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Ambient and Controlled Particle Exposures as Triggers for Acute ECG Changes

David Q. Rich, Annette Peters, Alexandra Schneider, Wojciech Zareba, Susanne Breitner, David Oakes, Jelani Wiltshire, Cathleen Kane, Mark W. Frampton, Regina Hampel, Philip K. Hopke, Josef Cyrys, and Mark J. Utell

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with a Critique by the HEI Health Review Committee

Research Report 186

Health Effects Institute

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ABOUT HEI

The Health Effects Institute is a nonprofit corporation chartered in 1980 as an independent research organization to provide high-quality, impartial, and relevant science on the effects of air pollution on health. To accomplish its mission, the institute

- Identifies the highest-priority areas for health effects research;
- Competitively funds and oversees research projects;
- Provides intensive independent review of HEI-supported studies and related research;
- Integrates HEI's research results with those of other institutions into broader evaluations; and
- Communicates the results of HEI's research and analyses to public and private decision makers.

HEI typically receives balanced funding from the U.S. Environmental Protection Agency and the worldwide motor vehicle industry. Frequently, other public and private organizations in the United States and around the world also support major projects or research programs. HEI has funded more than 330 research projects in North America, Europe, Asia, and Latin America, the results of which have informed decisions regarding carbon monoxide, air toxics, nitrogen oxides, diesel exhaust, ozone, particulate matter, and other pollutants. These results have appeared in more than 260 comprehensive reports published by HEI, as well as in more than 1000 articles in the peer-reviewed literature.

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All project results and accompanying comments by the Health Review Committee are widely disseminated through HEI's Web site (*www.healtheffects.org*), printed reports, newsletters and other publications, annual conferences, and presentations to legislative bodies and public agencies.

ABOUT THIS REPORT

Research Report 186, *Ambient and Controlled Particle Exposures as Triggers for Acute ECG Changes*, presents a research project funded by the Health Effects Institute and conducted by Dr. David Q. Rich of the University of Rochester Medical Center, Rochester, New York, Annette Peters of Helmholtz Zentrum München–German Research Center for Environmental Health, Neuherberg, Germany, and their colleagues. The report contains three main sections.

The HEI Statement, prepared by staff at HEI, is a brief, nontechnical summary of the study and its findings; it also briefly describes the Health Review Committee's comments on the study.

The Investigators' Report, prepared by Rich, Peters, and colleagues, describes the scientific background, aims, methods, results, and conclusions of the study.

The Critique, prepared by members of the Health Review Committee with the assistance of HEI staff, places the study in a broader scientific context, points out its strengths and limitations, and discusses remaining uncertainties and implications of the study's findings for public health and future research.

This report has gone through HEI's rigorous review process. When an HEI-funded study is completed, the investigators submit a draft final report presenting the background and results of the study. This draft report is first examined by outside technical reviewers and a biostatistician. The report and the reviewers' comments are then evaluated by members of the Health Review Committee, an independent panel of distinguished scientists who have no involvement in selecting or overseeing HEI studies. During the review process, the investigators have an opportunity to exchange comments with the Review Committee and, as necessary, to revise their report. The Critique reflects the information provided in the final version of the report.

HEI STATEMENT

Synopsis of Research Report 186

Particle Exposures as Triggers for Acute ECG Changes

BACKGROUND

A large number of epidemiologic studies have reported associations between higher exposure to particulate matter (PM) from combustion sources and increased cardiovascular mortality and hospitalization in vulnerable individuals, such as those with lung or heart disease and in older adults. Current and past research has aimed at identifying the pathophysiologic mechanisms responsible for these associations. Numerous studies have also shown that short-term exposures to PM and other pollutants are associated with changes in cardiac rhythm, such as HRV, and with alterations in the morphology of electrocardiogram (ECG) waveforms, providing insights into the interplay among pollutants, the heart, and the parasympathetic and sympathetic nervous systems.

APPROACH

The goal of the study by Rich, Peters, and colleagues was to reanalyze existing ECGs from four previous studies conducted by their teams to evaluate the associations between short-term (from 5 minutes to 6 hours) increases in exposure to fine PM (PM ≤ 2.5 µm in aerodynamic diameter [$PM_{2.5}$]) and ultrafine PM (PM ≤ 0.1 µm in aerodynamic diameter [UFP]) and changes in cardiac rhythm. The investigators were interested in assessing the effects of these particles on HRV and other ECG variables on shorter timescales than most previous studies. The ECGs were obtained using a portable recorder known as a Holter monitor, worn by the

What This Study Adds

- Rich, Peters, and colleagues analyzed the ECGs of more than 200 individuals from two completed panel studies and two completed controlled-exposure studies in relation to increases in exposure to ultrafine particles (UFPs) and ambient fine particles ($PM_{2.5}$) in the previous 6 hours. Through a statistical approach known as factor analysis, they identified three ECG variables that were common across the studies: SDNN, a marker of total heart rate variability (HRV); RMSSD, a marker of parasympathetic regulation; and T-wave complexity, a marker of repolarization.
- Increases in recent exposures (previous 2 to 5 hours) to UFPs and $PM_{2.5}$ were associated with changes in SDNN; increases in exposure to $PM_{2.5}$ over the same period were associated with changes in RMSSD. Very recent exposures (less than 1 hour before) were not associated with any ECG changes.
- The observed associations are not likely to be of clinical significance but provide evidence of particle-related subclinical physiological changes and increase our confidence in the use of HRV parameters as reproducible intermediate markers potentially relevant to the association between air pollution and cardiovascular outcomes.

This Statement, prepared by the Health Effects Institute, summarizes a research project funded by HEI and conducted by Dr. David Q. Rich at the University of Rochester School of Medicine and Dentistry, Rochester, New York; Dr. Annette Peters at the Institute of Epidemiology II, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany; and their colleagues. Research Report 186 contains both the detailed Investigators' Report and a Critique of the study prepared by the Institute's Health Review Committee.

subjects during the observation periods. The four previous studies were:

- The Augsburg panel study of individuals with diabetes or impaired glucose tolerance and individuals without the glutathione S-transferase M1 (*GSTM1*) gene (which is involved in the detoxification of oxidative-stress products);
- The Rochester panel study of patients with a history of acute coronary artery syndromes; and
- Two Rochester controlled-exposure studies of healthy volunteers and volunteers with diabetes.

Exposure Assessment

For the panel studies, the investigators estimated 1-hour average exposures to UFPs and $PM_{2.5}$ from ambient air measurements and, for the Augsburg study only, 5-minute average exposures to UFPs from personal air measurements. For the controlled-exposure studies, they estimated 1-hour and 5-minute average UFP exposures from chamber measurements of concentrated ambient UFPs or laboratory-generated elemental carbon particles. There were large differences between studies in the UFP concentrations.

Statistical Analyses

To reduce the number of variables in their statistical models, the investigators performed separate factor analyses of the hourly ECG variables and, for subsequent analysis, selected the three that had the highest correlation with a factor and were common to all four studies: SDNN (a marker of overall HRV), RMSSD (a marker of parasympathetic modulation), and T-wave complexity (a marker of repolarization).

The investigators used an additive mixed model as their basic statistical model, although the modeling approach varied somewhat across studies. They analyzed the three selected ECG variables (1-hour or 5-minute averages) in relation to the previous 1-hour average pollutant concentrations (up to 6 hours) or the previous 5-minute average pollutant concentrations (up to 60 minutes), reporting results as the percent change per interquartile increase in pollutant concentrations at each lag. They tested nine hypotheses in all: six about associations between 1-hour average UFP and $PM_{2.5}$ concentrations and 1-hour averages for the three ECG variables and three about associations between 5-minute average UFP concentrations and 5-minute averages for the same ECG variables.

RESULTS AND INTERPRETATIONS

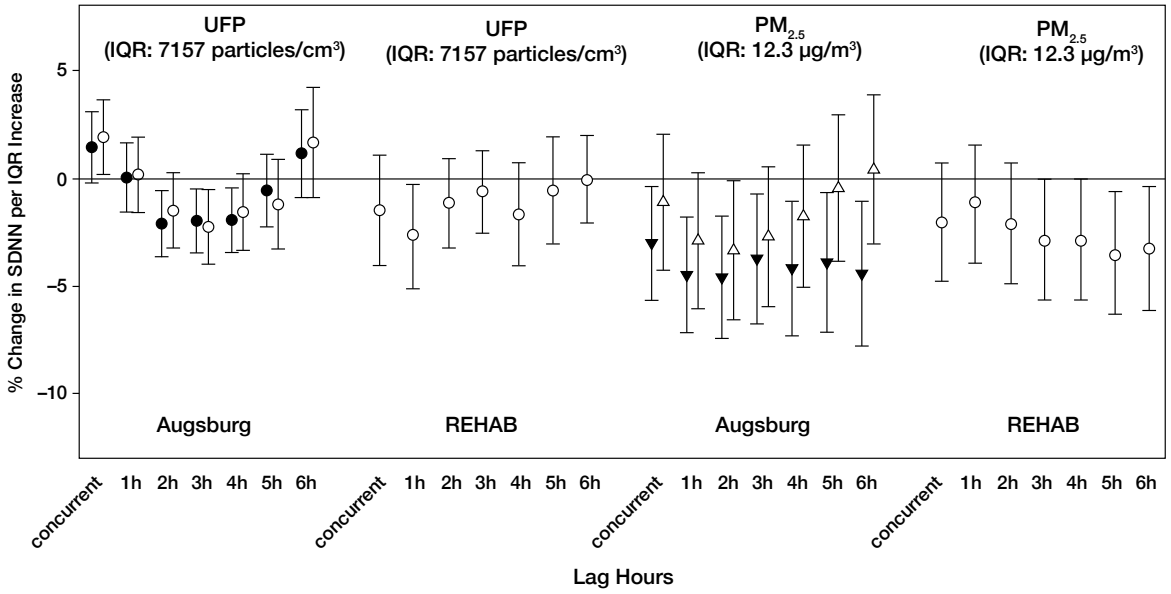
The analyses supported the hypotheses that higher exposures to UFPs and $PM_{2.5}$ are associated with lower total HRV (as assessed by SDNN) during the subsequent 2 to 5 hours (Figure 1) and that higher exposures to $PM_{2.5}$ are associated with lower RMSSD. These results are consistent with those reported by other studies. No associations were found with T-wave morphology. The study did not support the hypothesis that very recent (less than 1 hour) exposures to $PM_{2.5}$ or UFPs are associated with rapid ECG changes.

In its independent assessment of the study, the Health Review Committee concluded that the study was carefully conducted and made efficient use of existing data obtained from relevant populations to address important questions about associations between markers of cardiac function and recent exposure to PM. The investigators' inability to replicate many of their hypotheses across the four studies may have been caused, at least in part, by the pronounced differences in participant characteristics, exposure sources, compositions, concentrations, and study designs, coupled with the stringent criteria used to evaluate whether a hypothesis was replicated.

The Committee thought that the use of factor analysis to reduce the number of ECG outcome variables to model was novel and achieved its intended purpose. However, the use of a separate factor analysis for each study resulted in between-study differences in the number of factors and variables identified, raising doubts about the generalizability of this approach to other studies. The Committee also raised some concerns about the use of different statistical models to analyze the data from each of the studies.

The Committee agreed with the investigators' conclusion that recent exposures to UFPs and $PM_{2.5}$ are associated with subclinical alterations in markers of HRV and noted that the observed associations should not be interpreted to imply that ambient PM triggers the cardiac responses. These conclusions are broadly consistent with those of earlier studies, although the analyses presented in the current study are more detailed and extensive

than those in many of the earlier studies and represent an important addition to the literature. The Committee also agreed with the investigators that the observed associations are not likely to be of clinical significance but rather provide evidence of particle-related subclinical physiologic changes by which air pollution may increase the risk of acute cardiovascular events. The Committee did not think the investigators' conclusion that exposures to UFPs and $PM_{2.5}$ were independently associated with decreases in SDNN was clearly supported by the results. The combined results from the four studies increase confidence in the use of HRV parameters as reproducible intermediate markers potentially relevant to the associations between air pollution and cardiovascular outcomes.



Statement Figure 1. Percent change in SDNN (1-hour average) associated with each IQR increase in UFP and $PM_{2.5}$ concentrations in the concurrent hour and at lags 1 to 6 hours for the Augsburg and REHAB studies. For the Augsburg study, black symbols represent subjects in the group with diabetes or IGT, and white symbols represent healthy subjects in the group with a genetic susceptibility. For the REHAB study, the results were scaled to the same IQR increase as the Augsburg study. (1h = first hour before the measurement; 2h = second hour before the measurement, etc.)

INVESTIGATORS' REPORT

Ambient and Controlled Particle Exposures as Triggers for Acute ECG Changes

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ABSTRACT

INTRODUCTION

Previous studies have examined changes in heart rate variability (HRV*) and repolarization associated with increased particulate matter (PM) concentrations on the same and previous few days. However, few studies have examined whether these health responses to PM occur within a few hours or even less. Moreover, it is not clear whether exposure of subjects to ambient or controlled PM concentrations both lead to similar health effects or whether any of the subjects' individual characteristics modify any of their responses to PM. The aims of the current study were to investigate whether exposure to PM was associated with rapid changes (< 60 minutes or concurrent hour up to a delay of 6 hours) in markers of cardiac rhythm or changes in total antioxidant capacity (a marker of protection against oxidative stress) and whether any PM effects on cardiac rhythm markers were modified by total antioxidant capacity, age, obesity, smoking, hypertension, exertion, prior myocardial infarction (MI), or medication.

METHODS

We obtained data from a completed study in Augsburg, Germany (a panel study in $N = 109$ subjects, including a group with type 2 diabetes or impaired glucose tolerance [IGT; also known as prediabetes]) and a group of otherwise healthy subjects with a potential genetic susceptibility to detoxifying and inflammatory pathways (Hampel et al. 2012b), as well as three completed studies in Rochester, New York (the REHAB panel study of $N = 76$ postinfarction patients in a cardiac rehabilitation program [Rich et al. 2012b]; the UPDIABETES study of controlled exposure to ultrafine particles [UFPs, particles with an aerodynamic diameter < 100 nm] of $N = 19$ patients with type 2 diabetes [Stewart et al. 2010; Vora et al. 2014]; and the UPCON controlled-exposure study of concentrated UFP exposure in $N = 20$ young, healthy, lifetime nonsmokers). Data included 5-minute and 1-hour values for HRV and repolarization parameters from electrocardiogram (ECG) recordings and total antioxidant capacity measured in stored blood samples. Ambient concentrations of UFPs, accumulation-mode particles (AMP, particles with an aerodynamic diameter of 100–500 nm), fine PM ($PM_{2.5}$, particles with an aerodynamic diameter ≤ 2.5 µm), and black carbon (BC) were also available.

We first conducted factor analyses in each study to find subgroups of correlated ECG outcomes and to reduce the number of outcomes examined in our statistical models. We then restricted the statistical analyses to the factors and representative outcomes that were common to all four studies, including total HRV (measured as the standard deviation of normal-to-normal [NN] beat intervals [SDNN]), parasympathetic modulation (measured as the root mean square of the successive differences [RMSSD] between adjacent NN beat intervals), and T-wave morphology (measured as T-wave complexity). Next, we used additive mixed models to estimate the change in each outcome associated with increased pollutant concentrations in the

This Investigators' Report is one part of Health Effects Institute Research Report 186, which also includes a Critique by the Health Review Committee and an HEI Statement about the research project. Correspondence concerning the Investigators' Report may be addressed to Dr. David Q. Rich, University of Rochester School of Medicine and Dentistry, Department of Public Health Sciences, 265 Crittenden Boulevard, CU 420644, Rochester, NY 14642; email: *david_rich@urmc.rochester.edu*.

Although this document was produced with partial funding by the United States Environmental Protection Agency under Assistance Award CR–83467701 to the Health Effects Institute, it has not been subjected to the Agency's peer and administrative review and therefore may not necessarily reflect the views of the Agency, and no official endorsement by it should be inferred. The contents of this document also have not been reviewed by private party institutions, including those that support the Health Effects Institute; therefore, it may not reflect the views or policies of these parties, and no endorsement by them should be inferred.

* A list of abbreviations and other terms appears at the end of the Investigators' Report.

concurrent and previous 6 hours and with 5-minute intervals up to the previous 60 minutes, accounting for the correlation of repeated outcome measures for each subject and adjusting for time trend, hour of the day, temperature, relative humidity, day of the week, month, and visit number. Because multiple comparisons were an issue in our analyses, we used a discovery-and-replication approach to draw conclusions across studies for each research question.

RESULTS

In the Augsburg study, interquartile range (IQR) increases in UFP concentrations lagged 2 to 5 hours were associated with 1%–3% decreases in SDNN (e.g., lagged 3 hours in the group with a genetic susceptibility: –2.26%; 95% confidence interval [CI], –3.98% to –0.53%). In the REHAB study, similarly, IQR increases in UFP concentrations in the previous 5 hours were associated with < 3% decreases in SDNN (e.g., lagged 1 hour: –2.69%; 95% CI, –5.13% to –0.26%). We also found decreases in SDNN associated with IQR increases in total particle count (a surrogate for UFP) in the UPDIABETES study (lagged 1 hour: –13.22%; 95% CI, –24.11% to –2.33%) but not in the UPCON study.

In the Augsburg study, IQR increases in $PM_{2.5}$ concentrations in the concurrent hour and lagged 1–5 hours, AMP concentrations lagged 1 and 3 hours, and BC concentrations lagged 1–5 hours were associated with ~1%–5% decreases in SDNN (e.g., $PM_{2.5}$ lagged 2 hours in the group with diabetes or IGT: –4.59%; 95% CI, –7.44% to –1.75%). In the REHAB study, IQR increases in $PM_{2.5}$ concentrations lagged 5 and 6 hours and AMP concentrations in the concurrent hour and lagged up to 5 hours were associated with 1%–2% decreases in SDNN (e.g., $PM_{2.5}$ lagged 4 hours: –2.13%; 95% CI, –3.91% to –0.35%).

In the Augsburg study, IQR increases in $PM_{2.5}$ concentrations in the concurrent hour and BC lagged 1 and 6 hours were associated with 3%–7% decreases in RMSSD (e.g., $PM_{2.5}$ concurrent hour in the group with diabetes or IGT: –7.20%; 95% CI, –12.11% to –2.02%). In the REHAB study, similarly, increases in $PM_{2.5}$ concentrations lagged 4 to 6 hours — though not AMP or BC concentrations at any lag hour — were associated with ~2.5%–3.5% decreases in RMSSD (e.g., $PM_{2.5}$ lagged 5 hours: –3.49%; 95% CI, –6.13% to –0.84%). We did not find consistent evidence of any pollutant effects on T-wave complexity in 1-hour recordings. For 5-minute recordings, there was no consistent evidence of UFP effects on SDNN, RMSSD, or T-wave complexity at any 5-minute interval within 60 minutes.

We further concluded that these replicated hourly effects of UFP and $PM_{2.5}$ on short-term measures of SDNN and RMSSD generally did not differ between the groups in the studies (i.e., type 2 diabetes, pre-diabetes/IGT, post-infarction, and healthy subjects). Last, we found no consistent evidence of effects of any pollutant on total antioxidant capacity and no consistent evidence of modification of our $PM_{2.5}$–outcome associations by any of the potential effect modifiers.

CONCLUSIONS

Increased UFP concentrations were associated with decreased SDNN in both of the panel studies and one of the two controlled-exposure studies. We also found that decreased SDNN was associated with both increased $PM_{2.5}$ and AMP concentrations in the previous 6 hours in the panel studies and that decreased RMSSD was associated with increased $PM_{2.5}$ concentrations in the previous 6 hours in the panel studies. We therefore concluded that the research questions were replicated.

Our findings suggest that both UFPs and $PM_{2.5}$ are associated with autonomic dysfunction within hours of exposure, which may in part explain the previously reported risk of acute cardiovascular events associated with increased PM in the previous few hours. Despite the heterogeneity of the study populations and protocols, our findings provided consistent evidence for the induction of rapid pathophysiological responses by UFPs and $PM_{2.5}$. The absence of consistent associations between UFPs, $PM_{2.5}$, and these outcomes when examining shorter time intervals indicates that the 5- to 60-minute responses may be less pronounced than the responses occurring within hours. However, the findings from the 5-minute intervals may have been affected by the variety of protocols and conditions from study to study as well as by the potential effects of underlying diseases (e.g., healthy individuals versus individuals with diabetes or a recent coronary artery event), physical activity, circadian rhythms, stress, and/or medications.

INTRODUCTION AND SPECIFIC AIMS

Many studies have shown that increased concentrations of ambient air pollutants are associated with increased cardiovascular hospital admissions and mortality (e.g., Dominici et al. 2006; Peng et al. 2009). Many other studies have reported increased risks of MI in the few days following increases in ambient PM concentrations (e.g., D'Ippoliti et al. 2003; Peters et al. 2001, 2005;

Pope et al. 2006; Rich et al. 2010; Zanobetti and Schwartz 2005). A few have even suggested that ambient PM (Gardner et al. 2014; Peters et al. 2001) or exposure to traffic (Peters et al. 2004) might trigger MI within 1 or 2 hours. Similar immediate (i.e., within 1 hour) responses to ambient PM or traffic have also been reported for episodes of ventricular arrhythmia (Albert et al. 2007; He et al. 2011b).

Pathways thought to mediate PM effects on MI, ventricular arrhythmia, and other acute cardiovascular events (including autonomic dysfunction, systemic inflammation, endothelial dysfunction, and coagulation) have been discussed (Brook et al. 2004). Studies have also examined changes in HRV associated with increased PM concentrations in the previous few days (Baja et al. 2013; Gold et al. 2000; Pieters et al. 2012; Pope et al. 1999; Schneider et al. 2010a). Time- and frequency-domain analyses of HRV make it possible to assess impaired autonomic nervous activity noninvasively. Several authors have reported adverse cardiac health effects associated with decreased HRV (Brook et al. 2004, 2010; Xhyerhi et al. 2012). Decreased T-wave complexity, T-wave flattening, and other T-wave abnormalities might also precede adverse cardiovascular events (Greenland et al. 2003; Jacobsen et al. 2001; Lin et al. 2008; Rautaharju et al. 2013). Abnormalities in repolarization morphology, described by the fairly novel measurement of T-wave complexity, reflect the status of the myocardial substrate and have previously been found to be associated with an increased risk of cardiac events both in healthy subjects (Kors et al. 1998; Okin et al. 2002; Porthan et al. 2013) and in postinfarction patients (Zabel et al. 1998, 2000) in studies unrelated to the field of air pollution research. Only a few studies have examined whether these HRV responses to PM occur within 1 hour or even less than 1 hour. Among patients with coronary artery disease or diabetes, adverse changes in T-wave alternans and HRV (within 30 minutes to 2 hours) have been associated with increased BC concentrations (Zanobetti et al. 2009), with larger effects during times spent in traffic (Laumbach et al. 2010; Zanobetti et al. 2009, 2010). Moreover, HRV indices were associated with individual-level $PM_{2.5}$ exposures in the previous 1 to 6 hours (He et al. 2011a). The rapid responses in HRV to increases in air pollution were likely a consequence of the activation of the nervous system or a direct effect on the electrical system of the heart (Brook et al. 2010; Pope and Dockery 2006).

The effects of air pollutants on repolarization are less well explored. However, it is known that prolonged abnormalities in heart-rate-corrected QT interval (QTc) and T-wave amplitude, two markers of repolarization, might trigger the onset of arrhythmias (Roden 2008) or increase the risk for coronary deaths (Greenland et al. 2003). A few studies (Ghelfi et al. 2008; Henneberger et al. 2005; Liao et al. 2010; Zanobetti et al. 2009; Zareba et al. 2009) have investigated the relationship between elevated levels of PM air pollution and repolarization.

Other studies have provided evidence that oxidative stress may be a mechanism underlying air pollution effects on heart rate, HRV, and repolarization. These effects may also be different in one or more population subgroups with different host defenses against an oxidative stress challenge, because genetic polymorphisms have been linked to important differences in such defenses (Baja et al. 2010; Chahine et al. 2007; Hampel et al. 2012a; Park et al. 2006; Schwartz et al. 2005).

Previously, we demonstrated immediate (i.e., within 1 hour) triggering of MI by time spent in traffic (Peters et al. 2004), which if truly attributable to PM exposures requires very rapid cardiovascular responses. Indeed, Mills and colleagues (2007) found very immediate signs (within 1 hour) of ischemia in response to diluted diesel exhaust exposure while exercising, and Albert and colleagues (2007) found an increased risk of implantable cardioverter-defibrillator shock for ventricular tachycardia or ventricular fibrillation within 30 minutes after driving. We thus hypothesize that the potential mechanisms underlying these PM–MI and traffic–MI associations may act on time scales of 1 hour or less.

None of the studies mentioned above evaluated whether the immediate responses were affected by the subjects' antioxidant capacity, a blood marker indicative of oxidative stress or increased susceptibility to oxidative damage. The current study was intended to provide novel insights into the associations between air pollution and markers of immediate (i.e., 5 minutes to 1 hour) physiological responses. Given that many personal air pollution exposures are of short duration (e.g., while driving, riding on a bus or subway, or walking on the sidewalk close to a road), understanding the mechanisms by which particulate air pollution may affect cardiovascular health on these time scales is an important public health issue.

We used data from four existing studies that included controlled exposures to UFPs (using total particle number as a proxy for UFP) and ambient exposures to UFPs, AMP, $PM_{2.5}$, and BC as part of various ambient pollutant mixtures in Augsburg, Germany, and Rochester, New York. The cities' environments are characterized by local, mainly traffic-related fresh PM. In Germany, the proportion of diesel cars was considerably higher than in the United States, providing a unique opportunity to study the impact of $PM_{2.5}$ and UFPs containing varying amounts of diesel soot particles.

Our approach included studies of changes in cardiac rhythm associated with exposure to PM conducted in epidemiologic settings (the Augsburg panel study and Rochester REHAB panel study) and studies conducted in clinical settings with controlled UFP exposures in healthy (the Rochester UPCON study) and health-compromised individuals (the Rochester UPDIABETES study). We also examined the roles of selected patient characteristics (e.g., medication use, underlying disease, or age), exercise, and total antioxidant capacity as potentially modifying factors. All of these are in part related to subject behaviors and may shed light on the potential interactions of air pollution with various stressors.

Our study had the following three specific aims:

Specific Aim 1 To assess immediate ECG responses in association both with ambient air pollution and with controlled air pollution exposures.

1. To estimate changes in ECG outcomes within 1 hour associated with increases in ambient UFPs, AMP, $PM_{2.5}$, and BC and with controlled exposure to UFPs in the same hour and previous few hours both in the epidemiological panel studies (Augsburg and REHAB) and in the controlled-exposure studies (UPCON and UPDIABETES). We also estimated changes in ECG parameters within 5 minutes associated with increases in pollutant concentrations in the same 5 minutes and the previous 60 minutes.

2. To estimate these changes for the various disease groups (diabetes, IGT, and cardiac diseases) in the studies and to discuss similarities and differences among the results in the various studies and groups.

Specific Aim 2 To assess the ability of selected individual subject characteristics and physical exertion to modify the associations between air pollution and ECG parameters.

1. To assess if age, body mass index (BMI), smoking status, or previous diagnosis of hypertension modify the associations between air pollution concentrations and cardiac rhythm outcomes.

2. To assess if a high level of self-perceived exertion (as reported in an activity diary in the Augsburg study or as part of a cardiac rehabilitation exercise program in the REHAB study) by a subject is associated with a greater change in the ECG outcomes associated with increased pollutant concentrations than a low level of self-perceived exertion.

Specific Aim 3 To assess antioxidant capacity in association with air pollution, both as an outcome and as an effect modifier.

1. To estimate changes in total antioxidant capacity associated with increases in ambient PM concentrations in the same and previous few days (in the Augsburg and REHAB studies).
2. To assess the potential of total antioxidant capacity to modify the cardiac rhythm responses to UFPs and $PM_{2.5}$ examined in specific aim 1 (in the Augsburg and REHAB studies).

METHODS AND STUDY DESIGN

STUDY POPULATIONS, DESIGNS, AND PROTOCOLS

Our data analysis built on two epidemiological panel studies (the Augsburg and REHAB studies) and two controlled human exposure studies (the UPCON and UPDIABETES studies) that were funded from other sources. Results for three of the four studies have previously been published (Hampel et al. 2012a, 2012b; Rich et al. 2012b; Rückerl et al. 2014; Stewart et al. 2010; Vora et al. 2014; Wasserman et al. 2014).

Augsburg Panel Study The study population and protocol for the Augsburg panel study have been described previously (Hampel et al. 2012b). Briefly, between March 2007 and December 2008, we enrolled 110 participants, including 32 with type 2 diabetes, 32 with IGT, and 46 healthy participants with a potential genetic susceptibility to oxidative injury and inflammatory pathways. The potential genetic susceptibility was defined as having the null polymorphism for glutathione S-transferase M1 (*GSTM1*) and either two major alleles of the single-nucleotide polymorphism (SNP) rs1205 located in the C-reactive protein gene or at least one minor allele of the SNP *rs1800790* located in the fibrinogen gene *FGB*. The SNP *rs1800790* was selected based on studies by Jacquemin and colleagues (2008) and Peters and colleagues (2009), and the SNP *rs1205* was selected based on studies by Kolz and colleagues (2008) and Sunyer and colleagues (2008) that built on an earlier study by Peters and colleagues (2007). However, for the ECG parameters measured in the Augsburg study, the genetic susceptibility to inflammation was likely not relevant. Exclusion criteria were current smoking, intake of platelet-aggregation inhibitors other than acetylsalicylic acid, an MI or interventional procedure less than 6 months before the start of the study, chronic inflammatory diseases, an implanted pacemaker, atrial fibrillation, allergy to latex, and thrombosis or a

shunt in an arm. For each participant, data for a baseline characterization was collected, including information on health status; history of diabetes, pulmonary, or cardiac disease; medication; smoking history; and measurement of BMI. Participants were scheduled for up to five examinations every 4 to 6 weeks on the same day of the week between 7:30 A.M. and 3:00 P.M. In up to four of these examinations, each subject was outfitted in the study center with a continuous digital 12-lead ECG Holter recorder (Mortara Instruments, Milwaukee, WI). During the ECG recordings (mean duration 6 hours), participants were free to pursue their daily routines. Participants recorded all of their activities and locations in a diary. Each diary entry included a text description, location, and time (to the minute) of the activity. Participants also recorded whether they were indoors, outdoors, not in traffic (e.g., in a park), or in traffic. Descriptive analyses of the diary data were performed based on 2040 1-hour ECG intervals (diary information was missing for eight of the 1-hour intervals). For 64% of the 1-hour intervals, the participants were outdoors (in traffic or not) at least once during the hour. Fifty-seven percent of the 1-hour intervals were recorded while being in traffic at least once during the hour. In addition, venous blood samples were collected at every visit from every participant. Brief physical activities of the participants were recorded in their diaries in only 75 (4%) of the total 2048 1-hour intervals. The recordings were made in 50 visits (from a total of 356 visits) and for 34 of the 109 participants (one participant was ultimately excluded because of a missing blood sample). In total, 364 5-to-6-hour ECG measurements and 464 blood samples were then used in the analyses described below.

Rochester REHAB Study The study population and protocol for the REHAB panel study have been described previously (Rich et al. 2012b; Wasserman et al. 2014). Briefly, $N = 76$ patients participating in a cardiac rehabilitation program at the University of Rochester who were medically managed either with or without invasive intervention (e.g., coronary artery bypass grafting or angioplasty with intracoronary stent placement), who lived within 10 miles of our monitoring site, and who were otherwise medically cleared to participate in a rehabilitation exercise program, were enrolled. We intentionally did not recruit subjects who were current smokers or living in a household with a smoker; who had left bundle branch block on ECG, a pacemaker, or type 1 diabetes; or who had previously had a heart valve replacement, atrial arrhythmia, or anemia. The subjects were in early post-MI rehabilitation, post coronary bypass rehabilitation, or in rehabilitation for other conditions such as unstable angina. Sixty-nine of the 76 had undergone an intervention (either coronary artery bypass grafting or angioplasty with intracoronary stent placement). Approximately 70% of participants completed the program. The enrolled participants were involved in two rehabilitation sessions per week over a 10-week period (20 sessions per subject at most), each consisting of 30 to 45 minutes of exercise. Cardiovascular status and blood pressure were measured during each session, and continuous Holter ECG recordings were made. In addition to the regular electrocardiographically monitored treadmill exercise of the rehabilitation program, the subjects underwent 2- to 3-hour three-lead Holter ECG recordings (Vision Premier, Burdick, Milwaukee, WI), allowing evaluation of various ECG parameters at rest in a supine position before the supervised exercise, during the exercise, and during the immediate post-exercise recovery period. As part of the three-lead Holter recordings but before each exercise session, a 10-minute resting ECG was acquired to obtain baseline pre-exercise information. After completion of the resting ECG, the subjects undertook the routine exercise rehabilitation program. Venous blood samples were collected weekly.

We collected information on the exercise component of each subject's visit to the rehabilitation center during the study. At each visit, after all pre-exercise health measurements were made and the Holter monitor and leads were placed on the subject, each subject did 2 to 5 minutes of warm-ups, including gentle stretching. After the warm-ups, each subject chose his or her mode(s) of exercise (i.e., various bicycles, treadmills, or rowing machines), and the study coordinator then marked the beginning of the exercise session on the Holter. The subject then exercised for 30 to 45 minutes as the coordinator marked the subject's peak heart rate, blood pressure, and the time of each on the Holter recording. The subject's self-perceived exertion (on a scale of 6 for "No exertion at all" to 20 for "Maximum exertion") (Borg 1998) was also recorded. At the end of the exercise period, the coordinator marked the end of exercise and the start of the rest period on the Holter recording. Three subjects' recordings were inadequate for 1-hour analysis, leaving 73 subjects with ECG recordings and 657 blood samples for use in the analyses described below.

The study subjects wore the Holter monitors until the end of each rehabilitation session but did not wear them at any time before or afterward. Before and after each session, the subjects were free to pursue their normal everyday activities, and we did not collect information on these activities.

Rochester UPCON Study The UPCON study was a double-blind, randomized crossover study of 20 healthy lifetime nonsmokers. Subjects were admitted to the Clinical Research Center at the University of Rochester

Medical Center for an overnight stay before exposure in order to minimize confounding effects of ambient pollutant exposures. For 2 hours at rest, the subjects inhaled either outdoor ambient UFPs that were concentrated using a Harvard UFP concentrator system or filtered air. The mean particle number in the concentrated aerosol was $25 \pm 14 \times 10^4$ particles/cm^3, with a mean mass concentration of 158 ± 85 μg/m^3. The mean particle diameter was 94 nm, with a standard deviation of 8. No adverse effects or symptoms were associated with either of these exposures, and the subjects were unable to distinguish the pollutant exposures from the filtered-air exposures. For the analyses described below, particle number concentration (PNC) was used as a proxy for UFP.

During the exposures, each subject was monitored using a continuous digital 12-lead ECG Holter recorder (Mortara Instruments). Recordings started 2 hours before the exposures and lasted for 24 hours each. Five-minute supine resting ECG recordings were made before exposure, immediately after exposure, and 3.5 hours and 21 hours after exposure to evaluate ECG parameters in controlled conditions unaffected by physical activity or body position. One subject did not have adequate ECG recordings for either the UFP exposure or clean-air exposure, and three subjects had ECG recordings for only one exposure, leaving 19 subjects and 35 recordings (24 hours in length) for use in the 1-hour or 5-minute analyses described below.

Rochester UPDIABETES Study The study population and protocol for the UPDIABETES study have been described previously (Stewart et al. 2010; Vora et al. 2014). Briefly, the study was a double-blind, randomized crossover study of 19 subjects with type 2 diabetes according to World Health Organization criteria. Subjects were admitted to the Clinical Research Center at the University of Rochester Medical Center for an overnight stay before exposure in order to minimize confounding effects of ambient pollutant exposures. For 2 hours at rest, the subjects inhaled either freshly generated elemental carbon UFPs (50 μg/m^3, count median diameter 32 nm; total particle count concentration $10 \pm 1 \times 10^6$ particles/cm^3) or filtered air. For the analyses described below, total particle count was used as a proxy for UFP. Again, no adverse effects or symptoms were associated with the exposures, and the subjects were unable to distinguish the pollutant exposures from the filtered-air exposures. During the exposures, each subject was monitored using a continuous digital 12-lead ECG Holter recorder (Mortara Instruments). Recordings started 2 hours before each exposure and lasted for 48 hours each. Again, 5-minute supine resting ECG recordings were obtained before exposure, immediately after exposure, and 3.5 hours and 21 hours after exposure to evaluate ECG parameters in controlled conditions unaffected by physical activity or body position. One subject did not have ECG recordings for either exposure, and two subjects had ECG recordings for only 1 exposure, leaving 18 subjects and 34 recordings (48 hours in length) for use in the 1-hour or 5-minute analyses described below.

AMBIENT AIR POLLUTION MONITORING SITES AND MEASUREMENT METHODS

The focus of our study was to investigate whether changes in ECG outcomes were associated with changes in particulate air pollutant concentrations ($PM_{2.5}$, UFPs, AMP, and BC) in the previous few minutes and few hours. We therefore will describe only how these pollutants (and not gaseous pollutants, such as sulfur dioxide, nitrogen dioxide, carbon monoxide, or ozone) were measured at the Augsburg and Rochester monitoring sites.

