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Air Pollution Effects on Ventricular Repolarization

Robert L. Lux and C. Arden Pope III

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Air Pollution Effects on Ventricular Repolarization

Robert L. Lux and C. Arden Pope III

with a Critique by the HEI Health Review Committee

Research Report 141

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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 research and analyses to public and private decision makers.

HEI receives half of its core funds from the U.S. Environmental Protection Agency and half from the worldwide motor vehicle industry. Frequently, other public and private organizations in the United States and around the world also support major projects or certain research programs. HEI has funded more than 280 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 the peer-reviewed literature and in more than 200 comprehensive reports published by HEI.

HEI's independent Board of Directors consists of leaders in science and policy who are committed to fostering the public–private partnership that is central to the organization. The Health Research Committee solicits input from HEI sponsors and other stakeholders and works with scientific staff to develop a Five-Year Strategic Plan, select research projects for funding, and oversee their conduct. The Health Review Committee, which has no role in selecting or overseeing studies, works with staff to evaluate and interpret the results of funded studies and related research.

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 141, *Air Pollution Effects on Ventricular Repolarization*, presents a research project funded by the Health Effects Institute and conducted by Robert L. Lux of the University of Utah School of Medicine, and C. Arden Pope III of the Department of Economics, Brigham Young University. This 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 Lux and Pope, describes the scientific background, aims, methods, results, and conclusions of the study.

The Critique is prepared by members of the Health Review Committee with the assistance of HEI staff; it 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 141

Air Pollution Effects on Ventricular Repolarization

BACKGROUND

Epidemiologic studies have described an association between increases in cardiovascular morbidity and mortality and increases in ambient particulate matter (PM) concentrations, especially in susceptible populations such as the elderly and persons with pulmonary or cardiovascular disease. However, the biological mechanisms by which PM and other air pollutants may affect cardiovascular events are not well understood. Several studies have shown that air pollution may be associated with cardiac outcomes such as arrhythmias, suggesting that air pollutants may affect autonomic control of the heart.

Heart rate variability (HRV) is an electrophysiologic parameter that can be derived from an electrocardiogram (ECG). Low HRV is a marker of poor cardiac autonomic control and is associated with a higher risk of myocardial infarction and sudden cardiac death. Several cohort and panel studies have shown that increases in ambient PM concentrations are associated with decreased HRV, although it remains unclear how decreased HRV contributes to cardiac arrhythmia. Thus, the evaluation of additional ECG parameters, such as ventricular repolarization — the process of electrical recovery of the heart cells after contraction of the ventricles — is warranted.

Robert Lux of the University of Utah and Arden Pope of Brigham Young University proposed to study the association between air pollution and ventricular repolarization, using a novel approach developed by Dr. Lux to analyze ECG data previously collected by Dr. Pope. HEI funded the study because it considered the proposed evaluation of repolarization using novel electrophysiologic parameters to be an interesting approach to studying possible mechanisms underlying PM effects on cardiovascular morbidity and mortality.

APPROACH

Drs. Lux and Pope hypothesized that high levels of ambient PM might affect ventricular repolarization independent of heart rate and that such changes could lead to an increased risk of arrhythmia in elderly persons, who may be more susceptible to the effects of air pollution. They used the ECG data obtained previously in a study of elderly participants conducted by Dr. Pope, which had shown an association between ambient concentrations of particulate matter less than 2.5 μm in diameter ($PM_{2.5}$) and decreased HRV. In the current study, the investigators analyzed novel repolarization parameters in ECGs obtained from 61 participants. For each person, the ECG parameters were compared between days of high and low $PM_{2.5}$ concentrations. If there were more than 2 recordings for a participant, the ECG obtained on the day with the lowest $PM_{2.5}$ concentration served as the control.

Dr. Lux used custom software to calculate a root mean square (RMS) ECG, which captures the magnitude of the ECG wave forms. He then determined RMS ECG repolarization parameters such as RMS RT interval, RMS width of the T wave, and RMS QT interval, as well as their heart rate-corrected forms. Data were averaged over 30-minute periods ("epochs") and presented as a 24-hour time series. Two statistical approaches were used to evaluate differences between parameters on days with low versus high $PM_{2.5}$ concentrations: a nonparametric Kolmogorov-Smirnov test and within-subject regression modeling. Fifty-two participants were included in the Kolmogorov-Smirnov analysis; 61 participants were included in the regression modeling.

RESULTS AND INTERPRETATIONS

This study broadly confirmed previous findings that increased concentrations of $PM_{2.5}$ were

This Statement, prepared by the Health Effects Institute, summarizes a research project funded by HEI and conducted by Dr. Robert L. Lux at the Division of Cardiology, University of Utah School of Medicine, Salt Lake City, Utah, and Dr. C. Arden Pope III at the Department of Economics, College of Family, Home, and Social Sciences, Brigham Young University, Provo, Utah. Research Report 141 contains both the detailed Investigators' Report and a Critique on the study prepared by the Institute's Health Review Committee.

associated with decreased HRV. However, the novel approach to measuring ventricular repolarization did not find much evidence for an association between repolarization and $PM_{2.5}$ concentration using the Kolmogorov-Smirnov analysis, although some suggestive patterns were observed (e.g., a decrease in RT interval and an increase in T-wave width), which were of borderline significance when fixed-effects modeling was used. No significant changes were observed in the QT interval or its rate-corrected form, which is widely used in clinical settings and is associated with increased risk for arrhythmias.

As discussed by the investigators, there are several possible reasons for the lack of strong evidence for an association between $PM_{2.5}$ and ventricular repolarization in this study, one of which is that the study may have had insufficient statistical power to find subtle effects among large daily variations within and between participants. In addition, although state of the art, the method used in this study was developed based on 12-lead ECGs; however, in this study, only 2 leads were used in the ECG recordings, which may have affected the quality of the data. Further, as discussed by the investigators, the original study population was not controlled for diet, medication use, or activity patterns. Those factors are known to affect cardiac physiology and may contribute to considerable variation among participants.

The Health Review Committee considered the overall strategy in this study for statistical analysis — specifically, reducing the ECG data to 30-minute epoch summaries and subsequent analysis using regression methods — to be a good one. The regression modeling results are more robust than the Kolmogorov-Smirnov analysis and should be considered the primary statistical approach because they control for factors other than pollution that could affect ECG parameters, such as differences in participant characteristics and meteorologic measures. In contrast, results of Kolmogorov-Smirnov analysis on epoch data pooled across participants do not control for those factors and should be interpreted cautiously.

The Committee agreed with the investigators that the regression results do not provide clear support for an effect of air pollution on repolarization. However, the presence of significant associations of air pollution with some repolarization parameters suggests that repolarization should not be dismissed as a possible mechanism involved in the association between air pollution and cardiac effects. Further studies controlling for other sources of variability are needed to provide a more definitive answer.

INVESTIGATORS' REPORT

Air Pollution Effects on Ventricular Repolarization

Robert L. Lux and C. Arden Pope III

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ABSTRACT

We conducted a retrospective study of a set of previously published electrocardiographic data to investigate the possible direct association between levels of particulate air pollution and changes in *ventricular repolarization*—the cardiac electrophysiologic process that manifests itself as the T wave* of the electrocardiogram (ECG†) and that is definitively linked to and responsible for increased arrhythmogenesis. The published findings from this data set demonstrated a clear cardiac effect, namely, a reduction in heart rate variability (HRV) parameter values with increased levels of particulate air pollution (Pope et al. 2004), suggesting possible arrhythmogenic effects. Given this positive finding and the well-established sensitivity of cardiac repolarization to physiologic, pharmacologic, and neurologic interventions, and in light of emerging novel tools for assessing repolarization, we hypothesized that high levels of particulate air pollution would alter repolarization independent of changes in heart rate and, consequently, would increase arrhythmogenic risk. The likely mechanism of any deleterious effects on repolarization would be alteration of sodium, calcium, and potassium channels. The channel's structure, function, and kinetics are responsible for generating the cellular action potentials, which, when summed over the entire heart, result in the waves recorded by the ECG. A positive finding would provide evidence that increased levels of air pollution may be directly linked to increases in arrhythmogenic risk and, potentially, sudden cardiac death.

The study population consisted of 88 nonsmoking, elderly subjects in whom multiple, continuous, 24-hour, 2-channel ECG recordings were collected, along with blood samples to evaluate inflammatory mechanisms (not pursued in the current study). The concentration of fine particulate matter ($PM_{2.5}$, particulate matter with an aerodynamic diameter ≤ 2.5 μm) in daily samples was measured or estimated and used to trigger recording sessions for days considered to have "low" or "high" $PM_{2.5}$ concentrations. Each subject participated in one to five recordings over the study period, and all subjects lived within the greater Salt Lake Valley in Utah. We reanalyzed these recordings using custom software that incorporated a magnitude function of the ECG—the root mean square of all recorded leads (RMS ECG)—to determine the following for each beat in the 24-hour recording: cycle length (RR); RR dispersion; the interval between the RMS R- and T-wave peaks (RT), a robust estimate of mean duration of ventricular action potential; the width of the RMS T wave (TW), a robust estimate of the range of repolarization times that relates to repolarization dispersion and arrhythmogenesis; the RMS QT interval (QT) measured from the QRS onset to T-wave offset of the RMS ECG; and the regression slopes of RT versus RR, QT versus RR, and TW versus RR, which provide estimates of so-called repolarization restitution, or rate dependency of repolarization, which also is associated with arrhythmogenesis.

The study findings did not support the original hypothesis and demonstrated a lack of sensitivity of repolarization to changes in $PM_{2.5}$ concentrations. None of the repolarization variables showed a statistically significant change between days of low and high $PM_{2.5}$ concentrations, although we observed statistically significant differences for some variables using fixed-effects modeling. However, we did find a significant decrease in the standard deviation of cycle length, in concert with findings in the original study that showed a decrease in HRV parameter values. There

* Electrocardiogram waves are labeled P, Q, R, S, T, and U by convention.

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

This Investigators' Report is one part of Health Effects Institute Research Report 141, 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. Robert Lux, University of Utah, CVRTI, 95 South 2000 East, Salt Lake City, UT 84112-5000.

Although this document was produced with partial funding by the U. S. Environmental Protection Agency under Assistance Award CR–83234701 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.

was a slight but statistically insignificant increase in the width of the TW between recordings from days of low and days of high $PM_{2.5}$, suggesting that, in a setting of prolonged exposure to high levels of PM, the original hypothesis might be supported.

We conclude that in this study the short-term (day-to-day) differences in air pollution, specifically $PM_{2.5}$ concentration, did not affect ventricular repolarization. A likely explanation for the negative result is that the day-to-day variability of repolarization (arising from autonomic influences, activity, and heart rate) far outweighs the changes that might be induced by air pollution, if any. In addition, the study may have been underpowered. The findings do not refute the possibility of the deleterious repolarization effects of PM, particularly over prolonged periods of exposure, but suggest the need for exposure studies that provide better controls. In light of recent studies, it is also likely that in an at-risk population — for example, patients compromised with heart disease — repolarization changes may be more apparent.

INTRODUCTION

The deleterious health effects of air pollution have been known and studied for many years, but only in the last decade have studies focused specifically on the heart. A search of the Medline database reveals only 23 citation titles having both the words "air pollution" and "heart" in the years 1997 through 2000, but 128 between 2000 and 2007. A primary aim of many of these studies has been to find a positive association between increased levels of air pollution and adverse cardiac events, cardiac conditions, or worsening of cardiac disease. Pope and colleagues (1999) found significant changes in HRV measures in subjects who had multiple recordings across days having different (elevated or reduced) levels of ambient fine particulate pollution ($PM_{2.5}$). Berger and associates (2006) documented that elevated concentrations of fine and ultrafine particles increased the risk of arrhythmia in men with coronary artery disease. Dockery and colleagues (2005) found a moderate association between air pollution and episodes of ventricular arrhythmias, suggesting that air pollution per se could raise a patient's underlying arrhythmogenic risk. Most recently, in a large cohort of subjects, Pope and associates (2006) provided convincing data that link short-term particulate exposure to acute coronary events, especially among patients with preexisting coronary artery disease.

The emphasis of these studies has been primarily on establishing a causal link between air pollution and cardiovascular disease, rather than on determining or studying the mechanisms by which cardiac physiology and electrophysiology are altered or compromised. For example, many of the studies just mentioned have focused on HRV changes, which reflect variations in the statistical characteristics of an individual's heart rate and thereby represent autonomic changes more than direct changes to cardiac conduction or pacemaker function. Abnormal HRV parameter values have been associated with an increased risk of sudden cardiac death in heart failure patients, apart from air pollution effects. However, the direct effects of air pollutants on cardiac electrophysiology have not been studied extensively. The studies by Henneberger and colleagues (2005) and Zareba and colleagues (2001) assess air-pollution-induced changes in cardiac repolarization as a means of directly linking air pollution with changes in the structure and function of cardiac cells. These studies, which use QT and QT:RR slope, provide a rationale for pursuing the objectives of the work presented here, which focused on observed changes in ventricular repolarization.

