FINAL REPORT

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PERSONAL, INDOOR AND OUTDOOR PARTICULATE AIR POLLUTION AND HEART RATE VARIABILITY IN ELDERLY SUBJECTS WITH CORONARY ARTERY DISEASE

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ABSTRACT

Background: Decreased heart rate variability (HRV) is a marker of autonomic dysfunction and has been associated with future cardiac morbidity and mortality. HRV has been inconsistently associated with exposure to particulate matter (PM) air pollution. Fewer studies have examined whether air pollution exposure is a risk factor for cardiac arrhythmias. We aimed in this study to evaluate the relationships of heart rate variability and cardiac arrhythmias to PM exposures.

Methods: Heart rate variability and arrhythmias were measured with ambulatory electrocardiographs using available raw data collected in a cohort panel study the National Institutes of Health, National Institute of Environmental Health Sciences (funded by NIH NIEHS R01 ES-012243). Electrocardiograph data (8,952 person-hours) were collected in 50 nonsmokers with coronary artery disease age 71 years and older living in four retirement communities in the Los Angeles air basin. Exposures included hourly markers of traffic-related PM and secondary organic aerosols, daily size-fractionated particle mass, and hourly outdoor criteria gases. Subjects were genotyped for the glutathione S-transferase M1 and T1 null (risk) alleles. Repeated measures regression analyses were adjusted for actigraph-derived physical activity and heart rate, temperature, day of week, season, and community location.

Results: Risk of ventricular tachycardia was significantly increased with higher exposure to markers of traffic-related particles, secondary organic carbon, and ozone. We found stronger associations of ventricular tachycardia with OC and both its primary and secondary OC fractions among subjects who had the GSTM1 null genotype. Few consistent associations were observed for supraventricular tachycardia. PM exposure was significantly associated with decreased heart rate variability only in the 20 subjects using ACE inhibitors and in those with glutathione S-transferase M1 non-null (lower risk) genotype.

Conclusions: Results support the hypothesis that exposure to PM and ozone increases the risk of ventricular tachycardia among elderly subjects with coronary artery disease. These results are consistent with previous findings in this cohort for adverse effects of air pollution on systemic inflammation, blood pressure and electrocardiographic evidence of ischemia. However, decreased heart rate variability did not appear to be clearly affected except in subjects using ACE inhibitors.

EXECUTIVE SUMMARY

Background: Decreased HRV is considered to be a marker of autonomic dysfunction and has been inconsistently associated with exposure to PM air pollution. Fewer studies have examined whether air pollution exposure is a risk factor for cardiac arrhythmia. Both HRV and arrhythmias are associated with future cardiac morbidity and mortality. A limited number of studies have utilized repeated daily ambulatory electrocardiograph/electrocardiogram (ECG) data, opting instead to record short ECG strips at in-clinic visits, and most studies have relied on regulated particle mass concentration ($PM_{2.5}$ and PM_{10}). There are fewer data on ultrafine particles, which can carry large amounts of adsorbed or condensed toxic air pollutants. In the Los Angeles air basin mobile sources are the primary source of ultrafine particles, often found at high concentrations near busy traffic. We hypothesize that because of the high concentration of components with high oxidative potential (e.g., polycyclic aromatic hydrocarbons), exposure to traffic-related air pollution will be associated with decreased HRV.

Our objectives were to examine relationships between cardiovascular autonomic function as measured by HRV and exposures to air pollutants measured at retirement community sites of study subjects with diagnosed coronary artery disease. We hypothesized that exposure to ultrafine particles and traffic-related air pollution would be inversely associated with HRV, which will support the view that ultrafine particles and traffic-related air pollution lead to disturbances of cardiovascular autonomic function. We also evaluated effect modification of associations by polymorphisms in genotypes involved in oxidative stress as data was available from other ongoing work. The objectives of this study evolved to also evaluate relationships of cardiac arrhythmias to air pollutant exposures. We hypothesized that exposure to ultrafine particles and traffic-related air pollution would be positively associated with arrhythmias. We additionally evaluated the effects of ozone on both HRV and arrhythmias. The study research is important to the Board's regulatory function of protecting human health through ambient air quality standards.

Methods: We conducted a panel study with repeated measures to evaluate acute cardiovascular health effects of exposure to air pollution (NIH NIEHS R01 ES-012243). Valid data available for the present study included home air pollution and ambulatory ECG tracings in 50 elderly subjects with a history of coronary artery disease living in 4 retirement communities in the Los Angeles Air Basin. Subjects were monitored daily in their home by research technicians over two 5-day periods (sample size = 8,952 person-hours). Digital and paper diaries were used to monitor medication use. Actigraphs were used to monitor physical activity. The additional work in the present study was to analyze relationships of HRV and arrhythmias to air pollution exposure. Ambulatory ECG signals were analyzed and included 5-minute segments of ECG for time domain analysis of total and short-term variation in heart rate across individual hours, 24-hr periods, and daytime and nighttime periods. These HRV outcomes included: standard deviation of all normal R-R intervals in the time segment (SDNN), root mean square of the successive differences (r-MSSD), percentage of differences between adjacent normal RR intervals that are larger than 50 ms (pNN50). Arrhythmia outcomes included supraventricular and ventricular arrhythmias in non-sustained runs of $\geq$ 3 ectopic beats at a rate $\geq$ 120 beats per minute. During every person-day of ambulatory ECG monitoring, we collected 24-hr quasi-ultrafine ($PM_{0.25}$), accumulation ($PM_{0.25\text{-}2.5}$), and coarse mode ($PM_{2.5\text{-}10}$) particulate matter mass on Sioutas impactors. Hourly pollutant data included criteria gases, $PM_{2.5}$, total particle number, Aethelometer black carbon (BC), and Sunset Labs $PM_{2.5}$ elemental and organic carbon (EC, OC). We also estimated outdoor concentrations of primary and secondary OC. We conducted multiple regression analyses of HRV and arrhythmias using generalized estimating equations to account for within-subject correlations. Effect estimates were standardized per interquartile range increase in each air pollutant. Associations were adjusted for actigraph-derived physical activity and heart rate, temperature,

day of week, season, and community location. In addition to examining the relationship of outcomes to same day and exposures lagged several days, we conducted hourly analyses to determine whether there were acute effects. We also assessed subject susceptibility to decreased HRV and increased arrhythmias with increasing air pollutant exposures, including medication use (e.g., beta-blockers), sex, self-reported co-morbidities, and in subjects with vs. without GSTM1 and T1 null genotypes that potentially increase oxidative stress, which in turn can increase cardiovascular dysfunction.

Results: In regression analyses we found a 16 μg/m^3 increase in 24-hr average $PM_{2.5}$ was significantly associated with a 50% increase in the daily rate of ventricular tachycardia (VT) events (rate ratio 1.50). There were no significant associations for outdoor particle number and positive associations were observed for both $PM_{0.25}$ and $PM_{0.25\text{-}2.5}$. Analysis of $PM_{2.5}$ constituents indicated significant associations of daily VT with the last 24-hr average exposures to BC, EC, total OC, and primary OC. The last 24-hr average total OC had a notably strong estimated effect on daily VT (a 206% increased rate per 5.2 μg/m^3). The greatest apparent contribution was from primary as compared with secondary OC, which was of borderline significance. Ozone also had strong effects on daily VT (3-day O_3 average showed a 195% increased rate per 17.4 ppb). NO_x and the PM constituents BC, EC and OC, had the strongest VT effect estimates during the daytime in models analyzing hourly VT occurrence. Findings for indoor measurements were consistent with results for outdoor primary pollutants. This included the novel finding that positive associations of air pollutants with the daily count of VT was stronger and more significant for exposure to indoor EC of outdoor origin than for uncharacterized indoor EC, suggesting that outdoor fossil fuel combustion sources were important determinants of VT associations. There was little evidence of effect modification of VT associations with air pollutants by subject-specific characteristics or risk factors, other than nominally stronger associations of VT with OC and its primary and secondary OC fractions among subjects who had the GSTM1 null genotype. In contrast, relations between supraventricular tachycardia and air pollution were largely nonsignificant.

We found few significant associations beyond that expected by chance between the HRV outcomes and either indoor or outdoor hourly air pollutant exposures. We also found that 24-hour SDNN was not associated with daily averages of size-fractionated (gravimetric) PM mass concentrations or multi-day averages of the hourly air pollutants. However, we tested potential effect measure modification of outdoor air pollutant effects on HRV by medication use and found no significant differences except for ACE inhibitors. $PM_{2.5}$, EC, BC, OC (both primary and secondary fractions), and 4-hr and 8-hr ozone were significantly and inversely associated with hourly SDNN only among the 20 subjects taking ACE inhibitors and unexpectedly in those with glutathione S-transferase M1 non-null (lower risk) genotype.

Conclusions: The present study supports the hypothesis that exposure to ozone and to particulate air pollution including both ultrafine and accumulation mode fractions of $PM_{2.5}$ increases the risk of ventricular tachycardia in a population of elderly people with coronary artery disease. Results suggest that at higher particle and ozone concentrations arrhythmia rates increase to levels with potential clinical importance. Gene-environment interaction models suggest that lack of GSTM1 may enhance oxidative stress-induced arrhythmias from higher air pollutant exposures. However, decreased heart rate variability was not observed in relation to air pollutant exposures except in subjects taking ACE inhibitors. We speculate that ACE inhibitors may enhance respiratory irritant responses to air pollutants via stimulation of pulmonary vagal afferents by bradykinin.

BODY OF REPORT

1. CHAPTER ONE: INTRODUCTION

1.1 Background

Findings in cohort and time series studies suggest that environmental exposure to PM air pollution is associated with increases in cardiovascular hospitalization and mortality. Individuals at greatest risk include elderly individuals with preexisting cardiovascular or other diseases that place them at high risk for myocardial infarction or stroke (Pope and Dockery 2006). Pathophysiological mechanisms underlying the epidemiologic studies are emerging. There is a growing body of recent evidence that proinflammatory and pro-oxidant characteristics of PM may produce deleterious autonomic effects on cardiac function and vascular tone, which increase the risk of more adverse cardiovascular events (Brook et al. 2010). Particulate air pollution exposures can produce oxidative stress responses to reactive oxygen species generated by pro-oxidant PM components and subsequent inflammation may be an important mechanism of adverse cardiovascular responses (Brook et al. 2010).

