	12.9	17.1	21.3	38.7	8.4	6.3	0.93	0.80	1					
PNC [#/cm³]	176	11809 (±6253)	2764	7019	10447	14500	32528	7481	6975	0.37	0.36	0.32	1				
NO_2 [µg/m³]	248	40.7 (± 10.8)	15.4	33.1	40.0	47.0	71.9	14.0	9.6	0.64	0.67	0.55	0.52	1			
CO [mg/m³]	248	0.6 (±0.2)	0.3	0.4	0.5	0.7	1.7	0.2	0.2	0.58	0.55	0.56	0.74	0.63	1		
Air temperature [°C]	246	10.8 (±9.9)	-9.4	2.0	11.2	20.5	27.6	18.4	18.3	0.16	0.17	0.14	-0.64	0.11	-0.45	1	
Relative humidity [%]	246	69.1 (±14.5)	41.3	56.2	68.7	81.6	93.4	25.5	21.9	-0.16	-0.18	-0.15	0.29	-0.27	0.36	-0.77	1
Barom. pressure [hPa]	246	1018.2 (±6.9)	991.0	1014.7	1019.2	1023.0	1031.2	8.3	6.6	0.21	0.23	0.15	0.02	0.22	0.03	0.19	-0.32

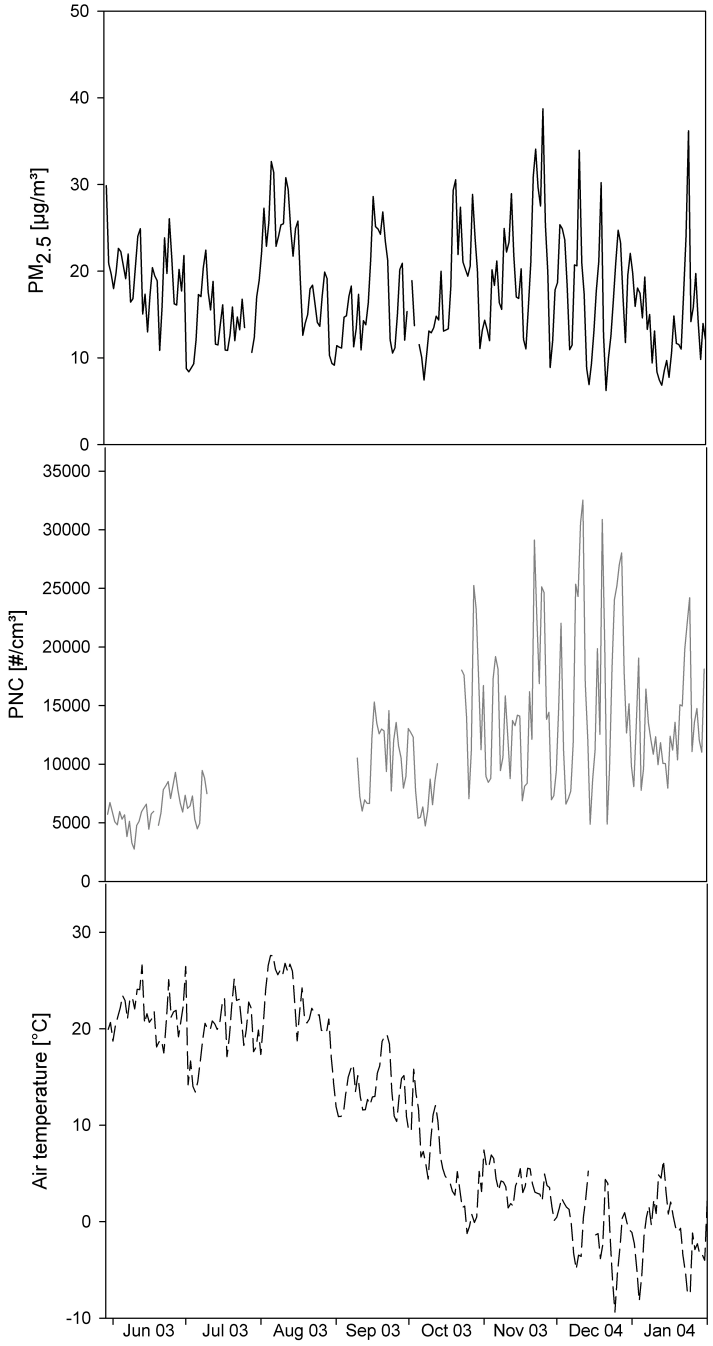
PM_{10}: Particulate matter with an aerodynamic diameter < 10 µm, $PM_{10-2.5}$: Coarse particles, $PM_{2.5}$: Particulate matter with an aerodynamic diameter < 2.5 µm, PNC: Particle number concentrations, NO_2: Nitrogen dioxide, CO: Carbon monoxide, SD: Standard deviation, IQR: Interquartile range of the 24h-average, IQR_5: Interquartile range of the 5-day average.

Supplemental Material, Table 2. SNPs with a significant influence on the variability of ECG parameters.

Outcome	Gene	SNP	Minor allele	Major allele	Distribution in study population N (%)			MAF %	p-value*
					Homozygous minor allele	Heterozygous	Homozygous major allele		
HR	*NFE2L2*	rs1962142	A	G	0 (0)	20 (30)#	67 (70)	14.9	1.57E-04
QTc	*NFE2L2*	rs10183914	T	C	9 (13)#	25 (37)	33 (49)	32.1	4.77E-04
	NFE2L2	rs2364725	G	T	19 (28)	28 (42)	20 (30)#	49.3	6.78E-07
	HFE	rs1799945	G	C	1 (2)	16 (24)	49 (74)#	13.6	2.11E-04
Tamp	*NFE2L2*	rs10183914	T	C	9 (13)	25 (37)#	33 (49)	32.1	1.59E-06
	NFE2L2	rs1806649	T	C	6 (9)	22 (33)#	39 (58)	25.4	4.44E-16
	NFE2L2	rs1962142	A	G	0 (0)	20 (30)#	67 (70)	14.9	7.15E-09
	NFE2L2	rs2364725	G	T	19 (28)	28 (42)	20 (30)#	49.3	3.04E-14
	GSTM1		-	-	-	-	-**	45.3***	4.44E-15
	GSTP1	rs4891	C	T	8 (12)	30 (45)#	29 (43)	34.3	1.31E-06
	GSTP1	rs1695	G	A	7 (10)	29 (29)#	31 (46)	32.1	2.92E-07
	GSTP1	rs6591256	G	A	15 (22)	31 (46)	21 (31)#	45.5	2.22E-16
	CYP1A1	rs2606345	C	A	4 (6)#	31 (46)	32 (48)	29.1	5.47E-06

SNP: Single nucleotide polymorphism, MAF: minor allele frequency, HR: Heart rate, QTc: Bazett-corrected QT-interval, Tamp: T-wave amplitude, #Indicates subgroup with highest variability in ECG parameter, *Taking multiple testing into account led to a significance level of 0.003125, **Homozygote carriers of deletion showed a higher variability in Tamp, **Percentage of homozygote carriers of deletion

Supplemental Material, Figure 1. Time series of $PM_{2.5}$, PNC and air temperature in Augsburg, Germany between May 30th 2003 and February 1st 2004 ($PM_{2.5}$: particulate matter with a diameter below 2.5µm, PNC: particle number concentration with a size range of 0.01 to 0.1µm in diameter).



6 Association between air pollution and heart rate variability is modified by SNPs involved in cardiac rhythm in individuals with diabetes or impaired glucose tolerance.

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Status: Ready for submission

Association between air pollution and heart rate variability is modified by SNPs involved in cardiac rhythm in individuals with diabetes or impaired glucose tolerance.