Repolarization is the return to excitability of cardiac cells after *depolarization*, which is the propagation of an electrical wave that triggers the mechanical contraction of the heart. The QRS of the ECG is the electrical manifestation of the depolarization of cardiac cells and muscle, a consequence primarily of calcium and sodium ionic currents flowing across cell membranes during the upstroke of cardiac action potentials. Similarly, the ST segment and T wave of the ECG are the electrical manifestation of repolarization and signify the electrical recovery of cells through the flow of potassium ions across the cell membranes during the plateau and downstroke phases of the cellular action potential. The complex process of repolarization is dependent on heart rate, autonomic tone, regional abnormalities of perfusions (ischemia), localized or diffuse disease, and even temperature — drinking hot or cold liquids can alter ventricular cellular action potentials in cardiac muscle proximal to the esophagus. Repolarization is also influenced by many pharmacologic agents (tricyclic antidepressants, antibiotics such as erythromycin, and many more), as well as cardiac drugs aimed at altering the duration of action potential in order to reduce arrhythmia risk.

It is important to note that the morphology and duration of action potential differ throughout the heart, and changes in the characteristics of action potential, caused by heart rate, pharmacologic intervention, or other influences, are themselves inhomogeneous. More than 40 years ago, Han and Moe and colleagues documented that heterogeneous excitability (i.e., inhomogeneous repolarization) is a primary factor in arrhythmogenesis and therefore may provide an important means to identify patients at risk of arrhythmias (Han and Moe 1964; Han et al. 1966). This connection between repolarization heterogeneity and arrhythmia risk

is the rationale in this study for assessing repolarization in subjects exposed to different levels of air pollution. We sought to explore the possibility that exposure to $PM_{2.5}$ may interfere with the potassium ion currents responsible for repolarization and hence increase arrhythmogenic risk.

The standard provided by the U.S. Food and Drug Administration (FDA) for approving pharmacologic agents dictates that in controlled clinical trials, the corrected QT interval (QTc) cannot change by more than 5 ms (U.S. FDA 2005). Since an increase in QTc is associated with an increase in repolarization heterogeneity (Han and Moe 1964), this represents a small increase in the dispersion of repolarization, for which there are as of yet no clinical measurement standards. Additionally, it is clear that the nature of the heterogeneity is important, with a disparity of repolarization across widely separated regions of the heart being considerably less arrhythmogenic than a disparity over adjacent regions, in other words, when there are steep spatial gradients of action potential duration or repolarization times themselves. Thus it is difficult to specify exactly what would be considered a significant change in repolarization relative to the effects of air pollution. From this perspective, a statistically significant change in either the time duration or the global disparity of repolarization, both assessed in this study, should be considered a starting point for future assessment.

A related perspective is that of the cardiologist, whose only assessment tool, at present, is QTc and T-wave morphology. The current standard states that a QTc of greater than 450 ms is abnormal (U.S. FDA 2005); however, there is no scale indicating a spectrum of arrhythmogenic risk associated with QTc. Similarly, there are no standards delimiting a "safe" T-wave width relative to the likelihood of arrhythmias. Nevertheless, clinicians are well aware that an abnormally long QTc and wide T waves are associated with increased risk of arrhythmias — for example, in cases of long QT syndrome and drug toxicity.

With the causal link between particulate air pollution and cardiovascular effects already established, we set out to investigate the possibility of establishing a mechanistic link between the two, namely, whether evidence could be found confirming that changes to ventricular repolarization are directly due to air pollutants, independent of heart rate or HRV changes already observed. In the present study, we reanalyzed data from the 2004 study by Pope and colleagues using published, novel, clinically applicable measures of repolarization that are potentially useful in characterizing repolarization heterogeneity and rate dependency in patients (Lux et al. 1999, 2001; Fuller et al. 2000a,b). Note that the measurements in this study were developed by our group (Dr. Lux and colleagues) based on experimental data directly relating electrocardiographic measurements to the characteristics of underlying myocardial action potential (Millar et al. 1985; Haws and Lux 1990).

SPECIFIC AIMS

The overall goal of this study was to measure and assess ventricular repolarization differences in previously recorded, continuous, 24-hour ECGs obtained from a cohort of nonsmoking elderly subjects. The recordings were made during periods when the subjects were exposed to low as well as high concentrations of ambient, airborne, fine PM — specifically $PM_{2.5}$. Most clinical analyses of ventricular repolarization have been based on the QT interval of the ECG. In this study, we used an emerging method based on interval measurements obtained from the RMS of recorded ECGs, namely, calculation of a magnitude function of the ECG, which delineates experimentally determined and validated measures of electrophysiologic parameters. The specific aims of the study were to

1. Measure — on a heartbeat-to-heartbeat basis — cycle length (RR); RR dispersion; the interval between the RMS R- and T-wave peaks (RT; an estimate of the mean duration of ventricular action potential); the width of the RMS T wave (TW; an estimate of ventricular repolarization dispersion); and the RMS QT interval (QT; measured from the QRS onset to the T-wave offset times of the RMS ECG) and corrected QT (QTc).
2. Compare statistically RR, RT, TW, and QTc measured during a 24-hour time series, and assess differences in RT:RR, QT:RR, and TW:RR dynamic regression.

We also proposed applying fixed-effects modeling to the data in order to assess causality of pollution in repolarization changes. Our underlying hypothesis is that air pollution (specifically high levels of ambient PM) deleteriously alters ventricular repolarization, independent of heart rate, which could lead to increased arrhythmogenesis.

METHODS AND STUDY DESIGN

STUDY POPULATION

The subjects in this study were 88 nonsmoking elderly residents of one of three Utah communities, who agreed to participate in 24-hour ambulatory ECG monitoring and blood testing. The communities were all part of the greater Salt Lake Valley and within 40 miles of the urban center of Salt Lake City. The age of the subjects, all of whom were retired, ranged from 54 to 89 years, and 57% were female. All enrolled subjects had no serious medical conditions,

and exclusion criteria included a history of diabetes, renal failure, Parkinson disease, mental illness, chronic alcohol abuse, oxygen therapy, abnormal heart rhythm, implanted pacemaker or defibrillator use, heart transplantation, or heart failure within the past 6 months. All subjects had read the research protocol and had read and signed the consent form previously approved by the Brigham Young University Institutional Review Board for the earlier study (Pope et al. 2004).

Since these data were studied retrospectively and since the principal investigator was blinded to patient information, the University of Utah Institutional Review Board classified the study as "exempt."

POLLUTION AND WEATHER DATA

Daily temperature and relative humidity data at the Salt Lake City, Utah, International Airport monitoring station were obtained from the National Climatic Data Center (*www.ncdc.noaa.gov*). Daily, 24-hour concentrations of $PM_{2.5}$ were obtained from the State of Utah Division of Air Quality, which used the U.S. Environmental Protection Agency's (EPA's) Federal Reference Method, and ranged from 1.7 to 74.0 $\mu g/m^3$ with a mean of 23.7 ± 20.2 $\mu g/m^3$. In addition to $PM_{2.5}$ measurements, an index of air stagnation called a *clearing index* was provided by the National Weather Service. This index, which is based on temperature, moisture, and wind, provides a relative measure of air stagnation, with low values indicating considerable stagnation and high values indicating little stagnation. Missing $PM_{2.5}$ data were estimated by extrapolation from available data from neighboring days if there were no large changes in the clearing index or by projection of data consistent with observed changes in the clearing index (for details, see Pope et al. 2004).

ECG DATA

ECG recording (as well as blood sampling) was triggered during relatively "high" and "low" concentrations of ambient airborne $PM_{2.5}$ as determined by measured data and observation of the clearing index. Health data were collected for as many as five different days on each subject using 24-hr Holter ECG recordings; as a result, subjects had different values for "high" and "low" levels of $PM_{2.5}$. For purposes of comparison, each subject served as his or her own control, and the health data of each subject collected from the day having the lowest $PM_{2.5}$ concentration were used as the "control" and then compared with data from all other days. In so doing, we hoped to observe changes in repolarization parameters in the recordings taken at higher concentrations.

The ECG recordings were made using Trillium 3000 recorders (Forest Medical, East Syracuse, NY), which continuously sampled data using 2 channels at 256 Hz per channel. These data were downloaded and stored on CDs for later processing. In addition to the ECG data, blood samples were drawn to assess differences in blood chemistry; however, these data were not analyzed as part of the present study. Of the 250 total recordings reported in the original publication (Pope et al., 2004), only 215 were analyzed in the present study; the other recordings were not used because of the technical inability to analyze the records, the presence of excessive noise, or the absence of the records from the set made available for the current study.

STUDY APPROACH

In the reanalysis of the data from the study conducted by Pope and colleagues (2004), our aim was to identify, if possible, any changes in repolarization parameter values between days of high and low $PM_{2.5}$ concentration. Given that there was no control for activity or diet, or any capture of timing information, this presented a challenge. Our approach in analyzing these ECG data was to (1) extract the study parameter values for each heartbeat; (2) determine the epoch statistics (averages, standard deviations, and ranges) for each of the study parameters; (3) compare the epoch statistics as a function of $PM_{2.5}$ concentration for each subject; and (4) summarize the observed differences. Epochs of 10, 30, and 60 minutes' duration were planned. Daily averages of the epoch averages for each variable and their standard deviations were also calculated. We also proposed to apply a fixed-effects analysis to the data in order to assess the effects of confounding information on any observed differences.

STATISTICAL METHODS AND DATA ANALYSIS

Processing of 24-Hour Holter Recordings

All digital recordings were downloaded from a DVD or CD into a custom program called ScalDyn (for Scalar Dynamics), which was developed by Dr. Lux. This program provides the capability to visualize long, continuous records of multilead ECG data; detect each beat and classify its wave as "normal sinus" or "other"; provide a noise figure of merit for each beat (for later culling of noisy data); and determine a variety of measurements for each beat, including RR and novel measurements of repolarization (described in the next section). These data were stored as text files for later statistical analysis and plotting. The time required for reading each 24-hour recording into the program, setting up the parameters for analysis, verifying the

quality of the recording and measurements, and printing the results was about 10 to 15 minutes.

Importantly, recording quality varied in terms of instantaneous noise; lead dropout; positional changes to the ECG; and intermittent changes in conduction and rhythm, including bundle branch block, atrial fibrillation, and premature atrial or ventricular contractions. However, for most recordings, a sufficient number of acceptable (non-noisy, sinus) beats were gleaned from the string of 80,000 to 120,000 beats recorded over 24 hours to provide reasonably robust epoch averages of the parameters used.

RMS ECG and Clinical Indices of Depolarization and Repolarization

The QT interval or its rate-corrected form (QTc) has been the measurement of choice for assessing ventricular repolarization. However, because the QT interval is lead dependent, it does not reflect local information about repolarization, and its use in assessing dispersion of repolarization (so-called QT dispersion) has been questioned. In a series of studies over the past two decades, Dr. Lux and his colleagues have developed a clinically applicable method for assessing repolarization based on direct experimental measurements of cardiac electrophysiology. The method can be applied to as few as two and as many as hundreds of simultaneously recorded ECGs and provides information about the mean depolarization and repolarization times as well as their dispersion. The method measures the times of waveform *peaks,* rather than the times of waveform onsets and offsets (used in the measurement of QT intervals, for example) and is much more robust because it has a higher signal-to-noise ratio.