Decreased HRV has been used as a marker of alterations in cardiac autonomic balance and has been associated with risk of cardiac morbidity and mortality (Liao et al. 1996; Task Force 1996; Tsuji et al. 1994, 1996). For these reasons, HRV has been used to assess the relation of cardiac autonomic control with exposure to air pollution (Link and Dockery 2010).

Decreased high frequency (HF) power spectra of heart rate is an HRV indicator of decreased vagal tone and has been associated with the extent of coronary atheromatosis in patients with coronary artery disease (CAD) (Airaksinen et al.1987; Huikuriet al. 1999). Associations have also been found between HRV and ischemia in CAD patients monitored with ambulatory ECGs, including asymptomatic ST segment depression indicative of cardiac ischemia (Bigger et al. 1990; Vardas et al. 1996). Given that many people with CAD already have decreased HRV, pollutant exposures that lead to further decreases are expected to precipitate adverse clinical outcomes.

For these reasons, a number of recent studies of HRV and air pollution have focused on subjects with CAD, but have reported mixed findings (Barclay et al. 2009, Folino et al. 2009; Hampel et al. 2010; Schneider et al. 2010; Zanobetti et al. 2010). Despite the mixed findings overall, a recent review of the literature on PM air pollution and cardiovascular health concluded that there was strong epidemiological evidence for association between decreased HRV and short-term exposures to PM, especially in older or clinically susceptible people (Brook et al. 2010). However, more research is needed to identify air pollutant sources and their components responsible for autonomic effects.

Ambient particulate air pollution has been associated with decreased HRV in humans, but few studies have used repeated daily ambulatory ECG, opting instead to record short ECG strips at in-clinic visits (reviewed by Delfino et al. 2005, and Pope and Dockery 2006). Furthermore, most studies have relied on regulated particle mass concentration ($PM_{2.5}$ and PM_{10}). However, there is sufficient reason to

believe that ultrafine particles (UFP, generally defined at < 0.1 µm in aerodynamic diameter) are capable of inducing the greatest amount of oxidative stress, inflammation, and other adverse effects per unit of PM mass compared with larger particle size fractions currently regulated by the US EPA (Delfino et al. 2005; Oberdörster 1995, 2001). This is due to major characteristics of UFP, including high pulmonary deposition efficiency, magnitudes higher particle number concentration than larger particles, and thus a much higher surface area (Utell 2000). Therefore, UFP can carry large amounts of adsorbed or condensed toxic air pollutants (oxidant gases, organic compounds and transition metals), which have been identified as having pro-inflammatory effects. Evidence has been presented that increased biological potency of UFP is related to the content of redox cycling organic chemicals and their ability to damage mitochondria (Araujo 2008; Li 2003). Systemic inflammatory responses leading to autonomic dysfunction could occur because of lung inflammation or translocation of UFP or pollutant components to the circulation (Nemmar 2002, 2004; Oberdörster 2002).

Mobile sources in the Los Angeles air basin are the primary source of exposure to UFP, which is often found at high concentrations near busy traffic (Fine 2004a, 2004b; Kim 2002; Zhu 2002a, 2002b, 2004). We hypothesize that traffic-related air pollution and UFP will be associated with decreased HRV because of their high concentration of pro-oxidant components.

Accurate exposure assessment methods are critical to assess the magnitude of human health effects from UFP in epidemiologic studies (Sioutas et al. 2005). A panel study by Timonen et al. (2006) has reported on the relationship between HRV and UFP, although other studies reviewed below have looked at total particle number concentrations, which is dominated by UFP. Timonen et al. (2006) followed a panel of elderly Europeans with CAD and showed increased ambient UFP (0.01-0.1 µm) particle number concentration was associated with a significant decrease in low-to-high frequency ratio (LF/HF) suggesting a change in sympathovagal balance towards increased vagal tone. LF HRV includes both sympathetic and vagal influences whereas HF indicates vagal tone. However, HRV data was not obtained using ambulatory (Holter) methods and particle data were from ambient monitoring sites far from subject locations. Also, given the high spatial variability of UFP, it is likely that UFP in Timonen et al. (2006) was less representative of personal UFP exposure than of $PM_{2.5}$ exposure, which has lower spatial variability (Sioutas et al. 2005). An experimental study in healthy young adults exposed to concentrated UFP also showed increased HRV suggestive of increased vagal activity (Samet et al. 2009). This finding, as well as the finding in Timonen et al. (2006), is counterintuitive since one expects a sympathetic stress response leading to decreased HRV. Huang et al. (2012), on the pother hand, found that experimental exposure of health human subjects to both NO_2 and concentrated fine particles increased the low frequency components of HRV and the LF/HF ratio suggesting a sympathetic stress response.

Chan et al. (2004) conducted a study to assess the relationship between HRV and personal particle number concentrations (dominated by UFP) for particles 0.02-1.0 µm in diameter ($NC_{0.02-1}$). They followed 9 young healthy adults and 10 elderly male subjects with obstructive lung function impairment. Subjects were monitored over only 10 daytime hours using a P-Trak Ultrafine Particle Counter (TSI Inc., Shoreview, MN) for $NC_{0.02-1}$. Subjects also wore ambulatory ECG monitors for continuous 5-min beat-to-beat intervals to assess HRV over the 10 hours. Using

linear mixed effects models they found decreases in HRV indices (SDNN and r-MSSD, defined below) were associated with exposure to one to four-hr moving averages of $NC_{0.02-1}$ before the 5-min HRV measurements. Associations were stronger for the elderly panel, with the strongest effects from two-hr average $NC_{0.02-1}$. These results suggest the effect of personal PM exposure on autonomic function is acute, although the monitoring period (10 hr) was too short in Chan et al (2004) to assess longer-term exposure impacts.

Few studies have looked at effect modification by genetic polymorphisms on the relationship between HRV and air pollution (for example: Chahine 2007; Park 2006; Schwartz 2005). This is important since some people may have a certain genetic predispositions to greater cardiovascular responses to air pollutant exposures. Genes of particular interest are those that code for proteins that protect cells from oxidative stress by detoxification of reactive oxygen/nitrogen species, electrophiles or reactive intermediates. A key example is the enzyme glutathione S-transferase (GST) that is represented by several isotypes (Hayes and Strange 2000). Two GST genes (M and T) can carry a homozygous null genotype leading to the absence of the enzyme that would otherwise protect cells by detoxification of electrophiles or reactive intermediates (e.g, polycyclic aromatic hydrocarbon epoxides) by conjugation with glutathione (GSH). In a small elderly cohort (the Normative Aging study), and using one measurement per subject, the HF component of HRV was inversely associated with ambient $PM_{2.5}$ only in subjects with the glutathione S-transferase M1 (GSTM1) null genotype (no enzyme is synthesized), unless subjects were taking statins, which was protective (Schwartz 2005). Similar to r-MSSD, HF represents parasympathetic influences in HRV. In a follow-up paper, three-way interaction between ambient $PM_{2.5}$, GSTM1 null and HO-1 long repeat (lower cytoprotective and anti-inflammatory functions) was associated with decreased SDNN, HF and LF (Chahine 2007). Park et al. (2006) found subjects in this cohort with certain polymorphisms in the hemochromatosis gene showed weaker associations between ambient $PM_{2.5}$ and decreased HRV, suggesting that the metabolism of redox active metals may be important.

In time series studies, hospital admissions and mortality from arrhythmias have often been associated with $PM_{2.5}$, but other studies of individuals, usually using implanted cardioverter-defibrillators, have reported mixed results as summarized by the comprehensive review of Brook et al. (2010). They concluded that there is some but limited or weak available epidemiological and mechanistic evidence linking air pollution exposure with risk of arrhythmias. One epidemiologic study of patients with implantable cardiac defibrillators (ICDs) found no association of VT with ambient air pollution (Metzger et al. 2007) whereas two others did (Dockery et al. 2005; Ljungman et al. 2008). Since the reviews were published, Mills et al. (2011) conducted a randomized controlled trial of dilute diesel exhaust vs. clean air exposure in 52 middle-aged participants and found no increased risk of arrhythmias.

A review of the literature by Link and Dockery (2010) provide evidence that PM might be more clearly associated with cardiac arrhythmias, particularly ventricular arrhythmias, in patients with underlying cardiac disease. Folino et al. (2009) studied CAD patients with ambulatory ECGs (similar to the present study) and found strong associations of PM with VT but not with HRV. Another study of CAD patients using ambulatory ECGs found associations of PM with VT but did not study HRV (Berger et al. 2006). However, more recently, He et al. (2011) studied 105 middle-aged

participants with and without cardiovascular disease using ambulatory ECGs and reported that personal $PM_{2.5}$ exposure was significantly associated with premature ventricular contractions only among participants without cardiovascular disease.

As pointed out by Brook et al. there is limited mechanistic data showing how PM effect risk of arrhythmia, but Link and Dockery (2010) propose that there may be a role for altered HRV, repolarization abnormalities, oxidative stress, and myocardial ischemia. Redox-active chemical components in PM may cause arrhythmias via lipid peroxidation, endothelial dysfunction, and other mechanisms involving oxidative stress (Griendling and FitzGerald 2003).

1.2. Scope and Purpose of the Project

This study sought to address gaps in the above-described literature on the relationship between HRV and air pollution. We performed a parallel analysis of risk of arrhythmias from air pollution exposures that could not be completed following the end of the parent grant (NIH NIEHS R01 ES-012243). The gaps investigated included:

1) data on the importance of ultrafine particles as compared with larger size fractions to decreased HRV and risk of arrhythmias;
2) data on the importance of hourly tracers of fossil fuel combustion and organic components to decreased HRV and risk of arrhythmias;
3) effect modification of HRV associations with air pollutants by variants in GST genes that are upregulated in response to oxidative stress.