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ABSTRACT

Background. Epidemiological studies have shown associations between particulate matter (PM) and heart rate variability (HRV).
Objectives. We investigated the effects of air pollution on the root mean square of successive differences in RR intervals (RMSSD) and the standard deviation of NN-intervals (SDNN) and effect modifications by single nucleotide polymorphisms (SNP).
Methods. Between March 2007 and December 2008 207 ECG recordings comprising 1153 1h-intervals were measured in 61 individuals with type 2 diabetes mellitus or impaired glucose tolerance (IGT) from Augsburg, Germany. Associations between 1h-averages of air pollutants (PM, sulphate, black carbon, and ultrafine particles) and ECG parameters were analyzed using additive mixed models. Genotypes of 139 SNPs supposed to be involved in cardiac rhythm were identified in the literature. Using regression

trees for longitudinal data, SNPs associated with ECG parameters were determined and included as potential air pollution effect modifiers.
Results. We observed concurrent and lagged decreases in SDNN by about 2-5% in association with all air pollutants, especially in participants with at least one minor allele of rs332229. Increases in PM$<$ 2.5µm ($PM_{2.5}$) were associated with 4h-lagged decreases of -6.6% [95%-confidence interval: -10.6;-2.6%] and -13.0% [-20.7;-5.1%] in SDNN in individuals with one or two minor alleles, respectively. We observed a -7.2% [-12.2;-1.8%] reduction in RMSSD associated with concurrent increases in $PM_{2.5}$. Individuals with at least one minor allele of rs2096767 or at most one minor allele of rs2745967 exhibited stronger $PM_{2.5}$ effects.
Conclusions. We identified a genetic predisposition in persons with diabetes or IGT making them potentially more susceptible to air pollutants with regard to changes in HRV.

INTRODUCTION

Initially, Pope et al. (1999), Peters et al. (1999), and Gold et al. (2000) reported associations between elevated air pollution levels and increases in heart rate (HR) and decreases in heart rate variability (HRV). Recent studies were able to confirm changes in heart rhythm. For instance, researchers observed decreased time and frequency domain parameters in association with elevated air pollution levels in patients with coronary artery disease (CAD) (Zanobetti et al. 2010), in individuals with metabolic syndrome (Min et al. 2009; Park et al. 2010), and in healthy participants (Wu et al. 2010). It is assumed that the observed findings might be a consequence of 1) an imbalance of the autonomic nervous system, 2) a direct affection of the electric system of the heart, or 3) a systemic inflammation and oxidative reaction promoting vascular dysfunction (Brook et al. 2010; Pope and Dockery 2006). Individuals with diabetes mellitus type 2 (DMT2) are supposed to be a susceptible population. Peel et al. (2007) and Zanobetti and Schwartz (2001), for example, observed an increased risk for emergency department visits and hospital admissions in association with air pollution increases in diabetes patients, respectively. Diabetes can cause autonomic neuropathy leading to a reduced HRV (Kudat et al. 2006); Park et al. (2005) observed a stronger reduction in some HRV parameters in association with particulate air pollution increases in DMT2 individuals compared to people without DMT2.
Ambient air pollution may act on the autonomic function via oxidative stress pathways (Brook et al. 2010). Therefore, not only people with DMT2 might be susceptible to air pollution but also people with genetic predispositions related to these pathways. Accordingly, authors observed modifications of

air pollution effects on HRV parameters by oxidative stress-related single nucleotide polymorphisms (SNP) (Chahine et al. 2007; Schwartz et al. 2005). It has also been reported that HR and HRV (Eijgelsheim et al. 2010; Newton-Cheh et al. 2007) as well as repolarization parameters (Chambers et al. 2010; Pfeufer et al. 2009) are modulated by variants in genes. We hypothesized that people with an altered cardiac rhythm due to genetic predispositions might also show different reactions to air pollution exposure. Therefore, we investigated the modification of air pollution effects on HRV parameters by SNPs involved in cardiac rhythm in potentially susceptible participants with DMT2 or impaired glucose tolerance (IGT) indicating an enhanced risk for DMT2. In contrast to already published studies (Chahine et al. 2007; Probst-Hensch et al. 2008; Schwartz et al. 2005) which used single candidate SNPs as potential effect modifiers we performed, in a first step, regression trees for longitudinal data in order to identify SNPs with an influence on repeated measurements of ECG parameters. Only these influential SNPs were then used as potential effect modifiers. The main advantage of this procedure was that we reduced the number of performed tests. Moreover, with this method we were able to select more than 130 SNPs from published genome-wide association studies (GWAS) and did not have to restrict our analysis to only a few candidate SNPs.

MATERIALS AND METHODS

Study design and study population

As part of the University of Rochester Particulate Matter Center investigations, a prospective panel study was conducted between March 19th 2007 and December 17th 2008 in Augsburg, Germany. Individuals with DMT2 or IGT were recruited from the KORA (Cooperative Health Research in the Region of Augsburg) F4 cohort which was conducted in the years 2006-2008 and also serves as study sample for genome-wide analysis (Holle et al. 2005; Wichmann et al. 2005). In our study, all individuals participated in up to four repeated ECG recordings scheduled every 4-6 weeks on the same weekday and at the same time of the day. Data on health status, medication as well as disease and smoking history were gathered at a baseline visit. Exclusion criteria were current smoking, intake of platelet aggregation inhibitors except for acetylsalicylic acid, a myocardial infarction (MI) and/or interventional procedure (PTCA, bypass surgery) less than six months before the start of the study, chronic inflammatory diseases, an implanted pacemaker, atrial fibrillation, allergy to latex, and thrombosis or shunt in an arm. All

participants gave written informed consent and the study protocol was approved by the Ethics Commission of the Bavarian Chamber of Physicians ("Bayerische Landesaerztekammer").

Clinical measurements

Participants were equipped with a 12-lead Mortara H12 digital Holter recorder (Mortara Instrument, Milwaukee, WI, USA). They left the study center to pursue their daily routines and returned after four to six hours. ECG parameters such as HR, repolarization, and HRV time and frequency domain parameters were determined on an hourly basis. Therefore, repeated ECG recordings for each participant and repeated 1h-averages of ECG parameters within one recording were available. Only individuals with at least one ECG recording with a duration of at least two hours were used for analysis.

Genotyping

Genome-wide data were available for each individual based on MACH imputation of Affymetrix 6.0 genotyped data (see Supplement Material). For our analysis we only used SNPs supposed to be involved in cardiac rhythm (e.g. HR, RMSSD, high and low frequency, and PR- and QT-interval) which were already identified in the literature and published before August 2010. SNPs were coded counting the number of minor alleles. We tested for evidence against the additive genetic model using a procedure introduced by Schaid et al. (Schaid 2004). For our analysis we excluded SNPs in case of a minor allele frequency below 5%, an imputation quality (observed vs. expected variance of the genotypes as returned as measure RSQR by MACH) below 0.6 potentially indicating a deviation from Hardy-Weinberg equilibrium (HWE), or a significant deviation from the additivity assumption. If less than three participants with DMT2 or IGT exhibited a homozygote minor allele frequency we combined the hetero- and rare homozygous frequency of the respective SNP into one group. See Supplemental Material for a more detailed description of the genetic data.

Air pollution and meteorology data

Amongst others, hourly means of air temperature, relative humidity, barometric pressure, particulate matter (PM) with an aerodynamic diameter be-

low 10µm or 2.5µm (PM_{10}, $PM_{2.5}$), ultrafine particles (UFP) with a size range of 0.01 to 0.1µm in diameter, and sulfate and black carbon (BC) mass concentration of $PM_{2.5}$ were measured at a central measurement site in Augsburg throughout the complete study period as described previously (Cyrys et al. 2008b; Pitz et al. 2008). For a more detailed description of the air pollution measurement and the replacement of missing values see Supplemental Material.

Statistical analysis

Influential SNPs

In a first step, we conducted a literature research and identified SNPs which have already been shown to modulate repolarization and HRV parameters. In order to determine the influence of SNPs on ECG parameters we used regression trees for longitudinal data implemented in the R package `REEMtree` (Sela and Simonoff 2010). This method alternates between estimating the regression tree, assuming that the previously estimated random effects of a mixed model are correct, and estimating the random effects, using the information of the regression tree performed in the prior step. In general, a regression tree is a non-parametric method which performs binary recursive partitioning dividing the outcome variable in homogeneous subgroups by using covariable information (Breiman et al. 1983). For each ECG parameter we estimated regression trees for longitudinal data always excluding one single ECG recording in order to check the robustness of the trees. SNPs which occurred at least in 75% of all trees were used to assess a potential modification of the air pollution effect.