The method is based on the calculation of a magnitude function of all measured leads — the RMS of all ECG signals after all signals have been adjusted to remove baseline signal. This is typically calculated by subtraction of the straight line between the adjacent T-P or P-R segments of each beat of the ECG recording. If $e_i(t)$ is the i^{th} ECG of N simultaneously recorded ECGs over time t, then the RMS ECG — represented as $E_{RMS}(t)$ — is calculated using the following equation:

$$E_{RMS}(t) = \sum_{i=1}^{N} e_i^2(t)$$

In Figure 1A, 8 traces (from leads I and II, and precordial leads V_1–V_6) from a standard 12-lead ECG are shown superimposed. The calculated RMS ECG for these traces combined is shown in Figure 1B. For the purpose of defining the intervals, we labeled the onset, peak, and offset of the RMS QRS as "Q," "R_{pk}," and "S," respectively. Since by definition the RMS ECG is always positive, it does not contain the negative deflections called Q and S that occur in conventional ECG traces, but our annotation is intended to identify times of QRS onset and offset in the RMS signal. Also labeled are the onset, peak, and offset of the RMS T wave, called T_{on}, T_{pk}, and T_{off}, respectively. Fuller and associates (2000a,b) documented in experimental canine studies that fiducial (temporal) measurements from this curve could be used to delineate some of the most important global electrophysiologic parameters on a beat-to-beat basis; specifically, peak times of the RMS QRS and T wave correspond to mean depolarization and repolarization times (mean times of action potential upstrokes and downstrokes, respectively) of the ventricles. The RT interval provides a robust measure of mean duration of ventricular action potential. Finally, the interval

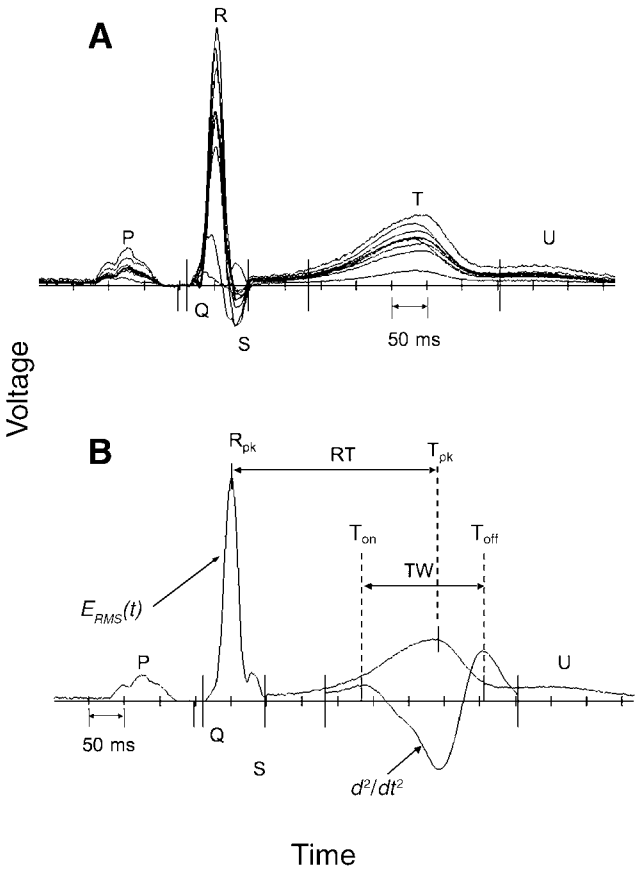

Figure 1. Example of one beat from a standard 12-lead ECG, with 8 "independent" leads. Tick marks on the x-axis (representing time) are drawn at 50-ms intervals. **A:** leads I and II, and precordial leads V_1–V_6 superimposed; **B:** the RMS ECG, $E_{RMS}(t)$, calculated from the 8 leads. Also shown is a second derivative (calculated using a parabolic-fit, least-mean-squared error estimate) of the RMS T wave (d^2/dt^2). In addition, the times of RMS QRS onset (Q), peak (R_{pk}), and offset (S) are represented by vertical lines, and the times of RMS T-wave onset (T_{on}), peak (T_{pk}), and offset (T_{off}) are indicated by broken vertical lines. RT and TW are the intervals between RMS R- and T-wave peaks and between T-wave onset and offset, respectively.

between RMS T-wave onset and offset times (T_{on}–T_{off}), called *RMS T width* and labeled "TW," provides a robust measure of the range of repolarization times (i.e., the range of all downstroke times for ventricular action potential). Each of the inflection points, Q, R_{pk}, S, T_{on}, T_{pk}, and T_{off}, can be calculated using a variety of detection techniques (see Fuller et al. 2000a,b for details). For the T-wave inflections, we calculated the second derivative using a parabolic-fit, least-mean-squared error estimate. This curve is shown as the triphasic wave labeled d^2/dt^2 and superimposed on the RMS T wave in Figure 1B; the time of the minimum d^2/dt^2 value during the RMS T wave was used as the time of the RMS T-wave peak (T_{pk}), and the times of the adjacent positive peaks were used as the times of the RMS T-wave onset and offset.

Feature Extraction

During the processing of each recording, the times of the QRS complex onset, peak, and offset, as well as T-wave onset, peak, and offset, were automatically measured in the RMS ECG for each beat, all relative to the time of QRS onset. Measurements required for analysis were then stored in a text file, an example of which is shown in Table 1. During the processing phase, we performed quality control of the data: each RMS ECG trace was reviewed to ensure that the automatically measured times reflected appropriate inflections in the observed waveforms. The full 24-hour recording and fiducial time measurements could be reviewed in a few minutes and reprocessed using altered program parameters if excessive noise prevented reliable measurements. Epoch averages of these data were determined, and time series strips were plotted for the epoch averages and the standard deviations of the following measurements (see Figure 2 for an example): RR; RMS RT; RMS TW; T_{on} and T_{off}; the linear regression slopes of RMS QT:RR and RT:RR; the standard error of the estimate of the RMS QT:RR and RT:RR regression slopes; correlation between RMS QT:RR and RMS RT:RR regression; and finally, RR dispersion, a measure of the rapidity of beat-to-beat heart rate changes derived from a Poincaré analysis of the heart rate. Additionally, we calculated patient-specific, rate-corrected RTs and TWs (RTc and TWc) using the regression calculations based on the epoch slopes and offsets for these variables (Table 2). In fact, the regression slopes of TW versus RR are near zero, one of the attractive features of this measurement. Thus, although TW is sensitive to repolarization change, it is not rate dependent, (unlike RT and QT). Since RMS-derived QT and RT are so closely related (and highly correlated), we used and report only the RT data.

Epoch Averaging

Since the data set for each recording amounted to approximately 100,000 heartbeats, some type of compression or

Table 1. Excerpt of Raw Measurements from One ECG Recording[a]

Beat	Sample	RR	R_{pk} (ms)	T_{on} (ms)	T_{pk} (ms)	T_{off} (ms)	QC
20990	2207 4182	1089	37.9	353.8	425.9	493.7	1
20991	2207 4457	1074	38.3	371.2	439.7	499.1	0
20992	2207 4712	996	37.6	370.2	437.0	498.4	1
20993	2207 4969	1003	38.1	372.4	436.4	496.6	1
20994	2207 5223	992	37.9	372.8	437.9	496.0	0
20995	2207 5469	960	39.1	371.2	437.7	497.0	1
20996	2207 5723	992	37.4	279.7	335.6	495.8	0
20997	2207 5986	1027	41.1	381.1	441.7	500.1	1
20998	2207 6236	976	41.6	373.5	439.1	498.1	1
20999	2207 6491	996	41.7	379.6	442.8	498.8	1
21000	2207 6779	1125	38.3	281.6	345.8	421.5	0
21001	2207 7065	1117	38.4	377.5	441.9	497.5	0
21002	2207 7348	1105	37.9	381.6	441.8	496.4	1
21003	2207 7678	1289	39.1	276.2	345.2	417.6	0
21004	2207 7993	1230	38.8	290.6	323.3	385.6	0

[a] Sample is the sample number in the recording; RR is the cycle length of the beat; R_{pk} is the time of RMS R peak; T_{on}, T_{pk}, and T_{off} are times of T onset, peak, and offset; and QC is the quality control index for the beat — 0 indicating a "clean" sinus rhythm beat and nonzero indicating a combination of noise or nonsinus rhythm beat.

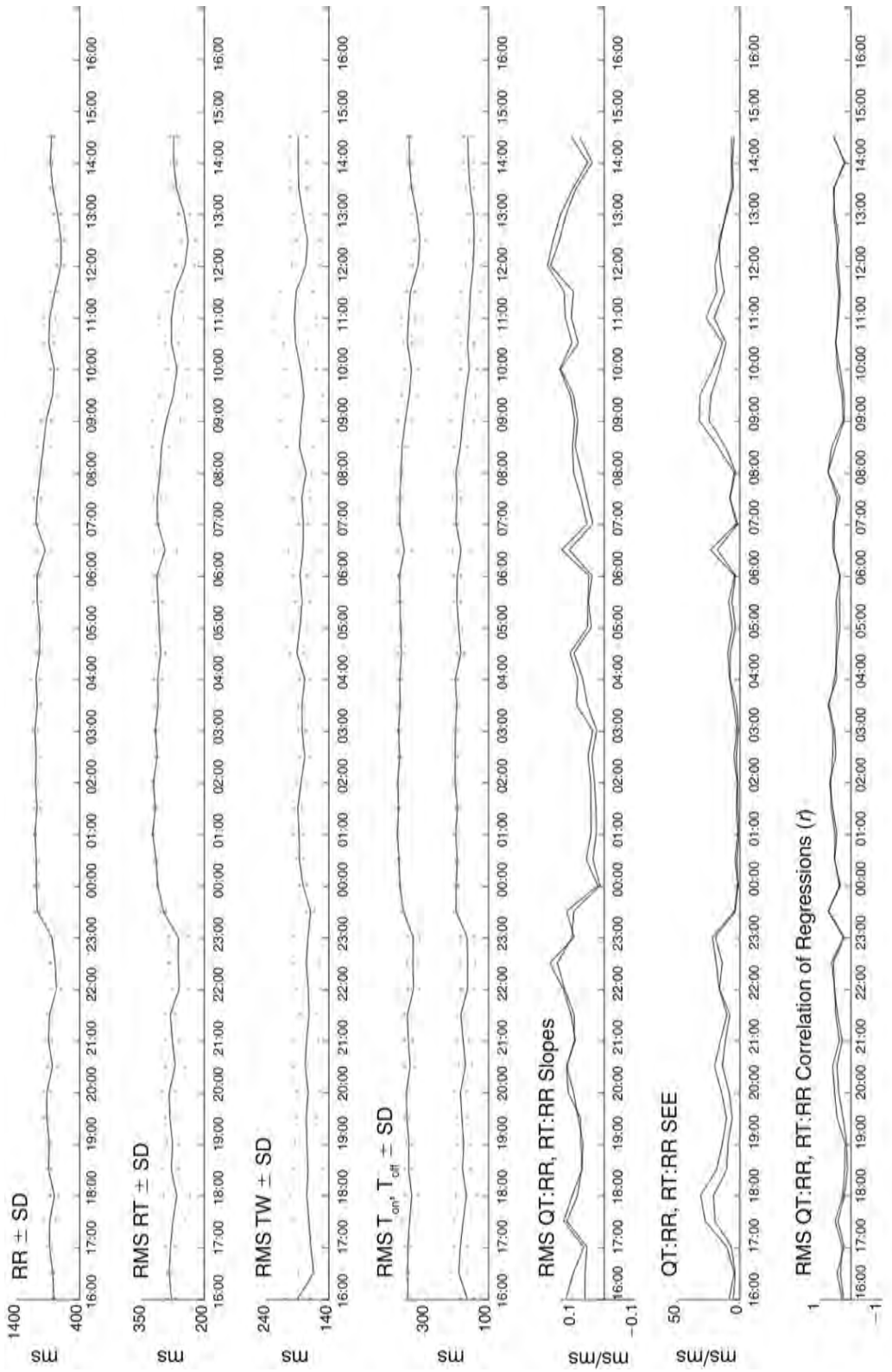

Figure 2. The 24-hour, 30-minute epoch averages of repolarization and heart rate parameters for one subject. From top to bottom: RR, RMS RT, RMS TW, RMS T_{on} and T_{off}; regression slopes of RMS QT:RR and RMS RT:RR; standard error of the estimate (SEE) of the RMS QT:RR and RMS RT:RR regression slopes; correlation (*r*) of RMS QT:RR and RMS RT:RR regressions; and finally, RR dispersion, a measure of how rapidly RR changes beat to beat. Standard deviations of the first four time series graphs are shown as "+" signs above and below the curves. The recording was less than 24 hours long.

Table 2. Study Variables

Variable	Explanation
RR	Cycle length
RT	Interval between R- and T-wave peaks of RMS ECG
QT	Interval between QRS complex onset and T-wave offset of RMS ECG
TW	Width of RMS T wave
RTc	Rate-corrected RT
TWc	Rate-corrected TW
RT:RR slope	Regression slope of RT vs. RR
TW:RR slope	Regression slope of TW vs. RR

averaging was necessary. We used 10-, 30-, and 60-minute epochs to assess and compare data sets. We settled on a 30-minute epoch length as a compromise between excessive and inadequate smoothing. For each epoch, we only used strings of beats that were "clean" (i.e., not noisy) and contained only normal sinus beats. In addition, we included only beats that were preceded and followed by normal sinus beats in order to guarantee that all cycle lengths were based on good beats. This ensured that the cycle lengths and other measurements averaged in each epoch would be representative and not biased by noise or spurious data. Most recordings resulted in roughly 80% acceptable data. Figure 2, included here for illustration purposes, shows 30-minute epoch averages (± standard deviations) of the study parameters for one subject's recording. There were a total of 215 such plots, one for each recording analyzed.

In addition to calculating 30-minute epoch averages for each subject, we calculated the 24-hour means and standard deviations of the epoch averages of all study variables. These were later compared using *z* scores (not shown).

Kolmogorov-Smirnov Test

Initially, we had thought of aligning recordings by time of day and using simple *z* scores and F-tests to compare the epoch averages and standard deviations of repolarization and heart rate. However, it soon became apparent that such an approach leads to large differences in outcomes throughout the day, even if the PM values are the same. This is a consequence of the fact that the heart *is* very different day to day because of differences in activity, autonomic tone, stress, and other factors, and a simple comparison of data from the same time of day could yield statistically significant differences for many possible reasons. This precludes using such an approach to determine differences arising from pollution effects alone. Thus, we resorted to a simpler approach in which we tested for differences in the parameter values using the Kolmogorov-Smirnov test, a nonparametric test that is robust and documents whether or not two sets of data are statistically significantly different. Since it is a nonparametric test, the data do not have to be normally distributed, and the method is valid regardless of the underlying distributions of the data. Clearly, in adopting this strategy, we intentionally discarded any sequential information — in other words, the method does not differentiate *when* differences occur, but rather *if* they occur over an entire data set.