In Augsburg Various ambient particle characteristics were measured at a fixed urban background monitoring site in Augsburg, Germany, throughout the entire time of the Augsburg study (Cyrys et al. 2008; Pitz et al. 2008a, 2008b). The monitoring site was on the campus of the University of Applied Sciences Augsburg, approximately 1 km southeast of the city center. The nearest major street was to the northeast, at a distance of 120 m. Particle mass concentrations of $PM_{2.5}$ were measured using a tapered element oscillating microbalance (TEOM; model 1400ab, Thermo Fisher Scientific, Waltham, MA). To correct the PM measurements for aerosol volatility effects, the TEOM was equipped with a filter dynamics measurement system (model 8500b, Thermo Fisher Scientific). Particle size distributions in the range of 10–500 nm were measured using a custom-built twin differential mobility particle sizer system consisting of two cylindrical Vienna-type differential mobility analyzers. Exiting monodispersed particles were counted in two condensation particle counters (models 3025a and 3010, TSI, Shoreview, MN) with diameter ranges of 10–23 nm and 18–500 nm, respectively. UFPs were calculated as the sum of the number of particles in the ranges of 10–30 nm, 30–50 nm, and 50–100 nm. AMP were calculated as the number of particles in the range of 100–800 nm. BC was measured using online methods (Aethalometer, series 8100, Thermo Fisher Scientific). The measured meteorological parameters were barometric pressure, global radiation, wind direction, wind speed, temperature, and relative humidity. All of the ambient pollutant data described above were available on an hourly basis.

In addition, personal measurements of particle number concentrations (PNCs)—as a surrogate for personal UFP exposures—were sampled using a portable condensation particle counter (model 3007, TSI) with a maximum count of 100,000 particles/cm^3. However, these personal PNC measurements were used only to analyze associations with ECG outcomes within 5 minute, not within 1 hour, because the measurements' duration of ~5–6 hours was too short to calculate 1-hour lags. Missing values for the particulate air pollutants and meteorological variables were not replaced, because < 1% of the 24-hour averages for them were missing or no parallel measurements made with other devices (for UFPs, AMP, or BC) were available.

In Rochester Particle size distributions for UFPs and AMP were measured using a wide-range particle spectrometer (model 1000XP, MSP, Shoreview, MN) at the Cardiac Rehabilitation Center from June 2006 to November 2009. The sample flow rate of this unit was 1.0 L/min. Measurements were made through a common switching valve alternately sampling indoor and outdoor air every 3.5 minutes. One size distribution sample was taken during each 3.5-minute interval. Outdoor concentration data were used in the statistical analyses described below. The measurements have been described previously (Wang et al. 2010). Although the size range used for the AMP (100–500 nm) was different from that by which AMP is usually defined (100–1000 nm), the majority of particles in these size ranges are smaller in mass and closer to the 100 nm cut-off value, and the use of the 100–500 nm size range was therefore expected to result in minimal differences in AMP concentrations. The monitoring site in Rochester is approximately 1500 m from an interstate beltway.

Hourly $PM_{2.5}$ mass and BC concentrations, wind speed and wind direction, ambient temperature, and relative humidity were measured at a New York State Department of Environmental Conservation site throughout the study period. $PM_{2.5}$ was measured using the TEOM. BC was measured using a single-wavelength Aethalometer until 2008 and a two-wavelength Aethalometer (both model #AE-22, Magee Scientific, Berkeley, CA) from then until November 2009. The New York State Department of Environmental Conservation site (latitude 43°09′56″ N, longitude 77°33′15″ W) is located on the east side of Rochester and is close to two major highways (I-490 and I-590) and to New York State Route 96, a state highway carrying traffic to and from downtown Rochester. The distance from the site to the nearest major street is 290 m.

The particle counter at the Cardiac Rehabilitation Center was down from September 3 to November 13, 2009, for mechanical reasons. We therefore imputed hourly UFP values at the Center site using the UFP measurements made at the Department of Environmental Conservation site. We used the hourly data from August 1 to September 3, 2009, and separately regressed the Department of Environmental Conservation site's 10–50-nm and 50–100-nm PNCs against the Cardiac Rehabilitation Center 10–50-nm and 50–100-nm PNCs. This regression result was then used to impute values for the missing hourly values during the shutdown period. They were then summed to estimate UFP concentrations for that hour.

These 2009 UFP PNC data followed the trend we have previously seen from 2005 onward (Wang et al. 2011a). The UFP trends also followed related reductions in pollutants over that time period, as observed at the nearby Department of Environmental Conservation site (Wang et al. 2011b). Part of the lower UFP values in 2009 were caused by the shutdown of the Russell 260 MW coal-fired power plant in spring 2008. Additional reductions were likely caused by the change in 2007 to ultralow sulfur on-road diesel fuel and then the gradual replacement of heavy-duty diesel fleet vehicles with new ones having catalytic regenerative traps on them.

In addition to monitoring size distributions at the Cardiac Rehabilitation Center and the New York State Department of Environmental Conservation site, a limited sampling campaign was conducted to assess the exposure of the subjects to UFPs at home and on their way to and from the Center. The subjects were provided with a water-based condensation particle counter (model 3781, TSI) that they took home from one exercise session and returned at their next session. Thirty subjects operated the condensation particle counters in their homes for generally 2 days; a few subjects (about five) operated it for 3 days. Seventeen subjects completed in-car measurements (during commutes averaging 13 minutes each way). However, these data were too fragmentary to use in any systematic manner in the assessment of the impact of UFPs on the subjects' health and were therefore not used in the analyses described below.

GENERATION OF CONTROLLED UFP EXPOSURES: UPDIABETES AND UPCON STUDIES

The UFP generation and exposures in the UPDIABETES study have been described previously (Chalupa et al. 2004). Briefly, particles were generated in argon using an electric spark discharge between graphite electrodes in a commercial aerosol generator (model GFG-1000; Palas, Karlsruhe, Germany) modified to prevent off-gassing of organic materials from inside the generator (McDonald et al. 2001). This procedure produced particles consisting of > 95% elemental carbon without metals. Particle mass

(measured using a TEOM [Rupprecht & Patashnick, Albany, NY]), particle number (measured using condensation particle counters [model 3022a, TSI]), and particle size distributions (measured using a Scanning Mobility Particle Sizer [model 3071, TSI]) were monitored on both the inspiratory and expiratory sides of the subject. During each 2-hour exposure, each subject inhaled from a mouthpiece and wore a nose clip.

For the UPCON study, exposures to concentrated ambient UFP or filtered air were generated using a Harvard ultrafine concentrated ambient particle system (HUCAPS), which has been described in detail elsewhere (Gupta et al. 2004). For the filtered-air exposures, a HEPA filter was used at the HUCAPS outlet. Briefly, the system consisted of a $PM_{2.5}$-size-selective inlet, a condensational growth unit using heated ultrapure water, a controlled supersaturation unit using precise cooling, a two-stage virtual impactor to concentrate the ultrafine fraction, a thermal drier to restore the ambient ultrafine particle distribution, an air cooler, and a final size-selective outlet to eliminate particles > 200 nm. Subjects were exposed at rest in a specially designed 100-ft^3 chamber made of plexiglass and stainless steel, maintained at 12 cm H_2O relative to atmospheric pressure (to draw airflow through the concentrator). The exposures occurred between November 2006 and June 2008. Approximately 50–60 L/min of HUCAPS output air was pulled into the exposure chamber by way of a venturi-type face mask (Hudson RCI, Teleflex Medical, Research Triangle Park, NC) covering the nose and mouth. PNCs (measured using the condensation particle counters) and size distributions (measured using the Scanning Electrical Mobility Particle Sizer or a Fast Mobility Particle Sizer [model 3091, TSI]) were monitored during exposures both outdoors at the HUCAPS intake approximately 20 meters from the roadway and at the face mask. Final exposure concentrations varied based on the ambient particle concentrations.

ECG OUTCOME MEASUREMENTS

ECG parameters from the Augsburg study were already available in 5-minute and 1-hour segments. For our current study, we reanalyzed the ECG data from the REHAB study into 1-hour segments and the UPCON and UPDIABETES studies into 5-minute and a 1-hour segments. For the REHAB study, no 5-minute segments were analyzed, because of the expected noise in the exercise periods and the expected difficulty of estimating 5-minute values for HRV and repolarization parameters. The methods we used to reanalyze the REHAB, UPCON, and UPDIABETES data are described below.

In the Augsburg, UPCON, and UPDIABETES studies, the ECG recordings were made using 12-lead Holter recorders (Mortara Instruments) and analyzed using HScribe software (Mortara Instruments). In the REHAB study, the recordings were made using 3-lead (modified V2, V5, and AVF) Holter recorders (Burdick Altair-DISC, Cardiac Science, Bothell, WA) and analyzed using Burdick Vision Premier Holter System software (Cardiac Science, Bothell, WA). All study Holter monitor recordings were first annotated automatically and then annotated by a trained technician using standard procedures. RR intervals were exported to a custom-made program that produced a set of HRV measures as well as measures of other ECG parameters. Deceleration capacity was analyzed using programs adapted from the authors of the methods (Bauer et al. 2006; Schmidt et al. 1999).

For each 5-minute or 1-hour segment in a recording, we measured time-domain HRV parameters, including the mean NN interval time between successive NN beats, inverse of the NN interval time (i.e., heart rate), SDNN, RMSSD, and percentage of NN intervals longer than 50 msec (PNN50). Based in part on work by Bigger and colleagues (1992), filtering criteria eliminated two RR intervals after premature ventricular or atrial beats. We did not apply preprocessing filtering to eliminate extreme values. We examined 5-minute segments during the resting period to standardize conditions for all HRV and repolarization parameters, requiring at least 200 beats for HRV analyses. For post-processing, we evaluated outliers and determined whether the values were valid or not based on intra-lab ranges developed during an earlier study (Schneider et al. 2010a). We measured deceleration capacity, a measure of heart rate dynamics (reflecting variability in heart rate during periods when the heart is slowing down), to complement information in the other HRV parameters (Bauer et al. 2008). Repolarization duration was analyzed using the QT-interval duration, which was measured manually (i.e., a technician evaluated three consecutive beats within each prespecified 2-minute period from the beginning of the resting ECG in lead II, taking the average QT for each time point) and corrected for heart rate using Bazett's formula (QTc). We measured T-wave amplitude using the eight original ECG leads I, II, and V1–V6, taking the median values from these leads for each beat and averaging them over each hour. For the data from the Augsburg, UPCON, and UPDIABETES studies, T-wave complexity—describing the morphology of the T-wave—was measured in each beat using principal component analysis based on the eight original leads and averaged over each hour using SuperECG software (Mortara Instruments) (Priori et al. 1997). For the data from the REHAB study, where only

three leads were available, T-wave complexity was analyzed using a custom-made COMPAS software program (Couderc et al. 2008; Vaglio et al. 2008). Using a fast Fourier technique, frequency-domain HRV parameters were computed, including high frequency (HF) power (0.15–0.40 Hz), low frequency (LF) power (0.04–0.15 Hz), very low frequency power (0.0033–0.04 Hz), and total power (0–0.5 Hz) (Malik and Camm 1995).

We performed analyses of continuous RR (NN) interval series for the entire 1-hour period to take advantage of the larger number of beats in the analyzed tachogram. A longer analyzed period should result in more stable and more representative estimates of HRV parameters, especially if the underlying data were acquired in nonsupine and nonresting conditions. HRV values coming from a continuous 1-hour period should therefore be more accurate in reflecting autonomic tone than those averaged over 12 values from individual 5-minute periods within the same hour.

TOTAL ANTIOXIDANT CAPACITY MEASUREMENTS

Using the $N = 464$ blood samples from the Augsburg study and the $N = 657$ blood samples from the REHAB study described above, we measured total antioxidant capacity. Our laboratory analysis method is described in Appendix A (available on the HEI Web site).

STATISTICAL METHODS AND DATA ANALYSIS

FACTOR ANALYSIS

Factor analyses were done for the hourly ECG outcomes in order to explore relationships among the various ECG parameters, find subgroups of correlated outcomes, and reduce the number of outcomes examined in the statistical models described below. Factor analyses were done separately for each of the four studies. For the Augsburg study, factor analysis was also done separately for the group with diabetes or IGT and the group with a genetic susceptibility. Our methods and results are presented in Appendix B. Briefly, an orthogonal factor model was applied to the correlation matrix of outcomes. Principal component analysis was used to estimate the factor loadings. Factor rotation was estimated using the varimax criterion. The number of factors used in the various models was determined by the number that could be interpreted in ways that corresponded to biological phenomena, as judged by Dr. Zareba (one of the authors of the current study). A factor loading of 0.6 or higher was considered to indicate that a variable was represented by that factor (factor loadings represent the degree of correlation between variables and their corresponding factors).

Based on these factor analyses, we identified four factors (and outcomes representing them to be used in our statistical analysis) for both the Augsburg study (overall HRV [represented by SDNN], parasympathetic modulation [RMSSD], T-wave morphology [T-wave complexity], and baroreflex sensitivity [LF]) and the REHAB study (overall HRV [SDNN], parasympathetic modulation [RMSSD], T-wave morphology [T-wave complexity], and heart rate [NN]). We identified five factors for both the UPCON study (overall HRV [SDNN], parasympathetic modulation [RMSSD], T-wave morphology [T-wave complexity], heart rate [NN], and repolarization [QTc]) and the UPDIABETES study (overall HRV [SDNN], parasympathetic modulation [RMSSD], T-wave morphology [T-wave complexity], heart rate [NN], and repolarization [QTc]). We then restricted our statistical analyses to the factors and representative outcomes that were common to all four studies. These factors were overall HRV (SDNN), parasympathetic modulation (RMSSD), and T-wave morphology (T-wave complexity). The statistical methods used to estimate the changes in these outcomes associated with increased particulate air pollutant concentrations are described below.

Table 1 shows the factor loadings of the ECG parameters for each resulting factor. Table 2 shows the Pearson correlation coefficients between the ECG parameters used in the factor analysis.

MAIN HOURLY ANALYSIS

Augsburg Study The associations between hourly air pollution and ECG parameters were analyzed using additive mixed models with random participant effects to accommodate repeated measures and to account for unexplained heterogeneity in the data. An appropriate covariance structure (first-order autocorrelation) was chosen in order to account for dependencies between repeated measurements. The confounder selection was conducted for each ECG parameter separately. Potential confounders were long-term time trend, time of day (morning versus afternoon), day of the week, air temperature, relative humidity, and barometric pressure. Possible lags considered for the meteorological variables were the 1-hour averages concurrent with the 1-hour ECG recordings, 1-hour averages lagged from 1 to 12 hours, and 24-hour averages (0–23 hours and 24–47 hours) before each 1-hour ECG interval. The confounders were included linearly or smoothly as penalized splines (P-splines) to allow for

Table 1. Factors and Respective Loadings of the ECG Parameters Resulting from Factor Analyses[a]

	Overall HRV				Parasympathetic Modulation				QT Interval		
	Augsburg	REHAB	UPCON	UPDIABETES	Augsburg	REHAB	UPCON	UPDIABETES	Augsburg	UPCON	UPDIABETES
HR/NN	−0.367	0.073	0.093	0.228	−0.340	0.225	0.317	−0.054		0.222	0.245
SDNN	0.959	0.902	0.884	0.869	0.128	0.067	0.354	0.237		−0.005	0.012
RMSSD	0.226	0.206	0.284	0.211	0.918	0.918	0.858	0.363		0.078	0.015
PNN50	—	0.091	0.252	0.218	—	0.893	0.842	0.321		0.048	−0.051
HF	−0.107	0.224	0.288	0.171	0.899	0.816	0.891	0.897		0.045	0.009
LF	−0.247	0.284	0.502	0.386	0.307	0.561	0.63	0.823		−0.087	0.076
VLF	0.956	0.882	0.894	0.92	−0.007	0.31	0.302	0.143		0.005	0.019
TP	0.968	0.924	0.952	0.928	−0.024	0.243	0.233	0.246		0.035	0.003
QTc	−0.077	—	0.009	0.021	−0.017	—	0.057	0.059		0.979	0.925
QTp SD	—	—	−0.044	−0.046	—	—	−0.097	−0.085		−0.075	0.003
Tamp	0.183	—	0.237	0.333	−0.017	—	0.228	0.072		−0.058	−0.278
Tcomp	0.017	−0.076	0.021	−0.007	0.089	0.060	0.014	0.004		−0.039	0.064
DC	0.260	0.107	0.280	0.323	−0.381	−0.063	0.26	0.211		−0.115	0.186
	T-wave morphology				Heart Rate				Baroreflex sensitivity		
HR/NN	0.502	0.088	−0.072	0.078		0.776	0.815	0.794	−0.019		
SDNN	−0.115	−0.071	−0.064	−0.085		−0.042	0.177	0.324	−0.033		
RMSSD	0.036	0.095	−0.126	−0.065		0.079	0.286	0.82	−0.149		
PNN50	—	0.005	−0.125	−0.056		−0.100	0.306	0.822	—		
HF	−0.017	0.02	−0.048	−0.044		0.307	0.112	0.286	0.182		
LF	−0.066	−0.01	−0.112	−0.081		0.463	0.217	0.192	0.836		
VLF	−0.149	−0.017	−0.109	−0.091		0.239	0.22	0.271	0.059		
TP	−0.081	−0.026	−0.048	−0.074		0.185	0.059	0.185	−0.073		
QTc	0.715	—	−0.059	0.158		—	0.063	0.195	0.018		
QTp SD	—	—	0.87	0.898		—	0.125	0.083	—		
Tamp	−0.733	—	−0.816	−0.638		—	0.283	0.298	0.276		
Tcomp	0.743	0.985	0.879	0.881		−0.058	−0.107	−0.057	−0.076		
DC	−0.267	−0.164	−0.090	−0.205		0.858	0.745	0.566	0.748		

Abbreviations: VLF = very low frequency, TP = Total Power, QTp SD = standard deviation of QTpeak interval, Tamp = T-wave amplitude, Tcomp = T-wave complexity, and DC = deceleration capacity.

[a] Gray highlights indicate absolute factor loadings > 0.6

Table 2. Pearson Correlation Coefficients for ECG Parameters Used in the Factor Analyses

	SDNN	RMSSD	PNN50	HF	LF	VLF	TP	QTc	QTp SD	Tamp	Tcomp	DC
Augsburg Study												
HR	−0.39	−0.30	−0.28	−0.20	−0.03	−0.38	−0.30	0.37	0.05	−0.32	0.14	−0.20
SDNN		0.34	0.36	0.01	−0.21	0.91	0.94	−0.16	0.19	0.25	−0.10	0.20
RMSSD			0.75	0.72	0.11	0.20	0.20	0.01	0.15	−0.03	0.07	−0.40
PNN50				0.66	0.16	0.23	0.21	−0.06	0.03	0.13	−0.02	−0.11
HF					0.36	−0.11	−0.11	0.00	−0.03	0.04	0.02	−0.17
LF						−0.14	−0.28	−0.08	−0.17	0.19	−0.11	0.34
VLF							0.93	−0.20	0.18	0.30	−0.12	0.29
TP								−0.13	0.19	0.22	−0.10	0.20
QTc									0.12	−0.33	0.26	−0.24
QTp SD										−0.43	0.41	−0.10
Tamp											−0.52	0.37
Tcomp												−0.24
REHAB Study												
NN	0.05	0.32	0.15	0.37	0.29	0.32	0.27	—	—	—	−0.01	0.44
SDNN		0.26	0.20	0.22	0.23	0.71	0.76	—	—	—	−0.10	0.11
RMSSD			0.82	0.75	0.54	0.47	0.40	—	—	—	0.14	0.05
PNN50				0.58	0.39	0.30	0.26	—	—	—	0.10	0.00
HF					0.64	0.45	0.40	—	—	—	0.04	0.18
LF						0.52	0.44	—	—	—	0.01	0.37
VLF							0.97	—	—	—	−0.09	0.23
TP								—	—	—	−0.10	0.44
QTc									—	—	—	—
QTp SD										—	—	—
Tamp											—	—
Tcomp												−0.17
UPCON Study												
NN	0.34	0.55	0.55	0.33	0.30	0.19	0.20	0.23	0.01	0.38	−0.15	0.48
SDNN		0.65	0.60	0.59	0.67	0.92	0.93	0.02	−0.08	0.39	−0.06	0.47
RMSSD			0.93	0.63	0.50	0.24	0.36	0.13	−0.14	0.44	−0.13	0.52
PNN50				0.59	0.49	0.24	0.34	0.10	−0.13	0.45	−0.12	0.50
HF					0.76	0.37	0.50	0.08	−0.03	0.24	−0.05	0.31
LF						0.58	0.64	0.04	−0.10	0.29	−0.06	0.43
VLF							0.90	0.03	−0.09	0.16	−0.03	0.19
TP								0.04	−0.06	0.21	−0.02	0.25
QTc									−0.11	0.01	−0.08	0.03
QTp SD										−0.62	0.59	−0.07
Tamp											−0.67	0.34
Tcomp												−0.10
UPDIABETES Study												
NN	0.41	0.57	0.54	0.26	0.19	0.38	0.30	0.32	0.14	0.16	0.00	0.52
SDNN		0.56	0.53	0.47	0.57	0.89	0.92	0.09	−0.13	0.42	−0.11	0.50
RMSSD			0.87	0.59	0.43	0.49	0.47	0.24	−0.06	0.36	−0.10	0.50
PNN50				0.52	0.33	0.48	0.44	0.18	−0.07	0.33	−0.10	0.51
HF					0.72	0.39	0.47	0.11	−0.07	0.23	−0.07	0.37
LF						0.53	0.54	0.07	−0.06	0.20	−0.05	0.33
VLF							0.95	0.06	−0.10	0.40	−0.10	0.45
TP								0.05	−0.10	0.38	−0.09	0.40
QTc									0.18	−0.20	0.22	0.14
QTp SD										−0.49	0.66	−0.14
Tamp											−0.49	0.31
Tcomp												−0.18

Abbreviations: HR = heart rate; VLF = very low frequency; TP = Total Power, QTp SD = standard deviation of QTpeak interval, Tamp = T-wave amplitude, Tcomp = T-wave complexity, and DC = deceleration capacity.

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, time of day, and day of the week were only selected in cases of model-fit improvement. Confounder models for all of the ECG parameters included time of day but not day of the week. For consistency, we decided to use the same confounder model for all ECG parameters. Because 1-hour averages of air temperature and relative humidity with a lag of up to 5 hours were selected for almost all ECG parameters, we included the 6-hour averages of these meteorological variables linearly in the uniform confounder model. The uniform confounder model also included a long-term time trend modelled as a P-spline and time of day. In order to ensure normally distributed residuals, RMSSD and T-wave complexity, but not SDNN, were log-transformed.

After assessing the confounder model, 1-hour averages of UFPs, $PM_{2.5}$, AMP, and BC concurrent with the 1-hour averages for the ECG measurements and up to 6 hours before the recordings were separately added to the confounder model, and the effects were estimated linearly. We also checked whether associations between particulate air pollution and the ECG outcomes differed between participants in the group with diabetes or IGT and those in the group with a genetic susceptibility by conducting a stratified analysis.

Next, we ran several sensitivity analyses to evaluate whether our findings were dependent on our analysis approach. In order to check the robustness of the effects of particulate air pollution, we specified different values of smoothness for the nonlinear components, especially for the time trend; and we included air temperature and relative humidity with various lag hours.

Then, because autocorrelation could be an issue in analyses dealing with 5-minute and 1-hour ECG data (which are naturally correlated with each other), we tried to check the robustness of our estimated effects using several more sensitivity analyses. We first compared the derived estimates of our a priori chosen fixed confounder model to an AIC-selected model for each outcome parameter separately. Second, we used different degrees of freedom for the trend variable to check for seasonality effects that could have an influence on autocorrelation in the data. Third, we replaced the random subject effects with fixed subject effects. Fourth, we assessed models with various covariance structures (e.g., cs, ar[1], ar[2], ar[3], and spatial). Fifth, we included the lagged outcome variable ($t - 1$) in the model, which is considered to be the strictest approach for autocorrelation and might therefore result in an overadjustment, leading to estimates that are too conservative. Finally, we checked the autocorrelation function (ACF) plots for the autocorrelation structure in the data. We then compared the beta coefficients and 95% CIs from these models with our main analysis described above.

Rochester REHAB Study We estimated the change in SDNN, RMSSD, and T-wave complexity associated with increased UFP, AMP, $PM_{2.5}$, and BC concentrations during the concurrent hour and previous 6 hours using additive mixed models. In the model building process, we started with a base model including visit number, hour of day, day of the week, and month of the year for each outcome. We used a forward selection approach, first selecting correlation structure, then selecting the most important confounders, and finally including the pollutant of interest. A separate analysis was run for each pollutant lag hour (concurrent hour and lag hours 1 to 6). To preserve comparability, we used the same correlation structure and covariates for each outcome, basing our choice on the preponderance of evidence from the various analyses at each step as assessed using AIC. For most outcomes, the compound symmetry structure outperformed the others considered (autoregressive and spatial power) and was therefore used in all subsequent analyses. We included as covariates in the model the visit number for that subject, hour of day, month of the visit, day of the week (Monday, Friday, or Tuesday–Thursday), mean hourly temperature lagged 5 hours, mean hourly relative humidity lagged 6 hours, and mean hourly carbon monoxide concentration lagged 4 hours. We modeled the mean relative humidity lagged 5 hours using a P-spline with three degrees of freedom.

As in the Augsburg study, we ran several sensitivity analyses evaluating our analytic options. These included a model similar to the main analysis, but in addition to using the compound symmetry covariance structure there was an additional variance term (autoregressive [1]) that estimated the variance across multiple measures within the same subject visit (Model #1 in Table 1 in Appendix Q). We next ran the same model as in the main analysis but only used the first hour of the subject visit in the analysis (Model #2 in Table 1 in Appendix Q, available on the HEI Web site). In another approach, generalized estimating equations were used to estimate the parameters of the model (Model #3 in Table 1 in Appendix Q). Last, we re-ran the main analysis model described above but modeled relative humidity with a linear term (Model #4 in Table 1 in Appendix Q) rather than a P-spline. We then compared the beta coefficients and 95% CIs from these models with our main analysis described above.

Rochester UPCON Study The statistical model used to analyze data for the UPCON study was again an additive

mixed model with a compound symmetry covariance matrix for repeated hours within the same subject (i.e., the same basic analysis as in the REHAB study). Each model also included indicator variables for hour of day (7:00 A.M. through 6:00 P.M.) and visit number (1 or 2). Separate models were estimated for each possible combination of hour-specific UFP measurement during the exposure and hour-specific endpoint measurement (i.e., outcome and UFP concentration both measured in the first hour of exposure, outcome measured in the second hour of exposure and mean UFP concentration measured over 2 hours of exposure, outcome measured in the first hour after exposure and mean UFP concentration measured over 2 hours of exposure, outcome measured in the second hour after exposure and mean UFP concentration measured over 2 hours of exposure, etc., through outcome measured in the sixth hour [lagged 1 hour to 6 hours] after exposure and mean PM concentration measured over 2 hours of exposure). The models contained responses for both particle exposure days and clean air exposure days.

Rochester UPDIABETES Study Our approach to the analysis of the data for the UPDIABETES study was similar to that for the UPCON study. However, the total particle count concentrations on the clean air exposure days were so small that they were essentially zero. The number 5 was used to replace all of the zero particle counts for the clean air days. Again, separate models were estimated for each lag hour (hour 1 of exposure, hour 2 of exposure, and lag 1 hour to lag 6 hours after exposure).

EFFECT MODIFICATION

Age (< 60 vs. ≥ 60 years of age), obesity (BMI < 30 vs. ≥ 30 kg/m^2), smoking status (never vs. former or occasional smoker), hypertension (yes versus no), and total antioxidant capacity (above vs. below median level) were then assessed as potential effect modifiers by including their corresponding interaction terms. For the Augsburg study, we also investigated potential effect modification by medication intake (statins or beta blockers) in the same way. For the REHAB study, prior MI and self-perceived physical exertion (above versus below median level) were assessed as modifiers in the same manner.

MAIN 5-MINUTE ANALYSES

Associations between 5-minute UFP concentrations and ECG parameters were also analyzed using additive mixed models with random participant effects and a first-order autocorrelation structure. A common confounder model was built for all ECG parameters. For the Augsburg study, 1-hour averages of air temperature and relative humidity measured in the same hour as the respective 5-minute ECG intervals, time of day (morning vs. afternoon), and long-term time trend as a linear term were included in all analytic models. RMSSD and T-wave complexity were log-transformed to ensure normally distributed residuals. For the UPCON and UPDIABETES studies, only the 5-minute mean total particle count was included in the models, and we did not log-transform RMSSD.

After assessing the confounder model, 5-minute averages of UFP (i.e., particle number concentrations [PNC]) concurrent with the 5-minute averages of ECG measurements (i.e., the pollutant concentration and outcome were from the same 5-minute time interval) and up to 60 minutes before the ECG recordings were separately added to the confounder model, with effects estimated linearly. For the Augsburg study, we checked whether associations between the pollutant concentrations and ECG outcomes differed between the subjects in the group with diabetes or IGT and those in the group with a genetic susceptibility by conducting a stratified analysis.

AIR POLLUTION AND TOTAL ANTIOXIDANT CAPACITY

We examined the associations between 24-hour averages of air pollution and total antioxidant capacity in the Augsburg and REHAB studies only. The statistical methods used for this analysis are described in Appendix A.

DISCOVERY-AND-REPLICATION APPROACH

Next, we applied a "discovery and replication" approach to draw conclusions about each of our 19 research questions (see below), using the results from the statistical analyses of the four studies described above. In short, for each research hypothesis/question to be confirmed/replicated, we needed to obtain the same response (e.g., increased pollutant concentration associated with an adverse change in the ECG parameter) for the question from the data [1] from both panel studies (Augsburg and REHAB) and from at least one of the controlled-exposure studies (UPCON or UPDIABETES) for questions that could be addressed in all four studies; [2] from the Augsburg study (see Table 3) and at least one of the controlled-exposure studies for questions that could only be addressed in these three studies; or [3] from both panel studies for questions that could only be addressed in these two studies. Table 3 shows the decision tree we used to score the degree of agreement among the studies for 15 of the research questions. For questions scoring ++++ (strongly agree) or +++ (agree), we concluded that the hypothesis

Table 3. Decision Tree

Studies Used and Degree of Agreement	Score	Conclusion	Replicated?
Study group 1: Augsburg (panel), REHAB (panel), UPCON, and UPDIABETES (1-hr UFP)			
All four studies agree	++++	Strongly Agree	Yes
Panel studies and one of two controlled-exposure studies agree	+++	Agree	Yes
Panel studies agree, but controlled exposure studies do not	++	Suggestive only	No
Panel studies do not agree	+	No agreement or association	No
Studies are contradictory	–	Contradictory	No
Study group 2: Augsburg (panel), UPCON, and UPDIABETES (5-min UFP)			
All three studies agree	++++	Strongly Agree	Yes
Augsburg and one of two controlled-exposure studies agree	+++	Agree	Yes
Controlled-exposure studies agree but Augsburg does not	++	Suggestive only	No
Panel studies do not agree	+	No agreement or association	No
Studies are contradictory	–	Contradictory	No
Study group 3: Augsburg (panel), and REHAB (panel) (1-hr $PM_{2.5}$)			
Panel studies agree	+++	Agree	Yes
Panel studies do not agree	+	No agreement	No
Panel studies are contradictory	–	Contradictory	No

underlying the question had been replicated. Table 4 shows all 19 research questions, including the 15 scored using the decision tree and the four (indicated by –) that could only be addressed in one study and hence could not be scored for degree of agreement. The questions were derived from our specific aims.

TWO-POLLUTANT ANALYSES, EXPOSURE RESPONSE FUNCTIONS, AND META-ANALYSES

For each replicated research question, two-pollutant models were used to examine the independent effects of the pollutant measurements. To avoid problems with collinearity, these analyses were only conducted when the pollutants' intercorrelation was ≤ 0.6. Second, we checked the exposure–response functions for the air pollutants and ECG parameters for deviations from linearity. The exposure–response functions were assessed using P-splines and then visual inspection to assess whether the smoothed exposure–response curve resembled a straight line. Third, we estimated the changes in heart rate (in the Augsburg study) and NN interval (the inverse of the heart rate; in the REHAB, UPCON, and UPDIABETES studies) associated with increases in UFP, $PM_{2.5}$, AMP, and BC concentrations in the same manner as for the other three outcomes (i.e., questions 1–9). This was done to evaluate whether our observed changes in the HRV and T-wave morphology parameters were not driven solely by changes in heart rate. Last, we combined study-specific effect estimates using meta-analysis methodology (van Houwelingen et al. 2002) to provide a single estimate of the percent change in SDNN associated with a standard incremental increase in pollutant concentration (percent change associated with each 1000 particles/cm^3 increase in UFP or AMP and with each 10 μg/m^3 increase in $PM_{2.5}$ concentrations). These methods are outlined in Appendix C.

For all data management and data analyses, we used statistical analysis software (SAS, version 9.2, SAS Institute, Cary, NC; and R, version 2.6.1, R Foundation for Statistical Computing, Vienna, Austria). This study was approved by the Ethics Committee of Bavaria (Bayerische Landesärztekammer) and the Research Subjects Review Board at the University of Rochester Medical Center in Rochester, New York.

Table 4. Research Questions Arising from Our Specific Aims

Aim #	Study Group # from Table 1	Question #	Research Question
1.1	1	1	Are adverse changes in **total HRV** associated with increased UFP in the previous 60 minutes or few hours?
	1	2	Are adverse changes in **parasympathetic modulation** associated with increased UFP in the previous 60 minutes or few hours?
	1	3	Are adverse changes in **repolarization or T-wave morphology** associated with increased UFP in the previous 60 minutes or few hours?
	3	4	Are adverse changes in **total HRV** associated with increased concentrations of the other pollutants ($PM_{2.5}$, AMP, and BC) in the previous few hours?
	3	5	Are adverse changes in **parasympathetic modulation** associated with increased concentrations of the other pollutants ($PM_{2.5}$, AMP, and BC) in the previous few hours?
	3	6	Are adverse changes in **repolarization or T-wave morphology** associated with increased concentrations of the other pollutants ($PM_{2.5}$, AMP, and BC) in the previous few hours?
	2	7	Are adverse changes in **total HRV** associated with increased UFP in < 60 minutes?
	2	8	Are adverse changes in **parasympathetic modulation** associated with increased UFP in < 60 minutes?
	2	9	Are adverse changes in **repolarization or T-wave morphology** associated with increased UFP in < 60 minutes?
1.2	—	10	Do the adverse ECG effects differ between study subgroups (prediabetes, coronary syndrome, and healthy)?
2.1	3	11	Are decreases in **total anti-oxidant capacity** associated with increased concentrations of any pollutants (UFP, $PM_{2.5}$, AMP, BC)?
2.2	3	12	Does **total antioxidant capacity** modify the associations between any ECG outcome (total HRV, parasympathetic modulation, or repolarization and T-wave morphology) and any pollutant (UFP, $PM_{2.5}$, AMP, or BC)?
3.1	3	13	Does **age** modify the associations between any ECG outcome (total HRV, parasympathetic modulation, or repolarization and T-wave morphology) and any pollutant (UFP, $PM_{2.5}$, AMP, or BC)?
	3	14	Does **body mass index** modify the associations between any ECG outcome (total HRV, parasympathetic modulation, repolarization and T-wave morphology) and any pollutant (UFP, $PM_{2.5}$, AMP, BC)?
	3	15	Does **smoking status** modify the associations between any ECG outcome (total HRV, parasympathetic modulation, or repolarization and T-wave morphology) and any pollutant (UFP, $PM_{2.5}$, AMP, or BC)?
	3	16	Does a **previous diagnosis of hypertension** modify the associations between any ECG outcome (total HRV, parasympathetic modulation, or repolarization and T-wave morphology) and any pollutant (UFP, $PM_{2.5}$, AMP, or BC)?
	—	17	***Augsburg Panel only*** Does any medication intake modify any of the demonstrated adverse ECG effects?
	—	18	***Rochester REHAB only*** Does having a priori myocardial infarction modify any of the demonstrated adverse ECG effects?
3.2	—	19	***Rochester REHAB only*** Does self-perceived physical exertion modify any of the demonstrated adverse ECG effects?

RESULTS

STUDY POPULATIONS

Characteristics of the study populations of each of the four studies are shown in Table 5.

In the Augsburg study, subjects in the group with diabetes or IGT were generally older (78% > 60 years of age; mean age = 66.1) male (66%) nonsmokers (41% never; 58% ex-smoker), of whom many were classified as being obese (47%) or as having hypertension (64%), but not as having a prior MI (9%) or coronary heart disease (6%). Subjects in the group with a genetic susceptibility were generally younger (36% age > 60 years of age; mean age = 55.5) male (60%) nonsmokers (51% never; 40% ex-smoker), of whom fewer were classified as being obese (18%) or as having hypertension (42%), a prior MI (0%), or coronary heart disease (7%). Because of the small number of subjects with recorded physical activity, it was not possible to examine the potential effect-modifying effects of physical exertion in the Augsburg study.