Air pollution effects

Air pollution effects were estimated with SAS statistical package (version 9.2; SAS Institute Inc., Cary, NC, USA) using additive mixed models with a random participant effect. A first order autoregressive covariance structure has been proved to be sufficient to account for the dependencies between the repeated ECG recordings.
A confounder selection was conducted for each ECG parameter separately. Potential confounders were long-term time trend, time of the day, day of the week, air temperature, relative humidity, and barometric pressure. Possible lags considered for meteorology were the 1h-averages concurrent to the 1h-ECG recordings, 1h-averages 1h up to 12h, and 24h-averages 0-23h and 24-47h before each 1h-ECG interval. The confounders were included linearly

or smoothly as penalized splines (P-Splines) to allow for a non-linear relationship. The lag and shape which minimized the Akaike Information Criterion (AIC) was selected. If a confounder was included as P-Spline, we checked whether a polynomial led to a smaller AIC. Barometric pressure, time of the day, and day of the week were only selected in case of model fit improvement. After assessing the confounder model, 1h-averages of air pollutants concurrent to the 1h-averages of ECG measurements and up to 6h before the ECG recordings were separately added to the confounder model and the effects were estimated linearly. In a further analysis, influential SNPs identified with regression trees were used as air pollution effect modifiers. Additionally, main effects and effect modifications on ECG parameters were calculated for participants with DMT2 and IGT separately.

Sensitivity analyses

As sensitivity analysis air pollution effects were estimated smoothly as P-Splines in order to check the linearity of the relationship between air pollutants and ECG parameters. Furthermore, we excluded ECG recordings of occasional smokers and of participants who reported that they have been exposed to environmental tobacco smoke (ETS) during the recording. We also excluded individuals with CAD, angina pectoris, or a MI. Additionally, we only included participants without intake of beta-adrenergic receptor blockers (beta-blockers).

RESULTS

Study population and clinical measurements

Overall, 61 participants with 207 valid ECG recordings (average duration: 5.6h) comprising 1153 1h-intervals were available for analysis. Table 1 describes the baseline characteristics of the non-smoking participants. Characteristics of participants with DMT2 and IGT did not differ except for glycosylated hemoglobin A1c, intake of antidiabetic medication, and intake of statins. Table 2 shows a description of the analyzed ECG parameters. Spearman correlation coefficients between ECG parameters were calculated for each ECG recording separately. According to the median of these correlation coefficients all ECG parameters were uncorrelated ($| r | < 0.5$). HR, RMSSD, and SDNN did not differ significantly between participants with DMT2 or IGT (data not shown).

Table 1: Description of the study population of 61 participants with type 2 diabetes or impaired glucose tolerance.

	All (N=61)		**Diabetes (N=31)**		**IGT (N=30)**		**p-value**
	Mean	**(SD)**	**Mean**	**(SD)**	**Mean**	**(SD)**	
Age (years)	65.7	(8.0)	66.7	(6.7)	64.8	(9.2)	0.37^a
BMI (kg/m^2)	30.2	(4.7)	30.9	(4.4)	29.5	(4.9)	0.27^a
	N	**(%)**	**N**	**(%)**	**N**	**(%)**	
Age (years)							
≤ 60	14	(23)	6	(19)	8	(27)	0.50^b
> 60	47	(77)	25	(81)	22	(73)	
BMI (kg/m^2)							
≤ 30	32	(52)	15	(48)	17	(57)	0.52^b
> 30	29	(48)	16	(52)	13	(43)	
Gender							
Male	40	(66)	23	(74)	17	(57)	0.15^b
Female	21	(34)	8	(26)	13	(43)	
Smoking							
Never smoker	26	(43)	10	(32)	16	(53)	0.16^c
Ex smoker	34	(58)	20	(65)	14	(47)	
Occasional smoker	1	(2)	1	(3)	0	(0)	
HbA1c							
$< 6.5\%$	46	(75)	17	(55)	29	(97)	$< 0.001^c$
$\geq 6.5\%$	15	(27)	14	(45)	1	(3)	
History of							
Coronary heart disease	4	(7)	3	(10)	1	(3)	0.61^c
Angina pectoris	5	(8)	2	(6)	3	(10)	0.67c
Myocardial infarction	6	(10)	5	(16)	1	(3)	0.20^c
Hypertension	40	(66)	20	(65	20	(67)	0.86^b
Medication use							
Antidiabetics	18	(30)	17	(55)	1	(3)	$< 0.001^c$
$\beta-$blockers	18	(30)	11	(35)	7	(23)	0.30^b
Statins	12	(20)	10	(32)	2	(7)	0.02^c

IGT: impared glucose tolerance, SD: standard deviation, BMI: body mass index, HbA1c: glycosylated hemoglobin A1c, $\beta-$blockers: Beta-adrenergic receptor blockers, ^{a}Student's t-test, ^{b}Chi-square test, ^{c}Fisher's exact test

Table 2: Description of the 1h-averages of ECG parameters.

	N	**Mean**	**SD**	**Min**	**25%**	**Median**	**75%**	**Max**	**IQR**
HR (beats/minute)	1153	79.3	14.5	46.5	69.3	78.9	87.9	132.9	18.7
RMSSD (ms)	1153	34.2	32.3	1.3	16.2	22.7	36.7	227.3	20.5
SDNN (ms)	1153	76.6	27.2	11.8	56.1	74.2	94.6	161.2	38.4

HR: heart rate, RMSSD: root mean square of successive differences in RR intervals, SDNN: standard deviation of all normal RR intervals, SD: standard deviation, IQR: interquartile range

Genotyping and influential SNPs

We identified 139 SNPs in the literature which are supposed to have an influence on repolarization and HRV parameters. In our data, seven SNPs were excluded either due to a MAF$<$ 5% or an imputation quality$<$ 0.6. For 56 SNPs the hetero- and minor homozygous genotype were combined to one group. Seven, eight, and ten SNPs did not have an additive effect on HR, RMSSD, and SDNN, respectively, and therefore were not used for the regression tree analysis. Seven, fourteen, and eleven SNPs were chosen using the tree selection procedure for HR, RMSSD, and SDNN, respectively. Supplemental Material, Table 1 contains information about the selected SNPs such as the position, MAF, the authors who initially reported modulations of ECG parameters by these SNPs, and their main effects on ECG parameters estimated with mixed models in our study. The regression trees for HR, SDNN, and RMSSD are given in the Supplemental Material, Figure 2.

Main effects of air pollutants

Figure 1 shows the percent changes of the mean ECG parameters per interquartile range (IQR: difference between the third and first quartile) increase in air pollutants together with 95%-confidence intervals (CI).

Increased BC levels were only marginally associated with an increase in HR (percent change: 0.9%, 95%-CI: [0.0;1.8%]) with a lag of 6h. We observed an association between increases in PM_{10} and $PM_{2.5}$ and a concurrent reduction in RMSSD (-5.3% [-9.3;-1.1%] and -7.2% [-12.2;-1.8%], respectively). Furthermore, RMSSD changed by -3.8% [-7.1;-0.5%] and -5.2% [-9.8;-0.4%] in association with elevated BC levels with a lag of 1h and 6h, respectively. Elevated $PM_{2.5}$ level led to concurrent (-3.3% [-6.0;-0.7%]) and lagged decreases in SDNN by about 3-4%. We observed similar effects of PM_{10} and sulfates. Increases in BC and UFP were only related with lagged decreases in SDNN showing the strongest associations with a lag of 2h (-3.7% [-5.6;-1.8%] and -1.9% [-3.4;-0.4%], respectively) .

Effect modification

We observed no significant air pollution effects on HR; therefore, we did not calculate effect modifications by SNPs for this ECG parameter. As $PM_{2.5}$ showed the strongest main effects on ECG parameters and PM variables were highly correlated with BC and sulfate but uncorrelated with UFP, we only present $PM_{2.5}$ and UFP effect modifications by SNPs.
rs333229 was the strongest effect modifier on SDNN (Figure 2). 34 partici-

Figure 1: Concurrent effects of 1h-averages of air pollutants on 1h-averages on heart rate (HR), root mean square of successive differences in RR intervals (RMSSD), and standard deviation of all normal RR intervals (SDNN).