We applied the Kolmogorov-Smirnov test to assess differences in RR, RMS RT, QT, and TW for different PM concentrations by comparing all parameter values for each subject and treating the recording associated with the lowest PM concentration as the "control."

Figure 3 shows probability distribution functions of RMS TW versus RR (left panel) and restitution scatterplots showing regressions of RT versus RR and TW versus RR (right panel) for one subject. Similar probability distribution function plots (not shown) were made for RR, RT, and QT for quality control of the measurements. For each plot, we also calculated the associated Kolmogorov-Smirnov "distances" (probabilities) and associated levels of significance for comparison of data at the lowest concentration of $PM_{2.5}$ with data at higher concentrations. The parameter D in the Kolmogorov-Smirnov test is the largest vertical separation (distance) between the pairs of estimated probability distribution curves. When coupled with the degrees of freedom (number of points in each of the two curves) the significance of the difference may be determined by calculation or table lookup. Probability distribution functions of RR, RT, QT, and TW were calculated, followed by linear regression of RT and TW *versus* cycle length for each set of data. We also calculated for each data set the linear regression offsets, slopes, standard errors, and correlations (data not shown). There were 88 such plots, one for each subject in the study.

Variable Comparisons

For individual subjects, we compared the study variables using the Kolmogorov-Smirnov test in order to assess the statistical significance of the differences. In addition to comparing data from the day having the lowest $PM_{2.5}$ (the control) with data from all other days, we stratified the data by days with the lowest and highest $PM_{2.5}$ and used the daily epoch averages of each study variable to compare data from all subjects. A total of 61 subjects of the original 88 were used for this analysis. The remaining 27 subjects were excluded because some of them had participated in only one recording and some recordings were not usable

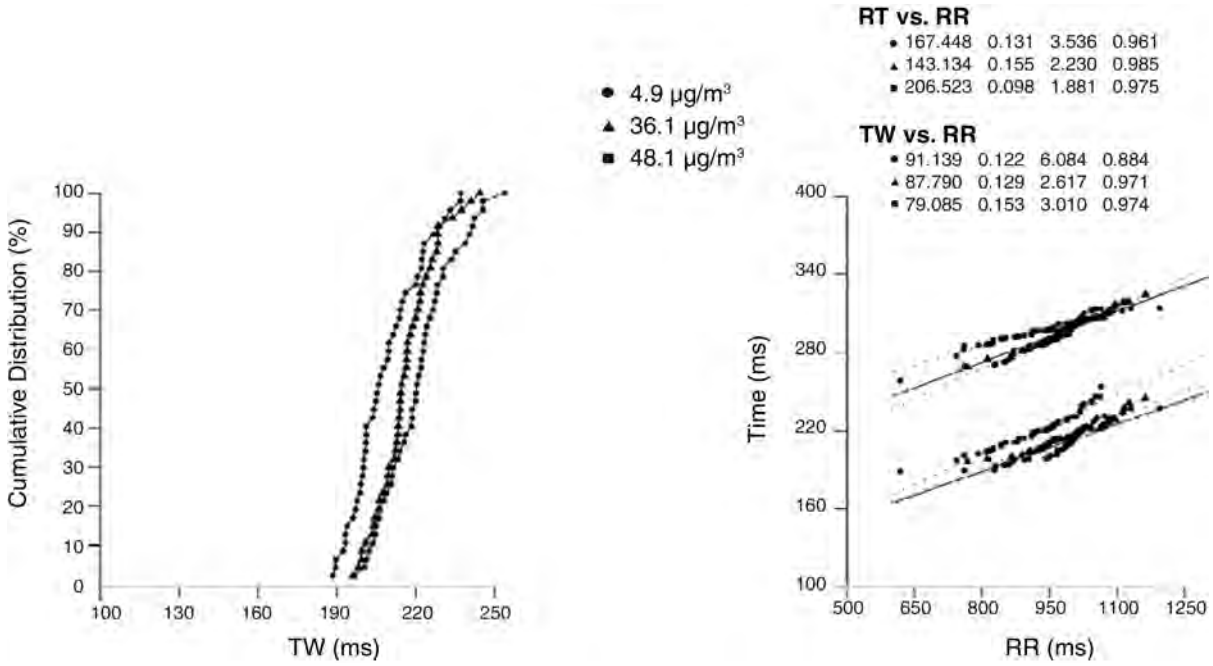

Figure 3. Probability distribution functions (left) of RMS TW versus RR and restitution scatterplots (right) showing RT versus RR (top) and TW versus RR (bottom) for one subject. TW values ranged from 190 to 250 ms (abscissa on the left); the cumulative probabilities are shown on the ordinate (on the left). Shown are data from recordings obtained for three different values of $PM_{2.5}$: 4.9, 36.1, and 48.1 µg/m^3. The TW for the lowest $PM_{2.5}$ concentration was significantly different from both the intermediate (D = 0.340; $P < 0.006$) and high (D = 0.383; $P < 0.001$) concentrations in this subject (Kolmogorov-Smirnov test). Regressions of RT versus RR and TW versus RR show a range of cycle lengths of about 700 to 1200 ms (abscissa on the right). The sets of values show regression intercept; regression slope; SEE; and r for three $PM_{2.5}$ concentrations.

for technical reasons (e.g., excessive noise or the inability to process the entire 24-hour recording).

Regression Modeling

Associations between $PM_{2.5}$ and various measures of cardiac repolarization were analyzed using two approaches. First, the correlation between each measure of repolarization and $PM_{2.5}$ was calculated for those participants who had multiple observations ($n = 61$). In order to explore the distribution of associations between $PM_{2.5}$ and repolarization and to identify obvious outliers, histograms that approximated the frequency distributions of these individual correlations were constructed. Then the data for each measure of repolarization were pooled, and two fixed-effects models were estimated. These models included subject-specific indicator variables. They evaluated time-dependent associations between measures of repolarization and $PM_{2.5}$ while controlling for subject-specific characteristics that did not vary over time. Model I included only $PM_{2.5}$ and the subject-specific fixed effects. Model II included $PM_{2.5}$; subject-specific fixed effects; and a bivariate, thin-plate smoothing spline of temperature and relative humidity (with six degrees of freedom). The models were run using all available observations and again after excluding observations from subjects whose measurements were extreme and out of range.

RESULTS

$PM_{2.5}$ CONCENTRATIONS

Figure 4 shows the cumulative probability distribution functions of $PM_{2.5}$ concentrations for the 61 subjects selected for the analysis; for each subject, the days with the lowest and highest $PM_{2.5}$ concentrations were included. Clearly the data do not overlap, and the low and high concentrations represent very different distributions ($P < 0.00001$).

RR AND RR STANDARD DEVIATIONS

The probability distribution functions in Figure 5 show that for the 61 subjects, there were no statistically significant differences in the distributions of RR between days with low and days with high $PM_{2.5}$ concentrations. This is consistent with the 2004 study by Pope and colleagues, which found no significant differences in heart rate per se with increased levels of PM. The data in Figure 6 show a significant ($P < 0.002$) difference in the standard deviation

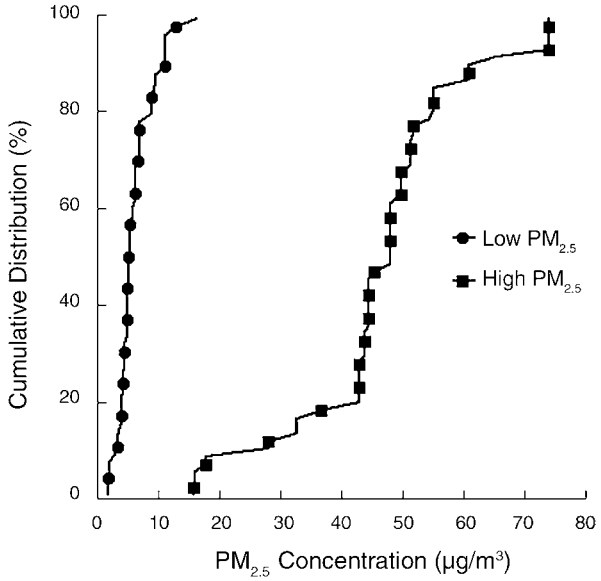

Figure 4. Probability distribution functions of low and high $PM_{2.5}$ concentrations on the recording days. Data from three subjects for which low values of $PM_{2.5}$ exceeded 30 µg/m^3 were excluded from these plots. The concentrations were significantly different ($P < 0.00001$, Kolmogorov-Smirnov test).

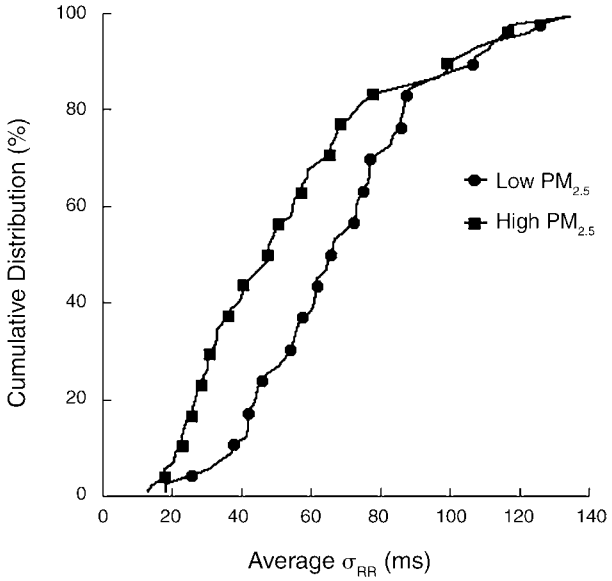

Figure 6. Probability distribution functions of standard deviations of the epoch averages of RR (σ_{RR}) for low and high $PM_{2.5}$ concentrations on the recording days. The decrease in overall RR variation on days with high versus low $PM_{2.5}$ concentrations — as estimated from the standard deviations of the epoch RR — was statistically significant ($P < 0.002$, Kolmogorov-Smirnov test).

of the epoch averages of RR (σ_{RR}) between days with high and low $PM_{2.5}$ concentrations. Although standard deviation is a gross measure of overall RR variation, once again this analysis confirms the HRV findings of Pope and colleagues (2004) from these data, which showed a decrease in specific HRV parameter values with increased $PM_{2.5}$ concentrations.

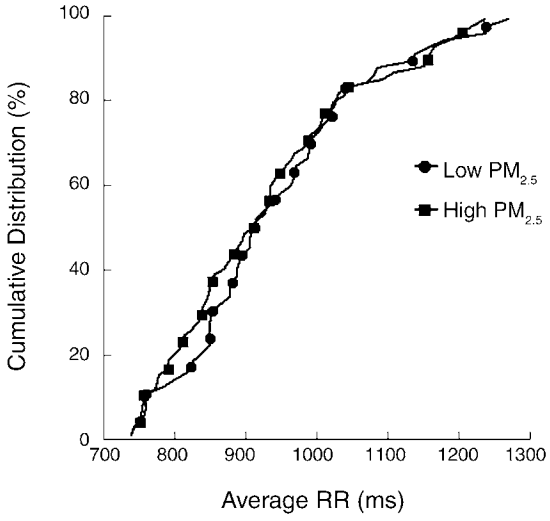

Figure 5. Probability distribution functions of epoch average RRs for low and high $PM_{2.5}$ concentrations on the recording days. There were no statistically significant differences between RR distributions across all subjects ($P > 0.05$, Kolmogorov-Smirnov test).

REPOLARIZATION CHANGES

Figures 7, 8, and 9 show the cumulative probability distribution functions of RT, TW, and RT:RR and TW:RR regression slopes, respectively, which compare the repolarization data of the 61 subjects from recordings during days of low $PM_{2.5}$ concentrations with those during days of high $PM_{2.5}$ concentrations. Clearly, for all of these repolarization variables, no significant differences were found, although there is a suggestion that TW increases slightly (but not statistically significantly) with increasing $PM_{2.5}$ concentrations (as indicated by the separation of the two curves in Figure 8). Differences in QT, QTc, and QT:RR slope were not statistically significantly different and have not been included in this report. Note that the slope of TW:RR in Figure 9 is near 0, indicating that TW is, for practical purposes, independent of heart rate.