In the REHAB study, the subjects were generally younger (mean age = 60.2) than those in the Augsburg group with diabetes or IGT and older than the Augsburg group with a genetic susceptibility. They were mostly male (67%) former smokers (53%), of whom a large proportion were classified as being obese (45%), and a moderate to large proportion were classified as having had a prior MI (58%), hypertension (59%), diabetes (23%), or percutaneous transluminal coronary angioplasty (85%). All were taking statins (100%). Most were taking beta blockers (90%), and many were taking angiotensin-converting enzyme inhibitors (68%). For each medication, subjects were either almost all taking or not taking the medication, resulting in too small a sample size in one of the groups to allow an examination of effect modifications of any pollutant–outcome associations by the medication.

In the UPCON study, the subjects were generally healthy middle-aged (mean age = 42.6 years; all < 60) adults who were about half male (53%) and only 37% obese. All were healthy never smokers with normal lung function, without evidence of cardiovascular disease or other organ dysfunction. Individuals with significant occupational pollutant exposures, those taking medications known to affect vascular function, or those with screening blood pressure greater than 140/90 mmHg were excluded from the study.

The UPDIABETES study subjects have been described previously (Stewart et al. 2010). Briefly, all had been diagnosed with type 2 diabetes and were without clinical cardiovascular disease, major organ dysfunction, uncontrolled hypertension, frequent hypoglycemia, statin-type lipid-lowering medications, platelet-active drugs including aspirin, and occupational exposure to particles. The subjects were of an age similar to that of the UPCON subjects (mean age = 45.7 years; all < 60) and were 50% male but had a much larger proportion classified as being obese (67%) (Table 5).

In summary, our analysis took advantage of the existing data from these four previously completed studies to evaluate associations between increases in various air pollutant concentrations and ECG outcomes (knowing full well that the study populations were different). Specifically, the Augsburg study included subjects with diabetes, IGT, or a genetic susceptibility to detoxifying and inflammatory pathways. The REHAB study included early postinfarction patients participating in a cardiac rehabilitation program. The UPDIABETES study included diabetic patients, and the UPCON study included young, healthy individuals without any of the conditions of the other study populations. Additional differences among the studies included the protocols described earlier and the air pollution mixtures and UFPs assessed in each study. Any inconsistencies or divergences in study findings across the studies for a research hypothesis could therefore easily be explained by one of these factors. At the same time, we embedded into our analysis plan a strategy to replicate and systematically assess the findings from the studies such that, if consistent results were found in the various studies, it would suggest that the results are generalizable to substantially larger portions of the population or to a given particle metric.

AMBIENT AIR POLLUTION AND CONTROLLED UFP EXPOSURES

The distribution and correlations of ambient and personal air pollutant concentrations are shown in Table 6 for the Augsburg study and in Table 7 for the REHAB study. The 1-hour and 24-hour concentrations of $PM_{2.5}$, UFPs, AMP, and BC were substantially higher in the Augsburg study than in Rochester during the REHAB Study.

Also shown in Table 7 are the distributions of total particle counts (as a surrogate for UFPs) during each exposure in the UPCON and UPDIABETES study used in our analysis. The total particle counts for these two controlled-exposure studies were an order of magnitude higher than the ambient UFP concentrations measured in the Augsburg and REHAB studies, although the duration of controlled exposure was only 2 hours. Further, the IQR values used to scale the changes in ECG outcomes in the UPCON and UPDIABETES studies were substantially

Table 5. Characteristics of Study Populations by Study

		AUGSBURG PANEL				ROCHESTER REHAB[a]		ROCHESTER UPCON		ROCHESTER UPDIABETES[b]	
		Diabetes + IGT $N = 64$		Gen.Susc. $N = 45$		$N = 73$		$N = 19$		$N = 18$	
		n	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)
Gender	Male	42	(66)	27	(60)	49	(67)	10	(53)	9	(50)
Age	< 60 years	13	(20)	28	(62)	33	(45)	19	(100)	18	(100)
	≥ 60 years	51	(80)	17	(38)	40	(55)	0	(0)	0	(0)
	Mean ± SD	66.1 ± 8.1		55.5 ± 13.2		60.2 ± 10.6		42.6 ± 10.7		45.7 ± 9.7	
Body mass index	< 30 kg/m²	34	(53)	37	(82)	40	(55)	12	(63)	6	(33)
	≥ 30 kg/m²	30	(47)	8	(18)	33	(45)	7	(37)	12	(67)
Employed	Yes	14	(22)	26	(58)	N/A		15	(79)	16	(89)
Smoking	Never	26	(41)	23	(51)	34	(47)	19	(100)	0	(0)
	Former	37	(58)	18	(40)	39	(53)	0	(0)	0	(0)
	Occasional	1	(2)	4	(9)	0	(0)	0	(0)	0	(0)
HbA1c	<6.5%	49	(77)	45	(100)	N/A		18	(100)[c]	4	(22)
	≥ 6.5%	15	(23)	0	(0)	N/A		0	(0)	14	(78)
Prior MI	Yes	6	(9)	0	(0)	42	(58)	0	(0)	0	(0)
Coronary heart disease	Yes	4	(6)	3	(7)	73	(100)	0	(0)	0	(0)
Angina pectoris	Yes	5	(8)	1	(2)	N/A	N/A	0	(0)	0	(0)
Stable angina						1	(1)	0	(0)	0	(0)
Hypertension	Yes	41	(64)	19	(42)	43	(59)	0	(0)	2	(11)
Diabetes	Yes	32	(50)	0	(0)	17	(23)	0	(0)	18	(100)
Coronary bypass surgery	Yes					3	(4)	0	(0)	0	(0)
PCTA	Yes					62	(85)	0	(0)	0	(0)
Anti-inflammatory medication	Yes	14	(22)	10	(22)	N/A		0	(0)	1	(6)
Corticosteroids	Yes	4	(6)	1	(2)	N/A		0	(0)	0	(0)
Statins	Yes	13	(20)	6	(13)	73	(100)	0	(0)	0	(0)
Beta blockers	Yes	19	(30)	9	(20)	66	(90)	0	(0)	3	(17)
Calcium channel blockers	Yes	8	(13)	3	(7)	7	(10)	0	(0)	1	(6)
Diuretics	Yes	25	(39)	11	(24)	20	(27)	0	(0)	1	(6)
Antithrombotic agents	Yes	14	(22)	6	(13)	N/A		0	(0)	1	(6)
Angiotensin receptor blockers	Yes	N/A		N/A		10	(14)	0	(0)	1	(6)
Angiotension-converting-enzyme inhibitor	Yes	N/A		N/A		50	(68)	0	(0)	4	(22)
Digitalis	Yes	N/A		N/A		1	(1)	0	(0)	0	(0)

Abbreviations: Gen. Susc. = participants with a genetic susceptibility, N/A = not available, HbA1c = glycated hemoglobin, and PCTA = percutaneous transluminal coronary angioplasty.

[a] Three subjects in the REHAB study were excluded because of inadequate Holter monitor data for hourly averages.

[b] One subject from UPDIABETES study was excluded because he or she lacked adequate Holter monitor data for hourly averages.

[c] Because HbA1c value was missing for one subject in the UPCON study, percentage with HbA1c < 6.5% is calculated based on 18 subjects with non-missing values.

Table 6. Distribution and Correlation of Augsburg Study Air Pollution Concentrations (March 19, 2007, to December 17, 2008)

Personal measurements of 5-min averages of particle number concentrations (particles/cm^3)

									Spearman correlation coefficients[a]					
	N	Mean	SD	Min	Q1	Median	Q3	Max	$PM_{2.5}$	UFP	AMP	BC	Temp	RH	BP
All	20,317	21,649	38,826	521	6,129	10,865	22,177	698,255	0.18	0.24	0.26	0.16	0.04	−0.06	0.08
Diabetes + IGT	11,815	20,836	39,304	521	6,331	11,124	22,017	698,255	0.11	0.25	0.24	0.08	0.08	−0.07	0.04
Gen. Susc.	8,502	22,778	38,125	657	5,873	10,487	22,452	462,591	0.26	0.24	0.29	0.25	−0.01	−0.03	0.13

Ambient air pollutants and meteorological parameters

									Spearman correlation coefficients					
	N	Mean	SD	Min	Q1	Median	Q3	Max	UFP	AMP	BC	Temp	RH	BP
1-hour averages														
$PM_{2.5}$ (μg/m^3)	15,461	13.7	11.2	0.0	5.8	10.9	18.1	106.5	0.42	0.75	0.73	−0.29	0.16	0.28
UFP (n/cm^3)	14,699	9,518	6,902	937	4,892	7,629	12,049	80,858		0.70	0.58	−0.14	0.00	0.23
AMP (n/cm^3)	14,699	2,060	1,535	88	1,020	1,657	2,615	17,377			0.76	−0.12	0.06	0.30
BC (μg/m^3)	13,359	1.8	1.5	0.3	0.9	1.3	2.1	21.4				−0.16	0.32	0.26
Air temperature (°C)	15,398	10.8	7.9	−8.4	4.7	10.8	16.5	33.8					−0.56	−0.12
Relative humidity (%)	15,398	76.9	18.3	21.0	63.3	81.3	92.8	100.0						0.02
Barometric pressure (hPa)	15,398	961	8	928	957	961	966	986						
24-hour averages														
$PM_{2.5}$ (μg/m^3)	644	13.7	10.0	1.6	6.7	11.3	17.8	65.8	0.50	0.76	0.73	−0.30	0.11	0.31
UFP (n/cm^3)	611	9,537	4,417	1,897	6,305	8,890	12,027	26,503		0.77	0.58	−0.09	−0.23	0.32
AMP (n/cm^3)	611	2,068	1,213	291	1,179	1,855	2,712	8,120			0.73	−0.06	−0.16	0.36
BC (μg/m^3)	550	1.8	1.1	0.4	1.1	1.5	2.2	7.3				−0.10	0.22	0.33
Air temperature (°C)	636	10.9	7.3	−5.8	5.0	11.2	17.0	27.0					−0.54	−0.11
Relative humidity (%)	636	77.0	12.6	32.4	68.1	77.6	86.9	100.0						−0.01
Barometric pressure (hPa)	636	961	8	934	957	961	966	984						

Abbreviations: BP = barometric pressure; Gen. Susc. = participants with a genetic susceptibility; IGT = impaired glucose tolerance; IQR = interquartile range; Q1 = 1st quartile; Q3 = 3rd quartile; RH = relative humidity; SD = standard deviation; Temp = temperature

[a] Between personal 5-min measurements of PNCs and ambient 1-hour air pollutants.

higher than those used in the Augsburg and REHAB studies (Table 7).

Table 8 shows the distribution of inspired-particle counts, masses, and sizes for the UFP exposures of the UPDIABETES and UPCON controlled-exposure studies. The median particle count concentration in the UPDIABETES study was approximately 50 times that of the UPCON study (which was approximately 65 times, in turn, that of the median UFP concentration in the REHAB study). However, the UPCON median particle mass concentration was approximately three times that of the UPDIABETES study, which was approximately seven times, in turn, that of the REHAB study.

ECG PARAMETERS

Distributions of 5-minute and 1-hour ECG parameters are shown for the Augsburg study in Appendix D (available on the HEI Web site), for the REHAB study in Appendix E, for the UPDIABETES study in Appendix F, and for the UPCON study in Appendix G. It should be added that for the Augsburg study it was not possible to analyze PNN50 in association with air pollution, because PNN50 = 0 for 62% of all included 1-hour intervals in the group with diabetes or IGT and for 48% of all included 1-hour intervals in the group with a genetic susceptibility.

The numerical values of the 5-minute and 1-hour ECG outcome variables that were based on mean values (e.g., heart rate and QTc) were similar. In cases where the ECG outcome variables represented the variance of cardiac rhythm (e.g., SDNN), the 5-minute values were generally lower than the 1-hour values (because longer observation times add variability and hence contribute to higher values for these measures).

When comparing 1-hour SDNN and RMSSD median values, the REHAB study postinfarction patients showed substantially higher values than those found in the other three studies. Repeated periods of exercise during the rehabilitation sessions in these patients likely contributed to the greater variation in their heart rate compared with values from periods of sedentary activity or mild exercise. The median SDNN in the REHAB study, for example, was 99 msec compared with 72–80 msec in the Augsburg, UPDIABETES, and UPCON studies. Similarly, the median RMSSD in the REHAB study was 56 msec compared with 23–29 msec in the other three studies. These observations further stress not only the diversity of our study populations but also the diversity of the studies' recording conditions.

Factor analysis allowed us to reduce the number of outcomes to be considered in our primary analysis from 11 to 4 in the Augsburg study, 10 to 4 in the REHAB study, and 13 to 5 in the UPCON and UPDIABETES studies.

- In the Augsburg study, the variables chosen to represent the four factors of parasympathetic modulation of the heart, overall HRV, T-wave morphology, and baroreflex sensitivity were RMSSD, SDNN, T-wave complexity, and LF, respectively.
- In the REHAB study, the variables chosen to represent the four factors of parasympathetic modulation of the heart, overall HRV, T-wave morphology, and heart rate were RMSSD, SDNN, T-wave complexity, and NN, respectively.
- In the UPCON and UPDIABETES studies, the variables chosen to represent the five factors of parasympathetic modulation of the heart, overall HRV, T-wave morphology, heart rate, and QTc were RMSSD, SDNN, T-wave complexity, NN, and QTc, respectively.

Again, Table 1 shows the factor loadings of the ECG parameters for each resulting factor, and Table 2 shows the Pearson correlation coefficients between the ECG parameters used in the factor analysis. Because only selected ECG parameters were used for further analyses, the correlation coefficients provide some basis for understanding how the results could be extrapolated to other variables. A more detailed description of these results can be found in Appendix B.

MAIN ANALYSIS: RESEARCH QUESTIONS

Questions 1–3: Are adverse changes in overall HRV, parasympathetic modulation, or T-wave morphology associated with increased UFP in the previous 60 minutes or few hours? We hypothesized that increased UFP concentrations in the same and previous 6 hours would be associated with decreased SDNN (Question 1 in Table 4). In the Augsburg study, IQR increases in UFP concentrations lagged 2 to 5 hours were each associated with 1–3% decreases in SDNN; the largest reduction, at lag 3 hours, was in the group with a genetic susceptibility (–2.26%; 95% CI, –3.98% to –0.53%) (Figure 1A and Appendix H [available on the HEI Web site]). Similarly, in the REHAB study, IQR increases in UFP concentrations in the concurrent and previous 5 hours were each associated with < 2% decreases in SDNN. The largest, at lag 1 hour, was 1.15% (95% CI, –2.19% to –0.11%) (Figure 1B and Appendix I). For comparison, results from the REHAB study scaled to the same IQR increases as those of the Augsburg study are also shown (Appendix J).

Table 7. Distribution and Correlation of Ambient Air Pollutant Concentrations in the REHAB Study and Controlled Particle Exposures in the UPCON[a] and UPDIABETES[a] Studies

REHAB—Ambient air pollutants and meteorological parameters (June 26, 2006, to November 25, 2009)									**Spearman Correlation Coefficient**				
	N	Mean	SD	Min	Q1	Median	Q3	Max	UFP	AMP	BC	Air Temp	Relative Humidity
Ambient 1-hour averages													
$PM_{2.5}$ (µg/m³)	26,618	8.7	7.3	0.0	3.7	7.0	11.5	64.0	0.21	0.65	0.62	0.01	0.09
UFP (particles/cm³)	29,671	4,050	3,704	12	1,931	3,183	5,136	154,980		0.56	0.38	0.00	−0.15
AMP (particles/cm³)	29,671	1,041	918	0	419	790	1,374	18,838			0.65	0.18	0.01
BC (µg/m³)	26,929	0.7	0.6	−0.2	0.3	0.5	0.9	11.7				0.18	0.21
Air temperature (°C)	29,957	11.3	10.9	−17.1	2.6	12.0	19.8	37.8					−0.31
Relative humidity (%)	29,941	64.8	19.9	0.0	50.9	67.7	81.6	99.2					
Ambient 24-hour averages													
$PM_{2.5}$ (µg/m³)	1,136	8.7	6.1	0.0	4.3	7.3	11.1	42.9	0.16	0.67	0.62	0.09	0.05
UFP (particles/cm³)	1,237	4,049	2,168	328	2,518	3,623	5,166	16,767		0.59	0.31	0.04	−0.33
ACP (particles/cm³)	1,237	1,041	782	20	504	858	1,371	6,314			0.67	0.26	−0.12
BC (µg/m³)	1,151	0.7	0.4	0.0	0.4	0.6	0.9	2.4				0.42	0.01
Air temperature (°C)	1,249	11.3	10.2	−13.2	3.1	12.5	20.2	31.1					−0.19
Relative humidity (%)	1,248	64.8	13.4	10.2	56.8	65.7	73.7	95.3					
UPDIABETES—1st hour of exposure: mean total particle count[b]													
UFP (EC)	17	9.8×10^6	0.64×10^6	9.13×10^6	9.43×10^6	9.71×10^6	10.15×10^6	11.57×10^6					
Clean Air Exposure	17	< 5	< 5	< 5	< 5	< 5	< 5	< 5					
UPCON—1st hour of exposure: mean total particle count[b]													
UFP (CAPS)	16	2.85×10^5	1.80×10^5	0.27×10^5	1.45×10^5	2.71×10^5	4.12×10^5	6.33×10^5					
Clean Air Exposure	14	346.4	768.9	35.3	57.7	116.2	254.4	2,996					

Abbreviations: SD = standard deviation, Q1: 1st quartile, Q3: 3rd quartile, EC = elemental carbon, CAPS = concentrated ambient particles.

[a] These subjects had particle count measurements for the first hour of the exposure.

[b] Mean total particle counts in the 1st hour of exposure for the sample used to examine ECG outcome changes associated with the 1st hour of total particle count.

Table 8. Distribution of Average Inspired-Particle Counts, Mass, and Size for the UPDIABETES and UPCON Controlled-Exposures Studies

	N	Mean	SD	Min	Q1	Median	Q3	Max
UPDIABETES[a]								
Count (particles/cm^3)	17	9,969,642	728,994	9,155,372	9,414,259	9,849,666	10,201,958	12,060,043
Mass (μg/m^3)	17	51	3	45	49	51	52	57
Size (nm)	17	32	2	30	31	31	32	36
UPCON[b]								
Count (particles/cm^3)	19	245,804	135,589	34,188	140,320	206,883	360,249	523,679
Mass (μg/m^3)	19	158	85	19	101	149	200	321
Size (nm)	19	94	8	76	87	95	99	109

Abbreviations: SD = standard deviation, Q1 = 1st quartile, and Q3 = 3rd quartile

[a] *N* = 17 subjects from the UPDIABETES study were used in one or more health analyses (i.e., had both particle measurements and ECG recording). *N* = 2 subjects did not have particle measurements during an exposure.

[b] *N* = 19 subjects from the UPCON Study were used in one or more health analyses (i.e., had both particle measurements and ECG recording).

We also found a significant reduction in SDNN associated with IQR increases in total PNCs in the UPDIABETES study (at lag 1 hour: –13.22%; 95% CI, –24.11% to –2.33%) (Figure 1C and Appendix K) but not in the UPCON study, where IQR increases in total particle count were generally associated with nonsignificant increases in SDNN (Figure 1C and Appendix L). Therefore, because increased UFP concentrations were associated with decreased SDNN in both of the panel studies and one of the two controlled-exposure studies, we scored the results as "Agree" and concluded that the hypothesis was replicated (Table 9) across the study populations.

We hypothesized, in addition, that increased UFP concentrations in the same and previous 6 hours would be associated with decreased RMSSD (Question 2 in Table 4). In the Augsburg study, IQR increases in UFP concentrations were not associated with decreased RMSSD, although we found nonsignificant decreases in RMSSD associated with increased UFP concentrations at most lag hours (Figure 2A and Appendix H). However, in the REHAB study, we did find decreases in RMSSD associated with IQR increases in UFP concentrations. The largest decrease was at lag 4 hours (–2.51%; 95% CI –4.04% to –0.98%) (Figure 2B and Appendix I). We did not find decreased RMSSD associated with IQR increases in total PNCs in either the UPDIABETES study (Figure 2C and Appendix K) or the UPCON study (Figure 2C and Appendix L). Therefore, because we did not find consistent effects across the studies, we scored the results as "No agreement" and concluded that the hypothesis was not replicated (Table 9).

Last, we hypothesized that increased UFP concentrations in the same and previous 6 hours would be associated with increased T-wave complexity (Question 3 in Table 4). In the Augsburg study, each IQR increase in UFP concentration lagged 6 hours was associated with a 2.03% increase in T-wave complexity (95% CI, 0.52% to 3.57%) in the group with diabetes or IGT (Figure 3A and Appendix H). In the REHAB study, however, we found a significant decrease in T-wave complexity associated with each IQR increase in UFP concentrations lagged 6 hours (–2.02%; 95% CI, –3.88% to –0.16%) (Figure 3B and Appendix I). In the UPCON study, each IQR increase in total PNC (i.e., UFP) was associated with decreases in T-wave complexity at all lags. The largest were at lag 3 hours (–23.14%; 95% CI, –60.41% to 14.12%) and at lag 4 hours (–22.99%; 95% CI, –44.07% to –1.91%) (Figure 3C and Appendix L). In the UPDIABETES study, non-significant 0.44% to 21.06% decreases in T-wave complexity were associated with IQR increases in total PNC (i.e., UFP) lagged 2 to 6 hours (Figure 3C and Appendix K). Therefore, because we found both increased and decreased T-wave complexity associated with increased UFP concentrations, we scored these results as "Contradictory" and concluded that the hypothesis was not replicated (Table 9). However, it is worth stressing that T-wave complexity is an ECG marker that is sensitive to changes in activity and body position and that a lack of agreement might therefore be related to the differences in conditions during the ECG recordings in the four studies.

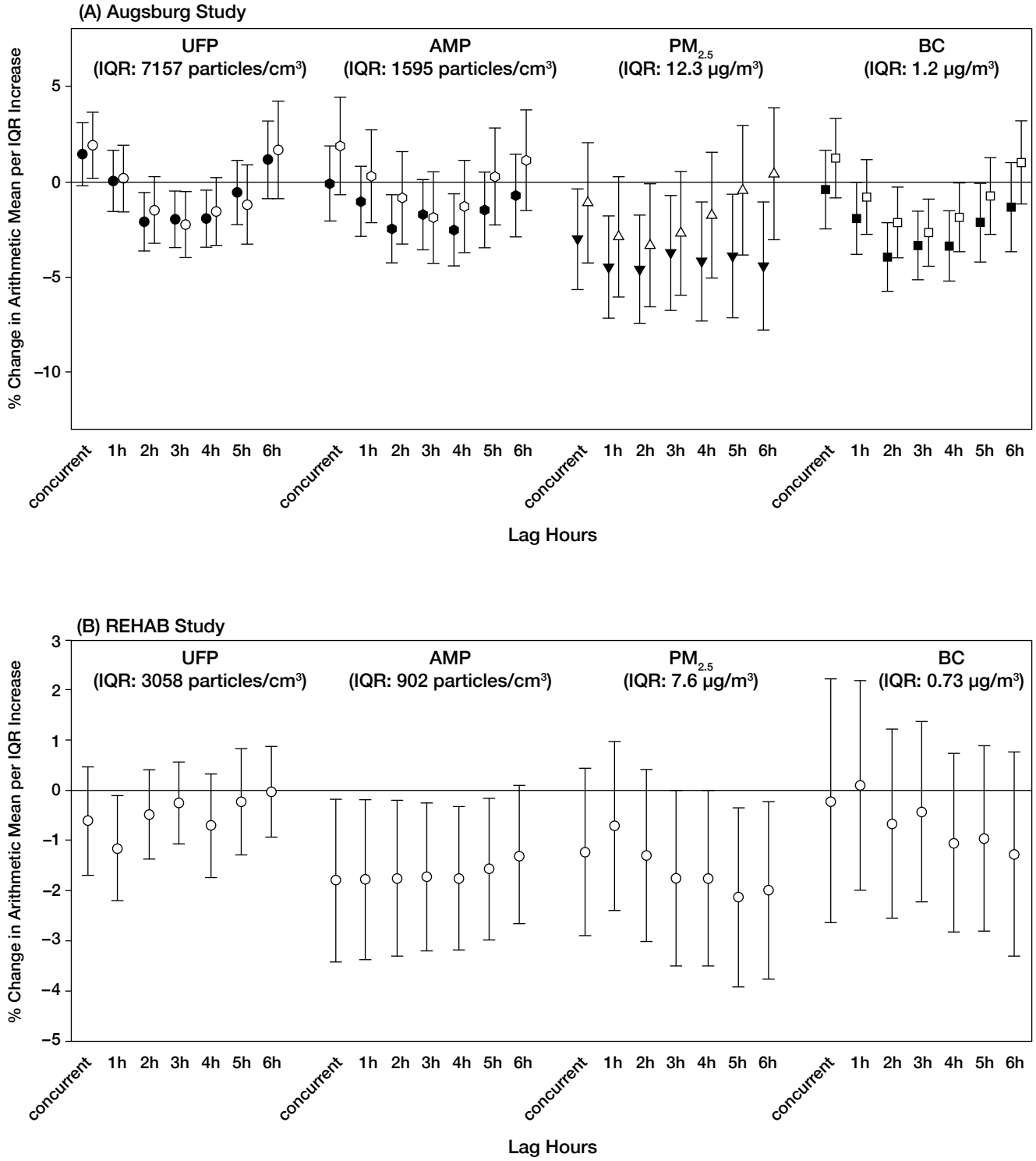

Figure 1. Percent change in SDNN (1-hour average) associated with each IQR increase in pollutant concentration in the concurrent hour and at lags 1 to 6 hours. (**A**) In the Augsburg study. Black symbols represent subjects in the group with diabetes or IGT. White symbols represent healthy subjects in the group with a genetic susceptibility; (**B**) In the REHAB study. (*Figure continues next page.*)

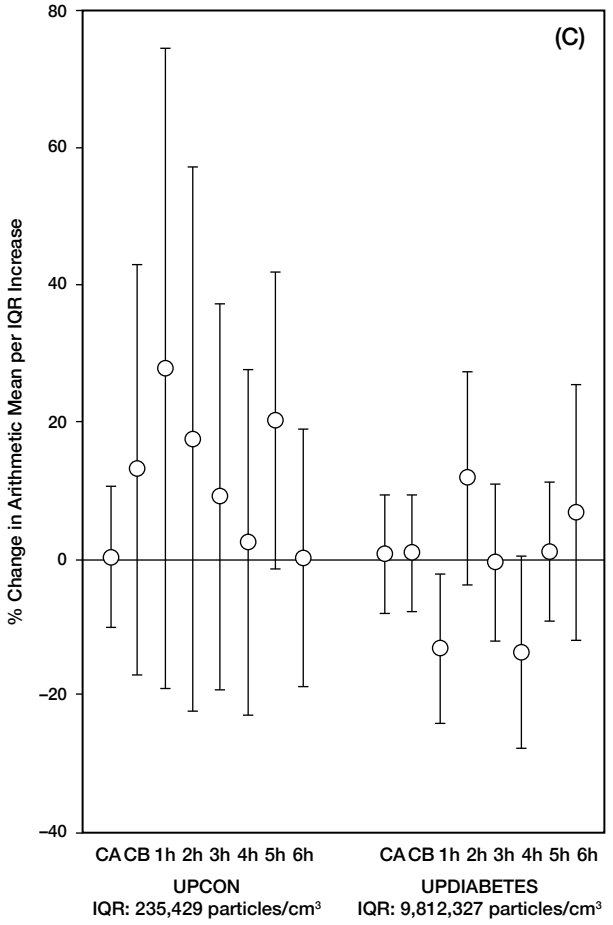

Figure 1 (*Continued*). Percent change in SDNN (1-hour average) associated with each IQR increase in pollutant concentration in the concurrent hour and at lags 1 to 6 hours. (**C**) In the UPCON and UPDIABETES studies. CA = first hour of exposure, CB = mean of 2-hour exposure, 1h = first hour after exposure, 2h = second hour after exposure, etc.

Questions 4–6: Are adverse changes in total HRV, parasympathetic modulation, or T-wave morphology associated with increased $PM_{2.5}$, AMP, or BC in the previous 60 minutes or few hours? We hypothesized that increased $PM_{2.5}$, AMP, or BC concentrations in the same and previous 6 hours would be associated with decreased SDNN (Question 4 in Table 4). In the Augsburg study, IQR increases in $PM_{2.5}$ concentrations for the concurrent hour and lagged 1 hour to 5 hours, AMP concentrations lagged 2 and 4 hours, and BC concentrations lagged 2 to 4 hours were each associated with significant 1%–5% decreases in SDNN. The largest reduction, associated with increased $PM_{2.5}$ concentrations lagged 2 hours, was in the group with diabetes or IGT (–4.59%; 95% CI, –7.44% to –1.75%) (Figure 1A and Appendix H). In the REHAB study, similarly, IQR increases in $PM_{2.5}$ concentrations lagged 5 and 6 hours and AMP concentrations in the concurrent hour and lagged 1 hour to 5 hours, but not BC concentrations at any lag hour, were associated with 1%–2% decreases in SDNN. The largest SDNN decrease was associated with each IQR increase in $PM_{2.5}$ concentrations lagged 5 hours (–2.13%; 95% CI, –3.91% to –0.35%) (Figure 1B and Appendix I). Therefore, because we found decreased SDNN associated with both increased $PM_{2.5}$ and AMP concentrations in the previous 6 hours in both studies, we scored the results as "Agree" and concluded that the hypothesis was replicated (Table 9).

Similarly, we hypothesized that increased $PM_{2.5}$, AMP, or BC concentrations in the same and previous 6 hours would be associated with decreased RMSSD (Question 5 in Table 4). In the Augsburg study, IQR increases in $PM_{2.5}$ concentrations in the concurrent hour and BC lagged 1 hour and 6 hours were each associated with significant 3%–7% decreases in RMSSD. The largest reduction, associated with increased $PM_{2.5}$ concentrations in the concurrent hour, was in the group with diabetes or IGT (–7.20%; 95% CI, –12.11% to –2.02%) (Figure 2A and Appendix H). In the REHAB study, similarly, IQR increases in $PM_{2.5}$ concentrations lagged 4 to 6 hours were associated with ~2.5% to ~3.5% decreases in RMSSD. The largest RMSSD reduction was associated with each IQR increase in $PM_{2.5}$ concentrations lagged 5 hours (–3.49%; 95% CI, –6.13% to –0.84%) (Figure 2B and Appendix I). Therefore, because we found decreased RMSSD associated with increased $PM_{2.5}$ concentrations in the previous 6 hours in both studies, we scored the results as "Agree" and concluded that the hypothesis was replicated (Table 9).

Last, we hypothesized that increased $PM_{2.5}$, AMP, or BC concentrations in the same and previous 6 hours would be associated with increased T-wave complexity (Question 6 in Table 4). In the Augsburg study, increased T-wave complexity was associated with increases in pollutant concentrations at lag 6 hours. The strongest effect, in BC, was in the group with diabetes or IGT (3.46%; 95% CI, 1.29% to 5.69%) (Figure 3A and Appendix H). In the REHAB study, however, we found no consistent pattern associated with any pollutant in the previous 6 hours (Figure 3B and Appendix I). We therefore scored the results as "No agreement" and concluded that the hypothesis was not replicated (Table 9).

Questions 7–9: Are adverse changes in total HRV, parasympathetic modulation, or repolarization and T-wave morphology associated with increased UFP in less than 60 minutes? We hypothesized that increased 5-minute averages of personally measured UFP concentrations (in the Augsburg study) or total PNCs in controlled exposures (as a proxy for UFP, in the UPCON and UPDIABETES studies) in the previous 30 and 60 minutes

Table 9. Research Questions, Scores, Conclusions, and Replication Judgements

Hypothesis #	Question #	Question	Score	Conclusion	Replicated?
1	1	Are adverse changes in **total HRV** associated with increased UFP in the previous 60 minutes or few hours?	+++	Agree	Yes
	2	Are adverse changes in **parasympathetic modulation** associated with increased UFP in the previous 60 minutes or few hours?	+	No association or agreement	No
	3	Are adverse changes in **repolarization/T-wave morphology** associated with increased UFP in the previous 60 minutes or few hours?	–	Contradictory	No
	4	Are adverse changes in **total HRV** associated with increased concentrations of the other pollutants ($PM_{2.5}$, AMP, or BC) in the previous few hours?	+++	Agree	Yes
	5	Are adverse changes in **parasympathetic modulation** associated with increased concentrations of the other pollutants ($PM_{2.5}$, AMP, or BC) in the previous few hours?	+++	Agree	Yes
	6	Are adverse changes in **repolarization or T-wave morphology** associated with increased concentrations of the other pollutants ($PM_{2.5}$, AMP, or BC) in the previous few hours?	+	No association or agreement	No
	7	Are adverse changes in **total HRV** associated with increased UFP in < 60 minutes?	–	Contradictory	No
	8	Are adverse changes in **parasympathetic modulation** associated with increased UFP in < 60 minutes?	–	Contradictory	No
	9	Are adverse changes in **repolarization or T-wave morphology** associated with increased UFP in < 60 minutes?	–	Contradictory	No
2	11	Are decreases in t**otal antioxidant capacity** associated with increased concentrations of any pollutants (UFP, $PM_{2.5}$, AMP, or BC)?	+	No association or agreement	No
	12	Does **total antioxidant capacity** level modify the associations between any ECG outcome (total HRV, parasympathetic modulation, or repolarization and T-wave morphology) and any pollutant (UFP, $PM_{2.5}$, AMP, or BC)?	+	No association or agreement	No

Table continues next page

would be associated with decreased 5-minute average SDNN (Question 7 in Table 4). In the Augsburg study, as hypothesized, each IQR increase in personal UFP concentrations in the concurrent 5 minutes was associated with significant decreases in the 5-minute mean SDNN both in the group with diabetes or IGT (–0.62%; 95% CI, –1.09% to –0.16%) and in the group with a genetic susceptibility (–0.93%; 95% CI, –1.40% to –0.46%). Increases in 5-minute mean UFP concentrations were also associated with smaller, statistically non-significant decreases in 5-minute average SDNN over the next 30 minutes (Figure 4A and Appendix M). In the UPDIABETES study, we found a significant decrease in 5-minute average SDNN associated with each IQR increase in total PNCs lagged 35–39 minutes (–10.00%; 95% CI, –17.20% to –2.81%). However, in the UPCON study, we found significant increases in 5-minute average SDNN associated with each IQR increase in total PNCs for almost all 5-minute averages up to a 60-minute lag. The largest increase in SDNN was associated with increased total PNCs lagged 45–49 minutes (4.62%; 95%

Table 9 (*Continued*). Research Questions, Scores, Conclusions, and Replication Judgements

Hypothesis #	Question #	Question	Score	Conclusion	Replicated?
3	13	Does **age** modify the association between any ECG outcome (total HRV, parasympathetic modulation, or repolarization and T-wave morphology) and any pollutant (UFP, $PM_{2.5}$, AMP, or BC)?	+	No association or agreement	No
	14	Does **body mass index** modify the associations between any ECG outcome (total HRV, parasympathetic modulation, repolarization and T-wave morphology) and any pollutant (UFP, $PM_{2.5}$, AMP, or BC)?	+	No association or agreement	No
	15	Does **smoking status** modify the associations between any ECG outcome (total HRV, parasympathetic modulation, or repolarization and T-wave morphology) and any pollutant (UFP, $PM_{2.5}$, AMP, or BC)?	+	No association or agreement	No
	16	Does **a previous diagnosis of hypertension** modify the associations between any ECG outcome (total HRV, parasympathetic modulation, or repolarization and T-wave morphology) and any pollutant (UFP, $PM_{2.5}$, AMP, or BC)?	+	No association or agreement	No
3	17	***Augsburg study only*** Does any medication intake modify any of the demonstrated adverse ECG effects?	—	—	No
	18	***REHAB study only*** Does self-perceived physical exertion modify any of the demonstrated adverse ECG effects?	—	—	No
	19	***REHAB study only*** Does having a priori MI modify any of the demonstrated adverse ECG effects?	—	—	No

CI, 2.70% to 6.54%) (Figure 4B and Appendix N). We therefore scored the results as "Contradictory" and concluded that the hypothesis was not replicated (Table 9).

Similarly, we hypothesized that increased 5-minute averages of personally measured UFP concentrations (in the Augsburg study) or total PNCs in controlled exposures (as a proxy for UFP, in the UPCON and UPDIABETES studies) in the previous 60 minutes would be associated with decreased 5-minute mean RMSSD (Question 8 in Table 4). In the Augsburg study, as hypothesized, each IQR increase in personal UFP concentration in each 5-minute segment within 30 minutes was associated with significant decreases in 5-minute mean RMSSD in the group with a genetic susceptibility, though not in the group with diabetes or IGT. The largest change in 5-minute RMSSD was associated with each IQR increase in the concurrent 5-minute UFP concentration (−0.95%; 95% CI, −1.48% to −0.43%) (Figure 5A and Appendix M). In the UPDIABETES and UPCON studies, however, we found consistent increases in 5-minute RMSSD associated with each IQR increase in 5-minute total PNCs (Figure 5B and Appendix N). We therefore scored the results as "Contradictory" and concluded that the hypothesis was not replicated (Table 9).