The presents study addressed these gaps by producing new HRV and arrhythmia data merged with available extensive exposure and health outcome assessments for a funded study that had been completed. This is an NIH, NIEHS-funded cohort panel study entitled "*Ultrafine Particulate Matter & Cardiorespiratory Health*" (grant no. ES-012243; NIEHS funding ended 7/31/2010). The NIEHS-funded study focused on measurements of blood biomarkers of inflammation and of cardiovascular function (blood pressure and electrocardiographic ST-segment depression). Briefly, exposure measurements include 24-hr size-fractionated gravimetric mass measurements (Monday through Friday at fixed retirement community sites) and indoor-outdoor particle number concentrations. The exposure assessment component of the NIH-funded study was greatly expanded with supplemental funding through California Air Resources Board contract no. 03-329 with co-funding by the South Coast Air Quality Management District (CARB/AQMD funding ended 5/30/2008). This included indoor and outdoor hourly concentrations of $PM_{2.5}$ EC, OC, BC, and mass, and criteria air pollutant gases. Funding from the Southern California Particle Center was added (US Environmental Protection Agency grant no. RD83241301) to measure biomarkers of antioxidant function and oxidative stress. The overall study based on these integrated funding sources is referred to as the Cardiovascular Health and Air Pollution Study (CHAPS). None of the specific aims of these studies addressed an analysis of HRV data, which required extensive processing of raw Holter data (461 24-hr Holters). At the end of the NIEHS study the arrhythmia data from the Holters had not been analyzed and therefore, is included in the present study for analysis.

The informative nature of this panel study prior to the present analysis are

evidenced in previous papers by Delfino et al. (2008, 2009, 2010a, 2010b, 2010c, 2011) that overall supported the following hypothesis (Figure 1) based in large part on data from epidemiologic studies and animal and controlled human exposure experiments (Brook et al. 2010; Link and Dockery 2010; Mills et al. 2009): Redox-active and other components in ultrafine particles from fossil fuel combustion affect airway and cardiovascular target sites leading to systemic inflammation and endothelial dysfunction, and thereby precipitate adverse cardiovascular responses including increased blood pressure and cardiac ischemia in humans. During a time of systemic inflammation, hypercoagulability, and endothelial dysfunction, the occurrence of arrhythmias would further enhance the risk of myocardial infarction. The possible role of autonomic dysfunction in this chain of events is unknown but decreased HRV has been associated with sudden arrhythmic death (Hartikainen et al. 1996; Odenmuyiwa et al. 1991).

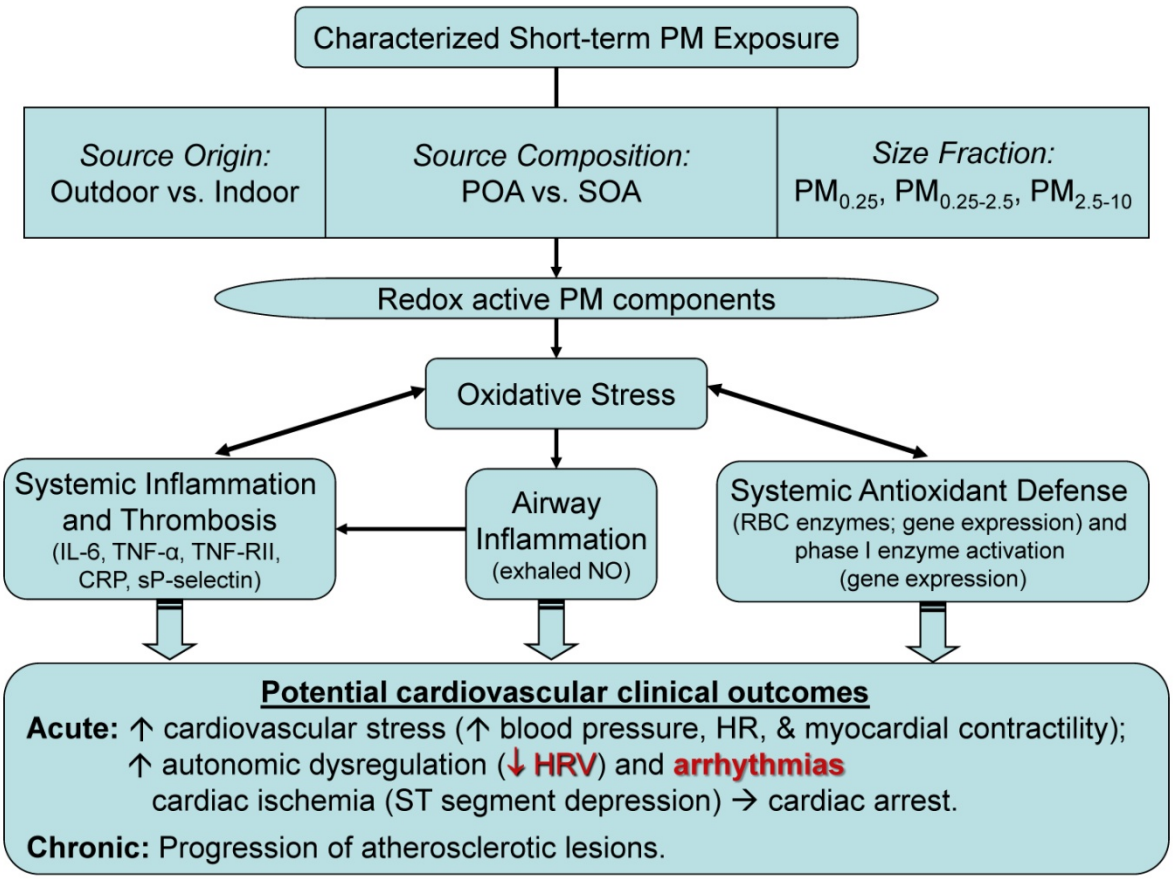

Figure 1. Hypothesized pathways from PM exposure to adverse cardiovascular health effects (in red) for evaluation in the present study.

Briefly, we conducted a longitudinal study with repeated measures to evaluate acute cardiorespiratory health effects of exposure to PM with a focus on UFP. Recruited subjects had CAD to ensure that clinically relevant changes in cardiovascular outcomes would occur during the study. Enrolled subjects in CHAPS were investigated in this study of HRV and arrhythmias. They include 50 subjects with valid ECG data for use in the analysis of HRV and arrhythmias. Subjects lived in four retirement communities in the Los Angeles Air Basin. Exposure assessment

was intensive and included daily PM mass (ultrafine, accumulation and coarse modes), and hourly indoor and outdoor home pollutant gases, EC, OC, BC, and total particle number concentration. Each subject also wore ambulatory ECG monitors for two 5-day periods (Sun through Fri) during two of their 12 weeks of follow-up. The two periods were separated by around a two-month rest period. During the ambulatory ECG monitoring they carried a roll-around air sampler for personal 24-hr PM mass (ultrafine, accumulation and coarse modes). However, the magnitude of missing data when merged with the ECG data precluded its use. DNA from stored blood samples of each subject was used for genotyping.

The study scope of work funded by NIEHS included an analysis of ambulatory ECG data that revealed significant positive associations of hourly ST segment depression ≥1 mm (an indicator of cardiac ischemia) with exposure to quasi-ultrafine particles and combustion-related pollutants (Delfino et al. 2011). The additional assessment of HRV parameters as outcomes representing autonomic function is important in that they are measurements across all monitored times for all subjects. In contrast, ST segment depression was only seen in around half of our subjects, and more sustained cardiac arrhythmias occured only in certain individuals as detailed below. Findings for HRV may also inform findings for arrhythmias that may be affected by changes in autonomic tone (Lahiri 2008).

The present work represents a substantial advancement in the study of relationships between ambulatory HRV and air pollution in that it has among the longest repeated measures of air pollution exposure and continuous ambulatory ECG data in individual subjects (up to 10 days per person). What is more important is that the study also included home microenvironmental exposures to a wide array of air pollutant measurements. . The availability of both hourly and daily air pollutant data with lags of 5 or more days enables us to assess the temporality of associations and to evaluate both very acute and multiday exposure impacts. In addition, detailed clinical characteristics and other outcomes have been collected in the parent study that allows us to test underlying susceptibility factors. This air pollutant and outcome data from CHAPS has allowed us to characterize pollutant exposure-response relations in ways that have already yielded clues to potentially important causal characteristics of particles (Delfino et al. 2008, 2009, 2010a, 2010b, 2010c, 2011). Briefly, key novel findings include the following:

- Increases in outdoor particulate matter (PM) air pollution were associated with increases in biomarkers of systemic inflammation and platelet activation in blood samples. Associations were strongest for measurements representing UFP.

- Circulating biomarkers of systemic inflammation were associated with markers of primary (combustion-related) organic aerosols but not with markers of secondary (photochemically-related) organic aerosols. Exhaled nitric oxide (a biomarker of airway inflammation) was associated with with markers of secondary organic aerosols and ozone suggesting that pro-oxidant products of photochemistry are important in airway inflammation.

- Associations of circulating biomarkers of systemic inflammation and platelet activation with outdoor PM were consistent with associations for indoor exposures to PM of outdoor origin, supporting findings that suggest adverse health effects of outdoor air pollutants occur even though people spend most of their time indoors.

- We found significant and strong positive associations of hourly ambulatory blood pressure with exposure markers of traffic-related combustion sources. Associations were stronger among obese subjects suggesting that this is a susceptible population.
- We found exposure to quasi-ultrafine particles and traffic-related air pollutants increased the risk of possible myocardial ischemia as indicated by ST segment depression > 1 mm recorded using ambulatory (Holter) electrocardiographs.

1.3. Tasks

The following tasks were completed:

1a We examined relationships between heart rate variability and exposures to indoor and outdoor home air pollutants in a panel of elderly people with a history of coronary artery disease.

We hypothesized that $PM_{0.25}$ and particle number concentration would be inversely associated with HRV, and support the conclusion that ultrafine PM exposure leads to disturbances of cardiovascular autonomic function involving an underlying sympathetic stress response. Ultrafine particles are emitted at high concentrations near traffic sources. We further hypothesize that exposure to traffic-related air pollution will be associated with decreased HRV. We measured HRV using the time domain measures SDNN, r-MSSD, and pNN50. We anticipated that the strongest associations would be for quasi-ultrafine PM, particle number concentration and markers of primary combustion aerosols (EC, BC and primary OC).

1b **We analyzed the relationship between the occurrence of cardiac arrhythmias and air pollution exposure**. We hypothesized that exposure to traffic-related air pollution will be associated with cardiac arrhythmias.