The Kolmogorov-Smirnov analysis of the TW and RT data showed highly significant differences between days of low and high $PM_{2.5}$ concentrations for each subject. However, analysis of the direction of the change across multiple subjects was inconclusive. For example, in a subset of 52 of the 61 subjects for which the individual Kolmogorov-Smirnov plots were acceptable (some plots had outliers or

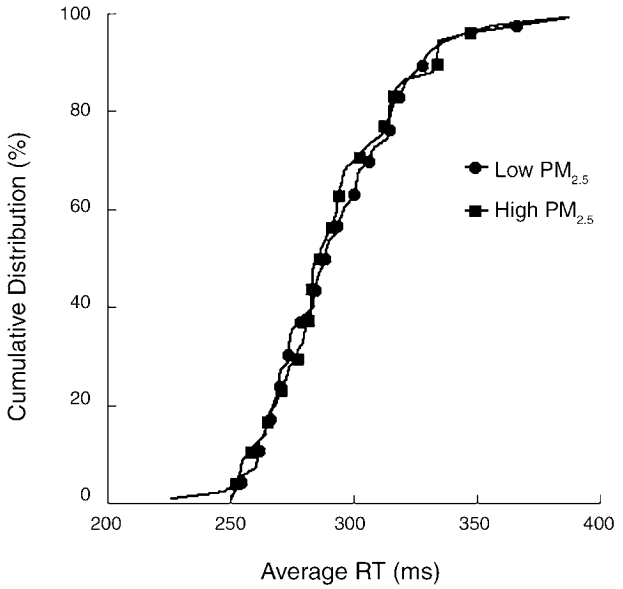

Figure 7. Probability distribution functions of epoch averages of RT for low and high $PM_{2.5}$ concentrations on the recording days. There was no apparent change in RT with $PM_{2.5}$ concentration ($P > 0.05$, Kolmogorov-Smirnov test).

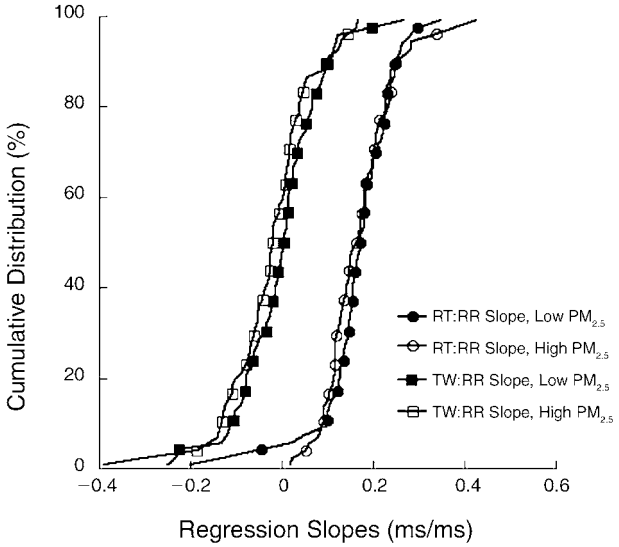

Figure 9. Probability distribution functions of epoch average slopes of TW:RR and RT:RR regressions for low and high $PM_{2.5}$ concentrations on the recording days. There was a slight but insignificant decrease in the rate dependency of TW and RT ($P > 0.05$, Kolmogorov-Smirnov test). Note the near-zero TW:RR slope compared with that of RT:RR, confirming that TW is nearly independent of heart rate.

a large imbalance in the number of samples in test pairs), 19 subjects showed a significant increase of TW, 20 subjects showed no difference, and 13 showed a significant decrease of TW between data from days with low and high $PM_{2.5}$ concentrations. These inconsistencies suggest that day-to-day TW variation apart from air pollution is substantial.

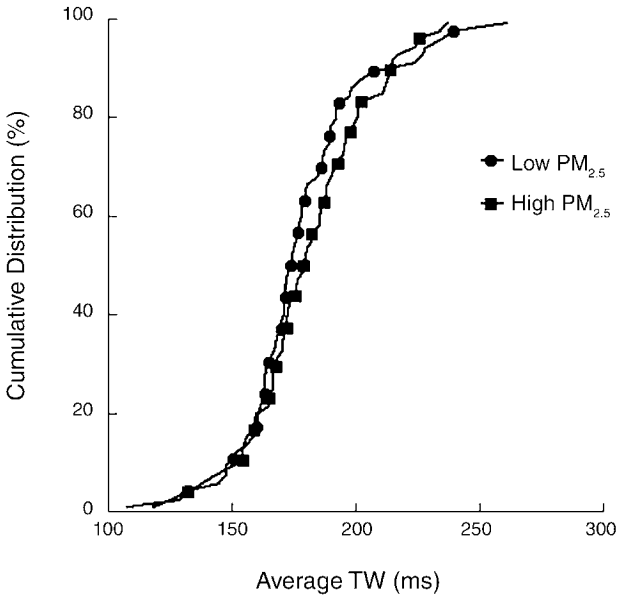

Figure 8. Probability distribution functions of epoch averages of TW for low and high $PM_{2.5}$ concentrations on the recording days. There was a small but statistically insignificant increase in TW between low and high $PM_{2.5}$ concentrations ($P > 0.05$, Kolmogorov-Smirnov test).

Table 3 summarizes the findings of the study and shows that for the 61 subjects selected for final analysis, none of the repolarization variables showed a significant difference between low and high $PM_{2.5}$ concentrations. It is important to note that the standard deviations of the repolarization variables are comparable, suggesting a consistency in the measurements and analyses. As a retrospective check

Table 3. Comparison of Study Variable Means for Low and High $PM_{2.5}$ Concentrations ($n = 61$)

	Low $PM_{2.5}$ ± SD	High $PM_{2.5}$ ± SD	P
PM ($\mu g/m^3$)	6.1 ± 2.9	46.3 ± 14.0	< 0.0001
RR (ms)	938.6 ± 129.6	926.9 ± 135.3	NS
σ_{RR} (ms)	67.9 ± 25.9	52.8 ± 29.5	< 0.01
RT (ms)	292.8 ± 28.3	291.2 ± 28.7	NS
RTc (ms)	306.5 ± 26.6	308.0 ± 25.4	NS
RT:RR slope	0.164 ± 0.0889	0.170 ± 0.077	NS
TW (ms)	177.7 ± 26.0	180.4 ± 25.5	NS
TWc (ms)	178.0 ± 35.6	178.0 ± 29.4	NS
TW:RR slope	−0.003 ± 0.093	−0.026 ± 0.099	NS

NS indicates not significant.

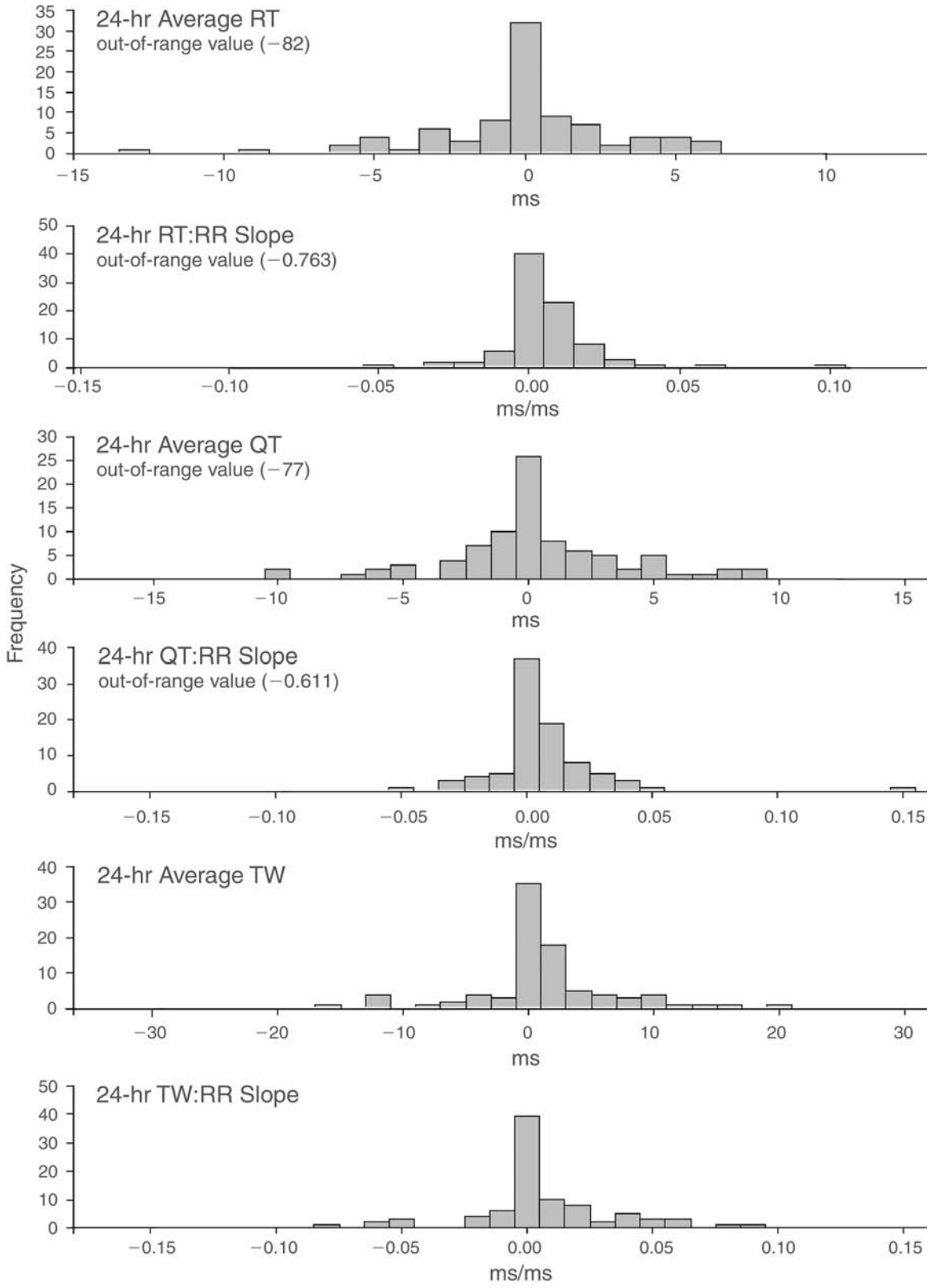

Figure 10. Frequency distributions of individual associations between cardiac repolarization measures (averaged over 24-hour period, midnight to midnight) and a 10-μg/m^3 increment change in $PM_{2.5}$. Out-of-range values that could not be included in the graph are indicated in the top left corner of the plots for RT, RT:RR slope, QT, and QT:RR slope.

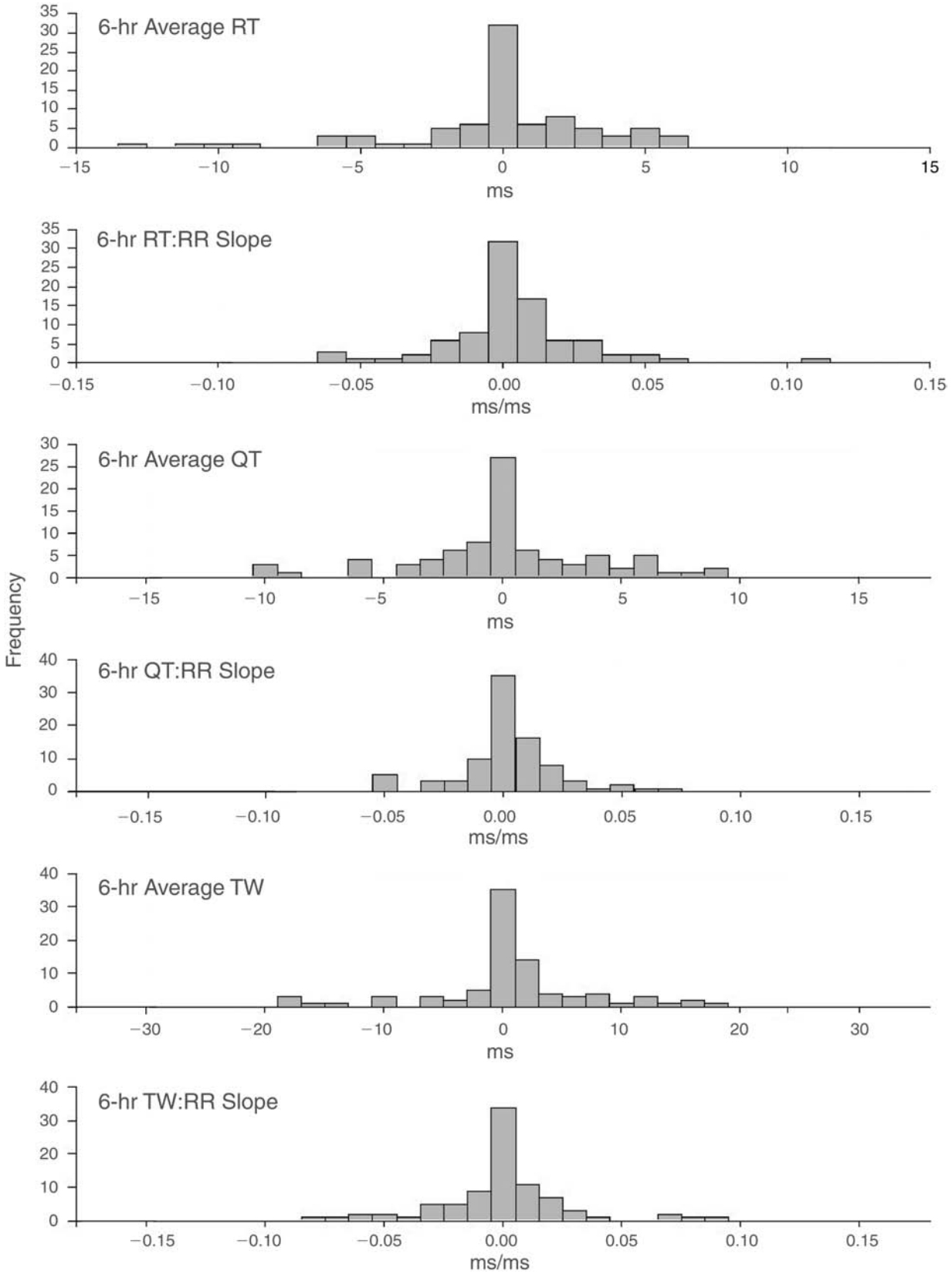

Figure 11. Frequency distributions of individual associations between cardiac repolarization measures (averaged over 6-hour period, midnight to 6 a.m.) and a 10-μg/m^3 increment change in $PM_{2.5}$.