Last, we hypothesized that increased 5-minute averages of personally measured UFP concentrations (in the Augsburg study) or total PNCs in controlled exposures (as a proxy for UFP, in the UPCON and UPDIABETES studies) in the previous 60 minutes would be associated with increased 5-minute mean T-wave complexity (Question 9 in Table 4). In the Augsburg study, as hypothesized, each IQR increase in personal UFP concentration in most 5-minute segments within 30 minutes was associated with a significant increase in 5-minute mean T-wave complexity in both the group with diabetes or IGT and the group with a genetic susceptibility. The largest effect

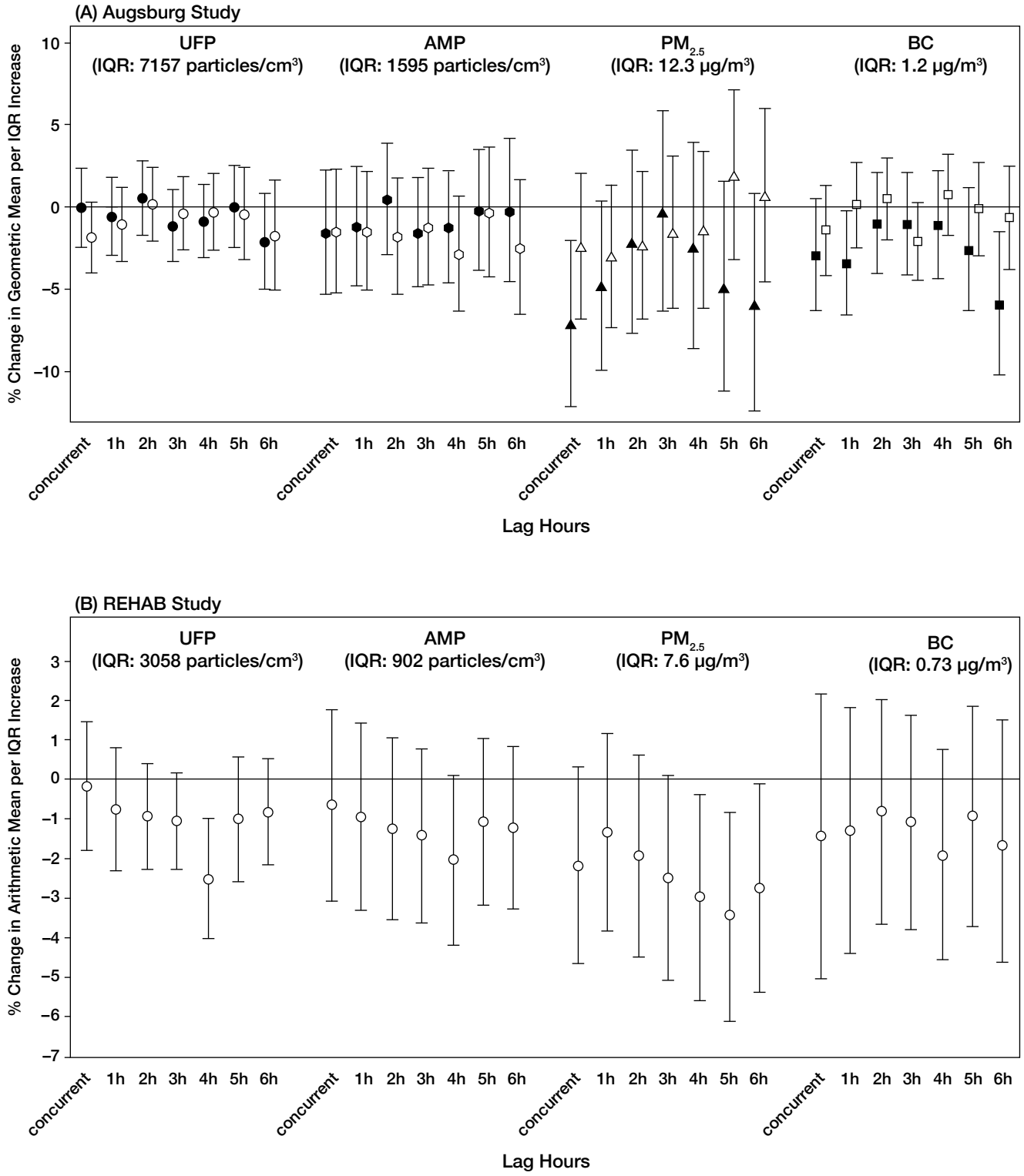

Figure 2. Percent change in RMSSD (1-hour average) associated with each IQR increase in pollutant concentration in the concurrent hour and at lags 1 to 6 hours. (**A**) In the Augsburg study. Black symbols represent subjects in the group with diabetes or IGT. White symbols represent healthy subjects in the group with a genetic susceptibility; (**B**) In the REHAB study. (*Figure continues next page.*)

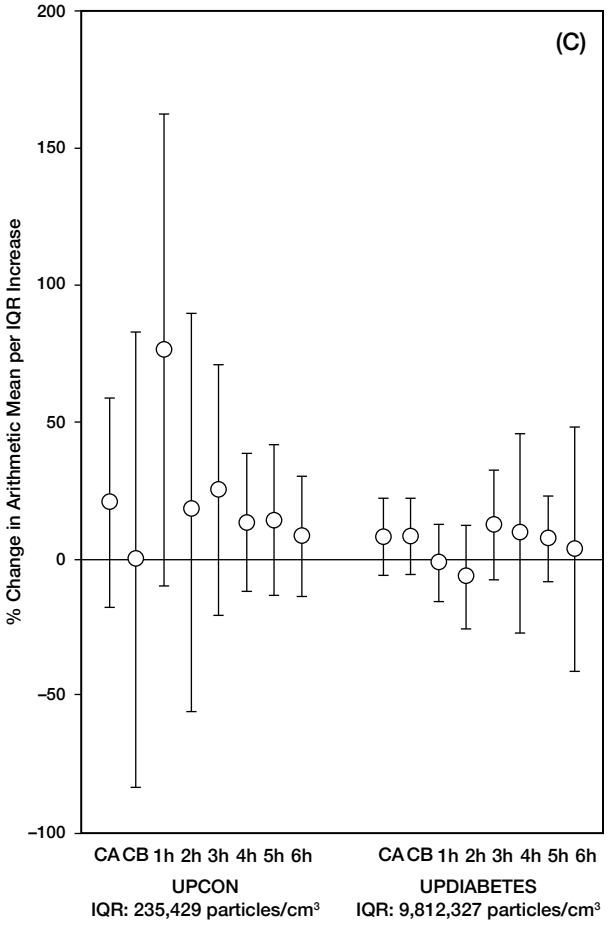

Figure 2 (*Continued*). Percent change in RMSSD (1-hour average) associated with each IQR increase in pollutant concentration in the concurrent hour and at lags 1 to 6 hours. (**C**) In the UPCON and UPDIABETES studies. CA = first hour of exposure, CB = mean of 2-hour exposure, 1h = first hour after exposure, 2h = second hour after exposure, etc.

found was a significant 0.40% increase (95% CI, 0.14% to 0.65%) in T-wave complexity associated with each IQR increase in UFP concentrations lagged 25–29 minutes (Figure 6A and Appendix M). In the UPDIABETES study, similarly, we found significant increases in 5-minute mean T-wave complexity associated with each IQR increase in 5-minute total PNCs within 60 minutes (largest at lag 25–29 minutes: 20.86%; 95% CI, 11.14% to 30.59%). In the UPCON study, however, we found significant decreases in 5-minute mean T-wave complexity associated with each IQR increase in 5-minute mean total PNCs in the previous 60 minutes (largest at lag 55–59 minutes: –5.48%; 95% CI, –7.51% to 3.45%) (Figure 6B and Appendix N). Therefore, because we found both increases and decreases in T-wave complexity associated with increased UFP concentrations across the studies, we scored the results as "Contradictory" and concluded that the hypothesis was not replicated (Table 9).

Question 10: Do the adverse ECG effects demonstrated here differ between the study subgroups (IGT, coronary symptoms, and healthy)? For questions 1–9, only question 1 (for UFPs with SDNN), question 4 (for $PM_{2.5}$ and AMP with SDNN), and question 5 (for $PM_{2.5}$ with RMSSD) were replicated, and associations were found in subjects with diabetes (both older subjects in Augsburg and younger subjects in Rochester), genetically susceptible subjects, and older subjects undergoing cardiac rehabilitation. For the research questions not replicated (3 and 6–9), effects were generally found in the hypothesized direction in the Augsburg study but not consistently in the other studies. No one study population or group was consistently scored as showing "No agreement" or an association in the direction opposite from that hypothesized. We therefore concluded that the adverse ECG effects demonstrated here do not differ between study subgroups consistently across the research questions.

Question 11: Are decreases in total antioxidant capacity associated with increased concentrations of UFPs, $PM_{2.5}$, AMP, or BC? Appendix A shows the methods, results, and interpretation of our analyses for Question 11. Briefly, we found no air pollution effects on total antioxidant capacity in either subject group of the Augsburg study and a reduced total antioxidant capacity in association with elevated concentrations of some air pollutants in the REHAB study (Appendix A). We therefore scored the results as "No agreement" and concluded that the hypothesis was not replicated (Table 9).

Question 12: Does total antioxidant capacity level modify the associations between any ECG outcome (total HRV, parasympathetic modulation, or T-wave morphology) and increased $PM_{2.5}$ concentrations? Appendix O outlines our methods and results for Question 12. Briefly, we did not find a pattern of modification of $PM_{2.5}$ effects by total antioxidant capacity levels across the studies. We therefore concluded that there was "No agreement" and that this hypothesis was not replicated (Table 9).

Questions 13–16: Do age, obesity, smoking status, or a previous diagnosis of hypertension modify the associations between any ECG outcome (total HRV, parasympathetic modulation, or repolarization and T-wave morphology) and increased $PM_{2.5}$ concentrations? We hypothesized that subjects ≥ 60 years of age (Question 13), who were obese (BMI ≥ 30 kg/m^2) (Question 14), who ever smoked (Question 15), or who had a previous diagnosis of hypertension (Question 16) would have larger decreases in SDNN or RMSSD or larger increases in T-wave complexity associated with each IQR increase in $PM_{2.5}$ concentrations

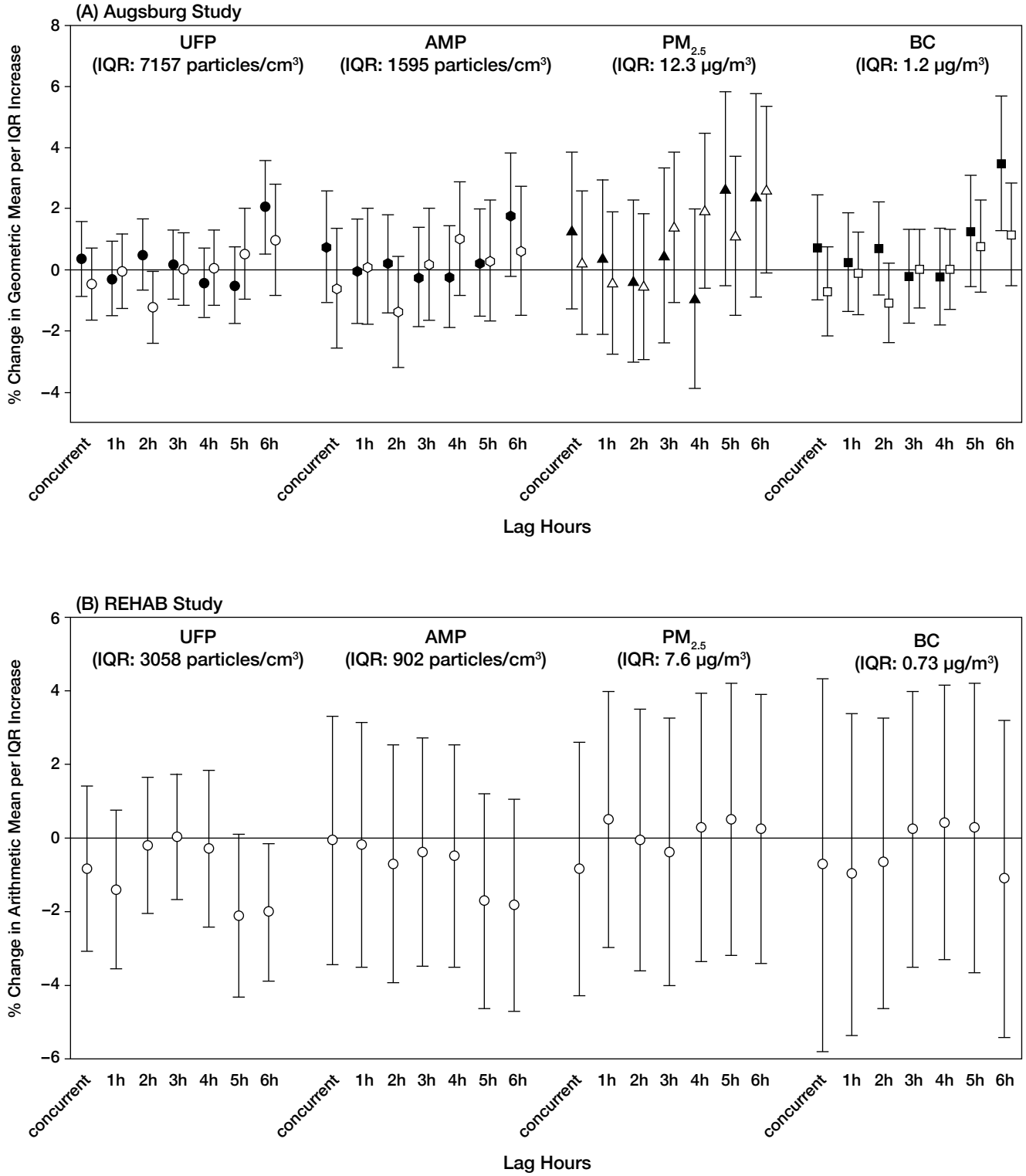

Figure 3. Percent change in T-wave complexity (1-hour average) associated with each IQR increase in pollutant concentration in the concurrent hour and at lags 1 to 6 hours. (**A**) In the Augsburg study. Black symbols represent subjects in the group with diabetes or IGT. White symbols represent healthy subjects in the group with a genetic susceptibility; (**B**) In the REHAB study. (*Figure continues next page.*)

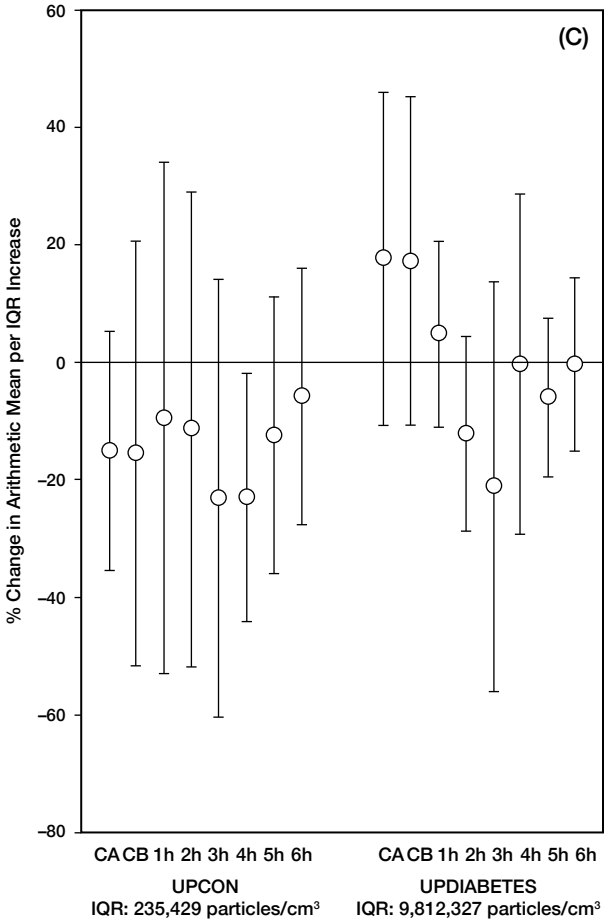

Figure 3 (*Continued*). Percent change in T-wave complexity (1-hour average) associated with each IQR increase in pollutant concentration in the concurrent hour and at lags 1 to 6 hours. (C) In the UPCON and UPDIABETES studies. CA = first hour of exposure, CB = mean of 2 hour exposure, 1h = first hour after exposure, 2h = second hour after exposure, etc.

compared with those < 60 years of age, who were not obese (BMI < 30 kg/m^2), who were never smokers, or who did not have a previous diagnosis of hypertension. For the Augsburg study, we evaluated $PM_{2.5}$ lagged 2 hours, because this was the lag for which we found the largest effects. For the Rochester study, for the same reason, we evaluated $PM_{2.5}$ lagged 5 hours.

We did not see consistently larger decreases in SDNN or RMSSD or larger increases in T-wave complexity associated with each IQR increase in $PM_{2.5}$ concentrations for subjects in either age group in either study. Further, none of the interaction terms were statistically significant (Table 10). We therefore concluded that there was "No agreement" and that the hypothesis was not replicated (Table 9).

Similarly, we did not see consistently larger decreases in SDNN or RMSSD or larger increases in T-wave complexity associated with each IQR increase in $PM_{2.5}$ concentration for subjects with BMI ≥ 30 kg/m^2 or BMI < 30 kg/m^2 in either study, and none of the interaction terms were statistically significant (Table 10). We therefore concluded that there was "No agreement" and that the hypothesis was not replicated (Table 9).

We did not see consistently larger decreases in SDNN or RMSSD or larger increases in T-wave complexity associated with each IQR increase in $PM_{2.5}$ concentration for subjects who were ever smokers or never smokers either in the Augsburg group with diabetes or IGT or in the REHAB study. However, in the Augsburg group with a genetic susceptibility, the ever smokers had a significantly (P = 0.016) greater decrease in RMSSD (–3.44%; 95% CI, –6.63% to –0.14%) than the never smokers (2.13%; 95% CI, –1.08% to 5.43%) (Table 10). Therefore, because this result was found in only one study, we concluded that there was "No agreement" and that the hypothesis was not replicated (Table 9).

And we did not see consistently larger decreases in SDNN or RMSSD or larger increases in T-wave complexity associated with each increase in $PM_{2.5}$ concentration for subjects who did or did not have a previous diagnosis of hypertension in either study. In contrast, in the Augsburg group with diabetes or IGT, those without hypertension had a significantly larger reduction in SDNN associated with each IQR increase in $PM_{2.5}$ concentration than those with hypertension (Table 10). Therefore, because we did not find a consistent pattern across studies, we concluded that there was "No agreement" and that the hypothesis was not replicated (Table 9).

Questions 17–19: Do medication intake, self-perceived exertion, or a prior MI modify the associations between any ECG outcome (total HRV, parasympathetic modulation, or repolarization and T-wave morphology) and increased $PM_{2.5}$ concentrations? We hypothesized that subjects taking statins or beta blockers (in the Augsburg study only) would have smaller decreases in SDNN or RMSSD or smaller increases in T-wave complexity associated with each IQR increase in $PM_{2.5}$ concentrations compared with those not taking statins or beta blockers (Question 17 in Table 4). However, both in the group with diabetes or IGT and in the group with a genetic susceptibility, the statins and beta blockers did not significantly modify our estimates of the changes in SDNN and RMSSD associated with each IQR increase in $PM_{2.5}$. Further, there was no clear pattern of a decrease in SDNN or RMSSD associated with increased $PM_{2.5}$ among those not taking statins or beta blockers and no change or a smaller

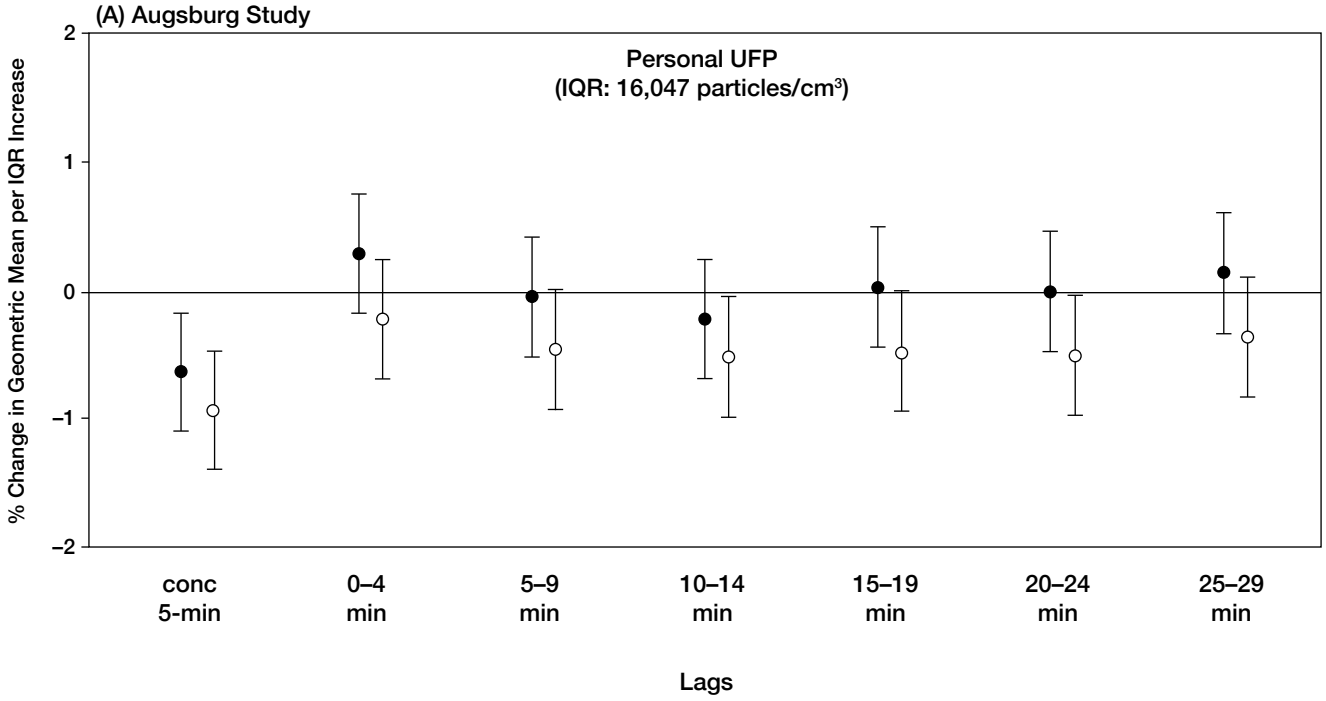

Figure 4. Percent change in SDNN (5-minute average) associated with each IQR increase in 5-minute mean pollutant concentrations. (**A**) In the Augsburg study for the previous 30 minutes. Black symbols represent subjects in the group with diabetes or IGT. White symbols represent healthy subjects in the group with a genetic susceptibility. Conc 5-min = concurrent 5 minutes; 0–4 min = 0 to 4 minutes before SDNN measurement; 5–9 min = 5 to 9 minutes before SDNN measurement; etc.). (*Figure continues next page.*)

decrease in SDNN or RMSSD among those taking the medications. Similarly, the use of statins or beta blockers did not appear to modify the association between T-wave complexity and increased $PM_{2.5}$ (Table 11). We therefore concluded that statins and beta blockers did not modify any association between the ECG outcomes we examined and increased $PM_{2.5}$ concentrations and that the hypothesis was not replicated (Table 9).

We next hypothesized that subjects undergoing cardiac rehabilitation (in the REHAB study only) reporting higher levels (i.e., the top 50%) of self-perceived exertion would have larger decreases in SDNN or RMSSD or larger increases in T-wave complexity associated with each IQR increase in $PM_{2.5}$ concentration compared with subjects reporting lower levels (i.e., the bottom 50%) of self-perceived exertion (Question 18 in Table 4). However, the percent changes in SDNN, RMSSD, or T-wave complexity associated with each IQR increase in $PM_{2.5}$ concentration lagged 5 hours were not larger for either the top or bottom half of the exertion values (Table 11). We therefore concluded that self-perceived exertion did not modify any association between the ECG outcomes we examined and increased $PM_{2.5}$ concentrations and that the hypothesis was not replicated (Table 9).

Last, we hypothesized that subjects with a prior MI (in the REHAB study only) would have larger decreases in SDNN or RMSSD or larger increases in T-wave complexity associated with each IQR increase in $PM_{2.5}$ concentration compared with those without a prior MI (Question 19 in Table 4). However, there was again no pattern of larger outcome changes associated with increased $PM_{2.5}$ concentration lagged 5 hours for either those with or without a prior MI, and none of the interaction terms were statistically significant (Table 11). We therefore concluded that having a prior MI did not modify any association between the ECG outcomes we examined and increased $PM_{2.5}$ concentrations and that the hypothesis was not replicated (Table 9).

SENSITIVITY AND ADDITIONAL ANALYSES

Sensitivity Analyses

We evaluated various modeling strategies to determine how sensitive our results were to the model structure chosen for analysis. Results from the sensitivity analyses done for SDNN in the Augsburg panel study are shown in Appendix P (available on the HEI Web site). For nearly all of these, the changes in SDNN associated with each IQR

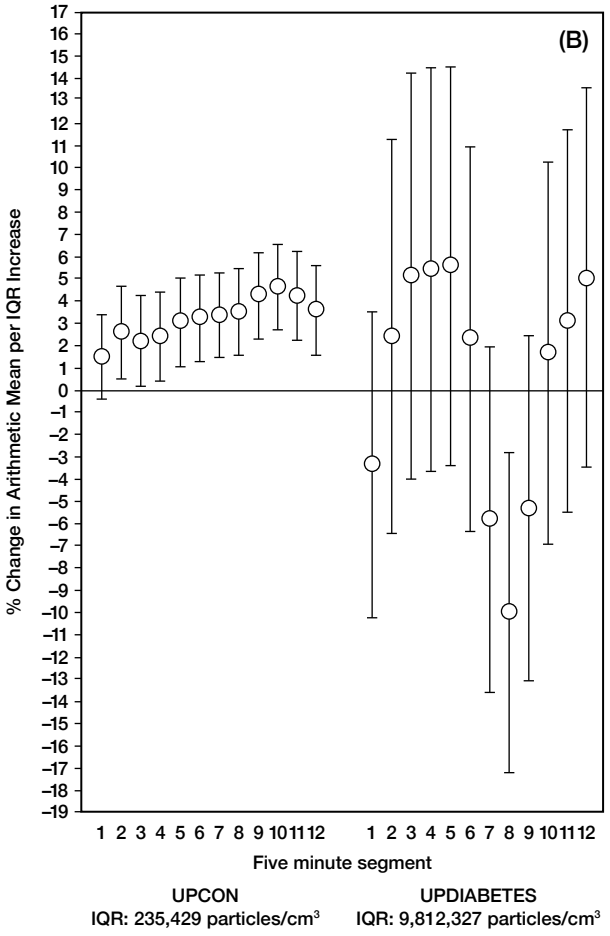

Figure 4 (*Continued*). Percent change in SDNN (5-minute average) associated with each IQR increase in 5-minute mean pollutant concentrations. (**B**) In the UPCON and UPDIABETES studies for the previous 60 minutes (in twelve 5-minute segments). 1 = 0 to 4 minutes before SDNN measurement; 2 = 5 to 9 minutes before SDNN measurement; 3 = 10 to 14 minutes before SDNN measurement, etc.

increase in UFP, $PM_{2.5}$, or BC concentrations were similar in size and direction to those from the main analysis, leading us to the same conclusions as before. In models that included the lagged outcome variable ($t - 1$), however, the SDNN changes were smaller in magnitude or even disappeared. However, these models are considered to be the strictest approach for autocorrelation and might therefore result in an over-adjustment, leading to overly conservative estimates.

Table 1 in Appendix Q shows the results from the sensitivity analyses done for SDNN in the REHAB study. For all analyses estimating the change in SDNN associated with each IQR increase in UFP, AMP, or $PM_{2.5}$ concentrations, beta coefficients and 95% CIs were generally similar in size and direction to those from the main analysis, leading us to draw the same conclusions. For Model #2, however, the beta coefficients were up to twice as large as those in the main analysis, suggesting that the effects of UFPs, AMP, and $PM_{2.5}$ on SDNN were even larger in the first hour of the clinic visit. For BC sensitivity analyses, the beta coefficients and 95% CIs for Models #3 and #4 were similar in size and direction to those from the main analysis. However, for Models #1 and #2, the beta coefficients were generally larger and the 95% CIs were wider than those in the main analysis. Further, several estimated SDNN changes associated with each IQR increase in BC were statistically significant in the sensitivity analyses (for the concurrent hour and lagged 1, 4, and 5 hours in Model #1 and lagged 4 and 5 hours in Model #2) that were not statistically significant in the main analysis.

We then re-ran our REHAB study analyses using the same covariates as in the Augsburg study to determine if our conclusions were robust to covariates included in the model. Table 2 in Appendix Q shows SDNN changes associated with increased UFP concentrations, SDNN changes associated with increased $PM_{2.5}$ concentrations, and RMSSD changes associated with increased $PM_{2.5}$ concentrations in the REHAB study. These are presented separately for a model including the original covariates (Model A covariates) and again using the same covariates as in the Augsburg study (Model B covariates). First, we generally found little difference in the size of these estimates when including Model A covariates versus Model B covariates. However, the size of the estimates for the SDNN analyses for $PM_{2.5}$ was generally smaller when including Model B covariates than when including Model A covariates. Second, when comparing the pattern of outcome changes associated with IQR increases in UFP and $PM_{2.5}$ concentrations between the REHAB study and Augsburg study when including the Model B covariates, there were still differences in the patterns of lagged responses between the studies. Thus, the covariates included in the study did not appear to be the primary determinant of differences in results between the studies.

In summary, across both of the panel studies, our sensitivity analyses did not produce consistently different results from our main analysis. Our results and inference therefore appeared to be robust to the modeling assumptions.

Two-Pollutant Models

For similar research questions whose hypotheses were replicated (e.g., "Are decreases in SDNN associated with increased UFPs?" and "Are decreases in SDNN associated with increased $PM_{2.5}$ or AMP in the previous few hours?"), we ran a series of two-pollutant models. Based on the

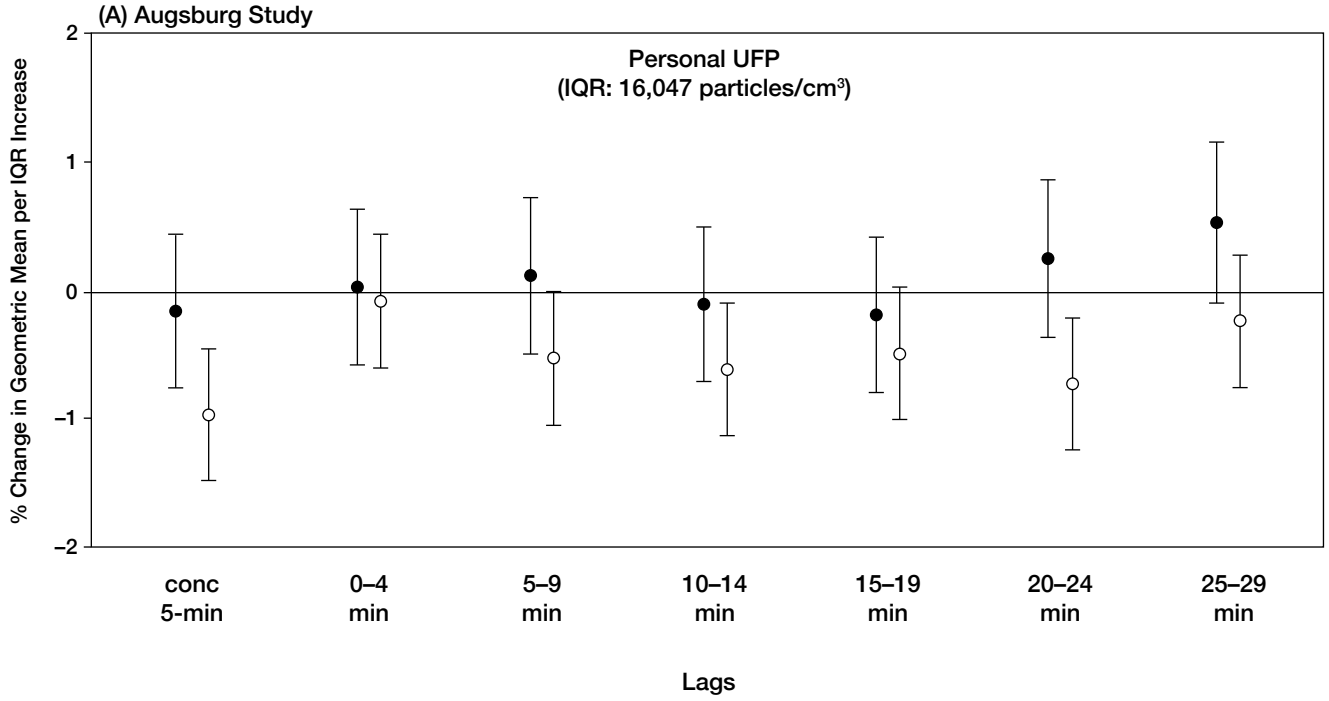

Figure 5. Percent change in RMSSD (5-minute average) associated with each IQR increase in 5-minute mean pollutant concentrations. (**A**) In the Augsburg study for the previous 30 minutes. Black symbols represent subjects in the group with diabetes or IGT. White symbols represent healthy subjects in the group with a genetic susceptibility. Conc 5-min = concurrent 5 minutes; 0–4 min = 0 to 4 minutes before RMSSD measurement; 5–9 min = 5 to 9 minutes before RMSSD measurement; etc. (*Figure continues next page.*)

pollutants significantly associated with decreased SDNN and the lag hours with the largest reductions in SDNN associated with a pollutant, we included all possible pairs of UFPs and $PM_{2.5}$ at lag 2 hours and then again at lag 3 hours for the Augsburg study as well as for UFPs, AMP, and $PM_{2.5}$ at lag 1 hour and 5 hours for the REHAB study. (Because the Spearman correlation coefficient between UFPs and AMP was 0.7 for the Augsburg study, we did not estimate two-pollutant models for this combination.)

In the Augsburg study, compared with the single-pollutant models estimating the changes in SDNN associated with each IQR increase in UFPs at lag 2 hours in the group with diabetes or IGT (–2.11%) and at lag 3 hours in the group with a genetic susceptibility (–2.26%), changes in SDNN associated with UFP when adjusting for $PM_{2.5}$ concentrations at the same lag hours generally decreased in both groups (–1.23% and –1.96%, respectively), or the changes were of a size similar to those in the single-pollutant models—and hence still suggestive of an effect (Table 12).

In the REHAB study, compared with the single-pollutant models estimating the changes in SDNN associated with each IQR increase in UFPs at lag 1 hour (–1.15%), AMP at lag 1 hour (–1.78%), or $PM_{2.5}$ lag 1 hour (0.71%), changes in SDNN associated with each pollutant (e.g., UFPs: –0.85%) when adjusting for another pollutant (e.g., AMP) were generally smaller than or of a size similar to those in the single-pollutant models—and hence still suggestive of an effect. The same pattern held for the two-pollutant models that included UFPs, AMP, or $PM_{2.5}$ lagged 5 hours. In none of the two-pollutant models did adjustment for one pollutant completely remove the effects of another pollutant (Table 12).