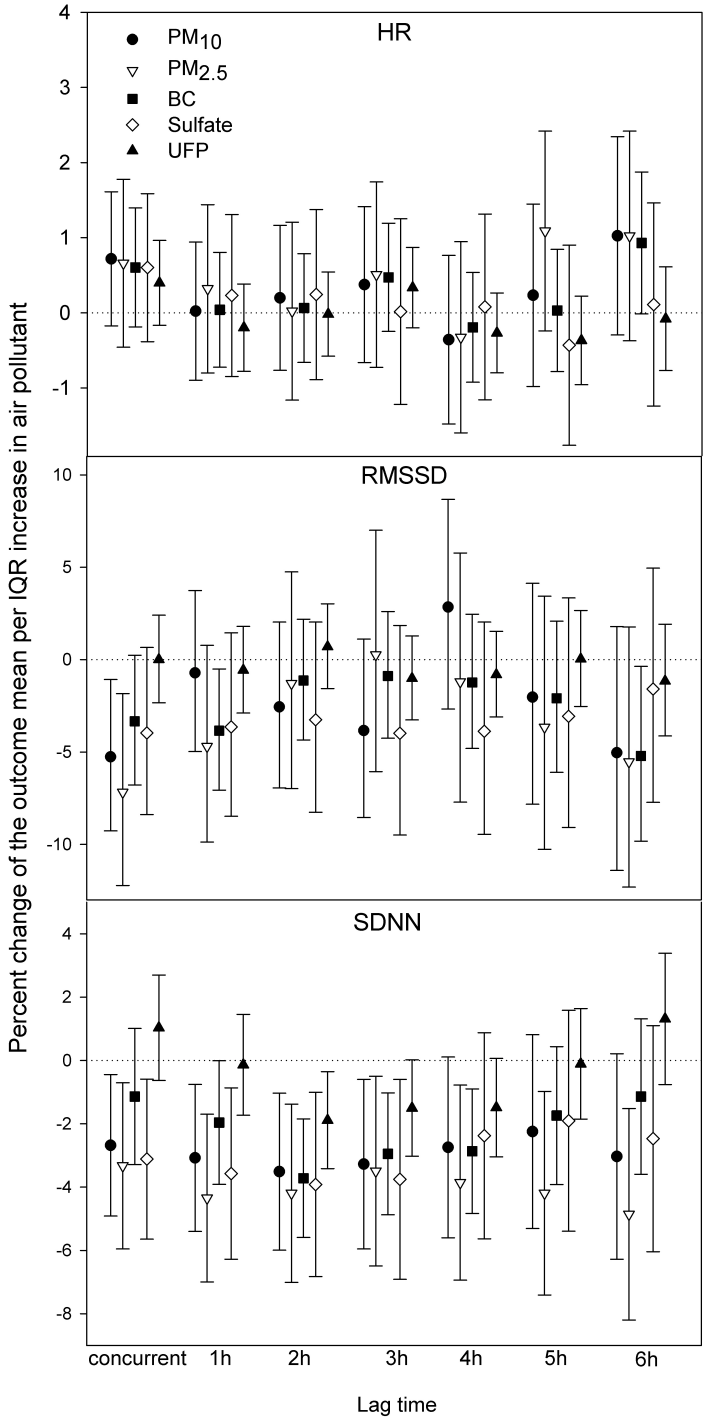
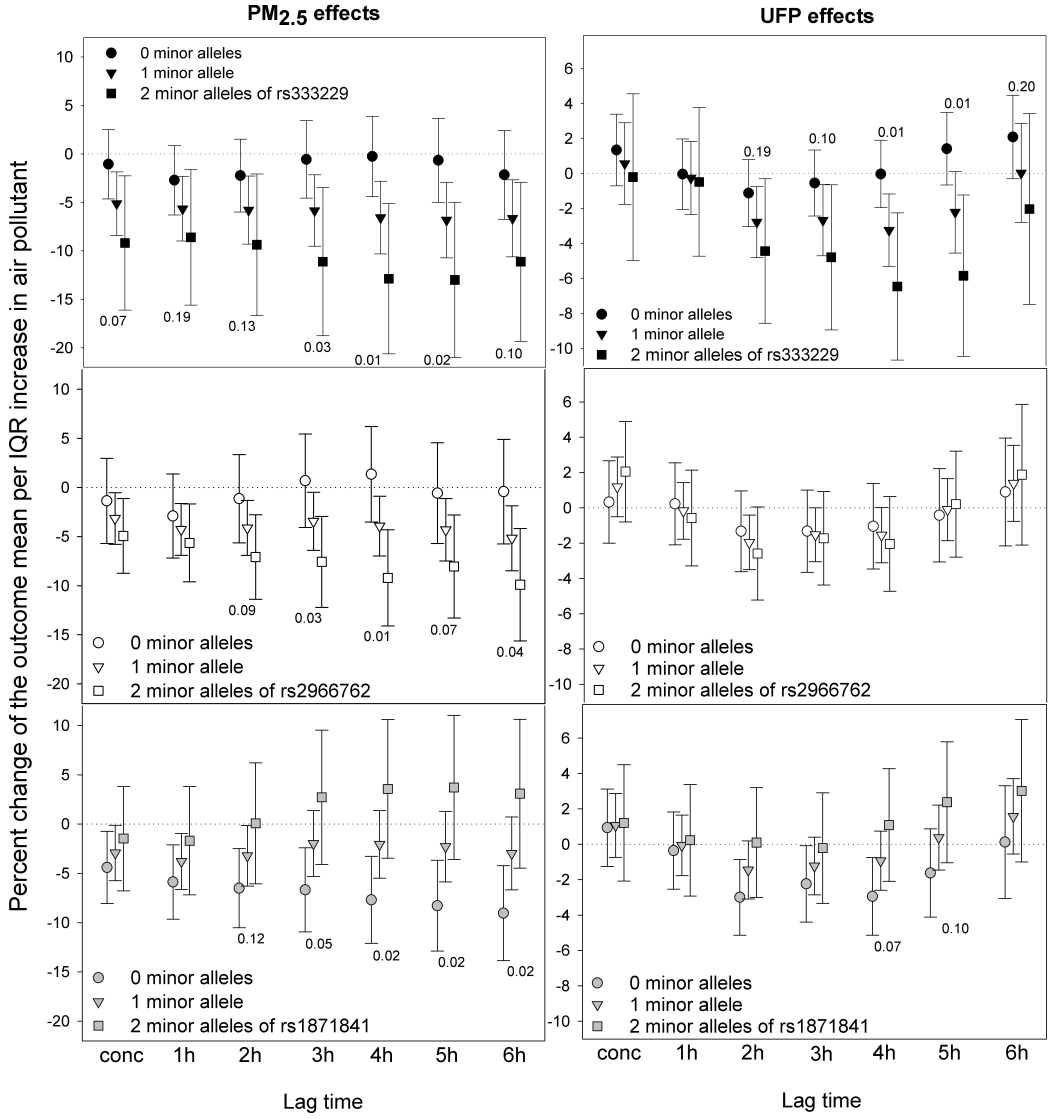


Figure 2: Effects of $PM_{2.5}$ and UFP on SDNN modified by the number of minor alleles of rs333229, rs2966762, and rs1871841. Interaction effects with a p-value < 0.2 are indicated.