Table 4. $PM_{2.5}$ Regression Coefficients × 10 (SE) for Various Regression Models[a]

	Model I		Model II	
	All Observations	Outliers Excluded	All Observations	Outliers Excluded
Subjects (*n*)	88	87	88	87
Observations (*n*)	208	206	208	206
24 hr				
Average RT	−0.785 (0.407)[b]	−0.773 (0.399)[b]	−0.956 (0.412)[c]	−0.969 (0.403)[c]
RT:RR slope	0.001 (0.002)	0.001 (0.002)	0.000 (0.002)	0.000 (0.002)
Average QT	−0.528 (0.421)	−0.517 (0.413)	−0.714 (0.424)[b]	−0.734 (0.417)[b]
QT:RR slope	0.001 (0.002)	0.001 (0.002)	0.002 (0.002)	0.002 (0.002)
Average TW	0.696 (0.617)	—	1.428 (0.672)[c]	—
TW:RR slope	0.001 (0.003)	—	0.007 (0.003)[c]	—
6 hr				
Average RT	−1.000 (0.500)[c]	—	−1.584 (0.500)[d]	—
RT:RR slope	0.001 (0.003)	—	−0.001 (0.003)	—
Average QT	−0.616 (0.510)	—	−0.978 (0.508)	—
QT:RR slope	−0.002 (0.002)	—	−0.002 (0.002)	—
Average TW	0.765 (0.991)	—	1.931 (0.998)[b]	—
TW:RR slope	−0.005 (0.005)	—	−0.002 (0.005)	—

[a] Model I includes $PM_{2.5}$ and subject-specific fixed effects. Model II includes $PM_{2.5}$, subject-specific fixed effects, and a bivariate thin-plate smoothing spline of temperature and relative humidity (6 df).

[b] $P < 0.10$.

[c] $P < 0.05$.

[d] $P < 0.01$.

on the potential lack of sufficient sample sizes, we performed a power calculation using the standard deviations of RT and TW, as measured in our data. Assuming a standard deviation of 30 ms in either RT or TW, and a power of 80 (i.e., an 80% chance to detect a difference), we calculated that an *N* of 111 samples would be required to detect a 10-ms change in either variable. For a power of 90, *N* would have to equal or exceed 154 samples. Thus, our study may have been underpowered.

FIXED-EFFECTS MODEL

Figure 10 presents the frequency distributions of the individual correlations between $PM_{2.5}$ concentration and the 24-hour measures of cardiac repolarization. For all measures, the correlation with $PM_{2.5}$ concentration is most frequently near zero, without evidence of predominately positive or negative associations. For average RT, RT:RR slope, average QT, and QT:RR slope, there was one subject in each data set with an extreme out-of-range value. Similar findings were observed for the 6-hour (midnight to 6 a.m.) measures (see Figure 11), except there were no extreme values.

The results of the fixed-effects regression models are presented in Table 4. The associations between estimated $PM_{2.5}$ concentration and repolarization are very small and not consistently statistically significant. There is some suggestion that $PM_{2.5}$ concentration may be negatively associated with average RT (and to a lesser extent with average QT). There is also some suggestion that $PM_{2.5}$ concentration is associated with RT:RR, QT:RR, and TW:RR slopes. The estimated associations are not much affected by excluding the subjects with extreme values.

DISCUSSION AND CONCLUSIONS

The objective of this study was to establish a direct link between increased levels of particulate air pollution, specifically particles with aerodynamic diameters of less than 2.5 μm, and changes in ventricular repolarization using a previously published set of ECG recordings. The rationale for this work is based on (1) the evidence that increases in $PM_{2.5}$ concentration result in decreased HRV parameter values; (2) the well-known and accepted evidence of

cardiac repolarization sensitivity to a wide variety of physiologic, autonomic, and pharmacologic stimuli as well as cardiac conditions and diseases; and (3) the well-established relationship between abnormalities of repolarization, particularly disparity or heterogeneity in temporal and spatial repolarization, and arrhythmogenic risk. We hypothesized that recordings from a cohort of nonsmoking, elderly subjects exposed to different levels of $PM_{2.5}$ on a daily basis would show evidence of induced changes in the characteristics of the electrocardiographic T wave.

Recent work by the pharmaceutical industry in the emerging field of cardiac safety has focused on developing methodology and testing protocols to detect small changes in the QT interval of the ECG. The rationale for such scrutiny is that prolongation of the QT interval is associated with increased risk of arrhythmias. In the present study, we focused on detecting changes in repolarization using novel and more sensitive measures than just the QT interval, which is fraught with problems.

The findings presented here did not support our hypothesis that day-to-day, low-to-high fluctuations of $PM_{2.5}$ concentrations influence ventricular repolarization independent of heart rate. None of the repolarization variables showed significant differences, when evaluated by either Kolmogorov-Smirnov or difference-of-means testing; however, we observed statistically significant differences for some variables using fixed-effects modeling. Correlations between $PM_{2.5}$ concentrations and raw ventricular repolarization study parameter values were near zero, suggesting that there was no apparent direct effect on repolarization. Fixed-effects modeling led to the same conclusion. Because the results from the 24- and 6-hour assessments were inconsistent, it is difficult to have confidence in the few differences observed using fixed-effects modeling.

The most obvious, but least interesting, explanation is that there is no effect of day-to-day changes in air pollution levels on ventricular repolarization. However, there are several other explanations that may shed light on why we did not observe statistically significant changes.

One explanation for the lack of evidence of statistically significant differences between study parameter values observed on days of low and high $PM_{2.5}$ concentration (and perhaps the most prominent one) is that the day-to-day differences in repolarization, apart from the potential effects of pollution, far exceed those differences that might arise from pollution effects. Day-to-day changes in repolarization are heavily influenced by autonomic tone, physiologic changes, and pharmacology, as well as simply the level of subject activity, and may far outweigh those that might be induced by increased levels of air pollution. In the present study, there was no way to control for these influences in these highly nonstationary data (discussed later in this section).

A second explanation is that the timing and duration of exposure relative to the timing and duration of the recording may not have been ideal. If there was a delay in the response, then the changes may have occurred after the observation period ended. Since the presumed mechanism of change in repolarization would almost certainly involve alterations in the structure and function of cardiac ion channels (calcium, sodium, and potassium), such a mechanism may take time to become activated; in other words, the effect may be cumulative. It is also possible that the duration of exposure may be important; in other words, had the subjects been subjected to prolonged periods of high $PM_{2.5}$ concentrations, would there have been an observable and significant effect on repolarization?

Another possible explanation is that the population in this study — nonsmoking elderly subjects — may not have been susceptible; it is possible that effects would be seen in an at-risk population. Indeed, a more recent study by Pope and associates (2006) documents the link between increased incidence of acute coronary events and short-term particulate exposures in patients with preexisting coronary artery disease. The mechanism of increased risk is probably not related to repolarization but rather to exacerbation of inflammation. Nevertheless, patients already compromised by heart disease or prone to arrhythmias (e.g., patients with coronary artery disease, congestive heart failure, cardiomyopathy, and perhaps even ventricular hypertrophy) would most likely show electrophysiologic changes in either conduction or repolarization.

Additionally, the failure to observe repolarization changes with different air pollution readings also raises the question of whether or not the parameters used are sufficiently sensitive to detect the changes, if and when they exist. To date, the QT interval and its rate-corrected form, QTc, have been the clinical standards for assessing repolarization change. More recently the QT:RR slope has been used as another meaningful repolarization measure in that it provides some estimate of subject-specific repolarization rate dependency. However, the problems with QT are many: the measurement relies on being able to delineate the "end of the T wave," which is sometimes problematic; it is lead dependent; it provides a gross interval (in essence, the interval between the earliest ventricular depolarization and latest ventricular repolarization) for the lead measured; and its rate-corrected form (Bazette correction) assumes that the QT interval behaves as a square root function of cycle length, which is based on an average pattern for large populations but which certainly does not fit patient-specific

patterns. The repolarization variables derived from the RMS ECG and used in this study — RT and TW — were confirmed experimentally (Fuller 2000a,b) to relate to mean duration of ventricular action potential and range of repolarization times. Furthermore, the measurements are robust in the sense that they do not rely on delineating the end of small and often poorly defined waves, but rather on identifying the peaks of waves and inflection points, using easy and robust estimates of waveform derivatives. Also, the TW index is nearly independent of heart rate, as can be seen in Figure 9. These novel methods are under development for use in cardiac safety studies since they show promise of improving the sensitivity and power of testing (R. L. Lux, unpublished observations, 1995–2008).

Another possible explanation is that the study was underpowered. Although power calculations can provide important guidance for designing prospective studies, they may also point out the failure of retrospective analyses to detect statistically significant changes in experimental variables. Our power calculation suggests that, given the large variation in two of the selected variables, RT and TW, an adequate sample size would necessitate recording data from more subjects than were available for our study. For this reason, we remain reluctant to dismiss the initial hypothesis that pollution affects repolarization.

Finally, it is important to note that data in this study represent some of the most difficult to analyze and compare, namely, *nonstationary time series data*. In the simplest terms, *nonstationary* means that the statistics of the data under study vary over time, and the tools available to determine the statistical significance of change in such data are limited. The classic approach to dealing with such data is to segment the data into epochs sufficiently short that the data statistics "stabilize," or become less time dependent — in other words, *stationary*. In this study, we divided the data into 30-minute epochs, which enabled a comparison of the data between epochs in the same time series. However, we also wished to compare epochs from two different time series, and although the epochs can be aligned by time of day, there is no guarantee that such an alignment is meaningful, given that the subject's activity, physical state, and other, uncontrollable variables will differ from day to day. On the one hand, statistically significant differences are likely to be found, but it will be difficult to determine which, if any, of these uncontrolled variables is a cause of the difference. On the other hand, as a consequence of the wide variation in data, epoch comparisons may show no significant difference. Our findings illustrate both these difficulties. Within subjects, comparison of RT and TW between days showed many examples of statistically significant differences, but these could occur between days that did not differ in level of $PM_{2.5}$ concentration. Conversely, some subjects showed no statistically significant differences in these variables between days having great differences in $PM_{2.5}$ concentration. To complicate the issue, the direction of change was arbitrary: elevated $PM_{2.5}$ concentration could lead to a significant increase or decrease in the variables or no change at all. The average epoch statistics and the fixed-effect modeling showed that, across all subjects and $PM_{2.5}$ concentrations, there appeared to be no consistent (same direction) effect of $PM_{2.5}$ concentration on these repolarization parameters. Since the approach used in this study did show a reduction in the standard deviation of overall cycle length — which confirms the reduced HRV documented in the original study (Pope et al. 2004) — we can claim that the method could detect heart rate changes. Thus, we are left with two possibilities: either there is no significant effect of short-term $PM_{2.5}$ exposure on ventricular repolarization in elderly subjects or there are effects, but our measures of repolarization or the analyses we used or both are insufficiently sensitive to detect them. New studies in controlled exposure settings may give the answer.

IMPLICATION OF FINDINGS

The primary implications of our findings are that repolarization changes occurring during increased levels of $PM_{2.5}$ are insignificant or small and thus do not appear to explain increased morbidity or mortality of elderly subjects during periods of high PM concentration and that they do not explain previously observed associations between $PM_{2.5}$ exposure and changes in HRV. Other potential mechanisms, including pulmonary and/or systemic inflammation, remain possible logical explanations. The limitations of our study, which include the lack of control for activity, behavior, diet, and other variables, as well as the small sample size, may have contributed to the fact that no statistically significant differences were observed between repolarization parameters that were observed on days of low and high $PM_{2.5}$ concentration. Repeating this study while controlling for exposure would offer an opportunity to reexamine the potentially important factors of increasing risk in elderly subjects and pre-existing heart disease.