Heart Rate Analyses

We next hypothesized that increased particulate pollutant concentrations in the same and previous few hours would be associated with increases in heart rate and decreases in NN interval. In the Augsburg study, IQR increases in pollutants were generally associated with small percentage increases in heart rate. The largest of these were associated with increased AMP in the concurrent hour (0.92%; 95% CI, 0.18% to 1.66%) and with BC lagged 6 hours (1.10%; 95% CI, 0.24% to 1.96%) in the group with diabetes or IGT (Appendix H). In the REHAB study, however, we saw a pattern of generally small

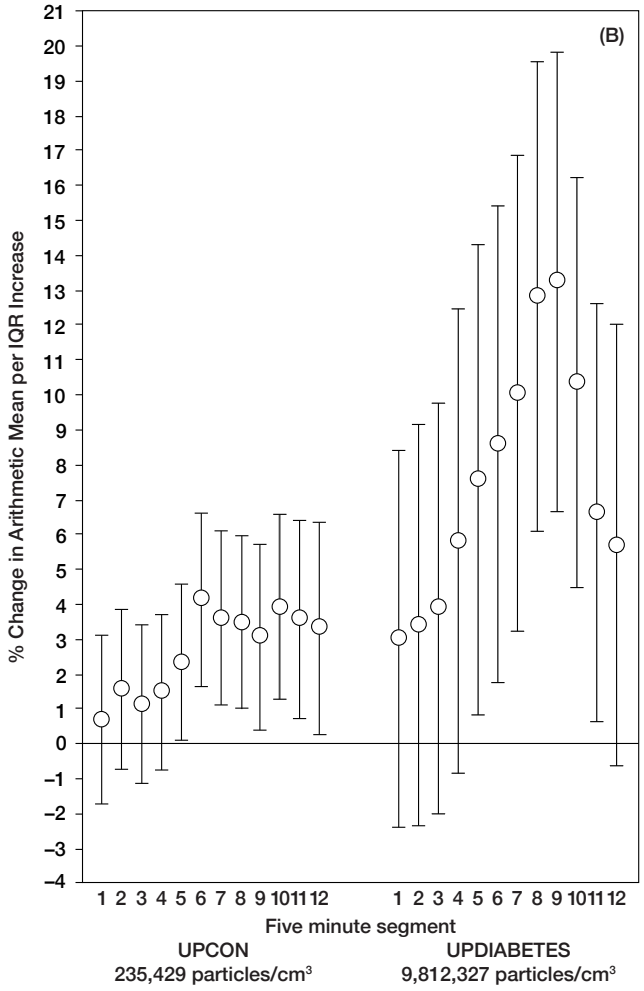

Figure 5 (*Continued*). Percent change in RMSSD (5-minute average) associated with each IQR increase in 5-minute mean pollutant concentrations. (**B**) In the UPCON and UPDIABETES studies for the previous 60 minutes (in twelve 5-minute segments). 1 = 0 to 4 minutes before SDNN measurement; 2 = 5 to 9 minutes before SDNN measurement; 3 = 10 to 14 minutes before SDNN measurement, etc.

percentage increases in NN interval (i.e., decreases in heart rate) associated with increased pollutant concentrations in the previous few hours. The largest of these was a 0.51% increase in NN interval (95% CI, 0.14% to 0.89%) associated with each IQR increase in UFP concentrations in the previous hour (Appendix I). There was therefore no clear pattern of pollutant effects on increased heart rate across the two panel studies.

Exposure–Response Functions

For each replicated research question, we checked the SDNN pollutant exposure–response functions for deviations from linearity. In the Augsburg study, these checks included SDNN and UFPs lagged 2 hours in the group with diabetes or IGT, $PM_{2.5}$ lagged 2 hours in the group with diabetes or IGT, and UFPs lagged 3 hours in the group with a genetic susceptibility, as well as $PM_{2.5}$ lagged 3 hours in the group with a genetic susceptibility. In the REHAB study, the checks included SDNN and UFPs lagged 1 hour, $PM_{2.5}$ lagged 1 hour, UFPs lagged 5 hours, and $PM_{2.5}$ lagged 5 hours. There did not appear to be deviations from linearity in either study. Examples of the linear exposure–response functions using P-splines are shown in Appendix R for the Augsburg study and Appendix S for the REHAB study.

Meta-Analyses

In a meta-analysis across both panel studies, we estimated that each 1000 particles/cm^3 increase in UFP concentrations lagged 4 hours was associated with a 0.22% decrease (95% CI, −0.34% to −0.09%) in SDNN and that each 10 μg/m^3 increase in $PM_{2.5}$ concentrations lagged 2 hours was associated with a 2.57% decrease (95% CI, −3.84% to −1.31%) in SDNN (Table 1B in Appendix C). It is noteworthy that the two panel studies from Augsburg and Rochester did not show much inter-study heterogeneity for UFP or $PM_{2.5}$ associations with SDNN, whereas when pooling UFP–SDNN associations across all four studies (Table 1A in Appendix C), there was often too much heterogeneity to develop a valid pooled estimate.

DISCUSSION AND CONCLUSIONS

Our goal was to provide novel insights into the associations between various air pollutants and markers of immediate (5-minute or 1-hour) cardiac physiological responses. Given that many personal air pollution exposures are of short duration (e.g., while driving, riding on a bus or subway, or walking on the sidewalk close to a road), understanding one or more mechanisms by which particulate air pollution may affect cardiovascular health on this time scale is an important public health issue. We therefore reanalyzed ECG recordings from four completed studies—a panel study in Augsburg, Germany, and a panel study and two controlled-exposure studies in Rochester, New York—to provide multiple suitable markers of HRV, heart rate, repolarization, and T-wave morphology. Increases in both UFP and $PM_{2.5}$ concentrations within 2 and 5 hours were independently associated with decreases in total HRV (i.e., SDNN). Increased $PM_{2.5}$ concentrations within 5 hours were also associated with decreased parasympathetic modulation (i.e., RMSSD).

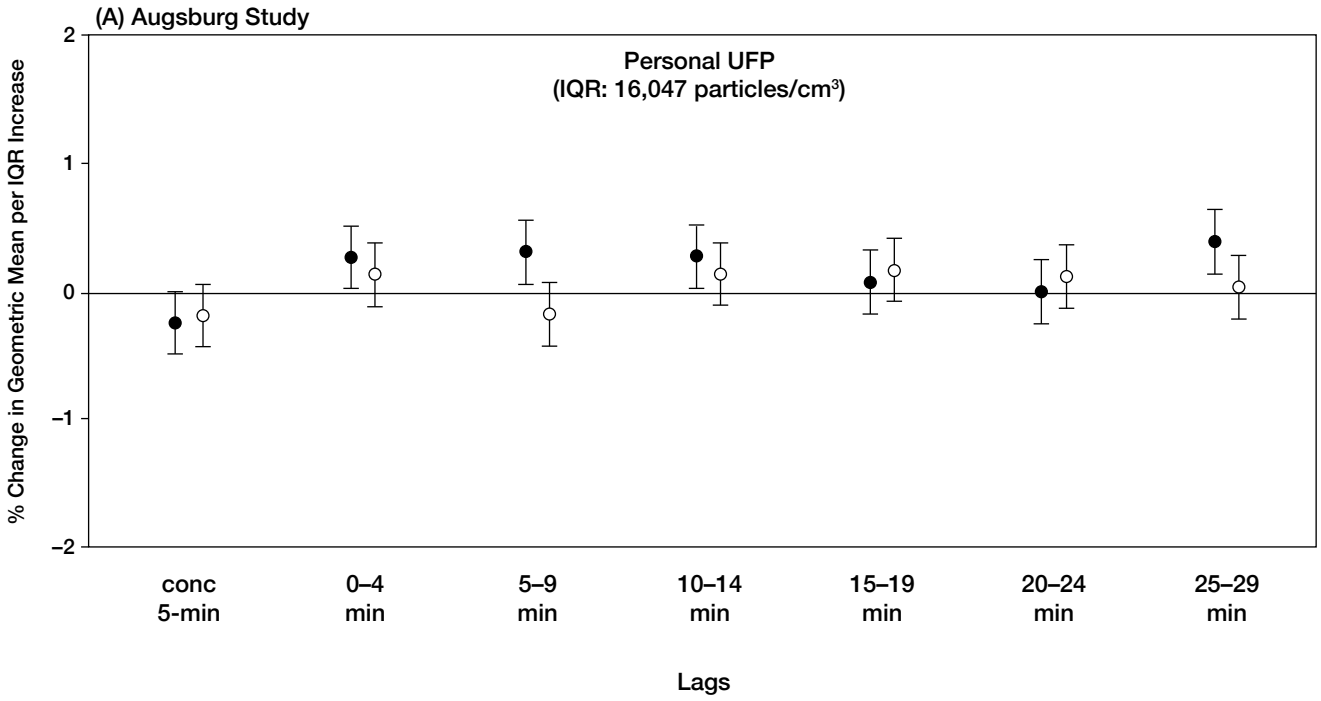

Figure 6. Percent change in T-wave complexity (5-minute average) associated with each IQR increase in 5-minute mean pollutant concentrations. (**A**) In the Augsburg study for the previous 30 minutes. Black symbols represent subjects in the group with diabetes or IGT. White symbols represent healthy subjects in the group with a genetic susceptibility. Conc 5-min = concurrent 5 minutes; 0–4 min = 0 to 4 minutes before T-wave complexity measurement; 5–9 min = 5 to 9 minutes before T-wave complexity measurement; etc. (*Figure continues next page.*)

Exposure–response functions appeared to be linear. However, we found no consistent evidence of UFP effects on parasympathetic modulation, no evidence of any pollutant effects on markers of T-wave complexity, and no consistent evidence of UFP effects on total HRV, parasympathetic modulation, or T-wave complexity at any 5-minute interval within 60 minutes. We also found that the effects of UFPs and $PM_{2.5}$ on SDNN and RMSSD generally did not differ between the various subgroups in the studies (i.e., subjects with diabetes or IGT, cardiac rehabilitation patients, and subjects who were older or younger but otherwise healthy). Based on our sensitivity analysis using heart rate (or NN) as an outcome, we can assume that the observed consistent pollutant effects on HRV (measured as SDNN and RMSSD) across studies were independent of any changes in heart rate associated with air pollution. Last, we found no consistent evidence of effects of any pollutant on total antioxidant capacity and no consistent evidence of effect modification of our $PM_{2.5}$–outcome associations by total antioxidant capacity, age, obesity, smoking, hypertension, exertion, or medications. Given the heterogeneity in study populations, ECG recording conditions, and study protocols, it is not surprising we could not replicate many of our hypotheses. However, the consistency of our findings for UFP–SDNN, $PM_{2.5}$–SDNN, and $PM_{2.5}$–RMSSD associations across these divergent studies is noteworthy.

The observed changes in SDNN and RMSSD associated with increased air pollutant concentrations in the previous few hours were small and may not be clinically significant. However, repeated small adverse changes in these parameters can affect homeostasis and thereby lead to manifestation of cardiovascular disease in the longer run. Moreover, the detected small changes give insight into the pathways that lead from inhalation of polluted air to a manifestation of disease.

It should be noted that the same basic statistical analysis was applied both to the two panel studies (Augsburg and REHAB) and to the two controlled-exposure studies (UPCON and UPDIABETES). For the interpretation of the results, it is important to bear in mind that the studies' designs and exposure scenarios were quite different. First, the pollutant exposures in relation to the timing of the outcomes in the two study designs were different. In the panel studies, for example, "moving," or "rolling," exposure periods (repeated hourly mean air pollutant

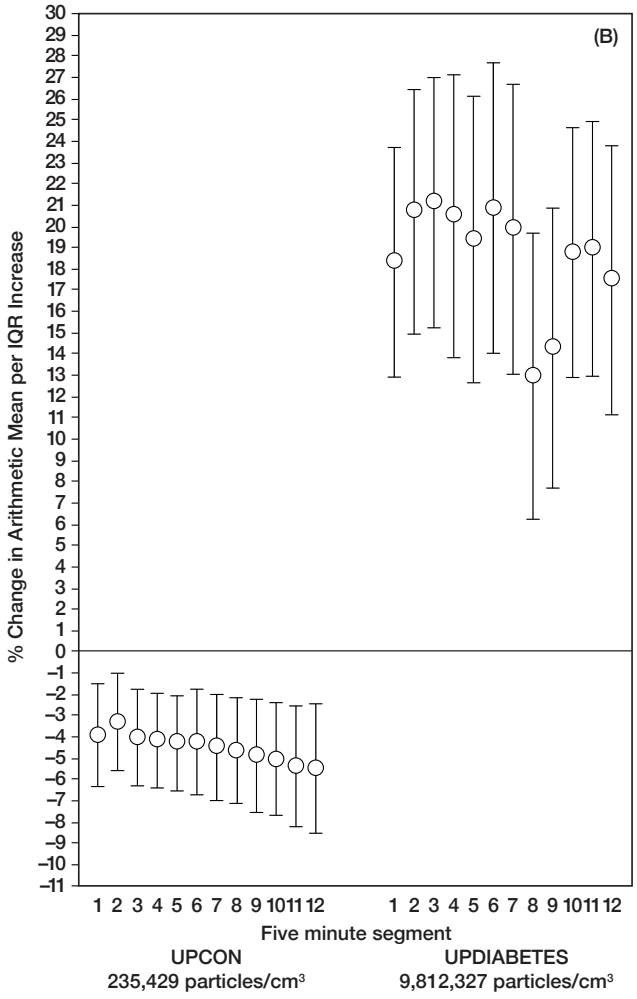

Figure 6 (*Continued*). Percent change in T-wave complexity (5-minute average) associated with each IQR increase in 5-minute mean pollutant concentrations. (**B**) In the UPCON and UPDIABETES studies for the previous 60 minutes (in twelve 5-minute segments). 1 = 0 to 4 minutes before SDNN measurement; 2 = 5 to 9 minutes before SDNN measurement; 3 = 10 to 14 minutes before SDNN measurement, etc.

concentrations) were associated with each outcome measurement obtained at specific time points (e.g., SDNN measured at the concurrent lag hour, lagged 1 hour, lagged 2 hours, etc.). In the controlled-exposure studies, the pollutant exposures were fixed in time and were only 2 hours long, and the outcome measurements were made only at increasing time lengths from the beginning and end of each exposure. Second, the controlled-exposure studies used UFP concentrations substantially higher than the ambient UFP concentrations used in the panel studies. Third, the compositions of the particles were different between the panel studies in Augsburg and Rochester. Fourth, the underlying characteristics of the study subjects varied.

Finally, there were differences in the subjects' activity levels during each study. Most HRV analyses performed for clinical and research purposes are made using 24-hour Holter recordings in ambulatory conditions, when subjects and patients are not restricted in their physical activity, meals, mental stressors, or sleep. However, all of these factors affect autonomic tone and heart rate. A longer recording, incorporating such factors, provides a broader range of heart rates and a wider scope of autonomic changes than shorter recordings do. In the Augsburg study, assessment of ECG changes while subjects went about their daily lives contributed to a better representation of the reactivity of the autonomic nervous system to various daily stimuli. In the Rochester REHAB study, the subjects were not assessed for ECG outcome changes in the midst of daily activities but rather while they participated in exercise sessions conducted during clinical visits as part of a cardiac rehabilitation program. Exercise is associated with increased sympathetic and decreased parasympathetic activity. However, these responses can vary by subject, depending on his or her underlying autonomic tone. Further, the responses can depend on individual autonomic responses to exercise that might be compromised in postinfarction patients. Even with all of these different designs, study populations, exposure scenarios, and exposure levels, we found consistent associations across the four studies for SDNN and, UFPs SDNN and $PM_{2.5}$, and RMSSD and $PM_{2.5}$. These associations may thus be rather robust to differences in the variable factors.

SDNN AND RMSSD

As discussed previously, there is concern that UFPs may cause adverse health effects (Devlin et al. 2014; Peters 2011). Because UFPs are very small, with little mass but high number and surface-area concentrations, they are deposited more efficiently in the alveolar region of the lungs and to a lesser extent in the larger airways (Kim and Jaques 2000). UFPs are cleared more slowly than larger particles (Choi and Kim 2007) because they are not well recognized by macrophages in the alveolar region. They cause oxidative stress (Araujo 2010; Li et al. 2003) and have been proposed as being able to exit the lung readily, potentially translocating into cells and entering the circulatory system, where they could affect other extrapulmonary tissues or organs (Nemmar et al. 2002, 2004; Oberdörster et al. 2002, 2004). Moreover, their motion is defined by diffusion rather than by aerodynamic properties, which could make the above-supposed translocation possible.

Previously, we demonstrated immediate (within 1 hour) triggering of MI by time spent in traffic (Peters

Table 10. Percent Change in SDNN, RMSSD, and T-Wave Complexity (T-Wave) Associated with Each IQR Increase in $PM_{2.5}$ Concentration (Augsburg, Lagged 2 Hr, 12.3 µg/m^3; Rochester, Lagged 5 Hr: 7.6 µg/m^3) by Study/Group with Effect Modification by Subject Characteristics

Modifier	Outcome	% Change	95% CI	% Change	95% CI	Interaction term *P*-value
AUGSBURG Study: Group with Diabetes or IGT (for 12.3 µg/m^3 $PM_{2.5}$ lagged 2 hr)						
		≥ 60 years of age		*< 60 years of age*		
Age	*SDNN*	−5.54%	(−8.97%, −2.10%)	−2.80%	(−7.61%, 2.01%)	0.36
	RMSSD	−2.06%	(−8.28%, 4.59%)	−2.74%	(−12.84%, 8.52%)	0.91
	T-wave	−0.82%	(−3.87%, 2.33%)	0.64%	(−4.17%, 5.69%)	0.62
		BMI ≥ 30 kg/m^2		*BMI < 30 kg/m^2*		
Obesity	*SDNN*	−3.66%	(−7.31%, −0.01%)	−5.86%	(−10.22%, −1.49%)	0.44
	RMSSD	0.69%	(−6.48%, 8.41%)	−6.33%	(−14.10%, 2.15%)	0.21
	T-wave	−0.35%	(−3.73%, 3.16%)	−0.53%	(−4.50%, 3.60%)	0.95
		Ever smoked		*Never smoked*		
Smoking	*SDNN*	−5.30%	(−8.83%, −1.77%)	−3.36%	(−7.96%, 1.25%)	0.51
	RMSSD	−3.60%	(−10.67%, 4.01%)	−0.64%	(−8.61%, 8.04%)	0.60
	T-wave	1.02%	(−2.42%, 4.59%)	−2.34%	(−6.20%, 1.69%)	0.21
		Yes		*No*		
Hypertension	*SDNN*	−2.46%	(−5.89%, 0.98%)	−9.07%	(−13.96%, −4.18%)	0.03
	RMSSD	−0.02%	(−6.84%, 7.30%)	−6.39%	(−14.77%, 2.83%)	0.27
	T-wave	−0.08%	(−3.30%, 3.25%)	−1.24%	(−5.54%, 3.27%)	0.68
AUGSBURG Study: Group with genetic susceptibility (for 12.3 µg/m^3 $PM_{2.5}$ lagged 2 hr)						
		≥ 60 years of age		*< 60 years of age*		
Age	*SDNN*	−4.44%	(−9.02%, 0.13%)	−2.25%	(−6.54%, 2.04%)	0.48
	RMSSD	−5.28%	(−11.56%, 1.45%)	−0.47%	(−6.23%, 5.65%)	0.28
	T-wave	0.26%	(−3.23%, 3.87%)	−1.27%	(−4.30%, 1.86%)	0.52
		BMI ≥ 30 kg/m^2		*BMI < 30 kg/m^2*		
Obesity	*SDNN*	−3.92%	(−14.65%, 6.81%)	−3.58%	(−6.97%, −0.18%)	0.95
	RMSSD	−3.45%	(−17.06%, 12.39%)	−2.72%	(−7.28%, 2.06%)	0.93
	T-wave	4.00%	(−3.96%, 12.61%)	−1.54%	(−3.51%, 1.46%)	0.24
		Ever smoked		*Never smoked*		
Smoking	*SDNN*	−1.16%	(−5.57%, 3.26%)	−5.62%	(−10.07%, −1.17%)	0.15
	RMSSD	−0.16%	(−6.73%, 6.87%)	−4.29%	(−9.89%, 1.65%)	0.35
	T-wave	−3.44%	(−6.63%, −0.14%)	2.13%	(−1.08%, 5.43%)	0.02
		Yes		*No*		
Hypertension	*SDNN*	−4.10%	(−8.53%, 0.34%)	−2.84%	(−7.22%, 1.54%)	0.68
	RMSSD	−4.47%	(−10.53%, 2.01%)	−0.63%	(−6.58%, 5.70%)	0.38
	T-wave	0.16%	(−3.15%, 3.58%)	−1.25%	(−4.42%, 2.02%)	0.55

Table continues next page

Table 10 (*Continued*).

Modifier	Outcome	% Change	95% CI	% Change	95% CI	Interaction term *P*-value
REHAB Study (for 7.6 µg/m^3 $PM_{2.5}$ lagged 5 hr)						
		≥ 60 years of age		*< 60 years of age*		
Age	*SDNN*	−2.36%	(−5.29%, 0.57%)	−0.05%	(−3.32%, 3.22%)	0.28
	RMSSD	−5.87%[a]	(−10.12%, −1.62%)	−1.26%	(−6.01%, 3.49%)	0.13
	T-wave	−0.94%	(−6.88%, 5.00%)	2.69%	(−4.03%, 9.41%)	0.40
		BMI ≥ 30 kg/m^2		*BMI < 30 kg/m^2*		
Obesity	*SDNN*	−0.32%	(−3.54%, 2.90%)	−2.20%	(−5.19%, 0.78%)	0.37
	RMSSD	−2.95%	(−7.64%, 1.74%)	−4.36%[b]	(−8.68%, −0.03%)	0.65
	T-wave	2.26%	(−4.29%, 8.81%)	−0.81%	(−6.88%, 5.25%)	0.48
		Ever smoked		*Never smoked*		
Smoking	*SDNN*	−1.99%	(−5.35%, 1.36%)	−0.89%	(−3.78%, 1.99%)	0.61
	RMSSD	−3.51%	(−8.37%, 1.35%)	−3.84%[c]	(−8.03%, 0.35%)	0.91
	T-wave	−2.47%	(−9.30%, 4.36%)	2.78%	(−3.09%, 8.64%)	0.23
		Yes		*No*		
Hypertension	*SDNN*	−0.46%	(−3.32%, 2.40%)	−2.71%	(−6.14%, 0.72%)	0.30
	RMSSD	−3.15%	(−7.31%, 1.02%)	−4.57%[c]	(−9.53%, 0.39%)	0.65
	T-wave	0.05%	(−5.75%, 5.85%)	1.44%	(−5.58%, 8.46%)	0.75

[a] $P < 0.01$.
[b] $P < 0.05$.
[c] $P < 0.10$.

et al. 2004), which, if truly attributable to particle exposures, requires very rapid cardiovascular responses. Further, we have previously reported that increased ambient $PM_{2.5}$ concentrations were associated with increased risks of MI over the next hour in both Boston (Peters et al. 2001) and Rochester (Gardner et al. 2014). Indeed, Mills and colleagues (2007) found very immediate signs (within 1 hour) of ischemia in response to diluted diesel exhaust exposure while exercising. Moreover, Albert and colleagues (2007) found an increased risk of implantable cardioverter-defibrillator shock for ventricular tachycardia or ventricular fibrillation within 30 minutes after driving. We thus hypothesized that the potential mechanisms underlying these PM–MI and traffic–MI associations might act on time scales of 1 hour or even less. We chose ECG-based outcome measures because we wanted to test the hypothesis that short-term exposures to PM induced changes in cardiac autonomic function—and indeed we were able to detect such changes across our panel and controlled-exposure studies associated with increased UFP, AMP, and $PM_{2.5}$ concentrations in the previous few hours.

According to Brook and colleagues (2010), there are three main potential biological pathways linking PM exposure to cardiovascular disease.

1. In the first pathway, circulating pro-oxidative or pro-inflammatory mediators (e.g., cytokines, acute phase reactants, and vasoactive hormones) released from the lungs are supposed possibly to induce a systemic chain reaction. This may lead to a change in vascular tone (endothelial dysfunction), adverse cardiac outcomes, and a pro-coagulation state with thrombus formation and ischemic response as well as promotion of atherosclerotic lesions, as suggested by Utell and colleagues (2002).

2. In the second pathway, particles deposited in the pulmonary tree may alter autonomic balance in the nervous system, leading to parasympathetic withdrawal or sympathetic activation. These effects can either be triggered directly, by an interaction of the particles with pulmonary receptors, leading to a stimulation of pulmonary neural reflexes (Widdicombe 1982;

Table 11. Percent Change in SDNN (msec), RMSSD (msec), and T-Wave Complexity (T-wave; %) Associated with Each IQR Increase in $PM_{2.5}$ Concentration (Augsburg, Lagged 2 Hr: 12.3 μg/m^3; Rochester, Lagged 5 Hr, 7.6 μg/m^3) by Study/Group with Effect Modification by Subject Characteristics

Modifier	Outcome	% Change	95% CI	% Change	95% CI	Interaction term *P*-value
Intake of Statins		*Yes*		*No*		
Augsburg group with diabetes or IGT	*SDNN*	−3.68%	(−10.79%, 3.43%)	−4.69%	(−7.76%, −1.62%)	0.80
	RMSSD	2.12%	(−11.19%, 17.41%)	−2.99%	(−8.80%, 3.18%)	0.51
	T-wave	3.91%	(−2.71%, 10.98%)	−1.22%	(−4.04%, 1.68%)	0.17
		Yes		*No*		
Augsburg group with a genetic susceptibility	*SDNN*	−5.62%	(−12.89%, 1.65%)	−2.85%	(−6.36%, 0.66%)	0.49
	RMSSD	−3.37%	(−13.35%, 7.75%)	−2.23%	(−6.98%, 2.77%)	0.80
	T-wave	1.83%	(−3.60%, 7.57%)	−1.09%	(−3.63%, 1.52%)	0.34
Intake of Betablockers		*Yes*		*No*		
Augsburg group with diabetes or IGT	*SDNN*	−5.59%	(−11.00%, −0.19%)	−4.27%	(−7.56%, −0.97%)	0.68
	RMSSD	0.41%	(−9.15%, 10.97%)	−3.56%	(−9.94%, 3.28%)	0.51
	T-wave	0.38%	(−4.33%, 5.32%)	−0.74%	(−3.84%, 2.45%)	0.70
		Yes		*No*		
Augsburg group with a genetic susceptibility	*SDNN*	−7.23%	(−13.88%, −0.58%)	−2.48%	(−6.06%, 1.10%)	0.21
	RMSSD	−1.21%	(−10.71%, 9.30%)	−2.80%	(−7.60%, 2.25%)	0.78
	T-wave	3.00%	(−2.15%, 8.41%)	−1.43%	(−4.00%, 1.21%)	0.13
Self-Perceived Exertion		*Top 50% of all values*		*Bottom 50% of all values*		
REHAB Study	*SDNN*	−0.84%	(−3.75%, 2.07%)	−2.05%	(−5.28%, 1.17%)	0.56
	RMSSD	−4.88%	(−9.11%, −0.65%)	−2.24%	(−6.91%, 2.43%)	0.38
	T-wave	−2.23%	(−8.20%, 3.73%)	3.89%	(−2.62%, 10.40%)	0.14
Prior MI		*Yes*		*No*		
REHAB Study	*SDNN*	−2.26%	(−5.05%, 0.53%)	0.18%	(−3.35%, 3.72%)	0.26
	RMSSD	−4.28%	(−8.33%, −0.22%)	−2.79%	(−7.92%, 2.33%)	0.64
	T-wave	2.31%	(−3.36%, 7.98%)	−2.31%	(−9.51%, 4.90%)	0.30

Widdicombe and Lee 2001), or indirectly, by provoking oxidative stress and inflammation in the lung. These alterations can contribute to the instability of a vascular plaque or initiate cardiac arrhythmias. Furthermore, altered ion-channel functions in myocardial cells could lead to cardiac malfunction triggered by air pollution (Schulz et al. 2005).

3. In the third pathway, mainly UFPs or soluble particle constituents may rapidly translocate from the pulmonary epithelium into the circulation and interact directly with the cardiovascular system (Nemmar et al. 2002, 2004; Oberdörster et al. 2002, 2004). This might not only affect the vascular endothelium and atherosclerotic plaques, but also provoke local inflammation and oxidative stress. Once in the circulation, UFPs might also have direct effects on the heart and other organs.

The confirmed effects of $PM_{2.5}$ (and AMP) of the previous few hours on SDNN and RMSSD suggest that some of the pathways described above are more likely than others. For particles of that size range, the second pathway seems plausible because the immediate associations, as seen in our data, point toward a direct stimulation of pulmonary receptors in the lung. Our findings might be mediated by a perturbation of the balance of the systemic autonomic

Table 12. Percent Change in SDNN Associated with Each IQR Increase in Concurrent and 1- to 6-hour Lagged UFP and $PM_{2.5}$ Concentrations in Two-Pollutant Models for Subjects in the Augsburg and REHAB Studies

Augsburg Study[a]

		UFP IQR = 7157 particles/cm^3		$PM_{2.5}$ IQR = 12.3 μg/m^3	
Group	Pollutant Averaging Time	% Change	95% CI	% Change	95% CI
Diabetes or IGT	Lag 2h (60–119 min)	−1.23%	(−2.96%, 0.51%)	−3.45%	(−6.73%, −0.17%)[c]
	Lag 3h (120–179 min)	−1.48%	(−3.18%, 0.21%)[b]	−2.12%	(−5.63%, 1.40%)
Genetic Susceptibility	Lag 2h (60–119 min)	−0.99%	(-2.88%, 0.90%)	−2.49%	(−6.13%, 1.15%)
	Lag 3h (120–179 min)	−1.96%	(−3.85%, −0.08%)[c]	−1.38%	(−5.10%, 2.35%)

[a] Spearman correlation between UFP and $PM_{2.5}$: $r = 0.42$.
[b] $P < 0.10$.
[c] $P < 0.05$.

REHAB Study[a]

		UFP[b] IQR = 1980 particles/cm^3		AMP[c] IQR = 902 particles/cm		$PM_{2.5}$[d] IQR = 7.6 μg/m^3	
Pollutant averaging time	*N*	% Change	95% CI	% Change	95% CI	% Change	95% CI
Lag 1h	2536	−0.85%	(−1.96%, 0.26%)	−1.33%	(−3.02%, 0.37%)	—	—
Lag 1h	2244	−0.78%	(−1.94%, 0.37%)	—	—	−0.60%	(−2.30%, 1.10%)
Lag 1h	2244	—	—	−2.10%[e]	(−4.29%, 0.09%)	0.58%	(−1.58%, 2.73%)
Lag 5h	2540	0.32%	(−0.85%, 1.48%)	−1.74%[f]	(−3.29%, −0.19%)	—	—
Lag 5h	2272	−0.45%	(−1.85%, 0.94%)	—	—	−2.11%[f]	(−3.91%, −0.31%)
Lag 5h	2272	—	—	−0.99%	(−2.83%, 0.86%)	−1.56%	(−3.69%, 0.57%)

[a] Spearman correlation between UFP and AMP: $r = 0.60$; between UFP and $PM_{2.5}$: $r = 0.16$; and between AMP and $PM_{2.5}$: $r = 0.67$.
[b] Model with UFP and AMP, both lagged 1 and 5 hours.
[c] Model with AMP and $PM_{2.5}$, both lagged 1 and 5 hours.
[d] Model with UFP and $PM_{2.5}$, both lagged 1 and 5 hours.
[e] $P < 0.10$.
[f] $P < 0.05$.

nervous system because of a stimulation of lung receptors or nerve endings in the human airways by inhaled particles (Brook et al. 2010). The confirmed effects of UFPs of the previous few hours on SDNN also make the third pathway seem likely, because the idea of UFPs travelling in the bloodstream through the body and affecting certain organs (such as the heart) directly would explain the rapid effects we have seen. A small fraction of UFPs may also pass through alveolar walls and affect the electric system of the heart directly (Peters et al. 2006), which might lead to reduced HRV. However, our hypotheses about PM effects on these ECG outcomes at time scales shorter than hours (e.g., our study's 5-minute analyses) were not confirmed. But the Augsburg study and one of the two controlled-exposure studies did provide hints that effects even more immediate than 1 hour are possible. For RMSSD, however, the effects of UFPs in the previous few hours could not be confirmed. RMSSD is an index of parasympathetic modulation, whereas SDNN reflects the variability of both sympathetic and parasympathetic activity. Because we confirmed a reduction in SDNN but not in RMSSD (only the results from the REHAB study agreed with the hypothesized effect size and direction) in association with UFP increases, we assume that the activation of the sympathetic

nervous system by UFPs is more pronounced than the vagal withdrawal. However, we realize that HRV parameters do not provide direct insight into sympathetic modulation of the heart, because LF power is believed to reflect mainly baroreflex sensitivity with only some of the sympathetic component rather than just sympathetic modulation of the heart. We therefore cannot fully disentangle why only the effects on SDNN, but not on RMSSD, could be confirmed across the study types and settings we examined. It is possible that SDNN has a somewhat stronger signal, because it reflects total HRV and not just parasympathetic modulation, as RMSSD does. Moreover, it is conceivable that for hourly ECG data analyses, and especially for 5-minute analyses, there is a possibility for greater variation among the analyzed segments in the uncontrolled conditions of the subjects' regular physical activities. For short recordings, it would be expected that resting supine (or sitting) recordings would provide more consistency of findings than uncontrolled conditions. SDNN and RMSSD, when derived from long recording periods (more than 24 hours or at least several hours) are more representative of changes in autonomic regulation than are shorter uncontrolled periods.

In summary, we hypothesize that $PM_{2.5}$ and AMP may activate different underlying intrinsic mechanisms than UFPs do. The changes in SDNN and RMSSD found in association with $PM_{2.5}$ and AMP may be associated with an activation of host defenses on an alveolar level, which may involve an immediate systemic oxidative stress response and a perturbation of the balance of the autonomic nervous system by direct stimulation of pulmonary receptors (Rajagopalan and Brook 2012). Short-term increases in UFP concentrations, however, may not only activate irritant receptors and therefore lead to changes in autonomic control, as suggested by Schulz and colleagues (2005), but may in addition be able to translocate rapidly from the pulmonary epithelium into the circulation and affect the heart directly, as suggested by our SDNN findings for time scales of 1 hour and less. Similarly, in further analyses of our work in Beijing, China, during the 2008 Summer Olympics (Gong et al. 2013; Huang W et al. 2012; Rich et al. 2012a; Zhang et al. 2013), Gong and colleagues (2014) examined the pattern of biomarker responses (reflecting hemostasis, pulmonary inflammation, oxidative stress, systemic inflammation, and oxidative stress) to UFPs and separately to $PM_{2.5}$ across lag days. Associations of some biomarkers with UFPs had different lag patterns compared with those for $PM_{2.5}$, suggesting that the ultrafine and fine size fractions may affect PM-induced pathophysiological pathways independently (Gong et al. 2014).

Animal data support the hypothesis of the alteration of the autonomic nervous system by air pollution (Elder et al. 2007; Godleski et al. 2000; Rhoden et al. 2005). Controlled-exposure studies in which human volunteers are exposed to concentrated fine or ultrafine particles also support these findings. Devlin and colleagues (2003) reported that elderly individuals experienced significant decreases in HRV immediately after exposure to concentrated air pollution particles 0.1–2.5 μm in diameter; the decreases persisted at least 24 hours after exposure for some HRV parameters. These findings agreed with those of an earlier study by Devlin and colleagues (2000) in which decreased HF power was reported in elderly participants exposed to fine concentrated air pollution particles. In another study, adult volunteers (healthy or with mild asthma) exposed to concentrated ambient UFPs for 2 hours experienced a decrease in LF power (Gong et al. 2008). In a more recent study, Devlin and colleagues (2014) exposed a potentially susceptible population with metabolic syndrome to concentrated ambient UFPs. The investigators found an associated decrease, 1 hour after exposure, in HF HRV normalized to heart rate as well as an increase in the ratio of LF to HF HRV, particularly in the *GSTM1* gene null subgroup. There was also an increase in LF HRV normalized to heart rate 1 hour after exposure. No HRV changes were associated with particle mass. In these studies, HF power was used as a marker of parasympathetic modulation of heart rate, LF power as a marker of sympathetic modulation of heart rate, and the ratio of the two (HF to LF) as an index of sympatho-vagal balance (a concept that has since changed, because LF is now believed to be influenced mostly by baroreflex sensitivity). The investigators' HF HRV findings can therefore be interpreted similarly to our RMSSD findings, and the use of the HF/LF HRV ratio is similar to our use of SDNN as a marker of total HRV, representing sympathetic and vagal stimulation as well as baroreflex sensitivity.

In controlled-exposure studies, positive associations between air pollutants and HRV parameters have been reported (e.g., Samet et al. 2009). However, while there have been some inconsistencies between studies, the general consensus is that HRV is impaired by exposure to air pollutants.

Several epidemiological studies have found inverse associations between short-term increases in ambient air pollution and HRV (Brook et al. 2010; Pieters et al. 2012; Rückerl et al. 2011), supporting the idea that air pollution might disturb the autonomic function of the heart. Evidence from epidemiological studies on the effects of ambient air pollution on HRV has suggested an increase in heart rate (Liao et al. 2004; Peters et al. 1999; Pope et al.

1999) and a decrease in SDNN, RMSSD, or HF power associated with increased PM concentrations in healthy elderly individuals, individuals with coronary artery disease (CAD), or individuals with diabetes (Adar et al. 2007; Baja et al. 2013; Creason et al. 2001; Gold et al. 2000; Holguin et al. 2003; Liao et al. 1999, 2004; Luttmann-Gibson et al. 2006; Park et al. 2005; Pope et al. 1999, 2004; Schneider et al. 2010a; Zanobetti et al. 2010). In controlled-exposure studies, some have found that decreased HF power is more pronounced in people with the *GSTM1* null allele (Chahine et al. 2007). Effects were seen mainly within hours (e.g., 4- and 6-hour averages) or on the day (24-hour averages) of exposure. In particular, Zanobetti and colleagues (2010) found a –1.5% [95% CI, –2.5 to –0.4%] decrease in RMSSD, but no changes in SDNN, associated with increased 1-hour concentrations of $PM_{2.5}$ directly preceding ECG recording in patients with CAD. A study conducted in taxi drivers in Beijing detected a –2.2% [95% CI, –3.8 to –0.6%] reduction in SDNN associated with increases in 30-minute concentrations of $PM_{2.5}$ measured inside the taxicab (S Wu et al. 2010). Furthermore, a study by Adar and colleagues (2007) showed that increases in 1-hour concentrations of traffic-related $PM_{2.5}$ led to decreased SDNN and RMSSD in elderly participants. They also found an increase in heart rate associated with elevated $PM_{2.5}$ levels. Recently, Sarnat and colleagues (2014) conducted a study in 42 adults performing two 2-hour scripted highway commutes during morning rush hour in metropolitan Atlanta, Georgia. The investigators found decreases in SDNN and RMSSD (time-domain HRV measures) 3 hours after the commute but no detectable changes in frequency-domain HRV measures.