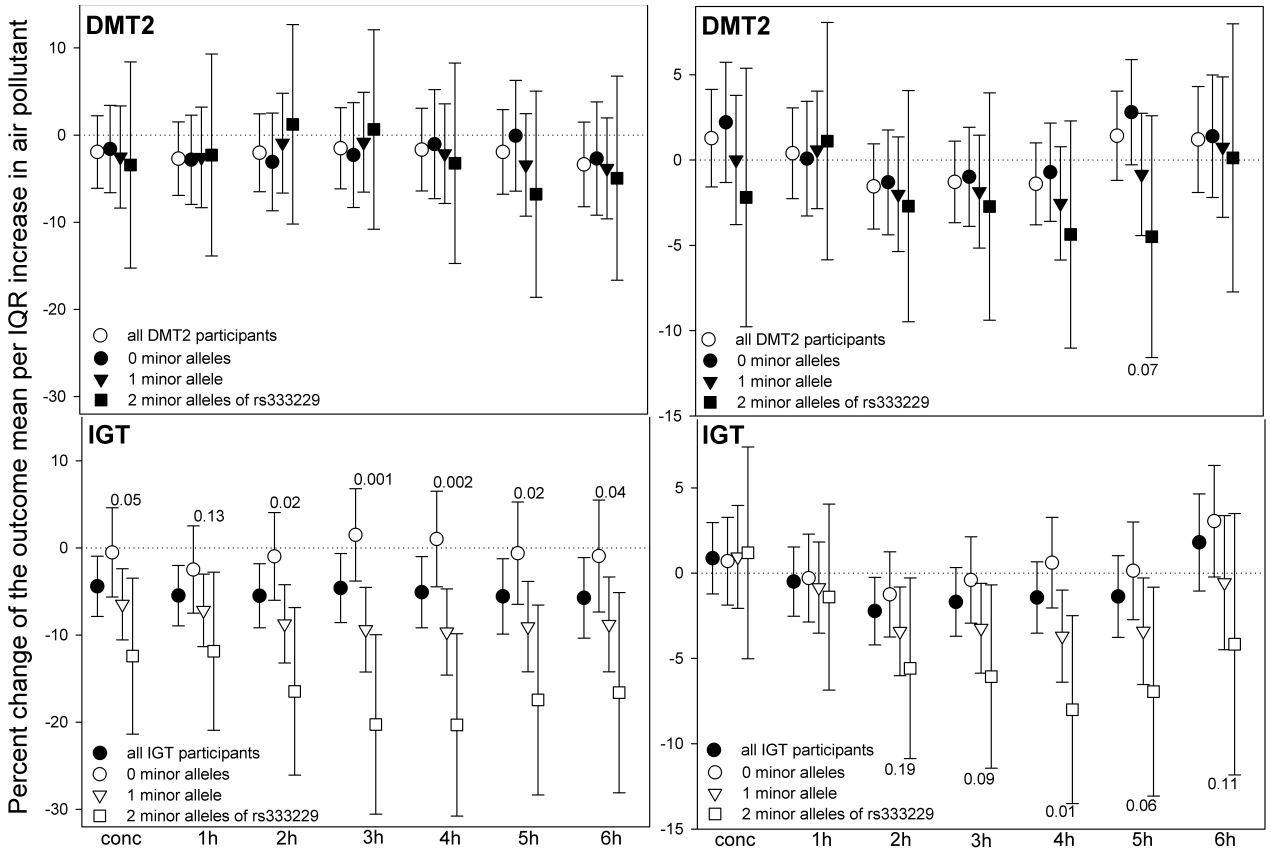
pants with 651 SDNN intervals were homozygous carriers of the major allele, 23 participants with 429 SDNN intervals were heterozygous, and 4 participants with 73 SDNN intervals were homozygous carriers of the minor allele. Throughout all lags only participants with at least one minor allele showed a reduction in SDNN in association with increases in $PM_{2.5}$. Individuals with no minor allele did not react to elevated $PM_{2.5}$ levels. We observed the strongest modification with a lag of 4h (p-value of interaction=0.01). Participants with one and two minor alleles exhibited a -6.6% [-10.3;-2.8%] and a -12.9% [-20.6;-5.1%] decreased SDNN, respectively. $PM_{2.5}$ effect modification by rs2966762 resulted in a similar pattern with weaker effects (Figure 2). Borderline and significant interaction effects between rs333229 and UFP on SDNN were detected with a lag of 2h to 5h. SDNN decreased by about 2-3% in individuals with one and about 4-6% in individuals with two minor alleles. Furthermore, an increase in $PM_{2.5}$ led to a 4-8% and to a 2-4% decrease in SDNN in participants with no or one minor allele of rs1871841 (Figure 2). The concurrent response of RMSSD to increases in $PM_{2.5}$ was modified by rs2096767 and rs2745967. Elevated $PM_{2.5}$ levels led to a -10.0% [-15.5;-4.1%] and a -13.2% [-23.3;-1.8%] reduction in RMSSD in individuals with one and two minor alleles of rs2096767, respectively. In contrast, people with no (-13.2% [-20.3;-5.6%]) and one minor allele (-6.4% [-11.5;-1.0%]) in rs2745967 exhibited a decrease in RMSSD in association with $PM_{2.5}$. We observed no effect modification by other SNPs selected with regression trees.
Analyzing air pollution effects on RMSSD and SDNN for participants with D2TM and IGT separately showed that significant main and interaction effects were only observed in individuals with IGT. Figure 3 illustrates the $PM_{2.5}$ and UFP effect modification by rs333229 on SDNN in the two panels.

An increase in $PM_{2.5}$ levels led to a 11-15% and to a 12-20% reduction in SDNN in participants with IGT having one or two minor alleles of rs333229, respectively. SDNN was reduced to a smaller amount (3-8%) in association with increases in UFP with a lag of 2h to 5h in individuals with IGT with at least one minor allele. We also observed effect modifications by rs2966762 and rs1871841 on SDNN only in participants with IGT (data not shown). No significant air pollution effects and effect modifications by SNPs were detected on SDNN and RMSSD in participants with DMT2.

Sensitivity analyses

There was no evidence for a deviation from linearity of the relationship between air pollutants and ECG parameters. Excluding 3 ECG recordings of one occasional smoker and 17 ECG recordings of 14 participants who have been exposed to ETS during the recording did not result in notable changes

Figure 3: Effects of $PM_{2.5}$ and UFP on SDNN modified by the number of minor alleles of rs333229 for DMT2 and IGT participants separately. Interaction effects with a p-value$<$ 0.2 are indicated.



in the air pollution effects of both the main analysis and the interaction analysis. The exclusion of 18 participants (66 ECG recordings) with an intake of beta-blockers and of 11 participants (37 ECG recordings) with CAD, angina pectoris, or MI led to similar but less significant air pollution effects on SDNN (data not shown).

DISCUSSION

Summary

We observed concurrent and lagged decreases in SDNN in association with elevated 1h-averages of PM and UFP levels in participants with metabolic disorders and especially in individuals with at least one minor allele of rs333229. Weaker effect modifications were observed for rs2966762 and rs1871841. A reduction in RMSSD was only associated with concurrent PM increases. These PM effects were stronger in individuals with at least one minor allele of rs2096767 or at most one minor allele of rs2745967. In general, air pollution effects seemed to be more pronounced in individuals with IGT than with DMT2. We observed no air pollution effects on HR.

Air pollution and HRV

Previous studies assessed changes in HRV in association with air pollution exposure calculated as 4h-, 6h-, or 24h-averages (Luttmann-Gibson et al. 2006; Schneider et al. 2010). Zanobetti et al. (2010) observed a -1.5% [-2.5;-0.4%] decrease in RMSSD but no changes in SDNN in association with elevated 1h-averages of $PM_{2.5}$ directly preceding the ECG recording in patients with CAD. In our study, we observed a concurrent reduction in RMSSD by -6.8% [-11.7;-1.7%] and in SDNN by -3.3% [-5.8;-0.7%]. However, BC effects were less pronounced in our study compared to the findings of Zanobetti et al. Similar to our findings, a study conducted in taxi drivers in Beijing detected a -2.2% [-3.8;-0.6%] reduction in SDNN in association with increases in 30min-averages of $PM_{2.5}$ measured inside the taxicab (Wu et al. 2010). Furthermore, a study by Adar et al. (2007) showed that increases in 1h-concentrations of traffic-related $PM_{2.5}$ led to a decreased SDNN and RMSSD in elderly participants. In contrast to our study, they also observed an increase in HR associated with elevated $PM_{2.5}$ levels. However, these studies only reported the air pollution effects directly preceding the ECG recording. Our findings of a rapid decrease in HRV in association with elevated PM-levels might be mediated by a perturbation of the balance of the systemic autonomic nervous system due to a stimulation of lung receptors or nerve end-

ings in the human airways by inhaled particles (Brook et al. 2010). RMSSD is an index of parasympathetic modulation, whereas SDNN reflects the variability of both sympathetic and parasympathetic activity. As we observed a stronger reduction in SDNN than in RMSSD in association with PM increases we assume that the activation of the sympathetic nervous system is more pronounced than the vagal withdrawal. However, we did not find air pollution effects on HR, an ECG parameter reflecting sympathetic activation. Additionally, we found a decrease in SDNN associated with elevated UFP levels. A small fraction of UFP may pass alveolar walls and affect the electric system of the heart directly (Peters et al. 2006) which might lead to a reduced HRV. We detected concurrent and delayed PM effects but only lagged UFP effects on SDNN. Therefore, we hypothesize that different effects of PM and UFP might reflect different biological pathways activated by different particle properties. It has also been shown that a reduced HRV might be a precursor of cardiovascular problems (Buccelletti et al. 2009; Lanza et al. 2006; Reed et al. 2005). Hence, our observed findings might be an intermediate step linking air pollution exposure to cardiovascular health.