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ABBREVIATIONS AND OTHER TERMS

σ_{RR}	cycle length standard deviation
CAPs	concentrated ambient particles
D	largest vertical separation (distance) between the pairs of estimated probability distribution curves (Kolmogorov-Smirnov test)
ECG	electrocardiogram
HRV	heart rate variability
ICAM	intercellular adhesion molecule
P, Q, R, S, T, U	ECG wave names, by convention
PM	particulate matter
$PM_{2.5}$	particulate matter with an aerodynamic diameter $\leq$ 2.5 μm
QC	quality control index for a beat
QRS	ECG complex that is the electrical manifestation of the depolarization of cardiac cells and muscle
QT	interval between the QRS complex onset and T-wave offset times of the RMS ECG
QTc	rate-corrected QT interval (based on QT:RR regression)
RMS	root mean square
RR	interval between adjacent R-wave peaks indicating the cycle length of a heart beat
RT	interval between R- and T-wave peaks of the RMS ECG
RTc	rate-corrected RT (based on RT:RR regression)
TW	width of the RMS ECG T wave as delineated by second derivative inflection points
TWc	rate-corrected TW (based on TW:RR regression)

Research Report 141, *Air Pollution Effects on Ventricular Repolarization*, R. L. Lux and C. A. Pope III

INTRODUCTION

Epidemiologic studies have described an association between increases in cardiovascular morbidity and mortality and increases in ambient particulate matter (PM*) concentrations, especially in susceptible populations such as the elderly and persons with pulmonary or cardiovascular disease (e.g., Burnett et al. 1995; Poloniecki et al. 1997; Goldberg et al. 2000; Samet et al. 2000; Hoek et al. 2001; Pope et al. 2002). There is some evidence for an association between air pollution levels and the occurrence of cardiac arrhythmias, which are associated with an increased risk of mortality (Peters et al. 2000; Dockery et al. 2005), although other studies did not find convincing support for such an association (Rich et al. 2004; Vedal et al. 2004; Peters et al. 2005; Metzger et al. 2007). However, the biological mechanisms by which PM and other air pollutants may affect cardiovascular events are not well understood. An increasing number of studies have investigated a range of possible biological mechanisms, such as effects on the autonomic nervous system or inflammatory responses triggering endothelial dysfunction, atherosclerosis, and thrombosis.

Low heart rate variability (HRV) is a marker of poor cardiac autonomic control and is associated with a higher risk of myocardial infarction and sudden cardiac death (e.g., Singer et al. 1988; Tsuji et al. 1996). At the time the study described in this Research Report was funded, several cohort and panel studies had shown that increases in ambient PM concentrations are associated with decreased HRV (e.g., Liao et al. 1999; Pope et al. 1999, 2004; Gold et al. 2000; Creason et al. 2001; Devlin et al. 2003), although it still remains unclear how decreased HRV contributes to cardiac arrhythmia. HRV is an electrophysiologic parameter that can be derived from an electrocardiogram (ECG); many other such ECG parameters can be derived, but their clinical relevance is uncertain. Which ECG parameters may be affected by exposure to certain air pollutants deserves further study (Zareba et al. 2001; Utell et al. 2002).

In 2004, the Health Effects Institute issued a request for preliminary applications (RFPA 04-6, "Health Effects of Air Pollution") to solicit proposals in novel and important aspects of the health effects of air pollution that fell outside the topics targeted in specific requests for applications that year. In response to RFPA 04-6, Robert Lux of the University of Utah and Arden Pope of Brigham Young University submitted an application to assess ventricular repolarization in ECG data previously obtained by Dr. Pope (Pope et al. 2004). Changes in ventricular repolarization, the process of electrical recovery of the heart, have been associated with an increased risk for life-threatening arrhythmias (e.g., Antzelevitch 2005). The ECG data were obtained from a panel of elderly participants and showed an association between ambient concentrations of particulate matter less than 2.5 μm in diameter ($PM_{2.5}$) and decreased HRV (Pope et al. 2004). Drs. Lux and Pope proposed to measure different parameters of ventricular repolarization based on 24-hour ECG recordings obtained during periods of high and low concentrations of ambient $PM_{2.5}$, using a novel way to analyze ECGs developed by Dr. Lux (Lux et al. 2001). HEI funded the proposal because the Health Research Committee considered the proposed use of novel electrophysiologic parameters to be an interesting approach to studying possible mechanisms underlying PM effects on cardiovascular morbidity and mortality.

This Critique is intended to aid HEI sponsors and the public by highlighting the strengths of the study, pointing out alternative interpretations, and placing the report into scientific perspective.

BACKGROUND

HRV and other ECG parameters have been the focus of attention in studies of air pollution and health for a number of years. At the time Lux and Pope began the current study, several studies had found evidence for an association between decreased HRV and increased concentrations of particulate air pollution (e.g., Liao et al. 1999;

Dr. Robert Lux's nine-month study, Air Pollution Effects on Ventricular Repolarization, began in July 2005 with total expenditures of $49,981. The Investigators' Report from Dr. Lux and co-investigator Dr. Arden Pope was received for review in June 2006. A revised report, received in January 2007, was accepted for publication in August 2007. During the review process, the HEI Health Review Committee and the investigators had the opportunity to exchange comments and to clarify issues in the Investigators' Report and in the HEI Health Review Committee's Critique.

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

Pope et al. 1999, 2004; Gold et al. 2000; Creason et al. 2001; Devlin et al. 2003). Many more studies have been conducted since, providing additional evidence for the observed association between HRV and air pollution in different locations and groups of people and extending it to other pollutants, such as long-term exposure to NO_2 (Felber Dietrich et al. 2008).

HEI has funded several human and animal studies of the potential mechanisms underlying the association between air pollution and cardiac electrophysiologic parameters. Among the first was a study by John Godleski and colleagues in a small number of dogs with or without induced coronary occlusion that were exposed to concentrated ambient particles (CAPs); they reported CAPs-induced ST segment elevation compared with unexposed controls as well as changes in heart rate, HRV, and decreases in T-wave alternans, but little or no change in inflammatory parameters (Godleski et al. 2000). (For an explanation of ECG terminology, see the sidebar, "Analyzing an Electrocardiogram.") In another animal study, Terry Gordon and colleagues measured heart rate and ECG intervals in rats with right-heart hypertrophy and pulmonary hypertension and in hamsters with a genetic cardiomyopathy but found little or no effect of CAPs on cardiac, pulmonary, or inflammatory measures in either species (Gordon et al. 2000).

More recently, Henry Gong and colleagues evaluated changes in cardiac electrophysiologic and blood parameters in healthy and asthmatic participants exposed to Los Angeles CAPs in a controlled laboratory setting (Gong et al. 2003). They reported modest changes in HRV and in blood levels of coagulation factor VII and soluble intercellular adhesion molecule-1; however, because the results were inconsistent and many other parameters were not changed, it is possible that those apparent changes occurred by chance. In another human study, Mark Frampton and colleagues measured pulmonary and cardiac function, as

ANALYZING AN ELECTROCARDIOGRAM

An electrocardiogram (ECG) measures the aggregate electrical potential of cells in the heart through several leads that are placed across a person's chest. The ECG trace shows peaks and troughs that reflect the activity of the heart's nodes, which send electrical signals to initiate contraction of the atria and ventricles. The different waves are marked "P" through "U" by convention (see Figure 1 of the Investigators' Report). The P wave signals depolarization as it travels from the sinoatrial node through the atria. The subsequent QRS complex represents the time required for *depolarization* (electrical inactivation) of the ventricles, which triggers their contraction. The T wave signals electrical recovery of the heart cells (*repolarization*). Sometimes a small U wave, which represents the last remnants of ventricular repolarization, may be seen following the T wave.

ECG traces are analyzed using computer programs that detect the onset, offset, and amplitude of the wave forms and also track abnormal sinus waves. An experienced physician or technician verifies the information by looking at individual traces. Only normal-to-normal (NN) heart beats are included in the analyses. Heart rate is calculated based on the interval between adjacent R-wave peaks (RR).

HEART RATE VARIABILITY

Heart rate variability (HRV) is the conventionally accepted term to describe the considerable long- and short-term fluctuations in heart rate that occur in normal people. A substantial body of evidence indicates that reduced HRV is associated with cardiac mortality after myocardial infarction (see the Investigators' Report and Critique). HRV can be determined through ECG recordings as short as 5 to 10 minutes, but 24-hour recordings are preferable. HRV data provide insight into the autonomic control of the heart.

The most established HRV parameters, and the simplest to obtain, are in the *frequency domain*: for example *ultra-* and *very low frequency* (ULF and VLF), *low frequency* (LF), and *high frequency* (HF), as well as the *LF/HF ratio.* These parameters are derived using standard statistical methods to quantify short- and long-term fluctuations in heart rate. ULF and VLF components occur less often than 3 per minute and are thought to be reflective of thermoregulation; LF components occur approximately 6 per minute and are related to baroreflex, a homeostatic mechanism for maintaining blood pressure; HF components occur at a rate equal to respiratory rate — between 9 and 24 breaths per minute. The LF/HF ratio has been proposed as an index of the balance between the regulatory influences of the sympathetic and parasympathetic nervous systems. One advantage of using the frequency domain parameters is that data can be obtained using relatively short recording intervals.

well as markers of coagulation and inflammation in blood and sputum, in healthy and mildly asthmatic participants who were exposed to concentrated ultrafine carbon particles in a controlled setting. They found few airway, systemic, or cardiac electrophysiologic changes associated with exposure to ultrafine carbon particles. However, they reported preliminary evidence of a reduction in QT interval duration with a concomitant increase in T-wave amplitude after ultrafine particle exposure in young healthy participants; they also reported a trend toward decreased HRV in mildly asthmatic participants, predominantly in males (Frampton et al. 2004).

HRV is one of the most frequently measured cardiac outcomes because it is noninvasive and relatively easy to obtain. HRV can be measured in a number of ways: frequently reported parameters are time-based, such as SDNN and rMSSD, or frequency-based, such as LF, HF, and the LF/HF ratio (see sidebar). HRV is best obtained when conditions are stationary, for example, by using metronomic breathing over short time periods. The predictive value of these parameters for cardiac events in the clinic remains difficult to assess, however. Although HRV is a useful marker for changes in autonomic control of the heart and has been associated with higher risk of myocardial infarction and sudden cardiac death, it is essentially a statistical approach that does not provide insight into the potential mechanisms underlying the observed association between air pollution and cardiac outcomes.

To better understand the effects of air pollution on the heart, it would be useful to study additional ECG parameters that directly reflect clinically relevant cardiophysiologic processes, such as the duration and amplitude of the electrical wave potentials that trigger contraction of the heart muscle. Ventricular repolarization is the process of electrical recovery of the heart cells after contraction of the ventricles and is represented by the ST segment and

In addition, *time-domain* parameters can be determined, such as the *standard deviation of NN intervals* (SDNN), a broad measure of HF and LF oscillations that reflects changes in autonomic tone; the *standard deviation of the mean values (averages) of NN intervals* in all 5-minute segments of a 24-hour recording (SDANN); and the *root mean square of successive differences* between adjacent NN intervals (rMSSD), a measure that corresponds to HF variability and reflects changes in cardiac vagal tone. Time-domain HRV parameters are more stable and reproducible than frequency-domain parameters.

ANALYZING WAVE FORMS

In addition to heart rate and HRV, other parameters commonly analyzed are the PR and ST segments, the QRS complex, and the QT interval. They reflect the condition of the myocardium (heart muscle). The length of the *PR segment* represents the time it takes for an electrical impulse to travel from the heart's sinoatrial node, where the normal electrical impulses of the heart are initiated, to the ventricles. The *QRS complex*, as defined earlier, represents the time required for electrical activation of the ventricles. The *QT interval* captures the time for both ventricular depolarization and repolarization; it represents the time between earliest ventricular depolarization and latest ventricular repolarization. Prolongation of the QT interval has been associated with an increased risk for ventricular arrhythmias in a variety of cardiac conditions. The QT interval is influenced by the heart rate; thus the *QT interval corrected for heart rate* (QTc) is often used to make comparisons between individuals. The *ST segment* starts at the end of the QRS complex and ends at the beginning of the T wave; it coincides with the plateau of the action potential. The ST segment is important in the diagnosis of ventricular ischemia and hypoxia because under those conditions, the ST segment can become either depressed or elevated. Elevation of the ST segment indicates early repolarization. Repolarization encompasses the entire ST-T part (ST segment plus T wave) of the ECG. *T-wave alternans* is a measure of beat-to-beat fluctuation in the magnitude and shape of the T wave.

NOVEL ECG PARAMETERS

Dr. Lux has pioneered a novel statistical method (Lux et al. 2001) to analyze ECG wave forms that uses a root mean square (RMS) of all recorded leads as an indicator of their magnitude. Novel parameters included in this report are the *RMS RT interval*, an estimate of mean ventricular action potential duration; the *RMS T width* (TW), an estimate of ventricular repolarization dispersion; and the *RMS QT* interval. In addition, Dr. Lux determined regressions of *RT versus RR*, *TW versus RR*, and *QT versus RR*, which provide estimates of the rate dependency of repolarization.