Unlike $PM_{2.5}$, UFPs are usually located in so-called hot spots (such as near busy roads). Because there are so far only a few monitoring networks in place anywhere in the world to measure particulate in this size range (Augsburg and Rochester being two such places), only a handful of epidemiology panel studies have assessed the potential adverse health effects associated with ambient UFP concentrations (Chan et al. 2004; Hampel et al. 2012a; Leitte et al. 2012; Rich et al. 2012b; Rückerl et al. 2006, 2014; Stölzel et al. 2007).

Most studies have used pollutant concentrations measured at central monitoring sites, rather than personal measurements, to estimate study subjects' air pollution exposures. Although central monitoring sites are assumed to be useful in epidemiological short-term studies, they still give only a rough background estimate of the PM exposures of a single individual. Studies examining the health effects associated with UFP concentrations, in which participants either live in or are taken to environments rich in UFPs, have primarily used personal measurements. Some of these studies have found positive associations between emissions from traffic and changes in cardiovascular end points (Delfino et al. 2008, 2010, 2011; Peters et al. 2004; Timonen et al. 2006). In general, only a few studies have investigated associations between personally measured particles (fine or ultrafine) or gaseous pollutants and ECG parameters, with inconsistent findings (Cárdenas et al. 2008; Chang et al. 2004, 2007; de Hartog et al. 2009; Fan et al. 2009; Folino et al. 2009; Huang et al. 2013; Langrish et al. 2012; Magari et al. 2002; Riojas-Rodríguez et al. 2006; Shields et al. 2013; Tarkiainen et al. 2003; Vallejo et al. 2006; Weichenthal et al. 2012; CF Wu et al. 2010; S Wu et al. 2010). In particular, a study by He and colleagues (2011a) found decreases in several HRV parameters and elevations in heart rate in association with personally measured 1- to 6-hour average concentrations of $PM_{2.5}$. However, none of these studies examined 5- to 60-minute PM–HRV associations, as we did in the current study.

Although many studies have reported a decrease in HRV associated with increasing PM concentrations and an exacerbation of these adverse effects in people with insulin resistance or diabetes (Park et al. 2005; Whitsel et al. 2009), positive associations between PM and HRV have also been reported (e.g., Meier et al. 2014; Riediker et al. 2004; Schneider et al. 2010a; Wheeler et al. 2006). However, there have also been studies that detected no (personal) air pollution effects on HRV (de Hartog et al. 2009; S Wu et al. 2010).

T-WAVE COMPLEXITY

Although we found consistently decreased SDNN and RMSSD in association with increased UFP or $PM_{2.5}$ concentrations, we did not find such associations for T-wave complexity (our marker of repolarization and T-wave morphology). However, previous work by our group reported adverse changes in markers of repolarization and T-wave morphology associated with increases in particle concentrations, including increases in ambient concentrations of $PM_{2.5}$, AMP, and organic carbon in the previous 24 hours associated with increased QTc (a marker of repolarization duration), increases in $PM_{2.5}$ concentrations in the previous 24 hours associated with increased T-wave complexity, and increases in UFPs, AMP, $PM_{2.5}$, and elemental carbon in the previous 6 hours associated with decreased T-wave amplitude in a panel of patients with pre-existing coronary heart disease. Effects were generally smaller for increases in these same pollutants in the previous 6 hours

(Henneberger et al. 2005). We have also reported changes in repolarization immediately and up to 4 days after increases in ambient PM concentrations (Schneider et al. 2010b) in a panel of patients with type 2 diabetes. Previously, in our original analysis of the Rochester REHAB study, we reported decreased RMSSD, SDNN, TpTe (a late repolarization marker), heart rate turbulence, and systolic and diastolic blood pressure associated with increased UFP, AMP, and $PM_{2.5}$ concentrations in the previous few days; only RMSSD was associated with increases in any particulate pollutant (i.e., AMP) in the previous 6 hours. We did not find associations for QTc with any pollutant (Rich et al. 2012b). In a controlled UFP exposure study, we did not find significant associations between markers of repolarization or HRV and 2-hour UFP exposures in healthy young subjects, although we found trends toward blunted QTc shortening and increased variability of T-wave complexity (Zareba et al. 2009). In healthy young subjects, again, we did not find changes in repolarization after controlled UFP exposures (Frampton et al. 2004). In a panel of patients with a prior MI, we found increased QTc associated with increased ambient $PM_{2.5}$ concentrations lagged 24–47 hours and both increased T-wave amplitude (lagged 0–23 hours) and decreased T-wave amplitude (lagged 48–119 hours) associated with increased $PM_{2.5}$ concentrations (Hampel et al. 2012b). Others have also examined these associations, with conflicting results (Devlin et al. 2014; Huang YC et al. 2012; Lux and Pope 2009; Samet et al. 2009; Sivagangabalan et al. 2011; Weichenthal et al. 2012; Xu et al. 2013; Zhang et al. 2009).

In the current study, we did not find consistent changes in T-wave complexity associated with either ambient or controlled exposures to any particulate pollutant (i.e., ambient PM in mixtures of various compositions and controlled exposures both with and without gaseous copollutants). Precisely repeated ECG recording conditions (e.g., in a supine position after 15 minutes of rest) are likely needed to replicate findings across centers and studies. The four studies we re-examined did not have such uniform recording conditions (their protocols included exercise or allowed regular activity), and it is therefore not surprising that there was no agreement across the studies about the effects of air pollution on repolarization and T-wave complexity.

SUSCEPTIBLE POPULATIONS

The subjects in our four studies ranged from patients with metabolic disorders (i.e., type 2 diabetes) to healthy adults with a genetic susceptibility on the detoxifying pathways (i.e., the *GSTM1* null polymorphism), postinfarction patients with underlying cardiovascular diseases, and healthy young subjects—being studied in two different locations (Augsburg and Rochester) using two different study designs (longitudinal panel studies versus controlled-exposure studies). Our reanalysis of these studies looked at selected susceptible subgroups to provide insight into the ways in which air pollution might precipitate death or acute cardiovascular events in persons with underlying heart disease or underlying metabolic disorders, on the hypothesis that particulate air pollution can alter cardiovascular function.

Genetic factors have also been shown in various studies (e.g., Devlin et al. 2014; Schneider et al. 2008, 2010a; Schwartz et al. 2005) to play a role in responsiveness to air pollutants, especially genes involved in oxidative stress pathways, such as *GSTM1*. *GSTM1* is a phase II enzyme that can scavenge oxygen free radicals, metabolize reactive oxygen species, and detoxify xenobiotics present in PM. It is therefore plausible that people with a *GSTM1* deletion (as in our Augsburg panel of subjects with a genetic susceptibility to detoxifying pathways) are not able to handle oxidative stress well and may be more responsive to agents such as PM that increase oxidative stress.

We also chose subjects with IGT (in addition to the subjects with diabetes) because people with metabolic disorders make up as much as 30% of the U.S. population and are at risk for developing diabetes and cardiovascular disease (Ford et al. 2004). Moreover, people with type 2 diabetes have been shown to be particularly susceptible to air pollution (Zanobetti and Schwartz 2001, 2002; Zeka et al. 2006). People with diabetes are known to have disproportional reactive oxygen species formation (Maritim et al. 2003), and we speculated that this might be also true for people with IGT, with the advantage that people with IGT are not on diabetes medication yet and also have lower intake rates of other influencing medications, such as statins or beta blockers. PM has been hypothesized to cause adverse health effects through the same mechanism of oxidative stress (Donaldson et al. 2005). Inhaled particles may on the one hand lead to local oxygen radical production in the lung because of organic components on the particles or by Fenton reactions catalyzed by transition metals. On the other hand, UFPs may be translocated into the blood circulation and produce oxygen radicals (Nemmar et al. 2002, 2004; Oberdörster et al. 2002, 2004) because of their surface-chemistry-related properties through redox-sensitive pathways (Donaldson et al. 2005). A study by Schwartz and colleagues (2005) concluded that the effects of $PM_{2.5}$ on HF HRV (another marker of parasympathetic modulation, comparable to RMSSD) seemed

to be mediated by reactive oxygen species, which would increase oxidative stress. Oxidative stress can initiate further cellular responses, thereby contributing to the pathogenesis of PM-induced disease (Xia et al. 2006). Diabetes and PM may therefore share common pathways and interact to enhance responsiveness to air pollutants.

Ischemic heart disease patients, MI survivors, and patients with CAD in general are of particular interest because their cardiovascular disease might make them especially sensitive to the effects of air pollution. At the same time, however, today's treatment of CAD may buffer potential responses to particles. Nevertheless, observed changes in CAD patients indicate the potential relevance of this adverse cardiac response to particles, because small changes in cardiac function may have even more dramatic effects in susceptible CAD patients than in healthy individuals.

EFFECT MODIFICATION BY PERSONAL CHARACTERISTICS

Previous work by our group and others has investigated whether the personal characteristics of study subjects modified any association between ambient PM concentrations or controlled PM exposure and cardiovascular outcomes, including biomarkers, clinical events or hospitalizations, and mortality. These exploratory analyses may provide insight into mechanisms by which PM may cause an adverse cardiovascular event or identify susceptible populations that may require a higher level of protection by way of clinical care or regulatory standards for ambient air quality. We and others have previously reported stronger associations between increased PM concentrations and cardiovascular events or biomarkers among hypertensive patients compared with nonhypertensive patients (Gardner et al. 2014; Park et al. 2005; Peel et al. 2007), while others have not (Dubowsky et al. 2006; Lee et al. 2008; Pope et al. 2006; Wellenius et al. 2006). Similarly, there has been inconsistent evidence of effect modifications for associations between PM cardiovascular events or biomarkers by smoking status (e.g., Diez Roux et al. 2008; Liao et al. 2009; O'Neill et al. 2007; Rückerl et al. 2007), older age (i.e., > 60 years or > 65 years) (e.g., Devlin et al. 2003; Gong et al. 2004; Rich et al. 2005; Stewart et al. 2010), obesity (e.g., Chen et al. 2007; Dubowsky et al. 2006; Schneider et al. 2008, 2010b; Schwartz et al. 2005), and use of medications, including statins and beta blockers (e.g., de Hartog et al. 2009; O'Neill et al. 2007; Schwartz et al. 2005). Given the differences in study population characteristics across our four studies (i.e., underlying disease, age, and having had a recent MI and thus taking multiple medications simultaneously), it is not surprising that we did not find consistent effect modifications across the studies by these characteristics. Furthermore, the power of our studies to detect significant differences in air pollution effects by these characteristics might have been insufficient. Future multicenter studies, done using consistent protocols in similar study populations, may be needed to more clearly evaluate whether our findings for UFP and $PM_{2.5}$ effects on SDNN (and other markers of total HRV) and RMSSD (and other markers of parasympathetic modulation) are modified by older age, obesity, comorbidities, or medications.

PM IN AUGSBURG, GERMANY, AND ROCHESTER, NEW YORK

In addition to the differences in study populations described above, we considered whether PM composition or sources were different between Augsburg and Rochester and whether these might have contributed to any divergent findings. The $PM_{2.5}$ data for all four studies were provided by TEOM measurements. However, a filter dynamics measurement system was used for this purpose in Augsburg, and a standard heated TEOM was used in Rochester. Because heating results in the loss of semivolatile species such as ammonium nitrate and organic compounds, the Rochester $PM_{2.5}$ values would be correlated with the actual concentrations but would be biased low. Although somewhat different instrumentation was used for the particle size distribution measurements, the measurement principles were the same and the measurements were comparable.

In Augsburg, the composition data were for PM with an aerodynamic diameter ≤ 10 μm (PM_{10}) (Gu et al. 2013), whereas in Rochester, the composition data were for $PM_{2.5}$ (Wang et al. 2012a, 2012b). Because source-apportionment studies have been conducted in both cities, a better basis of comparison might be to examine the sources and relative source contributions of the major source types in each location. The published source-apportionment studies, outlined in Appendix T, suggest that wood smoke is an important component of the winter aerosol in both cities. There are significant amounts of secondary inorganic species in both areas, with high nitrate in winter. Sulfate contributions to PM mass concentrations differ between the two locations. In Augsburg, high amounts of sulfate were found in winter; in Rochester, the amounts of sulfate were higher in summer. Although similar source types, such as motor vehicles, were identified in both cities, there are likely differences in the nature of the emissions. In the two cities' traffic fleets, for example, the fraction of diesel

light-duty vehicles is larger in Augsburg than in Rochester. Both locations had impacts from residential and commercial combustion, including the influence of wood smoke, but the impact of coal combustion decreased in Rochester after the shutdown of a local coal-fired power plant in spring 2008. Homes in Rochester are generally heated with natural gas; wood-burning appliances are used for discretionary heating. The use of coal for heating is not common in Germany. The power plants around Augsburg are either hydroelectric or powered by natural gas. Individual homes in Augsburg use natural gas or oil for heating. Thus, the sources, and likely the composition, of PM in Augsburg and Rochester are generally similar, with some differences mostly related to traffic-fleet composition and to the fossil fuels used to generate electricity and heat.

Studies of the temporal and spatial pollutant variability in both cities have also been conducted; they are summarized in Appendix T. There was reasonable uniformity in the concentrations of secondary inorganic species (nitrate and sulfate). However, significant variability in the impacts of the local sources (e.g., wood smoke and traffic) was found in both cities, such that the cities' single central monitoring sites could not fully represent exposures across the entire areas in which the study subjects lived. We therefore expect that there are similar degrees of exposure misclassification in both cities when using these measures to represent study subjects' exposures to PM.

STRENGTHS AND LIMITATIONS

Our study had several strengths, including the use of four completed studies with ECG recordings, multiple particulate pollution measurements, data on both ambient and controlled UFP concentrations and exposures, data on personally measured UFP concentrations for 5-minute analyses, factor analysis methods to reduce the number of outcomes assessed, and a discovery-and-replication approach to make it possible to draw conclusions from similar analyses across the studies. Further, the same research cardiology group, led by Dr. Wojciech Zareba, performed all the 1-hour and 5-minute analyses of ECG recordings from the four studies.

However, our study also had several limitations to consider. First, our analyses in the UPCON and UPDIABETES studies were based on small sample sizes, leading to reduced statistical power to detect significant associations between our outcomes and the 2-hour UFP exposures. As a result, large percentage changes were often not statistically significantly different from zero. However, we also drew conclusions based on the patterns of responses across lag hours, thereby lessening the impact of this limitation. Similarly, our assessment of susceptibility by subject characteristics in the studies was to some extent limited by the number of subjects with or without each characteristic, and we thus had insufficient power to detect effect modification by these characteristics.

Second, in the Augsburg and REHAB studies we assigned each study subject the value of UFPs, AMP, $PM_{2.5}$, and BC from our central monitoring site in each city regardless of how far the subject lived, spent time, or worked from that monitor. However, these exposure errors were likely a combination of Berkson and classical errors (Bateson et al. 2007; Zeger et al. 2000). Given the classical error, then, our effect estimates were likely underestimates.

Last, the Augsburg study assessed personal measurements of PNCs, a novel marker for personal exposure to fresh combustion particles. Using direct measurements of personal PNCs also provided different and novel information compared with that provided by studies of personal $PM_{2.5}$ or gaseous pollutants (Cárdenas et al. 2008; Chang et al. 2004, 2007; de Hartog et al. 2009; Fan et al. 2009; Folino et al. 2009; He et al. 2011a; Huang et al. 2013; Langrish et al. 2012; Magari et al. 2002; Riojas-Rodríguez et al. 2006; Shields et al. 2013; Tarkiainen et al. 2003; Vallejo et al. 2006; Weichenthal et al. 2012; CF Wu et al. 2010). However, the measurement devices used to characterize personal PNCs are usually operated by technical personnel and were not designed for use by study participants. As a consequence, we only measured 80% of the planned hourly measurements despite stringent subject training and review of the instruction sessions by audiotape. The missing measurements had no regular pattern and were unrelated to diligence in following the instructions by the study participants.

IMPLICATIONS OF FINDINGS

Previous studies have reported effects of increased PM concentrations on changes in HRV and repolarization over the next few days; only a few studies have investigated whether these responses to PM occur within a few hours or even less. We investigated whether both ambient PM and controlled PM exposures were associated with immediate changes (concurrent hour up to a delay of 6 hours and within 5 to 60 minutes) in markers of cardiac rhythm by reanalyzing ECG and air pollution data from two epidemiological panel studies (Augsburg and REHAB) and two controlled-exposure studies (UPDIABETES and UPCON). Across these four studies, we were able to confirm three hypotheses: (1) that reductions in total HRV (using SDNN as a marker) are associated with increased

UFP concentrations in the previous few hours, (2) that reductions in total HRV (using SDNN as a marker) are associated with increased $PM_{2.5}$ and AMP in the previous few hours, and (3) that reductions in parasympathetic modulation of the heart (using RMSSD as a marker) are associated with increased $PM_{2.5}$ concentrations in the previous few hours. We also concluded that these effects of UFPs and $PM_{2.5}$ on SDNN and of $PM_{2.5}$ on RMSSD generally did not differ between the subgroups in our studies (i.e., subjects with diabetes or IGT, cardiac rehabilitation patients, and older and younger otherwise healthy subjects). In our meta-analysis, we found significant decreases in SDNN in association with increased UFPs and $PM_{2.5}$, with low heterogeneity between results for the two panel studies. However, when pooling air pollution effects across all four studies, there was often too much heterogeneity to develop valid pooled estimates.

The autonomic nervous system continuously controls the behavior of the cardiovascular system. Autonomic imbalance is a major contributor to the triggering of cardiac arrhythmias, and as a consequence, changes may predispose susceptible individuals to sudden cardiac death during episodes of increased PM levels (Zareba et al. 2001). Alterations in autonomic tone might contribute to the instability of a vascular plaque or initiate cardiac arrhythmia (Schulz et al. 2005). It has been shown that decreased HRV might be a precursor of several cardiovascular problems (Buccelletti et al. 2009; Elder et al. 2007; Rhoden et al. 2005), and it is a well-known marker for cardiac mortality, especially in high-risk populations such as MI patients and individuals with heart failure (Task-Force 1996).

However, we found no consistent evidence of UFP effects on parasympathetic modulation (RMSSD), no evidence of any pollutant effects on markers of T-wave complexity, and no consistent evidence of UFP effects on total HRV (SDNN), parasympathetic modulation (RMSSD), or T-wave complexity at any 5-minute interval within 60 minutes. Therefore, our findings did not support the role of changes in these outcomes mediated by UFP or $PM_{2.5}$ concentrations over time intervals shorter than 1 hour. Measurement of these outcomes on a 5-minute basis, and perhaps measurement of T-wave morphology and repolarization on an hourly basis, in both epidemiological and controlled clinical studies where subjects are physically active may not be the appropriate manner in which to examine whether UFP and $PM_{2.5}$ concentrations and exposures are associated with such changes. For future studies examining these hypotheses, resting supine measurements of the outcomes may allow better statistical evaluation of these hypotheses. Further, it is not surprising that these responses across the groups were not always consistent, given the heterogeneity of the subjects' clinical health or disease states and varied underlying pathophysiologies. One might expect that exposure to ambient pollution causes a different physiological response in T-waves in exercising subjects recovering from a recent MI than in a healthy 30-year-old exercising in a chamber during controlled PM exposures.

The fact that we also found no associations between PM concentrations or exposures and total antioxidant capacity suggests that more work is needed to understand if increases in ambient air pollution affect people's ability to protect themselves against oxidative stress. Our findings also suggest that there was no consistent modification of associations between PM and ECG outcomes by total antioxidant capacity, age, obesity, smoking, hypertension, exertion, prior MI, or medications. The diversity in study populations, air pollutant concentrations and exposures, and ECG recording conditions are likely contributors to this lack of consistency. However, our finding of associations between UFP, $PM_{2.5,}$ SDNN, and RMSSD across the studies is noteworthy.

QUALITY ASSURANCE PROCEDURES

All standard operating procedures for our ECG analyses and total antioxidant capacity analyses as well as data management procedures in both Augsburg and Rochester are outlined in Additional Materials 1. Institutional review board approval was obtained from both the University of Rochester and Helmholtz Zentrum München.

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REFERENCES

Adar SD, Gold DR, Coull BA, Schwartz J, Stone PH, Suh H. 2007. Focused exposures to airborne traffic particles and heart rate variability in the elderly. Epidemiology 18:95–103.

Albert CM, Rosenthal L, Calkins H, Steinberg JS, Ruskin JN, Wang P, et al. 2007. Driving and implantable cardioverter-defibrillator shocks for ventricular arrhythmias: results from the TOVA study. J Am Coll Cardiol 50:2233–2240.

Araujo JA. 2010. Particulate air pollution, systemic oxidative stress, inflammation, and atherosclerosis. Air Qual Atmos Health 4:79–93.

Baja ES, Schwartz JD, Coull BA, Wellenius GA, Vokonas PS, Suh HH. 2013. Structural equation modeling of parasympathetic and sympathetic response to traffic air pollution in a repeated measures study. Environ Health 12:81.

Baja ES, Schwartz JD, Wellenius GA, Coull BA, Zanobetti A, Vokonas PS, et al. 2010. Traffic-related air pollution and QT interval: modification by diabetes, obesity, and oxidative stress gene polymorphisms in the normative aging study (nas). Environ Health Perspect 115:1617–1622.

Bateson TF, Coull BA, Hubbell B, Ito K, Jerrett M, Lumley T, et al. 2007. Panel discussion review: session three—issues involved in interpretation of epidemiologic analyses—statistical modeling. J Expo Sci Environ Epidemiol 17 Suppl 2:S90–96.

Bauer A, Malik M, Barthel P, Schneider R, Watanabe MA, Camm AJ, et al. 2006. Turbulence dynamics: an independent predictor of late mortality after acute myocardial infarction. Int J Cardiol 107:42–47.

Bauer A, Malik M, Schmidt G, Barthel P, Bonnemeier H, Cygankiewicz I, et al. 2008. Heart rate turbulence: standards of measurement, physiological interpretation, and clinical use: International Society for Holter and Noninvasive Electrophysiology Consensus. J Am Coll Cardiol 52:1353–1365.

Bigger JT, Fleiss JL, Steinman RC, Rolnitzky LM, Kleiger RE, Rottman JN. 1992. Correlations among time and frequency domain measures of heart period variability two weeks after acute myocardial infarction. Am J Cardiol 69:891–898.

Borg G. 1998. Borg's Perceived Exertion and Pain Scales. Champaign, IL: Human Kinetics.

Brook RD, Franklin B, Cascio W, Hong Y, Howard G, Lipsett M, et al. 2004. Air pollution and cardiovascular disease: a statement for healthcare professionals from the expert panel on population and prevention science of the American Heart Association. Circulation 109:2655–2671.

Brook RD, Rajagopalan S, Pope CA, III, Brook JR, Bhatnagar A, Diez-Roux AV, et al. 2010. Particulate matter air pollution and cardiovascular disease: an update to the scientific statement from the American Heart Association. Circulation 121:2331–2378.

Buccelletti E, Gilardi E, Scaini E, Galiuto L, Persiani R, Biondi A, et al. 2009. Heart rate variability and myocardial infarction: systematic literature review and metanalysis. Eur Rev Med Pharmacol Sci 13:299–307.

Cárdenas M, Vallejo M, Romano-Riquer P, Ruiz-Velasco S, Ferreira-Vidal AD, Hermosillo AG. 2008. Personal exposure to $PM_{2.5}$ air pollution and heart rate variability in subjects with positive or negative head-up tilt test. Enviro Res 108:1–6.

Chahine T, Baccarelli A, Litonjua A, Wright RO, Suh H, Gold DR, et al. 2007. Particulate air pollution, oxidative stress genes, and heart rate variability in an elderly cohort. Environ Health Perspect 115:1617–1622.

Chalupa DC, Morrow PE, Oberdörster G, Utell MJ, Frampton MW. 2004. Ultrafine particle deposition in subjects with asthma. Environ Health Perspect 112:879–882.

Chan CC, Chuang KJ, Shiao GM, Lin LY. 2004. Personal exposure to submicrometer particles and heart rate variability in human subjects. Environ Health Perspect 112:1063–1067.

Chang CC, Hwang JS, Chan CC, Wang PY, Hu TH, Cheng TJ. 2004. Effects of concentrated ambient particles on heart rate, blood pressure, and cardiac contractility in spontaneously hypertensive rats. Inhal Toxicol 16:421–429.

Chang LT, Tang CS, Pan YZ, Chan CC. 2007. Association of heart rate variability of the elderly with personal exposure to PM_1, $PM_{1-2.5}$, and $PM_{2.5-10}$. Bull Environ Contam Toxicol 79:552–556.

Chen JC, Cavallari JM, Stone PH, Christiani DC. 2007. Obesity is a modifier of autonomic cardiac responses to fine metal particulates. Environ Health Perspect 115:1002–1006.

Choi JI, Kim CS. 2007. Mathematical analysis of particle deposition in human lungs: an improved single path transport model. Inhal Toxicol 19:925–939.

Couderc JP, McNitt S, Hyrien O, Vaglio M, Xia X, Polonsky S, et al. 2008. Improving the detection of subtle i(kr)-inhibition: assessing electrocardiographic abnormalities of repolarization induced by moxifloxacin. Drug Saf 31:249–260.

Creason J, Neas L, Walsh D, Williams R, Sheldon L, Liao D, et al. 2001. Particulate matter and heart rate variability among elderly retirees: the Baltimore 1998 PM study. J Expo Anal Environ Epidemiol 11:116–122.

Cyrys J, Pitz M, Heinrich J, Wichmann HE, Peters A. 2008. Spatial and temporal variation of particle number concentration in Augsburg, Germany. Sci Total Environ 401:168–175.

de Hartog JJ, Lanki T, Timonen KL, Hoek G, Janssen NA, Ibald-Mulli A, et al. 2009. Associations between $PM_{2.5}$ and heart rate variability are modified by particle composition and beta-blocker use in patients with coronary heart disease. Environ Health Perspect 117:105–111.

Delfino RJ, Gillen DL, Tjoa T, Staimer N, Polidori A, Arhami M, et al. 2011. Electrocardiographic ST-segment depression and exposure to traffic-related aerosols in elderly subjects with coronary artery disease. Environ Health Perspect 119:196–202.

Delfino RJ, Staimer N, Tjoa T, Polidori A, Arhami M, Gillen DL, et al. 2008. Circulating biomarkers of inflammation, antioxidant activity, and platelet activation are associated with primary combustion aerosols in subjects with coronary artery disease. Environ Health Perspect 116:898–906.

Delfino RJ, Tjoa T, Gillen DL, Staimer N, Polidori A, Arhami M, et al. 2010. Traffic-related air pollution and blood pressure in elderly subjects with coronary artery disease. Epidemiology 21:396–404.

Devlin RB, Cascio WE, Kehrl H, Ghio AJ. 2000. Changes in heart rate variability in young and elderly humans exposed to concentrated ambient air pollution. Toxicologist 54:316–317.

Devlin RB, Ghio AJ, Kehrl H, Sanders G, Cascio W. 2003. Elderly humans exposed to concentrated air pollution particles have decreased heart rate variability. Eur Respir J Suppl 40:76s–80s.

Devlin RB, Smith CB, Schmitt MT, Rappold AG, Hinderliter A, Graff D, et al. 2014. Controlled exposure of humans with metabolic syndrome to concentrated ultrafine ambient particulate matter causes cardiovascular effects. Toxicol Sci 140:61–72.

Diez Roux AV, Auchincloss AH, Franklin TG, Raghunathan T, Barr RG, Kaufman J, et al. 2008. Long-term exposure to ambient particulate matter and prevalence of subclinical atherosclerosis in the multi-ethnic study of atherosclerosis. Am J Epidem 167:667–675.

D'Ippoliti D, Forastiere F, Ancona C, Agabiti N, Fusco D, Michelozzi P, et al. 2003. Air pollution and myocardial infarction in Rome: a case-crossover analysis. Epidemiology 14:528–535.

Dominici F, Peng RD, Bell ML, Pham L, McDermott A, Zeger SL, et al. 2006. Fine particulate air pollution and hospital admission for cardiovascular and respiratory diseases. JAMA 295:1127–1134.

Donaldson K, Tran L, Jimenez LA, Duffin R, Newby DE, Mills N, et al. 2005. Combustion-derived nanoparticles: a review of their toxicology following inhalation exposure. Part Fibre Toxicol 2:10.

Dubowsky SD, Suh H, Schwartz J, Coull BA, Gold DR. 2006. Diabetes, obesity, and hypertension may enhance associations between air pollution and markers of systemic inflammation. Environ Health Perspect 114:992–998.

Elder A, Couderc JP, Gelein R, Eberly S, Cox C, Xia X, et al. 2007. Effects of on-road highway aerosol exposures on autonomic responses in aged, spontaneously hypertensive rats. Inhal Toxicol 19:1–12.

Fan ZT, Meng Q, Weisel C, Laumbach R, Ohman-Strickland P, Shalat S, et al. 2009. Acute exposure to elevated $PM_{2.5}$ generated by traffic and cardiopulmonary health effects in healthy older adults. J Expo Sci Environ Epidemiol 19:525–533.

Folino AF, Scapellato ML, Canova C, Maestrelli P, Bertorelli G, Simonato L, et al. 2009. Individual exposure to particulate matter and the short-term arrhythmic and autonomic profiles in patients with myocardial infarction. Euro Heart J 30:1614–1620.

Ford ES, Giles WH, Mokdad AH. 2004. Increasing prevalence of the metabolic syndrome among U.S. adults. Diabetes Care 27:2444–2449.

Frampton MW, Utell MJ, Zareba W, Oberdörster G, Cox C, Huang LS, et al. 2004. Effects of exposure to ultrafine carbon particles in healthy subjects and subjects with asthma. Research Report 126. Boston, MA:Health Effects Institute.

Gardner B, Ling F, Hopke PK, Frampton MW, Utell MJ, Zareba W, et al. 2014. Ambient fine particulate air pollution triggers ST-elevation myocardial infarction, but not non-ST elevation myocardial infarction: a case-crossover study. Part Fibre Toxicol 11:1.

Ghelfi E, Rhoden CR, Wellenius GA, Lawrence J, Gonzalez-Flecha B. 2008. Cardiac oxidative stress and electrophysiological changes in rats exposed to concentrated ambient particles are mediated by TRP-dependent pulmonary reflexes. Toxicol Sci 102:328–336.

Godleski JJ, Verrier RL, Koutrakis P, Catalano P, Coull B, Reinisch U, et al. 2000. Mechanisms of morbidity and mortality from exposure to ambient air particles. Research Report 91. Boston, MA:Health Effects Institute.

Gold DR, Litonjua A, Schwartz J, Lovett E, Larson A, Nearing B, et al. 2000. Ambient pollution and heart rate variability. Circulation 101:1267–1273.

Gong H Jr, Linn WS, Clark KW, Anderson KR, Sioutas C, Alexis NE, et al. 2008. Exposures of healthy and asthmatic volunteers to concentrated ambient ultrafine particles in Los Angeles. Inhal Toxicol 20:533–545.

Gong H Jr, Linn WS, Terrell SL, Clark KW, Geller MD, Anderson KR, et al. 2004. Altered heart-rate variability in asthmatic and healthy volunteers exposed to concentrated ambient coarse particles. Inhal Toxicol 16:335–343.

Gong J, Zhu T, Kipen H, Wang G, Hu M, Guo Q, et al. 2014. Comparisons of ultrafine and fine particles in their associations with biomarkers reflecting physiological pathways. Environ Sci Technol 48:5264–5273.

Gong J, Zhu T, Kipen H, Wang G, Hu M, Ohman-Strickland P, et al. 2013. Malondialdehyde in exhaled breath condensate and urine as a biomarker of air pollution induced oxidative stress. J Expo Sci Environ Epidemiol 23:322–327.

Greenland P, Xie X, Liu K, Colangelo L, Liao Y, Daviglus ML, et al. 2003. Impact of minor electrocardiographic ST-segment and/or T-wave abnormalities on cardiovascular mortality during long-term follow-up. Am J Cardiol 91:1068–1074.

Gu J, Schnelle-Kreis J, Pitz M, Diemer J, Reller A, Zimmermann R, et al. 2013. Spatial and temporal variability of PM_{10} sources in Augsburg, Germany. Atmos Environ 71:131–139.

Gupta T, Demokritou P, Koutrakis P. 2004. Development and performance evaluation of a high-volume ultrafine particle concentrator for inhalation toxicological studies. Inhal Toxicol 16:851–862.

Hampel R, Breitner S, Schneider A, Zareba W, Kraus U, Cyrys J, et al. 2012a. Acute air pollution effects on heart rate variability are modified by snps involved in cardiac rhythm in individuals with diabetes or impaired glucose tolerance. Environ Res 112:177–185.

Hampel R, Breitner S, Zareba W, Kraus U, Pitz M, Geruschkat U, et al. 2012b. Immediate ozone effects on heart rate and repolarisation parameters in potentially susceptible individuals. Occup Environ Med 69:428–436.

He F, Shaffer ML, Li X, Rodriguez-Colon S, Wolbrette DL, Williams R, et al. 2011a. Individual-level $PM_{2.5}$ exposure and the time course of impaired heart rate variability: the APACR study. J Expo Sci Environ Epidemiol 21:65–73.

He F, Shaffer ML, Rodriguez-Colon S, Yanosky JD, Bixler E, Cascio WE, et al. 2011b. Acute effects of fine particulate air pollution on cardiac arrhythmia—the APACR study. Environ Health Perspect 119:927–932.

Henneberger A, Zareba W, Ibald-Mulli A, Rückerl R, Cyrys J, Couderc JP, et al. 2005. Repolarization changes induced by air pollution in ischemic heart disease patients. Environ Health Perspect 113:440–446.

Holguin F, Tellez-Rojo MM, Hernandez M, Cortez M, Chow JC, Watson JG, et al. 2003. Air pollution and heart rate variability among the elderly in Mexico City. Epidemiology 14:521–527.

Huang J, Deng F, Wu S, Lu H, Hao Y, Guo X. 2013. The impacts of short-term exposure to noise and traffic-related air pollution on heart rate variability in young healthy adults. J Expo Sci Environ Epidemiol 23:559–564.

Huang W, Wang G, Lu SE, Kipen H, Wang Y, Hu M, et al. 2012. Inflammatory and oxidative stress responses of healthy young adults to changes in air quality during the Beijing Olympics. Am J Respir Crit Care Med 186:1150–1159.

Huang YC, Rappold AG, Graff DW, Ghio AJ, Devlin RB. 2012. Synergistic effects of exposure to concentrated ambient fine pollution particles and nitrogen dioxide in humans. Inhal Toxicol 24:790–797.

Jacobsen MD, Wagner GS, Holmvang L, Macfarlane PW, Näslund U, Grande P, et al. 2001. Clinical significance of abnormal T waves in patients with non-ST-segment elevation acute coronary syndromes. Am J Cardiol 1;88(11):1225–1229.

Jacquemin B, Antoniades C, Nyberg F, Plana E, Muller M, Greven S, et al. 2008. Common genetic polymorphisms and haplotypes of fibrinogen alpha, beta, and gamma chains affect fibrinogen levels and the response to proinflammatory stimulation in myocardial infarction survivors: the AIRGENE study. J Am Coll Cardiol 52:941–952.

Kim CS, Jaques PA. 2000. Respiratory dose of inhaled ultrafine particles in healthy adults. Phil Trans R Soc A 358:2693–2705.

Kolz M, Koenig W, Muller M, Andreani M, Greven S, Illig T, et al. 2008. DNA variants, plasma levels and variability of C-reactive protein in myocardial infarction survivors: results from the AIRGENE study. Eur Heart J 29:1250–1258.

Kors JA, De Bruyne MC, Hoes AW, van Herpen G, Hofman A, van Bemmel JH, et al. 1998. T-loop morphology of cardiac events in the elderly. J Electrocardiol 31(suppl):54–59.

Langrish JP, Li X, Wang S, Lee MM, Barnes GD, Miller MR, et al. 2012. Reducing personal exposure to particulate air pollution improves cardiovascular health in patients with coronary heart disease. Environ Health Perspect 120:367–372.

Laumbach RJ, Rich DQ, Gandhi S, Amorosa L, Schneider S, Zhang J, et al. 2010. Acute changes in heart rate variability in subjects with diabetes following a highway traffic exposure. J Occup Environ Med 52:324–331.

Lee IM, Tsai SS, Ho CK, Chiu HF, Wu TN, Yang CY. 2008. Air pollution and hospital admissions for congestive heart failure: are there potentially sensitive groups? Environ Res 108:348–353.

Leitte AM, Schlink U, Herbarth O, Wiedensohler A, Pan XC, Hu M, et al. 2012. Associations between size-segregated particle number concentrations and respiratory mortality in Beijing, China. Int J Environ Health Res 22:119–133.