Genotypes and susceptibility to air pollution

Susceptibility to air pollution exposure might be partly affected by genetic predispositions as studies reported a modified HRV response by genotypes of oxidative stress-related SNPs (Chahine et al. 2007; Probst-Hensch et al. 2008; Schwartz et al. 2005). It is assumed that inhaled PM induces oxidative stress, an excess of reactive oxygen species, and a release of inflammatory mediators in the lung which might lead to an imbalance of the autonomic nervous system and hence to a decreased HRV (Brook et al. 2010). Thus, it is hypothesized that people with a reduced oxidative defence due to genetic predispositions might be especially susceptible to PM exposure. In our study, we investigated possible air pollution effect modifications by SNPs involved in changes of cardiac rhythm as reported in GWAS (Newton-Cheh et al. 2007). We hypothesized that people with an altered HRV due to genetic predispositions might also show different reactions to air pollution exposure. We detected the strongest air pollution effects on SDNN in individuals with at least on minor allele of rs333229 but no main effect of this SNP (Supplemental Material, Table 1). This SNP is located in the 3'untranslated region of the choline transporter gene (*CHT1*) (Neumann et al. 2005). *CHT1* encodes the high-affinity choline transporter (ChT) which carries choline into acetylcholine (ACh)-synthesizing neurons. ACh is a neurotransmitter of the sympathetic and parasympathetic system. Therefore, variations in *CHT1* may account for variations in ACh neurotransmission which might lead to

modulation of HR and HRV. However, we can only speculate that changes in ChT affect the response to air pollution. rs2966762, rs1871841, rs2096767, and rs2745967 modified the air pollution effects as well. Little is known about biological pathways of these SNPs and their influence on the cardiovascular system. rs2096767 is located in the matrix metallopeptidase 13 gene which is involved in a wide variety of physiological and pathological processes, including normal cell growth, differentiation, and cell regulation (Leeman et al. 2002). Newton-Cheh et al. (2007) observed a significant modulation of the PR-interval by rs2096767 in a GWAS. As all SNPs are located on different chromosomes and are uncorrelated (data not shown) we assume that the unknown underlying biological mechanisms could be different for each SNP.

Metabolic disorders and susceptibility to air pollution

The risk of developing CAD or suffering from an MI is increased in persons with DTM2 (Beckman et al. 2002). It has also been shown that insulin resistance might cause a reduction of the autonomic nervous system leading to a reduced HRV in both DMT2 and IGT groups (Perciaccante et al. 2006). Furthermore, authors reported QTc-prolongation, a marker of ventricular arrhythmias, and a higher risk for emergency department visits and hospital admissions for individuals with DMT2 compared to individuals without DMT2 in association with air pollution increases (Baja et al. 2010; Peel et al. 2007; Zanobetti and Schwartz 2001). Therefore, we assumed that participants with DMT2 or IGT might be especially susceptible to air pollutants. However, we observed no air pollution effects in individuals with DMT2 - only in IGT participants. By selecting subjects with IGT, we intended to study the impact of air pollutants in subjects with an enhanced risk for DMT2 but who were not heavily treated by beta-blockers, statins, or anti-diabetic medications as these medications may obliterate the effects of particle exposures. In our study 81% of participants with DMT2 took beta-blockers, statins, or anti-diabetic medication. These medications may have reduced the possibility to detect ambient air pollution effects on ECG-parameters for individuals with DMT2. People with diabetes are known to have disproportional reactive oxygen species formation (Maritim et al. 2003) and we speculate that this might be also true for individuals with IGT. PM has been hypothesized to cause adverse health effects through the same mechanism. Therefore, diabetes and PM may share common pathways and interact to enhance responsiveness to air pollutants.

Strengths and limitations

A strength of our study is the ability to analyze intra-individual variation in 1h-averages of ECG parameters measured repeatedly in up to four ECG recordings with an average duration of 5.6h. Models were adjusted for long-term time trend and meteorological variables to account for the possibility that the detected associations resulted from meteorological influences or seasonal differences alone. We controlled for circadian variation by design as the repeated ECG recordings started at the same time (±2h) for each participant, respectively, and models were adjusted for time of day in case of model fit improvement. A variety of outcome, exposure, and interaction variables have been used in our analyses; thus, some associations may have occurred only by chance. However, we reduced the number of performed tests by selecting SNPs with regression trees in a prior step and we did not include all 139 SNPs as potential air pollution effect modifiers. Furthermore, regression trees are not based on distributional assumptions. No additional tests were carried out in the SNP selection process. Nevertheless, we did not adjust our analyses for multiple testing as our analyses should be regarded as explorative. A limitation of our study is that only one central measurement site was used for the collection of ambient air pollution which poses a source for exposure misclassification as it assumes homogeneous exposure for the whole study area. Especially ultrafine particles are spatially heterogeneous and depend on distance from the roadway as they are mostly produced by local traffic. However, Cyrys et al. (2008a) investigated the temporal and spatial variation of particle number concentrations (PNC) at four background sites in Augsburg and reported high correlations ($r > 0.80$). Therefore, the use of one single ambient monitoring site is an adequate approach for characterizing exposure to ultrafine particles in time-series studies. A further strength of this study is the investigation of air pollution effects in a particularly susceptible subgroup. On the other hand, the results cannot be generalized to the whole population.

Conclusion

We observed a reduced HRV, predominantly SDNN, in association with concurrent and delayed increases in 1h-averages of PM and UFP exposure. These associations were modified by SNPs involved in cardiac rhythm. Therefore, we identified persons with a genetic predisposition making them potentially more susceptible to air pollutants with regard to HRV, a possible precursor of cardiac adverse events. Moreover, as the prevalence of diabetes is increasing worldwide it is important to conduct further investigations in this susceptible

population.

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ACKNOWLEDGEMENT

This research has been funded wholly or in part by the United States Environmental Protection Agency through STAR ("Science to Achieve Results") grant RD 832415 to the University of Rochester. It has not been subjected to the Agency's required peer and policy review and therefore does not necessarily reflect the views of the Agency and no official endorsement should be inferred. This study was supported in part by a grant from the German Federal Ministry of Education and Research (BMBF) to the German Center for Diabetes Research (DZD e.V.). The KORA research platform (KORA, Cooperative Health Research in the Region of Augsburg) and the MONICA Augsburg studies were initiated and financed by the Helmholtz Zentrum München, German Research Center for Environmental Health (formerly GSF, National Research Center for Environment and Health), which is funded by the German Federal Ministry of Education and Research and by the State of Bavaria. We thank Dr. Wolfgang Rathmann for performing the glucose tolerance tests and providing us with the HbA1c measurements.

COMPETING INTERESTS

The authors declare they have no competing financial interests.

SUPPLEMENTAL MATERIAL

Methods

Genotyping

Genome-wide data were available for each individual. DNA was extracted from ethylenediaminetetraacetic acid anticoagulated blood using a salting out procedure. Given Affymetrix 6.0 genotyping, the whole set of genotypes from HAPMAP release 22 were imputed with the Markov Chain Haplotype (MACH) software package version 1.16 (Li et al. 2009). For our analysis we only used SNPs supposed to be involved in cardiac rhythm (e.g. HR,

RMSSD, high and low frequency, and PR- and QT-interval) which were already identified in the literature and published before August 2010. For the selected SNPs, the allele dosages available from imputation were subsequently transformed into best guess genotypes counting the number of minor alleles, which is a typical coding if an underlying additive genetic effect model, i.e. a constant increase per copy of the minor allele, is assumed. We tested for evidence against the additive genetic model using a procedure introduced by Schaid et al. (Schaid 2004). Thereby, we compared a model only including a linearly coded SNP with a second model also including a variable indicating the heterozygous genotype. If the likelihood ratio test revealed a better model fit (p-value¡0.1) of the second model we assumed that the respective SNPs had no additive effect on the ECG-parameter. For our analysis we excluded SNPs in case of 1) a minor allele frequency below 5%, 2) an imputation quality (observed vs. expected variance of the genotypes as returned as measure RSQR by MACH) below 0.6 potentially indicating a deviation from Hardy-Weinberg equilibrium (HWE), or 3) a significant deviation from the additivity assumption. If less than three participants with DMT2 or IGT exhibited a homozygote minor allele frequency we combined the hetero- and rare homozygous frequency of the respective SNP into one group.