T wave in the ECG (see Figure 1 of the Investigators' Report). Changes in ventricular repolarization, such as elongation of the QT interval, elevation of the ST segment, and changes in T-wave morphology, have been associated with an increased risk for life-threatening arrhythmias (e.g., Antzelevitch 2005). Evidence has started to emerge that increased levels of air pollution may be associated with changes in ventricular repolarization (Gold et al. 2005; Henneberger et al. 2005; Yue et al. 2007). In cardiology, the QT interval or its heart rate-corrected form (QTc) has been the measurement of choice to assess repolarization. In addition, QT interval dispersion, or *inhomogeneity*, is of interest because it has been associated with an increased susceptibility to reentrant arrhythmia (i.e., arrhythmia originating in damaged heart tissue). However, because the QT interval is lead-dependent, it may not accurately reflect dispersion of repolarization (Lux et al. 1998); hence, Dr. Lux developed a novel approach to assess repolarization and its dispersion (Lux et al. 2001). Combining this approach with an interesting data set from the study conducted by Dr. Pope of a panel of 88 nonsmoking elderly participants provided a unique opportunity to assess whether measuring alternative ECG parameters could provide additional insight into the association between increased levels of air pollution and adverse cardiac outcomes.

SPECIFIC AIMS

In their study, Drs. Lux and Pope aimed to assess changes in ventricular repolarization of the heart based on 24-hr ECG recordings obtained from human participants during periods of low and high ambient airborne PM. They hypothesized that high levels of ambient PM might affect ventricular repolarization independent of heart rate; these changes could lead to an increased risk of arrhythmia in elderly persons, who may be more susceptible to the effects of air pollution.

The specific aims of this study were to

1. Measure on a heartbeat-to-heartbeat basis a number of standard and novel ECG parameters.
2. For each participant, compare several ECG parameters measured during a 24-hour time series on days of high and low $PM_{2.5}$ concentrations.

APPROACHES AND METHODS

Dr. Lux obtained existing ECG records from the panel study conducted by Dr. Pope (Pope et al. 2004), analyzed several novel ECG parameters of repolarization, and compared the parameter values between days of high and low $PM_{2.5}$ concentrations, with each person serving as his or her own control. Lux used two different statistical approaches to evaluate differences between parameter values.

PARTICIPANTS

In his earlier study, Dr. Pope evaluated a cohort of 88 nonsmoking elderly persons (ages 54–89, 57% female). Those with serious medical conditions, such as abnormal heart rhythm, pacemaker use, implanted defibrillator use, history of heart transplantation, or heart failure, within the previous 6 months were excluded. All participants lived in three communities in the Salt Lake Valley area in Utah and had been fitted with a Holter monitor to record 24-hour ambulatory ECGs. Dr. Lux obtained the ECGs from all 88 participants; the number of recordings varied from 1 to 5 per person. Data in one community were collected in the winter of 1999–2000 and summer of 2000; data in the other two communities were collected in the winter of 2000–2001. Because participants with only one recording and recordings of inferior quality were excluded, data from only 61 of the 88 participants were analyzed in this study. For more details on the participants, see Pope and colleagues (2004).

AIR QUALITY DATA

In his earlier study, Dr. Pope aimed at obtaining ECG recordings from each participant on both clear and polluted days. He used a clearing index — an indication of vertical and horizontal air mass stagnation — to determine clear and polluted days (i.e., days when an inversion would trap pollutants in the Salt Lake Valley). For days on which ECG recordings were made, Dr. Pope obtained daily, 24-hour $PM_{2.5}$ concentrations from the State of Utah Division of Air Quality; temperature and relative humidity were estimated based on data from the National Climatic Data Center for temperature and relative humidity in Salt Lake City. Any missing data were estimated based on an extrapolation of available data and by making use of the clearing index on consecutive days. For more details on air quality data, see Pope and colleagues (2004). $PM_{2.5}$ concentrations corresponding to each ECG recording were provided to Dr. Lux.

ECG ANALYSIS

Dr. Lux used custom software to calculate a root mean square (RMS) ECG, which captures the magnitude of the ECG wave forms. (Taking the RMS is a useful method when the numbers to be averaged include both positive and negative values, such as occurs in the positive and negative deflections of the wave forms in an ECG; because

the values are squared, all resulting data are positive.) Each RMS ECG was reviewed as part of a quality control procedure. Most recordings yielded about 80% acceptable data. A total of 215 ECGs out of 250 provided by Dr. Pope were included in this study.

For each ECG, standard parameters such as cycle length — the duration of one heart beat, from one R peak until the next (RR) — and its standard deviation σ_{RR} (a measure of HRV) were calculated. In addition, novel parameters of ventricular repolarization were calculated (see sidebar). Specifically, the investigators determined the RMS RT and RMS QT intervals and RMS TW, as well as their heart rate-corrected forms. They also determined the linear regressions of RT versus RR, TW versus RR, and QT versus RR, which are used to assess rate dependency. Data were averaged over 30-minute periods ("epochs") and presented as a 24-hour time-series (see Figure 2 of the Investigators' Report). All statistical analyses used data that had been reduced to average parameter values for these 30-minute epochs.

STATISTICAL ANALYSES

For each of the 61 persons included in the study, the investigators compared parameter values from ECGs obtained on days with high $PM_{2.5}$ concentrations with values from days with low $PM_{2.5}$ concentrations. If there were more than 2 recordings, the ECG obtained on the day with the lowest $PM_{2.5}$ concentration served as the control.

The investigators used two statistical approaches: (1) a nonparametric Kolmogorov-Smirnov test and (2) within-subject regression modeling. The Kolmogorov-Smirnov test assesses whether two distributions are statistically significantly different, regardless of the underlying distributional form of the data (i.e., the data do not have to be normally distributed). For each RMS ECG parameter, data from 30-minute epochs were plotted as probability distribution functions for "low" and "high" pollution days. The Kolmogorov-Smirnov test measured the strength of the evidence for a difference between the probability distribution curves. The investigators obtained these plots and the Kolmogorov-Smirnov tests for each participant individually and, for most analyses presented in the report, with data pooled from all participants. Fifty-two participants were included in the Kolmogorov-Smirnov analysis.

The regression modeling approach had two components: First, for each participant, the investigators calculated correlations between $PM_{2.5}$ concentrations and each ECG parameter value. They plotted histograms of the distributions of these correlations. Second, the investigators pooled data from all participants and performed regression analyses to assess the overall association between $PM_{2.5}$ concentration (the *x* variable) and each ECG parameter (the *y* variable). In the "Model I" regression analysis, they included a "fixed effect" for each of 61 participants in order to prevent any distortion of results by differences between participants (e.g., age and sex) that might otherwise confound the association between $PM_{2.5}$ concentrations and ECG parameters. In the "Model II" regression analysis, they also controlled for temperature and humidity by including a thin-plate spline for these variables in the model. The investigators ran regression analyses twice, once using 24-hour ECG parameter value summaries and then again using 6-hour summaries.

SUMMARY OF RESULTS

KOLMOGOROV-SMIRNOV TEST

As expected, $PM_{2.5}$ concentrations on days with high (average, 46 $\mu g/m^3$) concentrations were statistically significantly different from those with low $PM_{2.5}$ (average, 6 $\mu g/m^3$) concentrations, and the distributions did not overlap. Heart rate on high $PM_{2.5}$ days was not statistically significantly different from that on low $PM_{2.5}$ days; however, σ_{RR}, a measure of HRV, was significantly different. No statistically significant differences were observed in the distribution functions of the ventricular depolarization measures RMS RT, RMS TW, and RT:RR and TW:RR slopes on high $PM_{2.5}$ compared with low $PM_{2.5}$ days (see Table 3 of the Investigators' Report). No differences were observed for RMS QT, QTc, or QT:RR slope (data not shown).

FIXED-EFFECTS MODELS

Both regression models showed some evidence for an association between a decline in the average duration of ventricular depolarization and increasing $PM_{2.5}$ for 24-hour data (Model I: RT interval, $P < 0.10$; Model II: RT interval, $P < 0.05$; QT interval, $P < 0.10$; see Table 4 of the Investigators' Report). Model II, which included temperature and humidity, also showed a small, statistically significant increase in TW associated with a 10-$\mu g/m^3$ increment in $PM_{2.5}$ ($P < 0.05$).

HEI REVIEW COMMITTEE EVALUATION

The HEI Review Committee thought that this was an interesting project that investigated an important hypothesis regarding air pollution and cardiovascular health. Further strengths were the use of an existing data set that had shown an association between increased air pollution levels and decreased HRV, and the use of novel methods pioneered by Dr. Lux to assess ventricular repolarization.

This study broadly confirmed previous findings that increased concentrations of $PM_{2.5}$ are associated with decreased HRV. However, the novel approach to measure ventricular repolarization did not find much evidence for an association between repolarization and $PM_{2.5}$ concentrations, although some suggestive patterns were observed when using fixed-effects modeling (e.g., a decrease in RT interval and an increase in TW accompanied an increase in $PM_{2.5}$ concentration), which were of borderline statistical significance. No statistically significant changes were observed in the QT interval or its rate-corrected form, which is widely used in clinical settings and a prolongation of which is associated with an increased risk for arrhythmias. It remains difficult to interpret the importance of a decrease in RT interval compared with the lack of change in QT interval, which are closely correlated parameters. In addition, the observed change (decrease) in RT interval is opposite in direction to what would be expected based on the association of prolonged QT interval and heart disease. On the other hand, the suggestive finding of a longer T-wave duration supports clinical evidence of an association between changes in T-wave morphology and cardiac conditions (e.g., Antzelevitch 2005).

As discussed by the investigators, there are several possible reasons for the lack of strong evidence for an association between $PM_{2.5}$ concentration and ventricular repolarization in this study. One reason is that the study may have had insufficient statistical power to find subtle effects among large daily variations within and between participants. For example, ECG parameter values vary over the course of a day, complicating attempts to compare ECGs obtained in the same person on different days. The investigators attempted to align ECGs by time of day but concluded that it was not a useful approach. The fact that ECGs were not aligned may have contributed to additional variability. In addition, although the method used in this study was state of the art, it was developed based on 12-lead ECGs, while the investigators in this study used ECG recordings obtained with only 2 leads, which may have affected the quality of the data.

Furthermore, as discussed by the investigators, the original study population was not controlled for diet, medication use, or activity patterns. Those factors are known to affect cardiac physiology and may contribute to considerable variation among participants. The importance of taking medication use into account was underscored by a recent study that showed the strongest association between air pollution and reduced HRV in persons who were not using beta blockers (de Hartog et al. 2009). Although those with serious medical conditions were excluded from the study, it is possible that participants differed in their baseline HRV and ventricular repolarization parameter values, which may have affected their susceptibility to the effects of air pollution. Indeed, the investigators found considerable individual variation in the response to air pollution, but it remains unknown which factors may have contributed to a particular person's susceptibility.

The HEI Review Committee considered the overall statistical analysis strategy of reducing the ECG data to 30-minute epoch summaries and subsequently using regression methods to be a good one. However, results of Kolmogorov-Smirnov analyses on epoch data pooled across participants should be interpreted cautiously because this approach does not allow for determinants of ECG parameter values other than pollution, such as differences in individual participant characteristics and meteorologic measurements. The regression modeling results, which are more robust because the investigators did control for these factors, should be considered the primary ones.

The Committee agrees with the investigators that the regression results do not provide clear support for an effect of air pollution on repolarization. However, the presence of statistically significant associations of air pollution with some repolarization parameters suggests that repolarization should not be dismissed as a possible mechanism involved in the association between air pollution and cardiac effects. The observed association between air pollution and HRV (σ_{RR})—which confirmed the previous observations by Dr. Pope, although he used different HRV parameters—apparently was not subjected to the more stringent regression analysis in this study.

The regression modeling was similar to that used by Dr. Pope in his previous study, which evaluated five regression models that showed fairly consistent associations of decreased SDNN, SDANN, and rMSSD (see sidebar) with increased $PM_{2.5}$ concentrations (Pope et al. 2004). In that study, Dr. Pope also analyzed different lags of exposure to $PM_{2.5}$ for his fifth regression model, which included subject-specific fixed effects, interactive spline smoothing for temperature and relative humidity, and partial control for heart rate. The strongest associations were observed with $PM_{2.5}$ concentrations on the same day, becoming weaker at increasing lags and disappearing at lag 3 (Pope et al. 2004). The focus in the current study on same-day exposures was thus reasonable, although it would be interesting to analyze the novel ECG parameters explored in this study at longer lags as well.

Given the inclusion in the current study of observations during summer and winter, an evaluation of the role of season might also be interesting, both as a determinant in its own right (and hence a potential confounder of air pollution effects) and as a possible modifier of air pollution effects. The role of other factors, in particular preexisting

disease, in possibly modifying air pollution effects would also be an interesting focus of analysis, though in this study, power limitations would be substantial. As noted by the investigators, this study revealed substantial variation in ECG parameter values over time as well as between participants. Further effort to better understand the determinants of this variation might be of interest in its own right and might improve the ability to identify air pollution effects, if any.

In summary, the Health Review Committee concluded that, although this study provides little evidence for an effect of particulate air pollution on ventricular repolarization in elderly persons, such an effect cannot be ruled out. In particular, an effect might be seen in potentially more sensitive persons such as those with preexisting heart disease. Further studies controlling for other sources of variability are needed to provide a more definitive answer.

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