Li N, Sioutas C, Cho A, Schmitz D, Misra C, Sempf J, et al. 2003. Ultrafine particulate pollutants induce oxidative stress and mitochondrial damage. Environ Health Perspect 111:455–460.

Liao D, Creason J, Shy C, Williams R, Watts R, Zweidinger R. 1999. Daily variation of particulate air pollution and poor cardiac autonomic control in the elderly. Environ Health Perspect 107:521–525.

Liao D, Duan Y, Whitsel EA, Zheng ZJ, Heiss G, Chinchilli VM, et al. 2004. Association of higher levels of ambient criteria pollutants with impaired cardiac autonomic control: a population-based study. Am J Epidemiol 159:768–777.

Liao D, Shaffer ML, Rodriguez-Colon S, He F, Li X, Wolbrette DL, et al. 2010. Acute adverse effects of fine particulate air pollution on ventricular repolarization. Environ Health Perspect 118:1010–1015.

Liao D, Whitsel EA, Duan Y, Lin HM, Quibrera PM, Smith R, et al. 2009. Ambient particulate air pollution and ectopy—the environmental epidemiology of arrhythmogenesis in Women's Health Initiative Study, 1999–2004. J Toxicol Environ Health A 72:30–38.

Lin KB, Shofer FS, McCusker C, Meshberg E, Hollander JE. 2008. Predictive value of T-wave abnormalities at the time of emergency department presentation in patients with potential acute coronary syndromes. Acad Emerg Med 15(6):537–543.

Luttmann-Gibson H, Suh HH, Coull BA, Dockery DW, Sarnat SE, Schwartz J, et al. 2006. Short-term effects of air pollution on heart rate variability in senior adults in Steubenville, Ohio. J Occup Environ Med 48:780–788.

Lux RL, Pope CA 3rd. 2009. Air pollution effects on ventricular repolarization. Research Report 141. Boston, MA: Health Effects Institute.

Magari SR, Schwartz J, Williams PL, Hauser R, Smith TJ, Christiani DC. 2002. The association between personal measurements of environmental exposure to particulates and heart rate variability. Epidemiology 13:305–310.

Malik M, Camm AJ. 1995. Heart Rate Variability. Boston, MA:Wiley-Blackwell, 1–543.

Maritim AC, Sanders RA, Watkins JB 3rd. 2003. Diabetes, oxidative stress, and antioxidants: a review. J Biochem Mol Toxicol 17:24–38.

McDonald JD, Powell HQ, Bowen LE, Mauderly JL. 2001. Organic carbon content of aerosols produced from a Palas carbon generation system [Abstract]. Toxicologist 60:460.

Meier R, Cascio WE, Ghio AJ, Wild P, Danuser B, Riediker M. 2014. Associations of short-term particle and noise exposures with markers of cardiovascular and respiratory health among highway maintenance workers. Environ Health Perspect 122:726–732.

Mills NL, Tornqvist H, Gonzalez MC, Vink E, Robinson SD, Soderberg S, et al. 2007. Ischemic and thrombotic effects of dilute diesel-exhaust inhalation in men with coronary heart disease. N Engl J Med 357:1075–1082.

Nemmar A, Hoet PH, Vanquickenborne B, Dinsdale D, Thomeer M, Hoylaerts MF, et al. 2002. Passage of inhaled particles into the blood circulation in humans. Circulation 105:411–414.

Nemmar A, Hoylaerts MF, Hoet PH, Nemery B. 2004. Possible mechanisms of the cardiovascular effects of inhaled particles: systemic translocation and prothrombotic effects. Toxicol Lett 149:243–253.

Oberdörster G, Sharp Z, Atudorei V, Elder A, Gelein R, Kreyling W, et al. 2004. Translocation of inhaled ultrafine particles to the brain. Inhal Toxicol 16:437–445.

Oberdörster G, Sharp Z, Atudorei V, Elder A, Gelein R, Lunts A, et al. 2002. Extrapulmonary translocation of ultrafine carbon particles following whole-body inhalation exposure of rats. J Toxicol Environ Health A 65:1531–1543.

Okin PM, Devereux RB, Fabsitz RR, Lee ET, Galloway JM, Howard BV. 2002. Principal component analysis of the T wave and prediction of cardiovascular mortality in American Indians. Circulation 105:714–719.

O'Neill MS, Veves A, Sarnat JA, Zanobetti A, Gold DR, Economides PA, et al. 2007. Air pollution and inflammation in type 2 diabetes: a mechanism for susceptibility. Occup Environ Med 64:373–379.

Park SK, O'Neill MS, Vokonas PS, Sparrow D, Schwartz J. 2005. Effects of air pollution on heart rate variability: the VA normative aging study. Environ Health Perspect 113:304–309.

Park SK, O'Neill MS, Wright RO, Hu H, Vokonas PS, Sparrow D, et al. 2006. HFE genotype, particulate air pollution, and heart rate variability: a gene-environment interaction. Circulation 114:2798–2805.

Peel JL, Metzger KB, Klein M, Flanders WD, Mulholland JA, Tolbert PE. 2007. Ambient air pollution and cardiovascular emergency department visits in potentially sensitive groups. Am J Epidem 165:625–633.

Peng RD, Dominici F, Welty LJ. 2009. A Bayesian hierarchical distributed lag model for estimating the time course of risk of hospitalization associated with particulate matter air pollution. J R Stat Soc Ser C Appl Stat 58:3–24.

Peters A. 2011. Ambient particulate matter and the risk for cardiovascular disease. Prog Cardiovasc Dis 53:327–333.

Peters A, Dockery DW, Muller JE, Mittleman MA. 2001. Increased particulate air pollution and the triggering of myocardial infarction. Circulation 103:2810–2815.

Peters A, Greven S, Heid IM, Baldari F, Breitner S, Bellander T, et al. 2009. Fibrinogen genes modify the fibrinogen response to ambient particulate matter. Am J Respir Crit Care Med 179:484–491.

Peters A, Perz S, Doring A, Stieber J, Koenig W, Wichmann HE. 1999. Increases in heart rate during an air pollution episode. Am J Epidem 150:1094–1098.

Peters A, Schneider A, Greven S, Bellander T, Forastiere F, Ibald-Mulli A, et al. 2007. Air pollution and inflammatory response in myocardial infarction survivors: gene-environment-interactions in a high-risk group. Inhal Toxicol 19(suppl 1):161–165.

Peters A, Veronesi B, Calderón-Garcidueñas L, Gehr P, Chen LC, Geiser M, et al. 2006. Translocation and potential neurological effects of fine and ultrafine particles a critical update. Part Fibre Toxicol 3:13.

Peters A, von Klot S, Heier M, Trentinaglia I, Cyrys J, Hormann A, et al. 2005. Particulate air pollution and nonfatal cardiac events. Part 1. Air pollution, personal activities, and onset of myocardial infarction in a case-crossover study. Research Report 124. Boston, MA:Health Effects Institute.

Peters A, von Klot S, Heier M, Trentinaglia I, Hormann A, Wichmann HE, et al. 2004. Exposure to traffic and the onset of myocardial infarction. N Engl J Med 351:1721–1730.

Pieters N, Plusquin M, Cox B, Kicinski M, Vangronsveld J, Nawrot TS. 2012. An epidemiological appraisal of the association between heart rate variability and particulate air pollution: a meta-analysis. Heart 98:1127–1135.

Pitz M, Birmili W, Schmid O, Peters A, Wichmann HE, Cyrys J. 2008a. Quality control and quality assurance for particle size distribution measurements at an urban monitoring station in Augsburg, Germany. J Environ Monitor 10:1017–1024.

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

Pope CA, Dockery DW. 2006. Health effects of fine particulate air pollution: lines that connect. J Air Waste Manag Assoc 56:709–742.

Pope CA III, Hansen ML, Long RW, Nielsen KR, Eatough NL, Wilson WE, et al. 2004. Ambient particulate air pollution, heart rate variability, and blood markers of inflammation in a panel of elderly subjects. Environ Health Perspect 112:339–345.

Pope CA, Muhlestein JB, May HT, Renlund DG, Anderson JL, Horne BD. 2006. Ischemic heart disease events triggered by short-term exposure to fine particulate air pollution. Circulation 114:2443–2448.

Pope CA, Verrier RL, Lovett EG, Larson AC, Raizenne ME, Kanner RE, et al. 1999. Heart rate variability associated with particulate air pollution. Am Heart J 138:890–899.

Porthan K, Viitasalo M, Toivnen L, Havulinna AS, Jula A, Tikkanen JT, et al. 2013. Predictive value of electrocardiographic T-wave morphology parameters and T-wave peak to T-wave end interval for sudden cardiac death in the general population. Circ Arrhythm Electrophysiol 6(4):690–696.

Priori SG, Mortara DW, Napolitano C, Diehl L, Paganini V, Cantu F, et al. 1997. Evaluation of the spatial aspects of T-wave complexity in the long-QT syndrome. Circulation 96:3006–3012.

Rajagopalan S, Brook RD. 2012. Air pollution and type 2 diabetes: mechanistic insights. Diabetes 61:3037–3045.

Rautaharju PM, Zhang ZM, Haisty WK Jr, Prineas RJ, Kucharska-Newton AM, Rosamond WD, et al. 2013. Electrocardiographic predictors of incident heart failure in men and women free from manifest cardiovascular disease (from the Atherosclerosis Risk in Communities [ARIC] study). Am J Cardiol 112(6):843–849.

Rhoden CR, Wellenius GA, Ghelfi E, Lawrence J, Gonzalez-Flecha B. 2005. PM-induced cardiac oxidative stress and dysfunction are mediated by autonomic stimulation. Biochim Biophys Acta 1725:305–313.

Rich DQ, Kipen HM, Huang W, Wang G, Wang Y, Zhu P, et al. 2012a. Association between changes in air pollution levels during the Beijing Olympics and biomarkers of inflammation and thrombosis in healthy young adults. JAMA 307:2068–2078.

Rich DQ, Schwartz J, Mittleman MA, Link M, Luttmann-Gibson H, Catalano PJ, et al. 2005. Association of short-term ambient air pollution concentrations and ventricular arrhythmias. Am J Epidem 161:1123–1132.

Rich DQ, Kipen HM, Zhang J, Kamat L, Wilson AC, Kostis JB. 2010. Triggering of transmural infarctions, but not nontransmural infarctions, by ambient fine particles. Environ Health Perspect 118:1229–1234.

Rich DQ, Zareba W, Beckett W, Hopke PK, Oakes D, Frampton MW, et al. 2012b. Are ambient ultrafine, accumulation-mode, and fine particles associated with adverse cardiac responses in patients undergoing cardiac rehabilitation? Environ Health Perspect 120:1162–1169.

Riediker M, Cascio WE, Griggs TR, Herbst MC, Bromberg PA, Neas L, et al. 2004. Particulate matter exposure in cars is associated with cardiovascular effects in healthy young men. Am J Respir Crit Care Med 169:934–940.

Riojas-Rodríguez H, Escamilla-Cejudo JA, González-Hermosillo JA, Téllez-Rojo MM, Vallejo M, Santos-Burgoa C, et al. 2006. Personal $PM_{2.5}$ and CO exposures and heart rate variability in subjects with known ischemic heart disease in Mexico City. J Expo Sci Environ Epidem 16:131–137.

Roden DM. 2008. Keep the QT interval: it is a reliable predictor of ventricular arrhythmias. Heart Rhythm 5:1213–1215.

Rückerl R, Greven S, Ljungman P, Aalto P, Antoniades C, Bellander T, et al. 2007. Air pollution and inflammation (interleukin-6, C-reactive protein, fibrinogen) in myocardial infarction survivors. Environ Health Perspect 115:1072–1080.

Rückerl R, Hampel R, Breitner S, Cyrys J, Kraus U, Carter J, et al. 2014. Associations between ambient air pollution and blood markers of inflammation and coagulation/fibrinolysis in susceptible populations. Environ Int 70:32–49.

Rückerl R, Ibald-Mulli A, Koenig W, Schneider A, Woelke G, Cyrys J, et al. 2006. Air pollution and markers of inflammation and coagulation in patients with coronary heart disease. Am J Resp Crit Care Med 173:432–441.

Rückerl R, Schneider A, Breitner S, Cyrys J, Peters A. 2011. Health effects of particulate air pollution: a review of epidemiological evidence. Inhal Toxicol 23:555–592.

Samet JM, Rappold A, Graff D, Cascio WE, Berntsen JH, Huang YC, et al. 2009. Concentrated ambient ultrafine particle exposure induces cardiac changes in young healthy volunteers. Am J RespirCrit Care Med 179:1034–1042.

Sarnat JA, Golan R, Greenwald R, Raysoni AU, Kewada P, Winquist A, et al. 2014. Exposure to traffic pollution, acute inflammation and autonomic response in a panel of car commuters. Environ Res 133C:66–76.

Schmidt G, Malik M, Barthel P, Schneider R, Ulm K, Rolnitzky L, et al. 1999. Heart-rate turbulence after ventricular premature beats as a predictor of mortality after acute myocardial infarction. Lancet 353:1390–1396.

Schneider A, Hampel R, Ibald-Mulli A, Zareba W, Schmidt G, Schneider R, et al. 2010a. Changes in deceleration capacity of heart rate and heart rate variability induced by ambient air pollution in individuals with coronary artery disease. Part Fibre Toxicol 7:29.

Schneider A, Neas LM, Graff DW, Herbst MC, Cascio WE, Schmitt MT, et al. 2010b. Association of cardiac and vascular changes with ambient $PM_{2.5}$ in diabetic individuals. Part Fibre Toxicol 7:14.

Schneider A, Neas L, Herbst MC, Case M, Williams RW, Cascio W, et al. 2008. Endothelial dysfunction: associations with exposure to ambient fine particles in diabetic individuals. Environ Health Perspect 116:1666–1674.

Schulz H, Harder V, Ibald-Mulli A, Khandoga A, Koenig W, Krombach F, et al. 2005. Cardiovascular effects of fine and ultrafine particles. J Aerosol Med 18:1–22.

Schwartz J, Park SK, O'Neill MS, Vokonas PS, Sparrow D, Weiss S, et al. 2005. Glutathione-S-transferase M1, obesity, statins, and autonomic effects of particles: gene-by-drug-by-environment interaction. Am J Respir Crit Care Med 172:1529–1533.

Shields KN, Cavallari JM, Hunt MJ, Lazo M, Molina M, Molina L, et al. 2013. Traffic-related air pollution exposures and changes in heart rate variability in Mexico City: a panel study. Environ Health 12:7.

Sivagangabalan G, Spears D, Masse S, Urch B, Brook RD, Silverman F, et al. 2011. The effect of air pollution on spatial dispersion of myocardial repolarization in healthy human volunteers. J Am Coll Cardiol 57:198–206.

Stewart JC, Chalupa DC, Devlin RB, Frasier LM, Huang LS, Little EL, et al. 2010. Vascular effects of ultrafine particles in persons with type 2 diabetes. Environ Health Perspect 118:1692–1698.

Stölzel M, Breitner S, Cyrys J, Pitz M, Wölke G, Kreyling W, et al. 2007. Daily mortality and particulate matter in different size classes in Erfurt, Germany. J Expo Sci Environ Epidemiol 17:458–467.

Sunyer J, Pistelli R, Plana E, Andreani M, Baldari F, Kolz M, et al. 2008. Systemic inflammation, genetic susceptibility and lung function. Eur Respir J 32:92–7.

Tarkiainen TH, Timonen KL, Vanninen EJ, Alm S, Hartikainen JE, Pekkanen J. 2003. Effect of acute carbon monoxide exposure on heart rate variability in patients with coronary artery disease. Clin Physiol Funct Imaging 23:98–102.

Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. 1996. Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. Eu Heart J 17:354–381.

Timonen KL, Vanninen E, de Hartog J, Ibald-Mulli A, Brunekreef B, Gold DR, et al. 2006. Effects of ultrafine and fine particulate and gaseous air pollution on cardiac autonomic control in subjects with coronary artery disease: the ultra study. J Expo Sci Environ Epidemiol 16:332–341.

Utell MJ, Frampton MW, Zareba W, Devlin RB, Cascio WE. 2002. Cardiovascular effects associated with air pollution: potential mechanisms and methods of testing. Inhal Toxicol 14(12):1231–1247.

Vaglio M, Couderc JP, McNitt S, Xia X, Moss AJ, Zareba W. 2008. A quantitative assessment of T-wave morphology in LQT1, LQT2, and healthy individuals based on Holter recording technology. Heart Rhythm 5:11–18.

Vallejo M, Ruiz S, Hermosillo AG, Borja-Aburto VH, Cárdenas M. 2006. Ambient fine particles modify heart rate variability in young healthy adults. J Expo Sci Environ Epidemiol 16:125–130.

van Houwelingen HC, Arends LR, Stijnen T. 2002. Advanced methods in meta-analysis: multivariate approach and meta-regression. Stat Med 21:589–624.

Vora R, Zareba W, Utell MJ, Pietropaoli AP, Chalupa D, Little EL, et al. 2014. Inhalation of ultrafine carbon particles alters heart rate and heart rate variability in people with type 2 diabetes. Part Fibre Toxicol 11:31.

Wang Y, Hopke PK, Chalupa DC, Utell MJ. 2010. Long-term characterization of indoor and outdoor ultrafine particles at a commercial building. Environ Sci Technol 44:5775–5780.

Wang Y, Hopke PK, Chalupa DC, Utell MJ. 2011a. Effect of the shutdown of a coal-fired power plant on urban ultrafine particles and other pollutants. Aerosol Sci Tech 45:1245-1249.

Wang Y, Hopke PK, Chalupa DC, Utell MJ. 2011b. Long-term study of urban ultrafine particles and other pollutants. Atmos Eviron 45:7672–7680.

Wang Y, Hopke PK, Rattigan OV, Chalupa DC, Utell MJ. 2012a. Multiple-year black carbon measurements and source apportionment using delta-C in Rochester, New York. J Air Waste Manag Assoc 62:880–887.

Wang Y, Hopke PK, Xia X, Chalupa DC, Zhang Y, Utell MJ. 2012b. Source apportionment using positive matrix factorization on daily measurements of organic and inorganic speciated $PM_{2.5}$. Atmos Environ 55:525–532.

Wasserman EB, Zareba W, Utell MJ, Oakes D, Hopke PK, Frampton M, et al. 2014. Acute changes in ambient temperature are associated with adverse changes in cardiac rhythm. Air Qual Atmos Health 7:357–367.

Weichenthal S, Kulka R, Belisle P, Joseph L, Dubeau A, Martin C, et al. 2012. Personal exposure to specific volatile organic compounds and acute changes in lung function and heart rate variability among urban cyclists. Environ Res 118:118–123.

Wellenius GA, Schwartz J, Mittleman MA. 2006. Particulate air pollution and hospital admissions for congestive heart failure in seven United States cities. Am J Cardiol 97:404–408.

Wheeler A, Zanobetti A, Gold DR, Schwartz J, Stone P, Suh HH. 2006. The relationship between ambient air pollution and heart rate variability differs for individuals with heart and pulmonary disease. Environ Health Perspect 114:560–566.

Whitsel EA, Quibrera PM, Christ SL, Liao D, Prineas RJ, Anderson GL, et al. 2009. Heart rate variability, ambient particulate matter air pollution, and glucose homeostasis: the environmental epidemiology of arrhythmogenesis in the Women's Health Initiative. Am J Epidemiol 169:693–703.

Widdicombe JG. 1982. Pulmonary and respiratory tract receptors. J Exp Biol 100:41–57.

Widdicombe J, Lee LY. 2001. Airway reflexes, autonomic function, and cardiovascular responses. Environ Health Perspect 109 (Suppl 4):579–584.

Wu CF, Kuo IC, Su TC, Li YR, Lin LY, Chan CC, et al. 2010. Effects of personal exposure to particulate matter and ozone on arterial stiffness and heart rate variability in healthy adults. Am J Epidemiol 171:1299–1309.

Wu S, Deng F, Niu J, Huang Q, Liu Y, Guo X. 2010. Association of heart rate variability in taxi drivers with marked changes in particulate air pollution in Beijing in 2008. Environ Health Perspect 118:87–91.

Xhyheri B, Manfrini O, Mazzolini M, Pizzi C, Bugiardini R. 2012. Heart rate variability today. Prog Cardiovasc Dis 55:321–331.

Xia T, Kovochich M, Nel A. 2006. The role of reactive oxygen species and oxidative stress in mediating particulate matter injury. Clin Occup Environ Med 5:817–836.

Xu MM, Jia YP, Li GX, Liu LQ, Mo YZ, Jin XB, et al. 2013. Relationship between ambient fine particles and ventricular repolarization changes and heart rate variability of elderly people with heart disease in Beijing, China. Biomed Environ Sci 26:629–637.

Zabel M, Acar B, Klingenheben T, Franz MR, Hohnloser SH, Malik M. 2000. Assessment of QT dispersion for prediction of mortality or arrhythmic events after myocardial infarction. Circulation 102:1252–1257.

Zabel M, Klingenheben T, Franz MR, Hohnloser SH. 1998. Assessment of QT dispersion for prediction of mortality or arrhythmic events after myocardial infarction. Circulation 97:2543–2550.

Zanobetti A, Gold DR, Stone PH, Suh HH, Schwartz J, Coull BA, et al. 2010. Reduction in heart rate variability with traffic and air pollution in patients with coronary artery disease. Environ Health Perspect 118:324–330.

Zanobetti A, Schwartz J. 2001. Are diabetics more susceptible to the health effects of airborne particles? Am J Respir Crit Care Med 164:831–833.

Zanobetti A, Schwartz J. 2002. Cardiovascular damage by airborne particles: are diabetics more susceptible? Epidemiology 13:588–592.

Zanobetti A, Schwartz J. 2005. The effect of particulate air pollution on emergency admissions for myocardial infarction: a multicity case-crossover analysis. Environ Health Perspect 113:978–982.

Zanobetti A, Stone PH, Speizer FE, Schwartz JD, Coull BA, Suh HH, et al. 2009. T-wave alternans, air pollution and traffic in high-risk subjects. Am J Cardiol 104:665–670.

Zareba W, Couderc JP, Oberdörster G, Chalupa D, Cox C, Huang LS, et al. 2009. ECG parameters and exposure to carbon ultrafine particles in young healthy subjects. Inhal Toxicol 21:223–233.

Zareba W, Nomura A, Couderc JP. 2001. Cardiovascular effects of air pollution: what to measure in ECG? Environ Health Perspect 109(Suppl 4):533–538.

Zeger SL, Thomas D, Dominici F, Samet JM, Schwartz J, Dockery D, et al. 2000. Exposure measurement error in time-series studies of air pollution: concepts and consequences. Environ Health Perspect 108:419–426.

Zeka A, Zanobetti A, Schwartz J. 2006. Individual-level modifiers of the effects of particulate matter on daily mortality. Am J Epidemiol 163:849–859.

Zhang J, Zhu T, Kipen H, Wang G, Huang W, Rich D, et al. 2013. Cardiorespiratory biomarker responses in healthy young adults to drastic air quality changes surrounding the 2008 Beijing Olympics. Research Report 174. Boston, MA:Health Effects Institute.

Zhang ZM, Whitsel EA, Quibrera PM, Smith RL, Liao D, Anderson GL, et al. 2009. Ambient fine particulate matter exposure and myocardial ischemia in the environmental epidemiology of arrhythmogenesis in the Women's Health Initiative (EEAWHI) study. Environ Health Perspect 117:751–756.

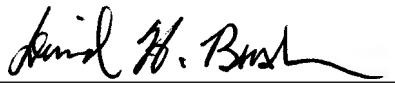
HEI QUALITY ASSURANCE STATEMENT

The conduct of this study was subjected to an independent audit by Mr. David Bush of T&B Systems, Inc. Mr. Bush is an expert in quality assurance for air quality monitoring studies and data management. The audit included a review of data quality for conformance to the study protocol as detailed in the final report. The dates of the audit are listed below, along with the phase of the study examined.

QUALITY ASSURANCE AUDITS

Date	Phase of Study
November/ December 2015	The final report was reviewed, including verification of data quality for each of the study components. The audit concentrated on the study's data management activities and included a review of the study's data sets. Several data points were traced through the entire data processing sequence to verify the integrity of the data sets. Recommendations resulting from the audit primarily dealt with resolving minor issues noted when comparing report content with study data sets. All issues were addressed by the authors.

Written reports of the audit were provided to the HEI project manager, who transmitted the findings to the Principal Investigator. The quality assurance audit demonstrated that the study was conducted by an experienced team with a high concern for data quality. Study personnel were responsive to audit recommendations, providing formal responses that adequately addressed all issues. The report appears to be an accurate representation of the study.



David H. Bush, Quality Assurance Officer

MATERIALS AVAILABLE ON THE WEB

The Appendices and Additional Materials 1 contain supplemental material not included in the printed report. They are available on the HEI Web site *http://pubs.healtheffects.org*.

APPENDICES (A THROUGH T)

Appendix A. Total Antioxidant Capacity

Appendix B. Factor Analysis Methods and Results

Appendix C. Meta-Analyses

Appendix D. Distribution of 5-Minute and 1-Hour ECG Parameters in Augsburg Study

Appendix E. Distribution of 1-Hour ECG Parameters in REHAB Study

Appendix F. Distribution of 5-Minute and 1-Hour ECG Parameters in UPDIABETES Study

Appendix G. Distribution of 5-Minute and 1-Hour ECG Parameters in UPCON Study

Appendix H. Percent Change in Augsburg ECG Outcomes Associated with Each IQR Increase in Concurrent and Lagged Air Pollutant Concentrations

Appendix I. Percent Change in REHAB ECG Outcomes Associated with Each IQR Increase in Concurrent and Lagged Air Pollutant Concentrations

Appendix J. Percent Change in REHAB ECG Outcomes Associated with Each Augsburg IQR Increase in Concurrent and Lagged Air Pollutant Concentrations in Participants in the REHAB Study

Appendix K. Percent Change in hourly UPDIABETES ECG Outcomes Associated with Each IQR Increase in Concurrent and Lagged Total PNCs

Appendix L. Percent Change in hourly UPCON ECG Outcomes Associated with Each IQR Increase in Concurrent and Lagged Total PNCs

Appendix M. Percent Change in Augsburg 5-Minute ECG Outcomes Associated with Each IQR Increase in Concurrent and Lagged 5-Minute Personal UFP Concentrations

Appendix N. Percent Change in 5-Minute ECG Outcomes Associated with Each IQR Increase in 5-Minute Mean Total PNCs in the UPDIABETES and UPCON Studies

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ABBREVIATIONS AND OTHER TERMS

AIC	Akaike information criterion
AMP	PM with an aerodynamic diameter of 100–500 nm; also known as accumulation-mode PM
BC	black carbon
BMI	body mass index
BRS	baroreflex sensitivity
CAD	coronary artery disease
CI	confidence interval
ECG	electrocardiogram
GSTM1	glutathione S-transferase M1
HF	high-frequency (0.15–0.40 Hz) power
HRV	heart rate variability
HUCAPS	Harvard ultrafine concentrated ambient particle system
IGT	impaired glucose tolerance (also known as prediabetes)
IQR	interquartile range
LF	low-frequency (0.04–0.15 Hz) power
MI	myocardial infarction
NN	normal-to-normal interval
PM	particulate matter
$PM_{2.5}$	PM with an aerodynamic diamater ≤ 2.5 μm; also known as fine PM
PM_{10}	PM with an aerodynamic diameter ≤ 10 μm
PNC	particle number concentration
PNN50	percentage of NN intervals longer than 50 msec
PNS	parasympathetic nervous system
P-spline	penalized spline
QTc	corrected QT interval
RMSSD	root mean square of the successive differences (between adjacent NN beat intervals)
RR	R-wave to R-wave (interbeat) interval
SDNN	standard deviation of NN beat intervals
SNP	single-nucleotide polymorphism
SNS	sympathetic nervous system
TEOM	tapered element oscillating microbalance
UFP	PM with an aerodynamic diameter ≤ 100 nm; also known as ultrafine PM
U.S. EPA	U.S. Environmental Protection Agency
VLF	very-low-frequency (0.0033 to 0.04 Hz) power

Research Report 186, *Ambient and Controlled Particle Exposures as Triggers for Acute ECG Changes*, D.Q. Rich and A. Peters et al.

INTRODUCTION

A large number of epidemiologic studies have reported associations between increases in exposure to particulate matter (PM*) from combustion sources and higher rates of cardiovascular mortality and hospitalization in health-compromised individuals, such as those with lung or heart disease, and in older adults.

An active area of research has aimed to identify the pathophysiologic mechanisms responsible for these epidemiologic associations. Evidence from panel and toxicologic studies has suggested that PM can affect many biologic pathways, including inducing low-grade systemic inflammation and oxidative stress, altering the control of cardiac rhythm (generally assessed through analyses of electrocardiograms [ECGs]), causing dysregulation of vascular function, and shifting the hemostatic balance toward a procoagulant state.

On the basis of the epidemiologic findings, many governmental agencies have set regulatory standards or guidelines for ambient PM classified by aerodynamic diameter. In the United States, the Environmental Protection Agency (EPA) has promulgated National Ambient Air Quality Standards for particles ≤ 2.5 µm in aerodynamic diameter ($PM_{2.5}$, also referred to as fine PM). Some scientists believe that the smallest particles within this range—ultrafine particles (UFPs), defined as having an aerodynamic diameter of ≤ 0.1 µm—have properties that may make them especially toxic. These properties include the ability to penetrate deep into the respiratory tree, a very high surface area for a given mass, and possibly the ability to cross the alveolar epithelial membrane and directly enter the circulation (reviewed in HEI Review Panel 2013). Thus, there is interest in understanding whether exposures to UFPs are associated with cardiovascular outcomes independently from exposures to $PM_{2.5}$.

In April 2011, in response to HEI's Request for Preliminary Applications 10-3: Health Effects of Air Pollution, Dr. Annette Peters (of Helmholtz Zentrum München) and Dr. David Rich (of the University of Rochester) submitted a proposal entitled "Ambient and Controlled Exposure to Ultrafine and Fine Particles as Triggers for Immediate Changes in Heart Rate, Heart Rate Variability, and Repolarization and the Role of Effect Modification by Antioxidants." The goal of the proposed study was to reanalyze ECGs from participants in earlier panel and controlled-exposure studies conducted by the applicants in Augsburg (Germany) and Rochester (New York) to examine the associations between recent exposure (i.e., within 5 minutes to 6 hours) to $PM_{2.5}$ and UFPs and changes in cardiac rhythm parameters. Another goal was to evaluate associations between levels of particles and the antioxidant capacity of stored blood samples from the same individuals. The HEI Health Research Committee was interested in the application because it would provide a cost-effective assessment of acute effects of fine and ultrafine PM on cardiac electrophysiology on a shorter timescale than most previous studies. However, the Committee was concerned about the lack of a detailed analytic plan and about the stability of the stored blood samples. Once the investigators addressed these concerns, the Committee recommended the study for funding. For practical reasons, the contract was set up with the University of Rochester, and Dr. Rich became the principal investigator of record.

This Critique is intended to aid the sponsors of HEI and the public by highlighting both the strengths and limitations of the study and by placing it into scientific perspective.

SCIENTIFIC BACKGROUND

The analysis of changes in ECGs parameters has been a focus of attention in studies of air pollution and health for

Drs. Rich and Peters's two-year study, "Ambient and Controlled Particle Exposures as Triggers for Acute ECG Changes and the Role of Antioxidant Status," began in March 2012. Total expenditures were $253,538. The draft Investigators' Report from Rich, Peters, and colleagues was received for review in August 2014. A revised report, received in May 2015, was accepted for publication in June 2015. During the review process, the HEI Health Review Committee and the investigators had the opportunity to exchange comments and to clarify issues in both the Investigators' Report and the Review Committee's Critique. (As a coinvestigator of the report, Dr. Mark Frampton was not involved in its evaluation by the Review Committee.)

* A list of abbreviations and other terms appears at the end of the Investigators' Report.

more than two decades. This section provides some background on what information can be obtained from an ECG and what is known about the associations between ECG changes and exposure to PM.

ANALYSIS OF AN ECG

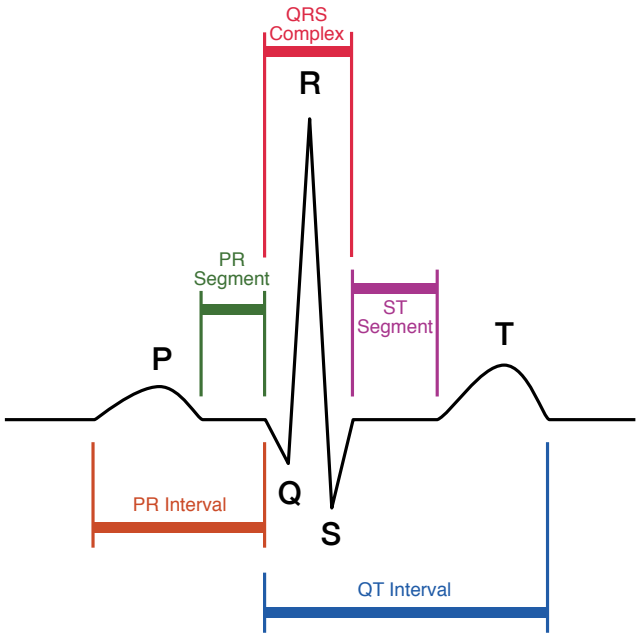
An ECG is a recording of the aggregate electrical activity of cells in the heart. The recording is made by way of several electrodes, or leads, that are placed on the skin across a person's chest, thus providing a noninvasive approach to monitoring the electrical activity of the heart. There is typically a close correspondence between the electrical activity of the heart, its structure, and its mechanical function. Thus, ECGs can be used to assess changes in cardiac structure or function over time in an individual, to help diagnose clinical cardiovascular disease, and to identify individuals at heightened risk of subsequent cardiovascular events. In the setting of environmental epidemiologic and controlled-exposure studies, specific parameters derived from ECGs can be used as intermediate cardiovascular outcomes and to shed light on the mechanisms by which environmental exposures may alter cardiovascular risk.

ECG tracings look like a series of peaks and troughs that are called waves and reflect the ensemble electrical activity of the heart over time. When a Holter monitor is used, the ECG runs continuously for a period of time, enabling the observations of changes. Critique Figure 1 shows the waves typically observed in association with a single heartbeat in a healthy adult. The P wave represents the electrical depolarization of the atria, the subsequent QRS complex represents the electrical depolarization of the two ventricles, and the T-wave signals the repolarization or electrical recovery of the ventricles. In the absence of disease, each of these electrical waves is followed a short time later by mechanical events corresponding to atrial and ventricular contraction, resulting in the pumping of blood through the systemic and pulmonary circulations. The clinical and research utility of ECGs comes from understanding the connections between cardiac structure, electrical function, and mechanical function. An abnormally wide or biphasic P-wave, for example, may indicate delayed conduction across the atria, suggesting certain forms of structural heart disease. Changes in the height of the ST segment may reflect abnormalities in ventricular repolarization and indicate heightened risk of life-threatening cardiac arrhythmias. The analysis of ECG recordings thus provides information on heart rate and heart rhythm. This section summarizes the most common ECG variables measured in environmental epidemiologic and human controlled-exposure studies and their interpretation. For a list of these variables see Critique Table 1.

ANALYSIS OF HEART BEAT

The autonomic nervous system is responsible for controlling the involuntary functions of the human body, such as those of the internal organs, including the heart. It consists of the parasympathetic nervous system (PNS), which is generally characterized as controlling the body's "rest-and-digest" functions, and the sympathetic nervous system (SNS), which is generally characterized as controlling the body's "fight-or-flight" responses. The heart is under the dual control of these two systems such that increased SNS activity or decreased PNS activity both generally result in higher heart rates.

Importantly, the frequency at which a heart beats is not constant but rather varies from beat to beat. This beat-to-beat variation is termed heart rate variability (HRV) and provides a marker of the relative balance between SNS and PNS activity. Lower HRV (i.e., less variation in heart rate from one beat to the next) can result from increased SNS activity or lower PNS activity and has been associated with increased risk of cardiovascular events in



Critique Figure 1. Schematic representation of an ECG curve with identification of specific waves and intervals. ("SinusRhythmLabels" created by Agateller [Anthony Atkielski], converted to svg by atom.-en:Image:SinusRhythmLabels.png. Licensed under public domain via Wikimedia Commons *https://commons.wikimedia.org/wiki/File:SinusRhythmLabels.svg#/media/File:SinusRhythmLabels.svg. https://en.wikipedia.org/wiki/ST segment.*)

Critique Table 1. Summary of the Principal ECG Variables

ECG variables[a]	Unit	Description
NN	ms	Interval between the QRS peaks of two successive normal heartbeats. Its reciprocal is a marker of instantaneous heart rate.
SDNN	ms	Standard deviation of all NN intervals. A marker of total heart rate variability.
RMSSD	ms	Square root of the mean of the square of the differences between adjacent NN intervals. Generally considered a marker of parasympathetic nervous system influence on the heart.
Deceleration capacity		An index calculated from selected NN intervals and thought to reflect baroreceptor reflex sensitivity.
LF	ms^2	Spectral power of HRV in the low-frequency range (0.04 to 0.15 Hz). Generally believed to reflect a combination of sympathetic nervous system influences on the heart and baroreceptor reflex sensitivity.
HF	ms^2	Spectral power of HRV in the high-frequency range (0.15 to 0.4 Hz). Generally considered a marker of parasympathetic nervous system influence on the heart and typically correlated with RMSSD.
Total power	ms^2	A marker of total heart rate variability. Highly correlated with SDNN.
QRS complex		Includes the Q, R, and S waves and represents electrical depolarization of the ventricles. Both the width and the shape of the QRS complex can provide clinical insights into cardiac pathophysiology.
QT interval (and QTc)	ms	The time interval from the beginning of the QRS complex to the end of the T-wave. Lengthening of the QT interval, adjusted for changes in heart rate (QTc), is associated with increased arrhythmia risk.
T-wave form	ms	Represents repolarization of the ventricles. The duration (msec), amplitude (uV), and morphology (referred to as "complexity," measured in each beat by principal component analysis and expressed as %) of this wave can provide useful insights into cardiac pathophysiology.
ST segment		The segment between the end of the QRS complex and the beginning of the T-wave. Deviations of the ST segment from the isoelectric line can indicate myocardial ischemia.