2. We evaluated effect modification of relationships in Task 1 by subject genotypes for proteins involved in oxidative stress or antioxidant responses to air pollutant exposures.

In this **exploratory aim**, we evaluated whether relationships of HRV with air pollutant exposures differ by germline variants in key antioxidant genes, GSTM1 and GSTT1. Genotyping of polymorphisms in these genes was done under other funding.

We hypothesized that GSTM1 and GSTT1 null genotypes would enhance HRV responses to air pollutant exposures. This exploratory aim could identify elderly subjects with CAD at potentially heightened susceptibility to the adverse effects of air pollution.

References:

Adkins S, Ganm KN, Mody M, La Du BN. Molecular basis for the polymorphic forms of human serum paraoxonase/arylesterase: glutamine or arginine at position

191, for the respective A or B allozymes. Am J Hum Genet 1993;52:598-608
Ahn J, Nowell S, McCann SE, Yu J, Carter L, Lang NP, Kadlubar FF, Ratnasinghe LD, Ambrosone CB. Associations between catalase phenotype and genotype: modification by epidemiologic factors. Cancer Epidemiol Biomarkers Prev 2006;15:1217-22.
Airaksinen, K.E.J., et al. Impaired vagal heart rate control in coronary artery disease. British Heart Journal 1987; 58:592-597.
Albert MA, Danielson E, Rifai N, Ridker PM. Effect of statin therapy on c-reactive protein levels: The pravastatin inflammation/CRP evaluation (PRINCE): A randomized trial and cohort study. JAMA 2001; 286:64-70.
Albrecht P, Cohen RJ. Estimation of heart rate power spectrum bands from real-world data: Dealing with ectopic beats and noisy data. Computers in Cardiology 1988; 15:311-314.
Ali-Osman F, Akande O, Antoun G, Mao JX, Buolamwini J. Molecular cloning, characterization, and expression in Escherichia coli of full-length cDNAs of three human glutathione S-transferase Pi gene variants. Evidence for differential catalytic activity of the encoded proteins. J Biol Chem. 1997;272:10004-12.
Araujo JA, Barajas B, Kleinman M, Wang X, Bennett BJ, Gong KW, Navab M, Harkema J, Sioutas C, Lusis AJ, Nel AE. Ambient particulate pollutants in the ultrafine range promote early atherosclerosis and systemic oxidative stress. Circ Res 2008;102:589-96.
Arhami M, Kuhn T, Fine PM, Delfino RJ, Sioutas C. Effects of sampling artifacts and operating parameters on the performance of a semi-continuous particulate EC/OC monitor. *Environ Sci Technol* 2006; 40:945-53.
Baea M-S, Schauer JJ, DeMinter JT, Turner JR, Smith D, Cary RA. Validation of a semi-continuous instrument for elemental carbon and organic carbon using a thermal-optical method. Atmospheric Environment, 2004; 38:2885–2893.
Barclay JL, Miller BG, Dick S, Dennekamp M, Ford I, Hillis GS, et al. 2009. A panel study of air pollution in subjects with heart failure: negative results in treated patients. Occup Environ Med 66:325–334.
Bastaki M, Huen K, Manzanillo P, Chande N, Chen C, Balmes JR, Tager IB, Holland N. Genotype-activity relationship for Mn-superoxide dismutase, glutathione peroxidase 1 and catalase in humans. Pharmacogenet Genomics 2006;16:279-86.
Bender, R; Lange, S. Adjusting for multiple testing--when and how? J Clin Epidemiol 2001; 54(4):343-9.
Berger A, Zareba W, Schneider A, Rückerl R, Ibald-Mulli A, Cyrys J, et al. 2006. Runs of ventricular and supraventricular tachycardia triggered by air pollution in patients with coronary heart disease. J Occup Environ Med 48:1149-1158.
Bigger, J.T., et al. Autonomic nervous system activity during myocardial ischemia in man estimated by power spectral analysis of heart period variability. American Journal of Cardiology, 1990; 66:497-498.
Brook RD, Rajagopalan S, Pope CA 3rd, Brook JR, Bhatnagar A, Diez-Roux AV, et al. 2010. Particulate matter air pollution and cardiovascular disease: An update to the scientific statement from the American Heart Association. Circulation 121:2331-78.
Buchheit M, Simon C, Viola AU, Doutreleau S, Piquard F, Brandenberger G. Heart rate variability in sportive elderly: relationship with daily physical activity. Med Sci Sports Exerc 2004;36:601-5.
Burr RL, Motzer SA, Chen W, Cowan MJ, Heitkemper MM. Logit50: a nonlinear

transformation of pNN50 with improved statistical properties. J Electrocardiol 2003;36:41-52.
Butkiewicz D, Grzybowska E, Phillips DH, Hemminki K, Chorazy M. Polymorphisms of the GSTP1 and GSTM1 genes and PAH-DNA adducts in human mononuclear white blood cells. Environ Mol Mutagen 2000;35:99-105.
Cabada JC, Pandis SN, Subramanian R, Robinson AL, Polidori A, Turpin B. Estimating the Secondary Organic Aerosol Contribution to PM2.5 Using the EC Tracer Method. Aerosol Sci Technol 2004;38:140–155.
Chahine T, Baccarelli A, Litonjua A, Wright RO, Suh H, Gold DR, Sparrow D, Vokonas P, Schwartz J. Particulate Air Pollution, Oxidative Stress Genes, and Heart Rate Variability in an Elderly Cohort. Environ Health Perspect 2007;115:1617-22.
Chan C-C, Chuang KJ, Shiao GM, Lin LY. 2004. Personal exposure to submicrometer particles and heart rate variability in human subjects. Environ Health Perspect 112:1063-1067.
Chen CL, Liu Q, Relling MV. Simultaneous characterization of glutathione S-transferase M1 and T1 polymorphisms by polymerase chain reaction in American whites and blacks. Pharmacogenetics 1996;6:187-91.
Cho AK, Sioutas C, Miguel AH, Kumagai Y, Schmitz DA, Singh M, Eiguren-Fernandez A, Froines JR. Redox activity of airborne particulate matter at different sites in the Los Angeles Basin. Environ Res 2005;99:40-7.
Crofts F, Taioli E, Trachman J, Cosma GN, Currie D, Toniolo P, Garte SJ. Functional significance of different human CYP1A1 genotypes. Carcinogenesis 1994;15:2961-3.

Delfino RJ, Gillen DL, Tjoa T, Staimer N, Polidori A, Arhami M, Sioutas C, Longhurst J. Electrocardiographic ST segment depression and exposure to traffic-related aerosols in elderly subjects with coronary artery disease. *Environ Health Perspect* 2011;119:196–202. PMID: 20965803.
Delfino RJ, Sioutas C, Malik S, Potential role of ultrafine particles in associations between airborne particle mass and cardiovascular health. *Environ Health Perspect*, 2005; 113:934-46.

Delfino RJ, Staimer N, Tjoa T, Arhami M, Polidori A, Gillen D, Kleinman MT, Schauer J, Sioutas C. Association of biomarkers of systemic effects with organic components and source tracers in quasi-ultrafine particles. *Environ Health Perspect*, 2010a;118:756-762.

Delfino RJ, Staimer N, Tjoa T, Arhami M, Polidori A, Gillen DL, George SC, Shafer MM, Schauer J, Sioutas C. Associations of primary and secondary organic aerosols with airway and systemic inflammation in an elderly panel cohort. *Epidemiology* 2010b;21:892-902. PMID: 20811287

Delfino RJ, Staimer N, Tjoa T, Gillen D, Polidori A, Arhami M, Kleinman MT, Vaziri N, Longhurst J, Sioutas C. Air pollution exposures and circulating biomarkers of effect in a susceptible population: clues to potential causal component mixtures and mechanisms. *Environ Health Perspect*, 2009;117:1232–1238. PMID: 19672402

Delfino RJ, Staimer N, Tjoa T, Polidori A, Arhami M, Gillen D, Kleinman MT, Vaziri N, Zaldivar F, Longhurst J, Sioutas C. Circulating biomarkers of inflammation, antioxidant activity, and platelet activation are associated with urban air pollution

in elderly subjects with a history of coronary artery disease. *Env Health Perspect*, 2008; 116:898–906. PMID: 18629312.

Delfino RJ, Tjoa T, Gillen D, Staimer N, Polidori A, Arhami M, Jamner L, Sioutas C, Longhurst J. Traffic-related air pollution and blood pressure in elderly subjects with coronary artery disease. *Epidemiology*, 2010c;21:396-404. PMID: 20335815

Diggle PJ, Heagerty P, Liang K-Y, Zeger SL. Analysis of Longitudinal Data, 2nd edition. Oxford: Oxford U. Press, 2002.

Dockery DW, Luttmann-Gibson H, Rich DQ, Link MS, Mittleman MA, Gold DR, et al. 2005. Association of air pollution with increased incidence of ventricular tachyarrhythmias recorded by implanted cardioverter defibrillators. Environ Health Perspect 113:670-674.

Fine, P.M., Chakrabarti, B, Krudysz M., Schauer J.J. and Sioutas, C. Seasonal, spatial, and diurnal variations of individual organic compound constituents of ultrafine and accumulation mode PM in the Los Angeles Basin. Environmental Science and Technology, 2004b; 38:1296-304.

Fine, P.M., Si, S., Geller, M.G., and Sioutas, C. Inferring the sources of fine and ultrafine PM at downwind receptor areas in the Los Angeles Basin using multiple continuous monitors. Aerosol Science and Technology, 2004a; 38:182-195.

Floro JN, Dunton GF, Delfino RD. 2009. Assessing physical activity in asthmatic children: accelerometer and electronic diary data. Res Q Exerc Sport 80:153-63.

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

Forsberg L, Lyrenas L, de Faire U, Morgenstern R. A common functional C-T substitution polymorphism in the promoter region of the human catalase gene influences transcription factor binding, reporter gene transcription and is correlated to blood catalase levels. Free Radic Biol Med. 2001 Mar 1;30(5):500-5.

Gastañaga VM, McLaren CE, Delfino RJ. Power calculations for generalized linear models in observational longitudinal studies: A simulation approach in SAS. *Comput Methods Programs Biomed,* 2006;84:27-33.