Air pollution and meteorology data

Ambient air pollution and meteorological variables such as air temperature, relative humidity, and barometric pressure were measured at a central measurement site in Augsburg throughout the complete study period as described previously (Cyrys et al. 2008; Pitz et al. 2008). Amongst others, hourly means of particulate matter (PM) with an aerodynamic diameter below 10µm or 2.5µm (PM_{10}, $PM_{2.5}$), ultrafine particles (UFP) with a size range of 0.01 to 0.1µm in diameter, and sulfate and black carbon (BC) mass concentration of $PM_{2.5}$ were available. Particle mass concentrations of $PM_{2.5}$ and PM_{10} were measured by two separate Tapered Element Oscillating Microbalance (TEOM, model 1400ab, Thermo Fisher Scientific Inc., USA) devices. To correct the PM measurements for aerosol volatility effects, each TEOM was equipped with a Filter Dynamics Measurement System (FDMS, model 8500b, Thermo Fisher Scientific Inc., USA). Particle size distributions (PSD) in the range from 3-900 nm were measured by a custom-built Twin Differential Mobility Particle Sizer (TDMPS) system consisting of two cylindrical, Vienna-type Differential Mobility Analyzers (DMA). Because of maintenance or plausibility checks of the TEOM devices approximately 8% of the hourly $PM_{2.5}$ and PM_{10} values were missing. Single missing 1h-averages of PM variables were replaced by the mean value of one hour before and after the missing value. In case of longer time intervals with missing values, PM vari-

ables were either replaced using a linear regression equation based on PSD measurements of one day before and after the missing time period or if no PSD measurements were available, the missing PM values were imputed by the mean values of two other official urban background measurement sites. The first one is located at the Bavarian Environment Agency approximately 3.5 kilometers south of the central measurement site and the second one is located at the Bourgesplatz, about 2.5 kilometers north of central measurement site. Missing values of all other air pollutants and of meteorological variables were not replaced as less than 1% of the 1h-averages were missing or no parallel measurements with other devices were available. Sulfate measurements started on April 25th 2007 and the device for BC was under repair between June 3rd and September 8th 2008.

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Li Y, Willer C, Sanna S, and Abecasis G. 2009. Genotype imputation. Annu.Rev.Genomics Hum.Genet. 10:387-406.

Pitz M, Schmid O, Heinrich J, Birmili W, Maguhn J, Zimmermann R et al. 2008. Seasonal and diurnal variation of $PM_{2.5}$ apparent particle density in urban air in Augsburg, Germany. Environ.Sci.Technol. 42:5087-5093.

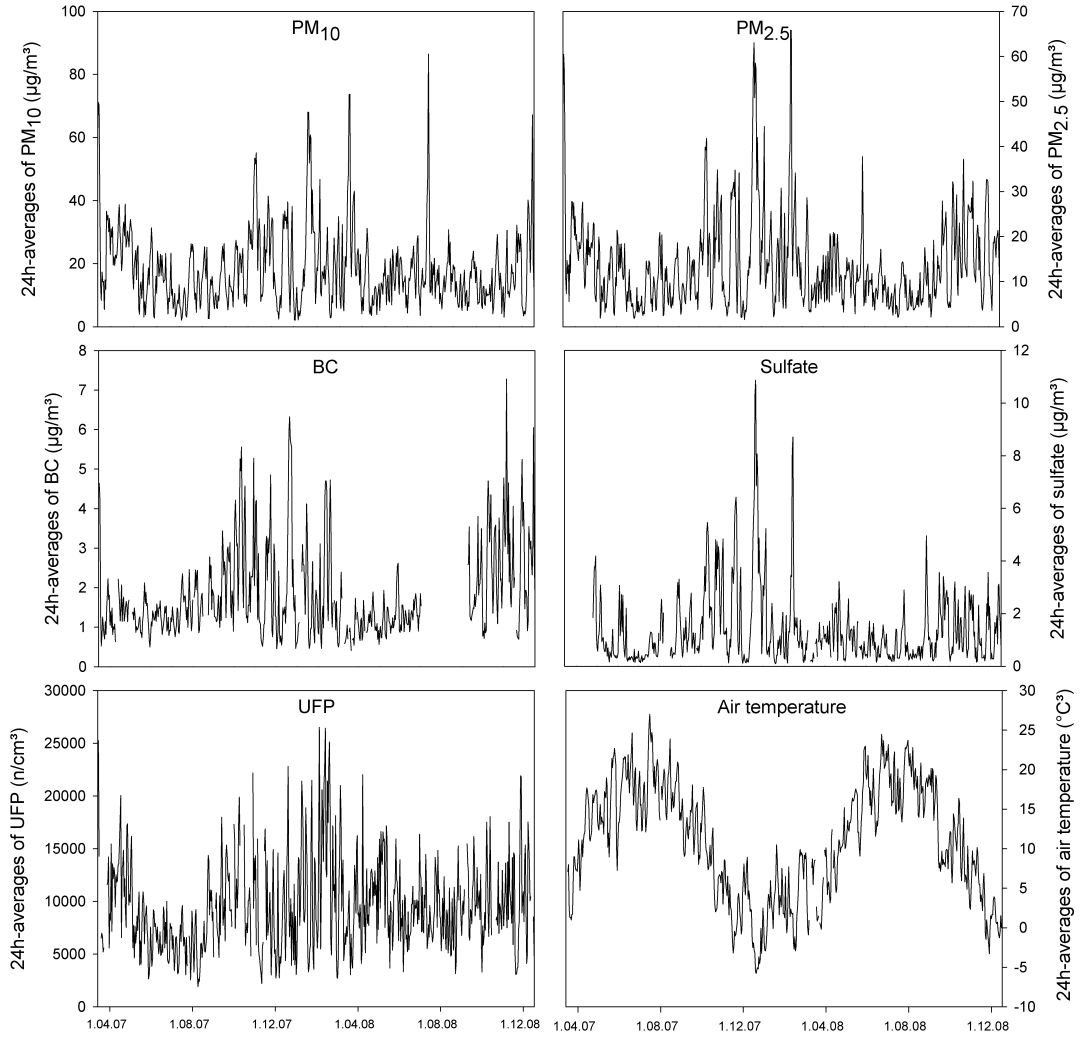
Schaid DJ. 2004. Evaluating associations of haplotypes with traits. Genet.Epidemiol. 27:348-364.

Supplemental Material, Table 1. SNPs which occurred in 75% of all regression trees performed for HR, SDNN, and RMSSD, respectively.