[a] All were measured in this study except ST segment.

multiple populations (Task Force 1996). As such, HRV provides a useful noninvasive marker of SNS–PNS balance, and reduced HRV may serve as an intermediate marker of cardiovascular risk.

Several indices of HRV can be derived from an ECG. The first step in the analysis of HRV is to create a time-series of all the interbeat intervals—that is, the time between the QRS complexes of every pair of sequential normal beats (RR intervals, typically termed normal-to-normal [NN] intervals to denote that only normal heart beats are to be included in the analyses). The reciprocal of an NN interval can be considered an instantaneous heart rate. The simplest measure of HRV is calculated by taking the standard deviation of all NN intervals (SDNN) in a given time period. SDNN provides a measure of total HRV. The root mean square of successive differences between adjacent NN intervals (RMSSD) is an index of HRV that is thought to primarily reflect PNS activity. SDNN and RMSSD are known as time-domain indices of HRV. Other indices of HRV can also be calculated based on partitioning the power spectra of the time-series of interbeat intervals (so-called frequency-domain indices). The high-frequency (HF) component of HRV is believed to reflect PNS activation of the heart; the low-frequency (LF) component is believed to be an indicator of SNS activity and baroreceptor reflex (or baroreflex) sensitivity (BRS) (Zareba et al. 2001). A number of other time- or frequency-domain indices have appeared in the literature, but heart rate, SDNN, RMSSD, LF, and HF remain the most commonly used ones.

Much of the beat-to-beat variation in heart rate is believed to be caused by the baroreflex, which helps maintain systemic blood pressure constant over short intervals. Arterial baroreceptors in the carotid sinus and arterial arch are sensitive to short-term changes in blood pressure,

such that a drop in blood pressure results in activation of the SNS and decreased PNS activity, which in turn increases heart rate and cardiac contractility, thereby restoring blood pressure. BRS is a measure of how much heart rate changes in response to a blood pressure drop of a given magnitude. A strong baroreceptor reflex (i.e., high BRS) is an indication of heightened parasympathetic activity and has been associated with lower risk of sudden death in postmyocardial infarction patients (La Rovere et al. 1998). HRV has been reported to be a predictor of mortality following acute myocardial infarction (Task Force 1996), and the combination of HRV and BRS may more accurately identify patients at high risk (La Rovere et al. 2001). The gold-standard method for assessing BRS involves intra-arterial administration of vasoactive drugs and is impractical in most epidemiologic studies. However, insights into BRS can be obtained noninvasively from beat-to-beat changes in heart rate (La Rovere et al. 2008; Parati et al. 2000).

Deceleration capacity and acceleration capacity are relatively new measures of HRV (calculated by processing sequences of NN intervals) that characterize the overall capacity of the heart rate to respond to autonomic nervous system changes. They have been shown to be stronger predictors of mortality after myocardial infarction than HRV (Bauer et al. 2006; Schneider et al. 2010) and are thought to reflect BRS.

All these variables are usually calculated over short recording periods (5 minutes) as well as over 24-hour periods.

ANALYSIS OF WAVEFORMS

All of the preceding measures are based on the interval between successive normal heartbeats. But ECGs also reveal that there are important variations between and within individuals over time in the morphology of ECG waveforms. Important ECG variables include the duration and morphology of the QRS complex, the QT interval (the section of the ECG between the start of the QRS and the end of the T-wave), the morphology of the T-wave, and the height of the ST segment.

During the cardiac cycle, arrhythmic risk is greatest during the phase of ventricular repolarization. Aberrant electrical currents or stimuli when the ventricles are incompletely repolarized can result in life-threatening cardiac arrhythmias such as ventricular tachycardia and ventricular fibrillation. A number of indices attempt to capture the heterogeneity in repolarization, including T-wave alternans (a marker of how much T-waves change from beat to beat) and QT-interval and T-wave heterogeneity (markers of how much repolarization varies in various parts of the ventricular myocardium.)

ASSOCIATIONS BETWEEN ECG CHANGES AND PM EXPOSURE

A large number of epidemiologic studies have shown an association between ambient concentrations of $PM_{2.5}$ and cardiovascular mortality and morbidity, including increased risk of myocardial infarction, cardiac arrhythmias, heart failure, ischemic heart disease, and stroke (Araujo and Brook 2011; Bhaskaran et al. 2011; Brook et al. 2010).

Based on these results, HRV and other EGC variables have been the focus of attention as subclinical changes that could provide insights into the pathways by which PM mediates adverse cardiovascular events. Other lines of research have focused on vascular function and prothrombotic markers (Brook et al. 2010; Donaldson et al. 2013).

The hypothesized pathways by which $PM_{2.5}$ and UFPs could act on the cardiac system include an indirect pathway involving oxidative stress and inflammation in the lung leading to subsequent systemic inflammation and a direct pathway through translocation of UFPs into the circulation or through the interaction of both $PM_{2.5}$ and UFPs with pulmonary sensory C-fibers (Brook et al. 2010; Donaldson et al. 2013). These fibers are unmyelinated afferent fibers of the vagus nerve and contain receptors that can respond to PM and other irritants (Ghelfi et al. 2008; Olshansky et al. 2008). This type of response would reflect a parasympathetic vagal response (Pieters et al. 2012).

There is an extensive literature on the associations between $PM_{2.5}$ and HRV. A recent meta-analysis of 29 epidemiologic studies (panel, cross-sectional, and repeated-measure studies) that met the criteria for inclusion concluded that increases in $PM_{2.5}$ in the previous 24 hours were associated with decreases in HRV as reflected in SDNN, RMSSD, HF, and LF (Pieters et al 2012.) The authors felt that these effects suggested an overall sympathetic response triggered by a systemic stress response. The analysis combined results from both 5-minute and 24-hour averages of the ECG recordings. A few studies (mostly panel studies) have investigated the associations between particle number (which largely represents UFP number) and ECG parameters. Overall the associations betwenn UFPs and HRV were not observed consistently, but when effects were present they were similar to those of $PM_{2.5}$ (HEI Review Panel 2013). Associations with other ECG variables in epidemiologic studies have seldom been reported. For UFPs most of the research has been conducted at the institutions of the two principal investigators of the study presented in this report.

In summary, the existing literaturature suggests that analysis of HRV is a useful tool for noninvasively investigating the modulation of the PNS and SNS systems by PM. Other ECG markers have also proved useful for gaining insights into the mechanisms by which inhaled PM can rapidly increase the risk of cardiac events.

AIMS

The overall goal of the study was to reanalyze existing EGCs from four completed studies to evaluate the associations between short-term increases (from 5 minutes up to 6 hours) in exposure to fine and ultrafine PM and changes in cardiac rhythm in various groups of individuals: healthy subjects, individuals with diabetes or impaired glucose tolerance (IGT) or without the glutathione S-transferase M1 (*GSTM1*) gene (which is involved in the detoxification of products of oxidative stress and xenobiotics), and patients with acute coronary artery syndromes. The investigators were interested in studying the effects of PM exposure of short durations on cardiac function changes in groups of individuals with different underlying health conditions. The primary aims to be addressed were as follows:

Aim 1. To assess immediate ECG responses in association both with ambient air pollution (UFPs and $PM_{2.5}$) and with controlled UFP exposure.

Aim 2. To assess the ability of selected individual subject characteristics and physical exertion to modify the associations between air pollution and ECG parameters.

Aim 3. To assess antioxidant capacity in association with air pollution, both as an outcome and as an effect modifier.

The third aim is discussed in Appendix A of the Investigators' Report and briefly discussed later in this Critique but is not presented in the Methods and Results.

STUDY DESIGN AND METHODS

The study relied on data collected in four past studies conducted by the two research groups: the Augsburg panel study (consisting of two subgroups of individuals, one with diabetes or IGT and one with genetic susceptibility to oxidative stress), the Rochester REHAB panel study (consisting of subjects with coronary artery disease), and two Rochester controlled-exposure studies (UPCON, with healthy subjects; and UPDIABETES, with subjects with diabetes). These are summarized in Critique Table 2.

EXPOSURE METRICS

For the two panel studies, the investigators included the following in their analyses:

- For $PM_{2.5}$ mass concentration, 1-hour averages of ambient measurements at a fixed monitoring site
- For UFP number concentrations, both 1-hour average concentrations measured at a central monitoring site (Augsburg) or outside the rehab clinic (Rochester) and 5-minute averages of personal exposure measurements based on the total number concentration (Augsburg only); these measurements included the total number of particles rather than just UFPs.

For the two controlled-exposure studies, the investigators included:

- Both 1-hour and 5-minute averages for UFP number concentrations during the 2-hour chamber exposures (no $PM_{2.5}$ measurements were available). The UPDIABETES UFPs consisted of elemental carbon and had an average size of 32 ± 2 nm; the UPCON UFPs consisted of concentrated ambient particles and had an average size of 89 ± 7 nm.

These metrics are also summarized in Critique Table 2.

The investigators also conducted some analyses of the Augsburg and Rochester panel studies using accumulation mode particles (particles between 100 and 1000 nm in aerodynamic diameter) and black carbon as the exposure metrics. The results of these analyses generally tracked the results with $PM_{2.5}$.

ANALYSES OF ECG PARAMETERS

The participants wore a Holter monitor during the study periods, which varied depending on the study. In the Augsburg study the 109 participants wore the Holter during a 6-hour period when they pursued their daily routines. In the Rochester REHAB study the 76 participants wore the Holter during the period when they were at the clinic for rehabilitation, including the exercise period (approximately 2–3 hours in total). In the two controlled-exposure studies the participants wore the Holter during a 24-hour period, some of which was spent at the clinic and some at home. The segments of the Holter

Critique Table 2. Summary of Studies Analyzed and Exposure Metrics

			Exposure metrics		
Study	Number and Type of Subjects	Length of Holter Recording	Pollutant Measures	Mean Number (n/cm^3) and Mass ($\mu g/m^3$) Concentration	IQR
Augsburg panel study	**109 patients total**: 64 with diabetes or IGT, (mean age 66.1 years) and 45 with genetic susceptibility (mean age 55.5) years	5- to 6-hour period while the subjects pursued their daily routine (up to 4 repeated visits per subject)	UFP number concentrations at a central monitoring site	9,518 (1-hr average)	7,157
			Particle number concentrations in personal air	21,649 (5-min average)	16,048
			$PM_{2.5}$ mass concentrations at a central monitoring site	13.7 (1-hr average)	12.3
REHAB panel study	**73 subjects** with coronary artery disease (mean age 60.2 years)	2–3 hours while at the rehab clinic: about 1 hour or more at rest and 30–45 minutes of exercise (up to 20 visits per subject)	UFP number concentrations outside the cardiac rehabilitation clinic	4,050 (1-hour average)	3,058
			$PM_{2.5}$ mass concentration at a central monitoring site	8.7 (1-hour average)	7.6
UPDIABETES controlled-exposure study	**18 subjects** with diabetes (mean age 45.7 year)	During 2 hours at rest in an exposure chamber and the following 22 hours	Elemental carbon total number concentration in the chamber	9,969,642 (2-hr average)	9,812,327
			Elemental carbon UFP mass concentration in the chamber	51 (2-hr average)[a]	
UPCON controlled-exposure study	**19 healthy subjects** (mean age 42.6 years)	During 2 hours at rest in an exposure chamber and the following 22 hours	Concentrated ambient total number concentration in the chamber	245,804 (2-hr average)	235,429
			Concentrated ambient UFP mass concentration in the chamber	158 (2-hr average)[a]	

[a] Not used in the analyses

recordings analyzed included the 2-hour exposure periods and the 6 hours after the end of the exposures.

The ECG traces from all four studies were analyzed by a team of researchers at the University of Rochester using a specialized computer program (Zareba et al. 2009). The ECG variables were averaged over sequential 5-minute or 1-hour recording periods (except for those from the REHAB study, for which the 5-minute averages were not used, because the subjects exercised, which introduced noise into the ECG parameter, making it difficult to estimate short-term values).

Factor Analysis

To reduce the number of variables to analyze in relation to air pollutants in the statistical models, the investigators performed separate factor analyses of all the hourly ECG parameters for each study and selected those that had a factor loading of 0.6 or higher and that were common to all four studies. Factor loadings represent the correlation of each variable with the given factor. Three factors were common to all four studies: overall HRV, parasympathetic modulation, QT interval, and T-wave morphology.

For each factor, the analyses yielded three common parameters (also referred to as outcomes): SDNN (a marker of overall HRV), RMSSD (a marker of parasympathetic modulation), and T-wave complexity (a marker of T-wave morphology and an indicator of repolarization). Although HF had a higher factor loading than RMSSD, as explained in the report, the investigators noted that there was a high degree of variability in HF (especially in the 5-minute averages), which would have made it difficult to detect effects of PM. The investigators thus decided to use RMSSD instead of HF.

STATISTICAL ANALYSIS

Rich, Peters, and colleagues used an additive mixed model as their basic model. The final model for each of the four studies (referred to as the confounder model) used different covariance structures and adjusted for different confounders. The confounders included various lags of temperature and relative humidity, barometric pressure, season, day of the week, and time of day. After building the confounder model, the investigators added to the model each ECG variable (1-hour or 5-minute averages) and either (a) the 1-hour average pollutant concentrations concurrent to the 1-hour ECG averages and up to 6 hours before or (b) the 5-minute average pollutant concentrations concurrent to the 5-minute ECG averages and up to 30 or 60 minutes before. The results are reported as the percent change per interquartile increases in pollutant concentrations at the various lags.

The investigators used an approach they referred to as discovery/replication to reach conclusions about whether their questions (hypotheses) were replicated, meaning that the association between a given pollutant and an ECG outcome was confirmed across studies. They stated that, in order for a research question to be replicated, two or more studies, depending on the question, needed to agree. When this condition was not met, they scored the results as suggestive, contradictory, or having no agreement.

SUMMARY OF KEY RESULTS

RESULTS FOR AIM 1

Aim 1 addressed nine specific questions (as detailed below). The results of each question are summarized under the specific hypotheses that were being tested and are listed based on the time over which the ECG variables and pollutant concentrations were averaged (i.e., 1 hour or 5 minutes). For the models including $PM_{2.5}$, only the two panel studies were analyzed, because $PM_{2.5}$ exposure data were not available for the controlled-exposure studies.

1-Hour Averages: Questions 1–6

Rich, Peters, and colleagues hypothesized that increases in UFP and $PM_{2.5}$ concentrations 1 to 6 hours before the ECG recording would be associated with decreases in SDNN and RMSSD and increases in T-wave complexity. Critique Table 3 summarizes the replicated findings.

Are adverse changes in SDNN associated with increases in concentrations of UFPs (Question 1) or $PM_{2.5}$ (Question 4) during the previous several hours? Hourly average concentrations of UFPs were associated with lower SDNN in the Augsburg study, the REHAB study, and the UPDIABETES study but, contrary to expectations, with higher SDNN in the UPCON study. Because the results were consistent across the two panel studies and one controlled-exposure study, the investigators concluded that Question 1 was replicated. Hourly average concentrations of $PM_{2.5}$ were associated with lower SDNN in the Augsburg and the Rochester REHAB panel studies. Thus, the investigator concluded that Question 4 was replicated.

Are adverse changes in RMSSD associated with increased concentrations of UFPs (Question 2) or $PM_{2.5}$ (Question 5) during the previous several hours? Across

Critique Table 3. Estimated Changes in 1-Hour Average SDNN and RMSSD per IQR Increase in 1-Hour Average UFP and $PM_{2.5}$ Concentrations (During the Previous 6 Hours)[a]

Study	**UFP and SDNN** Question 1	**$PM_{2.5}$ and SDNN** Question 4	**UFP and RMSSD** Question 2	**$PM_{2.5}$ and RMSSD** Question 5
Augsburg: Diabetes or IGT	↓ ~2% at lags 2, 3, and 4 hours	↓ 3.0%–4.6% at all lags	No statistically significant change	↓ 7.2% at concurrent hour
Augsburg: Genetic Susceptibility	↓ 1.9% and 2.3% at concurrent and 3-hour lag	↓ 3.3% at lag 2 hours	No statistically significant change	No statistically significant change
REHAB	↓ 1.15% at lag 1 hour	↓ ~2% at lags 5 and 6 hours	↓ 2.5% at lag 4 hours	↓ 2.8%–3.5% at lags 4 to 6 hours
UPDIABETES	↓ 13.2% at lag 1 hour	Not determined	No statistically significant change	Not determined
UPCON	No statistically significant change	Not determined	No statistically significant change	Not determined
Overall Conclusion	Agreement	Agreement	No agreement	Agreement

[a] ↓ = significant decrease per IQR increase in UFP or $PM_{2.5}$ concentrations.

the four studies, hourly average concentrations of UFPs were negatively associated with RMSSD only in the REHAB study, and the investigators concluded that Question 2 was not replicated. Hourly average concentrations of $PM_{2.5}$ were associated with lower RMSSD in the Augsburg study subjects with diabetes or IGT (but not in the subjects with genetic susceptibility) and in the REHAB study at some lags, and the investigators concluded that Question 5 was replicated.

Are adverse changes in T-wave complexity associated with increased UFP (Question 3) or $PM_{2.5}$ (Question 6) during the previous several hours? The results for associations between T-wave complexity and exposure to UFP or $PM_{2.5}$ were inconsistent across the studies, and the investigators therefore concluded that Questions 3 and 6 were not replicated.

5-Minute Averages: Questions 7–9

The investigators hypothesized that increases in 5-minute average UFP concentrations in the previous 30 to 60 minutes would be associated with lower 5-minute average SDNN and RMSSD and with higher 5-minute average T-wave complexity. Their analyses were restricted to measurements of personal exposure to UFPs and did not include the Rochester REHAB study. Results for SDNN and RMSSD are shown in Critique Table 4.

Are adverse changes in SDNN associated with increased concentrations of UFPs in the previous 60 minutes (Question 7)? Increases in personal 5-minute UFP counts were associated with decreases in 5-minute average SDNN in the Augsburg and UPDIABETES studies but with increases in the UPCON study, and the investigators concluded that the hypothesis was not replicated.

Are adverse changes in RMSSD associated with increased UFP in the previous 60 minutes (Question 8)? Increases in personal 5-minute UFP counts were associated with decreases in 5-minute average RMSSD in the Augsburg genetic-susceptibility panel but not in the Augsburg diabetes panel or REHAB panel. In the two controlled-exposure studies, increases in UFP counts were associated with increases in RMSSD. The authors concluded that this hypothesis was not replicated.

Are adverse changes in T-wave complexity associated with increased UFP in the previous 60 minutes (Question 9)? Increases in personal 5-minute particle counts were associated with increases in 5-minute average T-wave complexity in both Augsburg panels. Results in the two controlled-exposure studies differed. The authors thus concluded that the question was not replicated.

Two-Pollutant Model

To evaluate whether the observed associations between $PM_{2.5}$ and UFP concentrations and SDNN were robust to additional adjustment for the other pollutant, models including terms for both UFPs and $PM_{2.5}$ were applied to the Augsburg study (at lags 2 and 3 hours) and the REHAB study (at lags 1 and 5 hours). In the Augsburg study the effects "generally decreased in both groups … or … were of

Critique Table 4. Estimated Changes in 5-Minute Average SDNN and RMSSD per IQR Increase in 5-Minute Average UFP and $PM_{2.5}$ Concentrations (During the Previous 30–60 Minutes)[a]

Study	UFP and SDNN	UFP and RMSSD
Augsburg: Diabetes or IGT	↓ 0.62% in concurrent 5 minutes	No changes
Augsburg: Genetic Susceptibility	↓ At multiple lags within 30 minutes (largest was 0.93% in concurrent 5 minutes)	↓ At multiple lags within 30 minutes (largest was 0.95% in concurrent 5 minutes)
REHAB	Not analyzed	
UPDIABETES	↓ 10% at lag 35–39 minutes	↑ At almost all lags (largest was +13.2% at lag 40–44 minutes)
UPCON	↑ At all lags (largest was +4.6% at lag 45–49 minutes)	↑ At most lags (largest was +4.1% at lag 25–29 minutes)
Overall Conclusion	No agreement	No agreement

[a] ↓ = Significant decrease per IQR increase in UFP concentration; ↑ = Significant increase per IQR increase in UFP concentration.

a size similar to those in the single-pollutant models." In the REHAB study the changes were "generally smaller." The investigators concluded that the increases in both UFPs and $PM_{2.5}$ were independently associated with decreased SDNN.

RESULTS FOR AIM 2

Effect Modifications

The investigators hypothesized that age (< 60 versus > 60 years), obesity (BMI > 30), smoking status, or previous diagnosis of hypertension would modify the associations between $PM_{2.5}$ and SDNN, RMSSD, and T-wave complexity. However, there were no consistent differences in these associations by any of the groups, and none of the interaction terms were significant.

Sensitivity Analyses

The investigators conducted a number of sensitivity analyses and mostly confirmed the results of their main analyses. In one of these, the investigators applied to the REHAB study (analyses of SDNN with UFPs and $PM_{2.5}$ and of RMSSD with $PM_{2.5}$) the same model and confounders used in the analysis of the Augsburg study and concluded that there was generally "little difference in the size of these estimates" when including either set of model covariates."

HEALTH REVIEW COMMITTEE EVALUATION

In its independent review of the study, the Committee concluded that the study by Rich, Peters, and colleagues was carefully conducted and made efficient use of existing data obtained from relevant populations to address important questions about associations between markers of cardiac function and recent exposure to fine and ultrafine PM. The results were clearly presented and discussed. One of the notable study strengths was that the analyses of the ECG recordings from the separate studies were conducted by the same cardiology team. However, there were differences in the number of ECG electrodes and the software used to analyze the recordings across the studies.

The Committee agreed with the investigators' conclusions that exposure to both UFPs and $PM_{2.5}$ within 2 and 5 hours before the ECG recordings was associated with lower total HRV (i.e., 1-hour average SDNN) and that exposure to $PM_{2.5}$ within 5 hours was associated with lower parasympathetic modulation (i.e., 1-hour average RMSSD). The Committee noted that the conclusion about the $PM_{2.5}$ associations was based only on the analyses of data from the two panel studies. The magnitude of the associations was small but consistent with those reported in earlier studies. For example, the analyses of the Augsburg and Rochester studies described in the Investigators' Report showed a 2.57% decrease in SDNN for a 10-μg/m3 increase in $PM_{2.5}$ at lag 2 hours, which is larger than but in the same direction as the 0.12% decrease in SDNN for the same 10-μg/m3 increase in $PM_{2.5}$ (at lags ranging from 2 to 24 hours) reported in the recently published meta-analysis of 29 epidemiologic studies by Pieters and colleagues (2012).

With respect to the analysis of whether UFPs and $PM_{2.5}$ within 2 and 5 hours were independently associated with lower SDNN, the Committee thought that, while suggestive, the results did not convincingly establish the

presence of independent associations. The results were qualitatively similar in the one- and two-pollutant models, but the effect estimates tended to be attenuated, and several were no longer statistically significant in the two-pollutant models.

Results for T-wave complexity were for the most part null or in the opposite of the expected direction. The Committee noted that these results were difficult to interpret because the specific marker of T-wave morphology (i.e., T-wave complexity) used in the study has not previously been associated with an increased risk of adverse cardiac events or mortality. Other markers of repolarization, such as corrected QT interval (QTc) and deceleration capacity, have been associated with a risk of adverse cardiac events. These variables were measured in the current study's four underlying studies but were not chosen for analysis after the factor analysis, because they did not have significant loading. The investigators noted that in an earlier panel study in Erfurt, Germany, they observed associations between $PM_{2.5}$ and T-wave morphology, T-wave complexity, and QTc, and noted that both T-wave morphology and complexity reflect abnormalities in repolarization (Henneberger et al. 2005).

The associations between very recent increases (< 1 hour) in UFP levels and 5-minute averages of SDNN, RMSSD, and T-wave complexity were inconsistent across the studies analyzed. This could be because of the higher variability across shorter time periods, particularly in the settings where recording conditions were not standardized across studies, such as in the panel studies.

Although some earlier studies have reported different responses in subgroups of participants with underlying diseases, the current study found very few differences among the various groups. The Committee noted that some of the differences in the populations studied might have contributed to the heterogeneous results across the studies. One important finding to note is that the results from Augsburg were frequently different (qualitatively, at least) for the group with diabetes or IGT versus the healthy group with a genetic susceptibility. Other notable differences were evident, although few reached statistical significance. The number of subjects in the various subgroups was small, and the study may have lacked adequate power to detect any but the largest differences in effects across subgroups.

The Committee agreed with the investigators' assessment that the observed associations were not likely of clinical significance, but rather provided evidence of particle-related subclinical physiologic changes—and further insights into the pathophysiologic mechanisms by which air pollution may increase the risk of acute cardiovascular events. Specifically, SDNN and RMSSD are both measures of HRV that shed light on the relative activity of the sympathetic and parasympathetic autonomic nervous systems. A number of earlier studies have linked decreases in HRV with increased risk of cardiac arrhythmias and cardiovascular events, although the clinical relevance of changes in HRV from minute to minute or hour to hour remains unclear. The results presented in this report, in conjunction with the earlier literature, support the notion that short-term exposure to UFPs and $PM_{2.5}$ is associated with shifts in autonomic nervous system function, indicative of greater sympathetic activity and reduced parasympathetic activity. Although the investigators hypothesized that UFPs and $PM_{2.5}$ may affect different pathways acting at different timescales, the Committee thought that the results presented do not confirm or refute this hypothesis. Further, the Committee noted that the observed associations should not be interpreted to imply that ambient PM triggers the cardiac responses.

COMMENTS ON THE STUDY DESIGN

The Committee thought that a significant strength of the study was its novel use of previously collected ECG measurements and exposure data. However, the four underlying studies differed substantially in terms of participant characteristics and activities, as well as in the exposure atmospheres. In the Rochester REHAB study, for example, participants were indoors while wearing the Holter monitors and were exercising for part of the time. In the Augsburg study, participants performed their routine activities (both indoors and outdoors) while wearing the Holter monitors (the details of these activities were not reported). Finally, in the controlled-exposure studies, participants were exposed in a chamber during a period of 2 hours at rest.

By design, the UFP particle number and composition were vastly different in the controlled-exposure studies from those of the ambient exposures in the panel studies. The 1-hour average ambient UFP number concentrations in the Augsburg and REHAB studies ranged between 4,000 and 22,000 particles/cm^3 (depending on the location and whether it was measured in ambient or personal air). In comparison, the UPDIABETES study used laboratory-generated UFPs made of elemental carbon—much different in composition than ambient particles—with 2-hour average particle number concentrations of about 10,000,000 particles/cm^3. The average hourly and 5-minute concentrations did not vary with time. In the UPCON study the subjects were

exposed to concentrated ambient UFPs. Although these particles were more similar in composition to the ambient particles and there was some variability in concentrations during the exposure period, the 2-hour average particle number concentration was 250,000 particles/cm^3. In addition, the 2-hour average particle mass concentrations measured in the controlled-exposure studies (51 µg/m^3 in UPDIABETES and 158 µg/m^3 in UPCON) were much higher than the ambient $PM_{2.5}$ mass measured in the panel studies (between 8.7 and 13.7 µg/m^3). Given the large differences in composition and concentrations, direct comparison across studies implies untestable assumptions about the shape of the concentration–response curve over a wide range of exposure concentrations as well as about the etiologically relevant particulate constituents.

The inherent heterogeneity across the four studies represents both a strength of the current analyses and an important potential limitation. This heterogeneity is a strength because, for the hypotheses that were replicated, one can conclude that the associations observed are robust to differences in particle sources, composition, absolute levels, and duration of exposure, as well as being consistent across a range of participant characteristics. On the other hand, the Committee was concerned that the comparisons between panel and controlled-exposure studies (and formal conclusions about "replication") might have been too conservative given the very different designs (cohorts, types of exposures, and temporal aspects) of the studies. Consequently, the criterion that the results from both the panel and the controlled-exposure studies had to agree in order to replicate the questions/hypotheses may have been too rigid or represented too high a standard. However, the Committee commended the investigators for applying predefined criteria of concordance of results across the studies.

One of the original objectives of the study was to assess (1) whether air pollutant exposure was associated with higher total antioxidant capacity and (2) whether total antioxidant capacity modified the associations between air pollutants and ECG outcomes. The Committee noted that, in general, measuring oxidative stress is challenging, and the validity of available assays remains a controversial topic. With regard to the total antioxidant capacity assays used in the study, the Committee felt that insufficient information was presented about their variability and reproducibility and questioned whether the assays really provided a suitable marker of antioxidant capacity. Consequently, the Committee was not convinced that the results could be interpreted as intended and recommended moving them to an appendix of the report.

COMMENTS ON THE STATISTICAL ANALYSES

The Committee thought that the use of factor analysis to reduce the number of ECG outcome variables and the total number of analyses performed for modeling was novel and achieved the intended purpose, which was important for this study. However, the use of a separate factor analysis for each study resulted in between-study differences in the number of factors and representative outcomes for each factor, raising doubts about the generalizability of this approach to other studies where the correlation structure between outcomes may differ. In addition, as a result of factor analysis, associations with the wide range of available parameters—including some of growing interest, such as deceleration capacity (an emerging marker of BRS)—were not assessed. The Committee felt that the impact of this report on the broader literature on HRV might have been enhanced if the investigators had instead chosen a limited number of primary and secondary outcomes to consider across all studies. This alternative approach would still have alleviated potential concerns about multiple comparisons while providing results for a richer set of outcomes.

The Committee raised some concerns about the use of different statistical models to analyze the data from each of the four underlying studies. For example, different statistical models with a different set of potential confounders were used for the two panel studies, but the reasons for taking these different analytic approaches were not clear. In sensitivity analyses the investigators did apply the statistical model used in the Augsburg study to the REHAB study: the Review Committee noted that the associations between $PM_{2.5}$ and SDNN and RMSSD were attenuated compared with those of the initial REHAB study model and were no longer significant. Had the Augsburg statistical model been applied to the REHAB study in the main analyses, the conclusions of this report would have been somewhat different.

Finally, the Committee noted that the approach to the estimation of the effects of various exposure lags by fitting separate models with different lags, rather than using a constrained distributed lag model, may have missed the opportunity to show how a single "impulse" of the exposure affected the health outcome over the chosen time period.

CONCLUSIONS

This was a well-conducted study that addressed an important question about the associations between recent exposure to ultrafine or fine PM and changes in ECG parameters. The analyses supported the hypotheses that

higher exposures to UFPs and $PM_{2.5}$ were associated with lower total HRV (as assessed by SDNN) during the subsequent 2 to 5 hours and that higher exposures to $PM_{2.5}$ were associated with lower RMSSD, a marker of parasympathetic modulation. No associations were found with T-morphology, a marker of repolarization. The study did not support the hypothesis that very recent exposures (less than 1 hour) are associated with acute ECG changes.

The investigators' inability to replicate many of their findings across the four studies may have been caused, at least in part, by the pronounced differences in participant characteristics; exposure sources, compositions, and concentrations; and study designs—coupled with the stringent criteria used to evaluate whether or not a question was replicated.

Overall, the Committee agreed with the investigators' conclusion that recent (between 2 and 5 hours) exposures to UFPs and $PM_{2.5}$ are associated with subclinical alterations in markers of HRV. These conclusions are broadly consistent with a large body of earlier studies, although the analyses presented in the current study are more detailed and extensive than those in many of the earlier studies and represent an important addition to the literature. The Committee did not think that the investigators' conclusion that exposures to UFPs and $PM_{2.5}$ were independently associated with decreases in SDNN was clearly supported by the results.

The Committee also agreed with the investigators that the observed associations are not likely to be of clinical significance but rather provide evidence of particle-related subclinical physiologic changes and offer further insights into the pathophysiologic mechanisms by which air pollution increases the risk of acute cardiovascular events. The combined results from the four underlying studies increase our confidence in the use of HRV parameters as reproducible intermediate markers that are potentially relevant to the associations between air pollution and cardiovascular outcomes.

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REFERENCES

Araujo JA, Brook RD. 2011. Cardiovascular effects of particulate-matter air pollution: an overview and perspectives. In: Issues in Toxicology No. 8: Environmental Cardiology: Pollution and Heart Disease (Bhatnagar A, ed). Cambridge, UK:Royal Society of Chemistry, 76–104.

Bauer A, Kantelhardt JW, Barthel P, Schneider R, Mäkikallio T, Ulm K, et al. 2006. Deceleration capacity of heart rate as a predictor of mortality after myocardial infarction: Cohort study. Lancet 367:1674–1681.

Bhaskaran K, Hajat S, Smeeth L. 2011. Acute effects of particulate matter on the risk of myocardial infarction. In: Cardiovascular Effects of Inhaled Ultrafine and Nano-Sized Particles (Cassee F, Mills N, Newby D, eds). Hoboken, NJ:John Wiley & Sons, Inc., 23–43.

Brook RD, Rajagopalan S, Pope CA 3rd, Brook JR, Bhatnagar A, Diez-Roux AV, et al. 2010. Particulate matter air pollution and cardiovascular disease: an update to the scientific statement from the American Heart Association. Circulation 121:2331–2378.

Donaldson K, Duffin R, Langrish JP, Miller MR, Mills NL, Poland CA, et al. 2013. Nanoparticles and the cardiovascular system: a critical review. Nanomedicine 8:403–423.

Ghelfi E, Rhoden CR, Wellenius GA, Lawrence J, Gonzalez-Flecha B. 2008. Cardiac oxidative stress and electrophysiological changes in rats exposed to concentrated ambient particles are mediated by trp-dependent pulmonary reflexes. Toxicol Sci 102:328–336.

Henneberger A, Zareba W, Ibald-Mulli A, Ruckerl R, Cyrys J, Couderc JP, et al. 2005. Repolarization changes induced by air pollution in ischemic heart disease patients. Environ Health Perspect 113(4):440–446.

HEI Review Panel on Ultrafine Particles. 2013. Understanding the Health Effects of Ambient Ultrafine Particles. HEI Perspectives 3. Boston, MA:Health Effects Institute.

La Rovere MT, Bigger JT Jr, Marcus FI, Mortara A, Schwartz PJ, for the ATRAMI (Autonomic Tone and Reflexes After Myocardial Infarction) Investigators. 1998. Baroreflex sensitivity and heart-rate variability in prediction of total cardiac mortality after myocardial infarction. Lancet 351:478–484.

La Rovere MT, Pinna GD, Hohnloser SH, Marcus FI, Mortara A, Nohara R, et al. 2001. Baroreflex sensitivity and heart rate variability in the identification of patients at risk for life-threatening arrhythmias: implications for clinical trials. Circulation 103:2072–2077.

La Rovere MT, Pinna GD, Raczak G. 2008. Baroreflex sensitivity: Measurement and clinical implications. Ann Noninvasive Electrocardiol 13:191–207.

Olshansky B, Sabbah HN, Hauptman PJ, Colucci WS. 2008. Parasympathetic nervous system and heart failure: pathophysiology and potential implications for therapy. Circulation 118:863–871.

Parati G, Di Rienzo M, Mancia G. 2000. How to measure baroreflex sensitivity: from the cardiovascular laboratory to daily life. J Hypertens 18:7–19.

Pieters N, Plusquin M, Cox B, Kicinski M, Vangronsveld J, Nawrot TS. 2012. An epidemiological appraisal of the association between heart rate variability and particulate air pollution: a meta-analysis. Heart 98:1127–1135.

Schneider A, Hampel R, Ibald-Mulli A, Zareba W, Schmidt G, Schneider R, et al. 2010. Changes in deceleration capacity of heart rate and heart rate variability induced by ambient air pollution in individuals with coronary artery disease. Part Fibre Toxicol 7:29.

Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. 1996. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Circulation 93:1043–1065.

Zareba W, Couderc JP, Oberdorster G, Chalupa D, Cox C, Huang LS, et al. 2009. ECG parameters and exposure to carbon ultrafine particles in young healthy subjects. Inhal Toxicol 21:223–233.

Zareba W, Nomura A, Couderc JP. 2001. Cardiovascular effects of air pollution: what to measure in ECG? Environ Health Perspect 109 Suppl 4:533–538.

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