Geisler SA, Olshan AF. GSTM1, GSTT1, and the risk of squamous cell carcinoma of the head and neck: a mini-HuGE review. Am J Epidemiol 2001;154:95-105.

Griendling KK, FitzGerald GA. 2003. Oxidative Stress and Cardiovascular Injury Part II: Animal and Human Studies. Circulation 108: 2034-2040.

Grossman P, Taylor EW. Toward understanding respiratory sinus arrhythmia: relations to cardiac vagal tone, evolution and biobehavioral functions. Biol Psychol 2007;74:263-85.

Grossman P, Wilhelm FH, Spoerle M. Respiratory sinus arrhythmia, cardiac vagal control, and daily activity. Am J Physiol Heart Circ Physiol 2004;287:H728-34.

Guénégou A, Leynaert B, Bénessiano J, Pin I, Demoly P, Neukirch F, Boczkowski J, Aubier M. Association of lung function decline with the heme oxygenase-1 gene promoter microsatellite polymorphism in a general population sample. Results from the European Community Respiratory Health Survey (ECRHS), France. J Med Genet 2006;43:e43.

Hampel R, Schneider A, Bruske I, Zareba W, Cyrys J, Rucker R, et al. 2010. Altered Cardiac Repolarization in Association with Air Pollution and Air Temperature among Myocardial Infarction Survivors. Environ Health Perspect 118:1755–1761.

Hartikainen JEK, Malik M, Stauton A, Poloniecki J, Camm J. 1996. Distinction between arrhythmic and nonarrhythmic death after acute myocardial infarction based on heart rate variability, signal-averaged electrocardiogram, ventricular arrhythmias and left ventricular ejection fraction. J Am Coll Cardiol 28:296-304.

Hassett C, Aicher L, Sidhu JS, Omiecinski CJ. Human microsomal epoxide hydrolase: genetic polymorphism and functional expression in vitro of amino acid variants. Hum Mol Genet 1994;3:421-8. Erratum in: Hum Mol Genet 1994;3:1214.

Hatzis C, Godleski JJ, Gonzalez-Flecha B, Wolfson JM, Koutrakis P. 2006. Ambient particulate matter exhibits direct inhibitory effects on oxidative stress enzymes. Environ Sci Technol 40:2805-2811.

Hayashi S, Watanabe J, Kawajiri K. Genetic polymorphisms in the 5′ flanking region change transcriptional regulation of the human cytochrome P450 IIE1 gene. J Biochem 1991;100:559–560

Hayes JD, Strange RC. Glutathione S-transferase polymorphisms and their biological consequences. Pharmacology 2000;61:154-166.

He F, Shaffer ML, Rodriguez-Colon S, Yanosky JD, Bixler E, Cascio WE, et al. 2011. Acute Effects of Fine Particulate Air Pollution on Cardiac Arrhythmia: The APACR Study. Environ Health Perspect 119:927–932.

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

Huikuri HV, Jokinen V, Syvanne M, Nieminen MS, Airaksinen KE, Ikaheimo MJ, Koistinen JM, Kauma H, Kesaniemi AY, Majahalme S, Niemela KO, Frick MH. Heart rate variability and progression of coronary atherosclerosis. Arterioscler Thromb Vasc Biol 1999; 19(8):1979-85.

Kim S, Shi S, Zhu Y, Hinds WC, Sioutas C. Size distribution, diurnal and seasonal tends of ultrafine particles in source and receptor sites of the Los Angeles Basin. J Air Waste Manage Assoc, 2002; 52:174-185.

Kiyohara C, Yoshimasu K, Takayama K, Nakanishi Y. NQO1, MPO, and the risk of lung cancer: a HuGE review. Genet Med 2005;7:463-78.

Kleiger RE, Stein PK, Bigger JT Jr. Heart rate variability: measurement and clinical utility. Ann Noninvasive Electrocardiol. 2005;10:88-101.

Knoebel SB, Crawford MH, Dunn MI, Fisch C, Forrester JS, Hutter AM Jr, Kennedy HL, Lux RL, Sheffield LT, Fisch C, et al. Guidelines for ambulatory electrocardiography. A report of the American College of Cardiology/American Heart Association Task Force on Assessment of Diagnostic and Therapeutic Cardiovascular Procedures (Subcommittee on Ambulatory Electrocardiography). Circulation 1989;79(1):206-15.

Kop WJ, Verdino RJ, Gottdiener JS, O'Leary ST, Bairey Merz CN, Krantz DS. Changes in heart rate and heart rate variability before ambulatory ischemic events(1). J Am Coll Cardiol 2001;38:742-9.

Lahiri MK, Kannankeril PJ, Goldberger JJ. Assessment of autonomic function in cardiovascular disease: physiological basis and prognostic implications. Am Coll Cardiol. 2008;51:1725-33.

Lefer, AM; Scalia, R; Lefer, DJ**.** Vascular effects of HMG CoA-reductase inhibitors (statins) unrelated to cholesterol lowering: new concepts for cardiovascular disease. Cardiovasc Res 2001; 49:281-7.

Leviev I, Deakin S, James RW. Decreased stability of the M54 isoform of paraoxonase as a contributary factor to variations in human serum paraoxonase

concentrations. J Lipid Res 2001;42:535-82.
Li N, Sioutas C, Cho A, Schmitz D, Misra C, Sempf J, Wang M, Oberley T, Froines J, Nel A. Ultrafine particulate pollutants induce oxidative stress and mitochondrial damage. Environ Health Perspect 2003; 111:455-60.
Liao D, Cai J, Rosamond WD, Barnes RW, Hutchinson RG, Whitsel EA, Rautaharju P, Heiss G. Cardiac autonomic function and incident coronary heart disease: a population-based case-cohort study. The ARIC Study. Atherosclerosis Risk in Communities Study. Am J Epidemiol 1997; 145(8):696-706.
Lindstrom MJ, Bates DM. Newton-Rhaphson and EM algorithms for linear mixed-effects models for repeated-measures data. J Am Stat Assoc 1988; 83:1014-1022.
Link MS, Dockery DW. 2010. Air pollution and the triggering of cardiac arrhythmias. Curr Opin Cardiol 25:16–22.
Ljungman PLS, Berglind N, Holmgren C, Gadler F, Edvardsson N, Pershagen G, et al. 2008. Rapid effects of air pollution on ventricular arrhythmias. Eur Heart J 29: 2894–2901.
Lough, G.C. 2004. Sources of Metals and NMHC from Motor Vehicle Roadway. Ph.D. Thesis. University of Wisconsin-Madison, Madison, WI. Major Professor: James J,. Schauer.
Metzger KB, Klein M, Flanders WD, Peel JL, Mulholland JA, Langberg JJ, et al. 2007. Ambient Air Pollution and Cardiac Arrhythmias in Patients With Implantable Defibrillators. Epidemiology 18: 585-592.
Mills NL, Donaldson K, Hadoke PW, Boon NA, MacNee W, Cassee FR, Sandström T, Blomberg A, Newby DE. Adverse cardiovascular effects of air pollution. Nat Clin Pract Cardiovasc Med. 2009;6:36-44
Mills NL, Finlayson AE, Gonzalez MC, Törnqvist H, Barath S, Vink E, et al. 2011. Diesel exhaust inhalation does not affect heart rhythm or heart rate variability. Heart 97: 544-550.
Misra, C.; Singh, M.; Shen, S.; Sioutas, C.; Hall, P. A. Development and evaluation of a personal cascade impactor sampler (PCIS). *J. Aerosol Sci.* 2002, *33*, 1027-1047.
Münzel T, Sinning C, Post F, Warnholtz A, Schulz E. Pathophysiology, diagnosis and prognostic implications of endothelial dysfunction. Ann Med 2008;40:180-96.
Nemmar A, Hoet PH, Vanquickenborne B, Dinsdale D, Thomeer M, Hoylaerts MF, et al. 2002. Passage of inhaled particles into the blood circulation in humans. Circulation 105:411-414.
Nemmar A, Hoylaerts MF, Hoet PH, Nemery B. 2004. Possible mechanisms of the cardiovascular effects of inhaled particles: systemic translocation and prothrombotic effects. Toxicol Lett 149:243-253.
Nolte C G, Schauer JJ, Cass G R, Simoneit BRT. 1999. Highly Polar Organic Compounds in Meat Smoke. Environmental Science and Technology. 33:3313-3316.
Nygårds ME, Hulting J. An automated system for ECG monitoring. Comput Biomed Res 1979 Apr, 12(2):181-202.
Oberdörster G, Gelein RM, Ferin J, Weiss B. 1995. Association of particulate air pollution and acute mortality: involvement of ultrafine particles? Inhal Toxicol 7:111-124.
Oberdörster G, Sharp Z, Atudorei V, Elder A, Gelein R, Lunts A, et al. 2002. Extrapulmonary translocation of ultrafine carbon particles following whole-body inhalation exposure of rats. J Toxicol Environ Health A 65:1531-1543.