Known association to	SNP	%-change 95%-CI	Chromosome	Position	Gene	Major	Minor	MAF	Coding	Initial traits	Publication
HR	rs1801133	-1.9 (-9.0;4.8)	1	11778965	*MTHFR*	G	A	34	linear	HF, LF, SDNN	Baccarelliet al. 2008
	rs2932529	-1.3 (-8.2;5.3)	1	112994887	CAPZA1	A	G	30	linear	RR	Newton-Cheh et al. 2007
	rs10494366	-4.7 (-11.7;1.3)	1	160352309	*NOS1AP*	T	G	33	linear	QT	Arkinget al. 2006, Marroniet al. 2009, Lehtinenet al. 2008, Marjamaaet al. 2009, Postet al. 2007, Tobinet al. 2008
	rs4657178	-1 (-8.7;6.5)	1	160477234	NOS1AP	C	T	24	linear	QT	Pfeufer et al. 2009
	rs2745967	5.6 (0.0;12.3)	1	206195345		A	G	36	linear	RR	Eijgelsheim et al. 2010
	rs10495588	13 (5.5;23)*	2	12120724		G	A	20	categorial	QT	Newton-Cheh et al. 2007
	rs882300	2.8 (-3.6;9.8)	2	136692725		T	C	34	linear	PR	Newton-Cheh et al. 2007
	rs11970286	2.1 (-3.8;8.4)	6	118787067	*PLN*	C	T	35	linear	QT	Pfeufer et al. 2009, Eijgelsheim et al. 2010
	rs757092	2.1 (-4.6;9.3)	11	2455754	KCNQ1	A	G	34	linear	QT	Pfeufer et al. 2005
	rs4488182	-6.6 (-16.2;1.6)	11	41032137		T	G	22	categorial	PR	Newton-Cheh et al. 2007
	rs2096767	-3 (-10;3.5)	11	102296609	*MMP13*	C	G	30	linear	PR	Newton-Cheh et al. 2007
	rs885389	-2.1 (-9.9;5.3)	12	130187715	GPR133	G	A	28	linear	RR	Marroni et al. 2009
	rs2074518	-9.6 (-16.9;-4.2)*	17	30348495	LIG3	C	T	48	linear	QT	Newton-Cheh et al. 2009
	rs1805127	3.8 (-1.6;9.9)	21	34743691	KCNE1	C	T	37	linear	T-wave alternans	Koskela et al. 2008
RMSSD	rs1801133	-4.8 (-25.6;21.8)	1	11778965	*MTHFR*	G	A	34	linear	HF, LF, SDNN	Baccarelli et al. 2008
	rs12090585	4.3 (-17.8;32.4)	1	160305400	*NOS1AP*	G	A	28	linear	QT	Chambers et al. 2010
	rs1932933	23.1 (-2.7;55.9)	1	160384670	*NOS1AP*	C	T	32	linear	QT	Chambers et al. 2010
	rs1407709	58 (16;115.2)	1	186329302		C	T	19	categorial	TP	Newton-Cheh et al. 2007
	rs2745967	-21.4 (-36.7;-2.2)	1	206195345		A	G	36	linear	RR	Eijgelsheim et al. 2010
	rs1866559	5.3 (-15.6;31.5)	2	27189090	*CGREF1*	T	C	38	linear	SDNN	Newton-Cheh et al. 2007
	rs2643191	3.9 (-18.5;32.4)	3	165861387		C	G	46	linear	RR	Newton-Cheh et al. 2007
	rs10515199	-10.6 (-45.8;47.3)	5	74135390		C	T	7	categorial	TP	Newton-Cheh et al. 2007
	rs286751	7.7 (-14;34.9)	5	107430837	*FBXL17*	T	C	44	linear	SDNN	Newton-Cheh et al. 2007
	rs11970286	-9.9 (-27.5;11.9)	6	118787067	*PLN*	C	T	35	linear	QT	Pfeufer et al. 2009, Eijgelsheim et al. 2010
	rs463924	0.2 (-21.7;28.1)	11	2674256	*KCNQ1*	C	T	33	linear	QT	Pfeufer et al. 2005
	rs2096767	18.9 (-5.9;50.4)	11	102296609	*MMP13*	C	G	30	linear	PR	Newton-Cheh et al. 2007
	rs3825214	-9.9 (-35.3;25.5)	12	113279826	*TBX5*	A	G	20	categorial	QRS, PR, QT	Holm et al. 2010
	rs1558139	-9.5 (-32.4;21.2)	19	15858564	*CYP4F2*	A	G	43	linear	QT	Newton-Cheh et al. 2007
SDNN	rs10494366	3.5 (-5.3;12.4)	1	160352309	*NOS1AP*	T	G	33	linear	QT	Arking et al. 2006, Marroni et al. 2009, Lehtinen et al. 2008, Marjamaa et al. 2009, Post et al. 2007, Tobin et al. 2008
	rs4669749	-8 (-17.4;1.5)	2	11610115	*GREB1*	A	C	43	linear	LF/HF	Newton-Cheh et al. 2007
	rs333229	-4.1 (-13.8;5.6)	2	107997816	*CHT1*	G	T	25	linear	HF	Neumann et al. 2005
	rs2966762	11.2 (2.9;19.5)	5	109411118		C	T	44	linear	SDNN	Newton-Cheh et al. 2007
	rs281868	5.9 (-2.3;14)	6	118680754	*SLC35F1*	A	G	42	linear	RR	Eijgelsheim et al. 2010
	rs1871841	-8.7 (-18.1;0.8)	8	13674417		C	G	40	linear	LF/HF	Newton-Cheh et al. 2007
	rs9297393	12.3 (3.8;20.7)	8	108356753	*ANGPT1*	T	G	38	linear	SDNN	Newton-Cheh et al. 2007
	rs10509700	6.8 (-1.6;15.3)	10	97884521	*ZNF518*	G	C	43	linear	LF/HF	Newton-Cheh et al. 2007
	rs4488182	13.8 (2.2;25.5)	11	41032137		T	G	22	categorial	PR	Newton-Cheh et al. 2007
	rs7188697	-9.2 (-22.0;3.6)	16	57179679	*NDRG4*	A	G	18	categorial	QT	Pfeufer et al. 2009
	rs17779747	3.2 (-6.0;12.4)	17	66006587	*KCNJ2*	G	T	33	linear	QT	Pfeufer et al. 2009

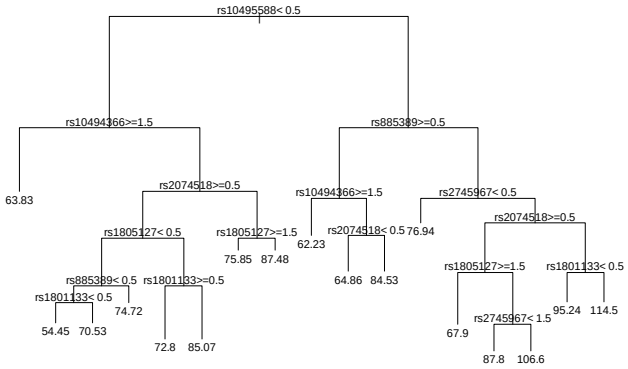
HR: Heart rate, RMSSD: root mean square of successive differences in RR intervals, SDNN: standard deviation of all normal RR intervals, HF: high frequence, LF: low frequency, TP: total power, SNP: single nucleotide polymorphism, CI: confidence interval MAF: minor allele frequency; *SNP effects are still significant when using a Bonferroni adjusted p-value=0.05/14=0.00357.

Supplemental Material, Figure 1. 24h-averages of air pollutants and air temperature measured during the study period from March 14th 2007 to December 17th 2008.

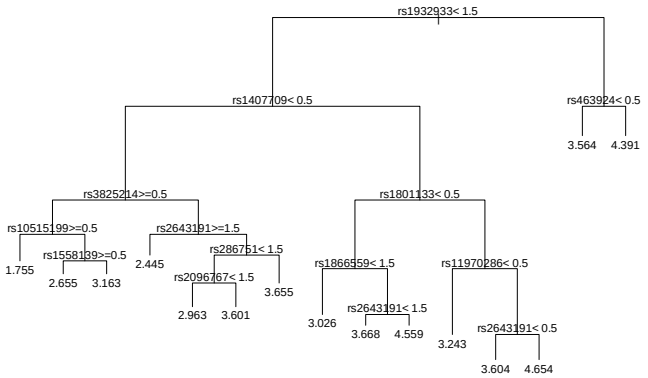


Supplemental Material, Figure 2. Regression trees with the selected SNPs for HR (beats/minute), RMSSD (ms), and SDNN (ms), respectively. The mean values of the ECG parameter within each node are given below.

HR

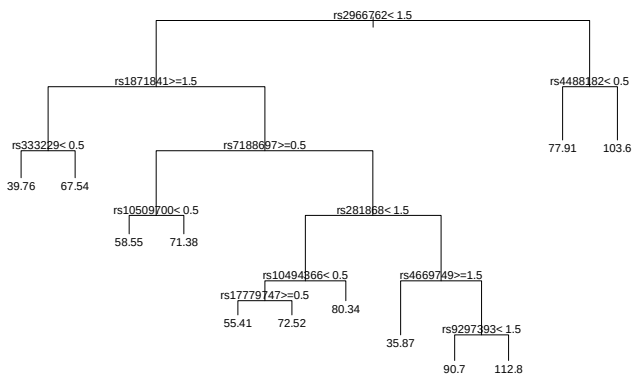


RMSSD*



*rs12090585 and rs2745967 appear in 75% of all conducted trees always excluding a single ECG recording. These SNPs do not appear in this tree using all ECG recordings.

SDNN



ACKNOWLEDGEMENT

- First, I would like to thank my supervisor PD Dr. Annette Peters, Director of the Institute of Epidemiology II at Helmholtz Zentrum München, who provided the idea of this thesis. Despite her tight schedule she found time to support my work and to give helpful advice.

- I also want to thank Prof. Dr. Dr. H.-Erich Wichmann, Director of the Institute of Epidemiology I at Helmholtz Zentrum München and Chair of Epidemiology Institute of Medical Information Processing, Biometry and Epidemiology of Ludwig-Maximilians-University of Munich for making this work possible.

- Special thanks go to my co-supervisor Dr. Alexandra Schneider, Team leader of the Research Unit "Environmental Risks", who really gave me an understanding of environmental epidemiology. She always had time for questions and helped me if epidemiological issues seemed to be too difficult for a statistician. She patiently guided me through my work and always had fruitful comments and suggestions.

- Further, I would like to thank Dr. Susanne Breitner who always had a sympathetic ear if statistical questions occurred.

- Thanks to all my nice colleagues and roommates who made it worth to travel the long way to Neuherberg every day. In particular, Dr. Kathrin Wolf who not only shared her knowledge about statistics and programming with me but also her everyday thoughts and chocolate.

- Biggi, Christine, Maria, Sandra, and Martin.

Hiermit erkläre ich, Regina Hampel, 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 Herkunft 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 05.04.2011

(Regina Hampel)