Oberdörster G. 2001. Pulmonary effects of inhaled ultrafine particles. Int Arch Occup Environ Health 74:1-8.
Odenmuyiwa O, Malik M, Farrell T, Bashir Y, Poloniecki J, Camm J. 1991. Comparison of the predictive characteristics of heart rate variability index and left ventricular ejection fraction for all-cause mortality, arrhythmic events and sudden death after acute myocardial infarction. Am J Cardiol 68:434-439.
Park SK, O'neill MS, Wright RO, Hu H, Vokonas PS, Sparrow D, Suh H, Schwartz J. HFE Genotype, Particulate Air Pollution, and Heart Rate Variability: A Gene-Environment Interaction. Circulation 2006;114:2798-805.
Patterson SM, Krantz DS, Montgomery LC, Deuster PA, Hedges SM, Nebel E. Automated physical activity monitoring: validation and comparison with physiological and self-report measures. Psychophysiology 1993; 30:296–305.
Pigeolet E, Corbisier P, Houbion A, Lambert D, Michiels C, Raes M, et al. 1990. Glutathione peroxidase, superoxide dismutase, and catalase inactivation by peroxides and oxygen derived free radicals. Mech Ageing Dev 51:283-297.
Polidori A, Arhami M, Delfino RJ, Allen R**,** Sioutas C. Indoor-outdoor relationships, trends and carbonaceous content of fine particulate matter in retirement communities of the Los Angeles basin. J Air Waste Manage Assoc 2007;57:366-379.
Pope CA 3rd, Dockery DW. Health effects of fine particulate air pollution: lines that connect. J Air Waste Manag Assoc 2006;56:709-42.
Redmond DP, Hegge FW. Observations on the design and specification of a wrist-worn human activity recording system. Behavior Research Methods, Instruments and Computers, 1985; 17:659–669.
Ridker PM. Role of inflammatory biomarkers in prediction of coronary heart disease. Lancet 2001; 358:946-48.
Sagai M, Saito H, Ichinose T, Kodama M, Mori Y. 1993. Biological effects of diesel exhaust particles. I. In vitro production of superoxide and in vivo toxicity in mouse. Free Radical Biol Med 14:37-47.
Samet JM, Rappold A, Graff D, Cascio WE, Berntsen JH, Huang YC, Herbst M, Bassett M, Montilla T, Hazucha MJ, Bromberg PA, Devlin RB. Concentrated ambient ultrafine particle exposure induces cardiac changes in young healthy volunteers. Am J Respir Crit Care Med. 2009;179:1034-42.
Sammel, M; Lin, X; Ryan, L. Multivariate linear mixed models for multiple outcomes. Stat Med 1999; 18:2479-92.
Schauer JJ and Cass GR. Source apportionment of wintertime gas-phase and particle-phase air pollutants using organic compounds as tracers. Environ. Sci. Technol. 2000; 34:1821-1832.
Schauer JJ, Rogge WF, Hildemann, LM, Mazurek, MA, Cass GR. Source apportionment of airborne particulate matter using organic compounds as tracers. Atmos. Environ. 1996; 30:3837-3855.
Schmieder RE, Hilgers KF, Schlaich MP, Schmidt BM. Renin-angiotensin system and cardiovascular risk. Lancet 2007;369:1208-19.
Schneider A, Hampel R, Ibald-Mulli A, Zareba W, Schmidt G, Schneider R, et al. 2010. Changes in deceleration capacity of heart rate and heart rate variability induced by ambient air pollution in individuals with coronary artery disease. Particle and Fibre Toxicology 7:29.
Schwartz J, Park SK, O'Neill MS, Vokonas PS, Sparrow D, Weiss S, Kelsey K. Glutathione-S-transferase M1, obesity, statins, and autonomic effects of particles: gene-by-drug-by-environment interaction. Am J Respir Crit Care Med

2005;172:1529-33.
Schwartz J. The distributed lag between air pollution and daily death. Epidemiology 2000;11:320-326.
Shapiro D, Goldstein IB. Wrist actigraph measures of physical activity level and ambulatory blood pressure in healthy elderly persons. Psychophysiology 1998; 35:305-12.
Sheesley RJ, Schauer JJ, Chowdhury Z, Cass GR, Simoneit BRT. 2003. Characterization of organic aerosols emitted from the combustion of biomass indigenous to South Asia. Journal of Geophysical Research-Atmospheres, 108(D9): art. no.-4285.
Sheffield LT, Berson A, Bragg-Remschel D, Gillette PC, Hermes RE, Hinkle L, Kennedy H, Mirvis DM, Oliver C. AHA special report. Recommendations for standards of instrumentation and practice in the use of ambulatory electrocardiography. The Task Force of the Committee on Electrocardiography and Cardiac Electrophysiology of the Council on Clinical Cardiology. Circulation 1985;71(3):626A-636A.
Sheppard L, Slaughter JC, Schildcrout J, Liu LJ, Lumley T. 2005. Exposure and measurement contributions to estimates of acute air pollution effects. J Expo Anal Environ Epidemiol 15:366-376.
Singh M, Misra C, Sioutas C. Field evaluation of a personal cascade impactor sampler (PCIS). Atmos Environ 2003;37:4781-4793.
Sioutas C, Delfino RJ, Singh M. Exposure assessment for atmospheric ultrafine particles (UFP) and implications in epidemiological research. *Environ Health Perspect*, 2005; 113:947-55.
Steenland, K; Bray, I; Greenland, S; Boffetta, P. Empirical Bayes adjustments for multiple results in hypothesis-generating or surveillance studies. Cancer Epidemiol Biomarkers Prev 2000; 9:895-903.
Sutton A, Khoury H, Prip-Buus C, Cepanec C, Pessayre D, Degoul F. The Ala16Val genetic dimorphism modulates the import of human manganese superoxide dismutase into rat liver mitochondria. Pharmacogenetics 2003;13:145–157.
Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, Heart rate variability: Standards of measurement, physiological interpretation, and clinical use. Circulation 1996; 93:1043-1065.
Timonen KL, Vanninen E, de Hartog J, Ibald-Mulli A, Brunekreef B, Gold DR, Heinrich J, Hoek G, Lanki T, Peters A, Tarkiainen T, Tiittanen P, Kreyling W, Pekkanen J. Effects of ultrafine and fine particulate and gaseous air pollution on cardiac autonomic control in subjects with coronary artery disease: the ULTRA study. J Expo Sci Environ Epidemiol 2006;16:332-41.
Traver RD, Siegel D, Beall HD, Phillips RM, Gibson NW, Franklin WA, et al. Characterization of a polymorphism in NAD(P)H: quinone oxidoreductase (DT-diaphorase). Br J Cancer 1997; 75:69-75.
Treuth MS, Adolph AL, Butte NF. Energy expenditure in children predicted from heart rate and activity calibrated against respiration calorimetry. Am J Physiol 1998; 275(1 Pt 1):E12-18.
Tsuji H, Larson MG, Venditti FJ Jr, Manders ES, Evans JC, Feldman CL, Levy D. Impact of reduced heart rate variability on risk for cardiac events. The Framingham Heart Study. Circulation 1996; 94(11):2850-55.
Tsuji H, Venditti FJ Jr, Manders ES, Evans JC, Larson MG, Feldman CL, Levy D. Reduced heart rate variability and mortality risk in an elderly cohort. The

Framingham Heart Study. Circulation 1994; 90(2):878-83.
Turpin BJ, Cary RA, Huntzicker JJ. An in situ, time-resolved analyzer for aerosol organic and elemental carbon. Aerosol Science and Technology 1990;12:161–171.
Utell MJ, Frampton MW. Acute health effects of ambient air pollution: the ultrafine particle hypothesis. J Aerosol Med 2000 ; 13:355-59.
Vardas, P.E., et al. Spectral analysis of heart rate variability before and during episodes of nocturnal ischaemia in patients with extensive coronary artery disease. European Heart Journal, 1996; 17:388-393.
Wang LI, Miller DP, Sai Y, et al. Manganese superoxide dismutase alanine-tovaline polymorphism at codon 16 and lung cancer. J Natl Cancer Int 2001;93:1818–21.
Watson MA, Stewart RK, Smith GB, Massey TE, Bell DA. Human glutathione Stransferase P1 polymorphisms: relationship to lung tissue enzyme activity and population frequency distribution. Carcinogenesis 1998;19:275-280.
Weiss ST, Ware JH. Overview of issues in the longitudinal analysis of respiratory data. Am J Respir Crit Care Med 1996; 154:S208-S211.
Wong MY, Day NE, Luan JA, Chan KP, Wareham NJ. The detection of gene-environment interaction for continuous traits: should we deal with measurement error by bigger studies or better measurement? Int J Epidemiol 2003;32:51-7.
Yamada N, Yamaya M, Okinaga S, Nakayama K, Sekizawa K, Shibahara S, Sasaki H. Microsatellite polymorphism in the heme oxygenase-1 gene promoter is associated with susceptibility to emphysema. Am J Hum Genet 2000;66:187–195.
Zanobetti A, Gold DR, Stone PH, Suh HH, Schwartz J, Coull BA, et al. 2010. Reduction in Heart Rate Variability with Traffic and Air Pollution in Patients with Coronary Artery Disease. Environ Health Perspect 118:324–330.
Zeger SL, Thomas D, Dominici F, Samet JM, Schwartz J, Dockery D, Cohen A. Exposure measurement error in time-series studies of air pollution: concepts and consequences. Environ Health Perspect 2000; 108:419-26.
Zerbe GO, Murphy JR. On multiple comparisons in the randomization analysis of growth and response curves. Biometrics 1986; 42:795-804.
Zheng, M., Cass, G.R., Schauer, J.J. and Edgerton, E.S., 2002. Source apportionment of PM2.5 in the southeastern United States using solvent-extractable organic compounds as tracers. Environmental Science & Technology, 36(11): 2361-2371
Zhu Y, Hinds WC, Kim S, Shen S, Sioutas C. Seasonal trends of concentration and size distributions of ultrafine particles near major freeways in Los Angeles. Aerosol Science and Technology 2004;38(Suppl 1):5-13.
Zhu Y, Hinds WC, Kim S, Shen S. and Sioutas, C. Study on ultrafine particles and other vehicular pollutants near a busy highway. Atmos Environ 2002b 36:4375-4383.
Zhu Y, Hinds WC, Kim S, Sioutas C. Concentration and size distribution of utrafine particles near a major highway. J Air Waste Manage Assoc 2002a; 52:1032-42.

2. CHAPTER TWO: TASK 1

2.0 INTRODUCTION

We conducted a cohort panel study of acute cardiovascular outcomes with home-based ambient air pollution monitoring in the Los Angeles air basin from July 2005 through February 2006 and July 2006 through February 2007. Given the urban study location, our interest was on the effects of traffic-related ultrafine particles since they have been shown to be enriched in chemical components that have a pro-oxidant effect on cells (Ayres et al. 2008, Verma et al. 2009). A chain of events may occur following exposure to pro-oxidant chemical that can cause arrhythmias via lipid peroxidation, endothelial dysfunction, and other mechanisms involving oxidative stress (Griendling and FitzGerald 2003). The present study used ECG and air pollution data from this panel study to evaluate whether increased frequency of cardiac arrhythmias and decreased heart rate variability are associated with exposure to PM air pollution.

2.1 MATERIALS AND METHODS

2.1.1 Study Population.

We recruited elderly subjects from four retirement communities in the Los Angeles air basin who were to be followed with repeated 24-hr ambulatory ECG (Holter) measurements. Eligibility criteria included age of 65 years or older, confirmed history of coronary artery disease, being a nonsmoker, and no passive exposure to tobacco smoke. Study cardiologists confirmed coronary artery disease diagnoses with a medical records review (Delfino et al., 2011). Screen eligible subjects were consented and then clinically evaluated by study cardiologists and nurses at their retirement community site in our mobile medicine clinic. This included 105 potentially eligible subjects, 21 of whom were determined to not be eligible to participate in the overall study. In addition, 18 dropped out of the ambulatory ECG monitoring portion of the study, and four had an insufficient number of ambulatory ECG monitoring hours (< 72 hours). Twelve subjects with pacemakers also were excluded since arrhythmia and HRV data in the presence of paced beats are not valid, leaving 50 subjects and 8,952 hours of data. The paced ECG data were confirmed using baseline ECG and ambulatory ECG data.

Subjects lived in four retirement communities with around 200 or more residents in areas of the Los Angeles Air Basin with high air pollutant concentrations (three communities in the east and west parts of the San Gabriel Valley and one community in Riverside). Sites were selected that were impacted by both local traffic sources as well as receptors of transported pollutants. All communities prohibited smoking in common areas and had air conditioning throughout. Details describing each community cannot be publically revealed as per IRB agreements. Although results may have differed between the four communities, subject data were pooled to gain sufficient statistical power for analysis.

We studied two retirement communities in 2005-2006 and two retirement communities in 2006-2007. Subjects were followed in two periods to increase variability in exposures to particle components (including primary and secondary organic aerosols) and in size distribution by season (Sioutas et al. 2005). In each community, we collected data during a period of higher photochemical activity (July to mid-October) and during a cooler

period when traffic-related primary air pollutants are likely to increase at ground level (mid-October through February). Subjects were studied with ambulatory ECG monitoring in two periods each for five consecutive days (one 5-day period in the first seasonal period and one 5-day period in the second seasonal period). The 5-day ambulatory monitoring series started on Monday morning and ended on Friday afternoon. Research assistants visited each subuject's home daily to download electronic data and set a new day's run for including ambulatory ECG, actigraphs, and personal digital assistant diaries.

Daily medication use was monitored using paper diaries. Stress and anxiety may confound associations since their occurrence has the potential to stimulate arrhythmias (Ziegelstein 2007). Electronic diaries were used to assess hourly occurrence and severity of anxiety and stress as described in Delfino et al. (2010b).

This study was approved by the institutional review board of the University of California, Irvine, in 2003. All participants gave informed consent.

2.1.2 Ambulatory Data: Electrocardiograph and Personal Accelerometer.

Electrocardiograph/electrocardiogram (ECG): Subjects wore an ambulatory ECG (Holter) monitor for two 5-day periods during two of their 12 weeks of follow-up. Monitoring took place from Sunday through Friday. We used the Burdick model 92513 Compact Digital Holter Recorder and Scanner Software System (Burdick Inc., Deerfield, WI). The Burdick is a 7-lead 3-channel Holter ECG with a data acquisition speed of 200 Hz. Each of the follow-up days in the morning, the subject removed leads and bathed before a trained research assistant arrived at the subject's home to download ECG data and setup the ECG for a new day of continuous data collection. The Holter ECG signals from the 3 channels were read and analyzed using the Burdick Vision Premier Holter Analysis System. This software system includes algorithms for QRS labeling, arrhythmia detection, artifact identification and data correction. These data were further edited for artifacts and outliers by a Holter technician from the Noninvasive Laboratory of the University of California Medical Center. Notations of abnormalities, arrhythmias, ST segment changes and flagged ECG regions were reviewed by cardiologists.

Arrhythmia outcomes included supraventricular and ventricular arrhythmias in non-sustained runs of $\geq$ 3 ectopic beats at a rate $\geq$ 120 beats per minute (Berger et al. 2006). For each subject we identified the duration of arrhythmias and the number of arrhythmia events in each hour. We also counted the number of daily arrhythmia events with reference to the gravimetric particle mass sampling times (afternoon to afternoon). The Holter output for each episode of supraventricular (atrial) tachycardia (SVT) and ventricular tachycardia (VT) were extracted. Atrial fibrillation was observed for only two subject-hours, atrial flutter for only two subject-hours, and atrioventricular block only 12 subject-hours. This was too infrequent to be able to analyze those arrhythmia outcomes. Therefore, SVT only included paroxysmal supraventricular tachycardia (N=460 subject-hours) among 39 participants. Accelerated idioventricular rhythm (N=50 subject-hours) also was observed too infrequently analyze. VT included both single focus ventricular tachycardia (N=69 subject-hours) and multifocal ventricular tachycardia (N=232 subject-hours). The 301 total VT events (out of 8,952 monitored hours) were observed in 18 participants.

For the HRV outcomes, we used the Burdick Vision Series HRV software to analyze the QRS annotation file (described above). HRV outcomes were derived from 5-min epochs using acceptable normal-to-normal (NN) intervals (Task Force

1996). Only beats classified as normal and not preceded by ectopic beats or prolonged RR intervals were included in the NN interval estimation. An interpolation algorithm using cubic splines was used to replace missing beats including the removed ectopic beats and artifacts, premature ventricular contractions and other non-normal R-R areas (Albrecht 1988). NN intervals <300 and or >3,000 ms or with NN ratios between < 0.8 or > 1.2 were rejected. Time segments in which more than 20% of RR intervals required replacement were excluded from the analysis.

Only time domain indexes were utilized because no stationary recording periods are available as it is not possible to standardize the conditions during 24-hr Holter recording. The following three HRV outcome variables consisted of standard time domain variables (Task Force 1996) measured at the hourly level:

SDNN: Standard deviation of all normal R-R intervals in the time segment. It is a measure of overall HRV representing the total amount of variability present in the selected time interval, and is influenced by changes in both sympathetic and parasympathetic tone. It is therefore a non-specific measure of sympathovagal balance (Task Force 1996).

r-MSSD: Root mean square of the successive differences. It is the square root of the mean of the sum of the squares of differences between the adjacent normal R-R intervals over the selected time segment. The selected time segment will be each hour as this is the averaging time period for the continuous air samplers. The r-MSSD is a measure of short-term variation in heart rate representing high frequency components of HRV (Task Force 1996).

pNN50: Percentage of differences between adjacent normal RR intervals that are larger than 50 ms. This is an alternative measure of short-term variation to r-MSSD (Task Force 1996). The pNN50 is believed to be a better estimate of short-term HRV and an index of the degree of respiratory sinus arrhythmia. Because it is a percentage measure of each excursion greater than 50 ms, it is much less susceptible to large amplitude outliers as are other HRV measurements (Burr 2003). Both pNN50 and rMSDD and are measurements of parasympathetic (vagal) activity.

Our analysis was primarily focused on the full 24-hour ECG recording periods. In analyses of daily PM filter data, we will primarily focus on 24-hour ECG recordings spanning the gravimetric PM mass sampling periods. This will include the SDNN outcome and arrhythmia event counts for the lag 0 day 24-hour PM sampling period. However, day/night differences in NN intervals can contribute to a major fraction of the SDNN magnitude (Kleiger et al 2005). Therefore, stratified analyses for daytime periods and nighttime periods also were conducted for air pollutants measured hourly.

The distributions of SDNN, r-MSSD and pNN50 were moderately right skewed as expected (Burr 2003). Therefore, as previously described by Zanobetti et al. (2010) we used three times the median of the absolute differences as a cutpoint for outlier exclusion. A few outliers for SDNN and r-MSSD (up to 152 subject-hours, 1.7%) were extreme (> 3 SD) and were first removed before calculating the median of the absolute differences. The median of the absolute differences was then calculated from the remaining 98.3 % of hourly records. Using median absolute difference criteria, 11% and 13% of the hourly SDNN and r-MSSD records were removed, respectively.. The logit transformation was used for pNN50 because it

exhibited typical nonlinear saturation effects near 0% and 100%, resulting in a loss of 201 hours (pNN50 = 0) or 2.27% of the data.

Personal Accelerometer Data: It is possible that associations between air pollution and ambulatory HRV could be confounded or modified by different activity levels, especially respiratory sinus arrhythmia as reflected by r-MSSD or pNN50. This is because of the inverse relationship between physical activity and vagal influences on the heart during high levels of activity (Grossman 2004, 2007). Therefore, we electronically monitored physical activity continuously for each 24-hr period with a personal motion logger (Mini-motionlogger, Ambulatory Monitoring, Inc., Ardsley, NY), a piezoelectric accelerometer. It operates automatically, requires limited cooperation, and does not disrupt a subject's activity or routine (Shapiro 1998). The sensor is a piezoelectric beam that detects all 3 axes of movement. Movement is translated into an electric signal stored on a memory chip. The device is small (4.4 x 3.3 x 1.0 cm) and lightweight (57 g). It records the frequency or duration of movements with high test–retest reliability and validity (Patterson 1993). We recently published data from 62 school children with asthma showing moderate validity between Actigraph and electronic diary data on physical activity collected over 10 days (Floro et al. 2010).

We placed the Actigraph at waist level with the ECG to better capture whole body movements rather than the typical wrist-worn location. The accelerometer recorded objective measurements of movement in the unit's high sensitivity proportional integrating measure mode. This mode measures movement intensity as opposed to frequency, which is useful in discriminating waking activities. The mode also sums the absolute value of deviations from zero volts each 0.1 sec during a series of one-minute epochs and stores the value at the end of each epoch.

We discarded nominal amount (a few minutes) of accelerometer data at the beginning and end of each sampling period to avoid movement artifacts. Valid accelerometer data was present for 92.8% of all hourly ECG records. We normalized 1-minute data by subject-specific Z-scores, and then determined average movement intensity for hourly periods that matched the HRV and arrhythmia data.

2.1.3 Exposures

Measured air pollutant exposures:

Four retirement communities were selected for the recruitment of subjects and monitoring of air pollution to enhance the accuracy of exposure assessments. This was accomplished by using outdoor home air monitoring in a fully equipped trailer provided by CARB. It was parked on the property of each community site and away from main roadways. A parallel set of indoor data were collected on instruments placed in the main community areas of each retirement community.

Measurements of hourly particle exposures for the analysis of both hourly and 24-hr HRV outcomes included the following:

Total particle number (PN) concentrations (Condensation Particle Counter model 3785; TSI Inc, Shoreview, MN),

$PM_{2.5}$ black carbon (BC, Aethalometer; Magee Scientific, Berkeley, CA),

$PM_{2.5}$ organic and elemental carbon (OC-EC Analyzer model 3F; Sunset Laboratory Inc, Tigard, OR) (Arhami et al. 2006), and

$PM_{2.5}$ mass using a Beta-Attenuation Mass (BAM) Monitor (model 1020; Met One instruments Inc, Grants Pass, OR).

Measurements of hourly continuous air pollutant gases included ozone (O_3), and markers of fossil fuel combustion, namely carbon monoxide (CO) and nitrogen oxides (NO_x), were measured using federal reference methods. BC and EC are similar but not identical measurements of carbon linked to fossil fuel combustion.

We used the Sioutas™ Personal Cascade Impactor Sampler (SKC, Inc., Eighty Four, PA), to measure daily size-fractionated PM mass concentrations for the analysis of 24-hr outcomes (Misra et al. 2002). The analysis included particles 0-0.25 μm in diameter ($PM_{0.25}$), accumulation mode particles, 0.25-2.5 μm in diameter ($PM_{0.25\text{-}2.5}$), and coarse mode particles, 2.5-10 μm in diameter ($PM_{2.5\text{-}10}$). $PM_{0.25}$ is considered "quasi-ultrafine" because the traditional cutpoint for the ultrafine mode is around 0.1 to 0.2 μm. Meteorological data were also collected at the air sampling trailer and at the indoor monitoring location. Because particle mass measurements were conducted only during the five days of the week that the ambulatory ECG data collection was underway, we could not assess lags beyond one day since the data loss would be too great. Therefore, for gravimetric mass measurements, all 24-hour averaged outcomes were analyzed in relation to lag 0 day, lag 1 day and 2-day average PM mass.

<u>Estimated air pollutant exposures:</u>

We estimated the mass of total organic carbon (OC) attributed to secondary OC (SOC, a surrogate of photochemically-derived OC) and the mass of OC attributed to primary OC (OC_{pri}, attributable to primary combustion sources, mostly traffic in the study regions). These estimates were based on the EC tracer method that uses EC as a tracer of primary combustion generated OC, and are described elsewhere (Polidori et al. 2007; Cabada et al. 2004; Lim et al. 2003; Turpin et al. 1995). OC_{pri} and EC are assumed to be emitted from the same combustion sources. Data points primarily during rush hour traffic are characterized by high CO and NO peaks and are thus used to identify times dominated by primary sources with less formation of secondary aerosols. The primary OC/EC ratio that characterizes each month of study was determined by regressing the OC and EC data we collected during these periods. Deming linear least-squares regression (Cornbleet and Gochman 1979) was used since the uncertainties in OC and EC were assumed equal. OC_{pri} and SOC was estimated by the following expressions:

$$OC_{pri} = a \times EC + b$$

$$SOC = OC - OC_{pri},$$

where $a = (OC/EC)_{pri}$, which is the characteristic primary OC/EC ratio for the study area, and b = non-combustion primary OC. Usually, the SOC values estimated by this method vary with season and sampling location and are higher during afternoon hours in the warm seasons with photochemical smog episodes.

We also estimated indoor PM exposures of outdoor origin using the following methods that were also used in Delfino et al. 2008. We found in that study that associations were stronger for indoor exposures to PM of outdoor origin than uncharacterized indoor exposures. We concluded that outdoor home measurements

are sufficient to capture the cardiovascular health impacts of outdoor air pollutants even though people spend most of their time indoors.

First, air exchange rates and infiltration factors (F_{inf}) at each site were determined. The average AERs calculated during CHAPS at the four retirement communities ranged from 0.21 to 0.40 hr^{-1}(see Arhami et al. 2009 for more details). Estimated F_{inf} and measured particle concentrations were then used in a single compartment mass balance model to assess the contributions of indoor and outdoor sources to measured indoor EC, OC_{pri}, SOC, and PN (Polidori et al. 2007). Indoor exposures to PM of outdoor origin are relevant to personal PM exposures given that people generally spend most of their time indoors. The combined indoor and outdoor exposure data we use here is that data where we were able to estimate indoor concentrations of outdoor origin (PN, EC, OC_{pri}, and SOC) from data from our work with Constantinos Sioutas and colleagues at the University of Southern California (Polidori et al. 2007).

A single compartment mass balance model (Meng et al. 2005; Polidori et al. 2006; Wallace 1996) was used to assess the mean contributions of indoor and outdoor sources to measured indoor OC, EC, $PM_{2.5}$ and PN concentrations. Under the assumption of perfect instantaneous mixing and that the factors affecting the indoor concentrations are constant or change slowly with time, the steady state indoor concentration of any particulate species can be described by the following eq:

$$C_{in} = \frac{P(AER)C_{out}}{AER+k} + \frac{Q_i / V}{AER+k} = F_{inf}C_{out} + C_{ig} = C_{og} + C_{ig}$$

where, C_{in} is the indoor concentration of the species of interest (µg/m^3), C_{out} is the corresponding outdoor concentration (µg/m^3), F_{inf} is the corresponding infiltration factor (dimensionless), C_{ig} is the indoor-generated concentration for the same species found indoors and C_{og} is the outdoor-generated concentration for the same species found indoors. Typically, in the mass balance model C_{ig} is expressed by $Qi/V(a+k)$, where Q_i is the indoor source strength (µg/h), and V is the house volume (m^3).

The infiltration factor (F_{inf}, defined as the equilibrium fraction of ambient particles that penetrate indoors and remain suspended) is a key determinant of the indoor concentrations of particulate species. F_{inf} is described by the following eq:

$$F_{inf} = P(\mathrm{AER})/(\mathrm{AER}+k)$$

where, P is the penetration coefficient (dimensionless). F_{inf} for particles varies with particle composition, particle size and volatility, surface to volume ratio of the indoor sampling location and indoor air-speed. F_{inf} is typically highest for non-volatile species such as EC (Lunden et al. 2003; Sarnat et al. 2006). F_{inf} for OC, EC, and PN were estimated from the corresponding indoor/outdoor concentration ratios. In particular, hourly indoor/outdoor ratios (I/O) for each particulate species were determined at times when no indoor particle sources, such as cooking or cleaning, were likely to be present (i.e. only I/O ratios ≤ 1 were considered). Daily F_{inf} estimates were then obtained by averaging these segregated hourly I/O ratios. Mean F_{inf} for each group and phase of the study were also determined by averaging the corresponding daily values. To verify these results the same analysis of the I/O concentration ratios was then repeated by using only nighttime data (from 00:00 to

06:00 am), for at this time resident activities causing indoor particle generation were expected to be minimal.

The indoor-outdoor air exchange rates (AER; h^{-1}) at each community site were estimated from indoor CO measurements collected during periods affected by a dominant indoor source. We considered in our calculations only time-periods when the CO concentration peaked at values significantly higher than the background CO level and that was followed by a non-source period (mostly observed in the morning and probably associated with cooking activities). Assuming an exponential decay of particles, that AER and outdoor concentrations are constant during the decay period, and that indoor concentrations are well mixed, then:

$$C_t = e^{-(AER+k)t} C_0$$

or

$$\ln C_t = -(AER+k)t + \ln C_0$$

where, C_t is the indoor CO concentration after time t (after the decay period), C_0 is the initial peak CO concentration (right after CO emission) and k is the indoor loss rate for particles or gases (h^{-1}) (Abt et al 2000). Since k is rather negligible for CO, it was possible to estimate the AERs for the sites directly from the above-mentioned eq (2) by regressing $\ln C_t$ over $\ln C_0$.

2.1.4 Statistical Analysis

We used regression models to investigate associations between each air pollutant and the HRV and arrhythmia outcomes adjusting for confounding variables (described below). All estimates of effect are reported per interquartile range (IQR) of the pollutant. Generalized estimating equations (implemented in SAS PROC GENMOD) were used to account for within-subject correlations induced by the repeated measures design. For continuous HRV outcomes SDNN, rMSSD, and pNN50A, we assumed a Gaussian variance structure (i.e., a constant error variance unrelated to the mean).

For the arrhythmia outcomes (VT and SVT) analyzed on a daily basis by daily counts we assumed a Poisson variance and estimated the rate ratio (RR, alternatively called the relative rate). The SAS PROC GENMOD program for these models includes the maximum likelihood estimate of the dispersion parameter in the standard error estimates. For the analysis of the hourly absence vs. presence of VT or SVT events we assumed a binomial variance structure and estimated the odds ratio (OR). Modeling an autoregressive correlation for residual errors led to the best model fit given that observations were repeated on the same individual. For daily counts, VT events for one subject with >19 events per day during the warm season phase were highly influential (Cook's D > 0.3) in our models and were removed from all VT analyses. Other large value outliers were 3 standard deviations above the mean (8 observations) and were also removed due to an overly positive influence on VT regression parameters.

We estimated odds ratios for the occurrence of one or more arrhythmia hourly events in relation to air pollutant exposures using hourly exposure-outcome data. For this analysis we could only use the air pollutant variables that were measured hourly (e.g., EC and OC). Pollutant averaging times prior to each outcome measurement were 1 hour, 4 hours, 8 hours, 24 hours, 3 days, and 5 days. The

shorter averaging times are justified since cardiovascular effects may be very acute (Langrish et al. 2012). We also estimated rate ratios using daily counts of the arrhythmia data in relation to air pollutant exposures using both hourly pollutant data that were averaged daily and daily gravimetric size-fractionated PM data. For size-fractionated PM mass, we analyzed current day 24-hr average, lag day (25-48-hour average) and 2-day averages were used because of the limitation of PM filter collection only during the 5 days of ECG monitoring. This PM mass data was analyzed in relation to matched 24-hr counts of VT/SVT and 24-hr SDNN. Effect modification for VT by daytime/nighttime exposure hours was an exploratory analysis so for hourly analyses we fit models stratified by this variable and conducted formal hypothesis tests using interaction terms. Significant interaction (product) terms are considered at the nominal p-value < 0.1. Although the approach was exploratory, it is based on expectations that both risk of VT (e.g., Lampert et al. 1994) and air pollutant exposures in our study (Polidori et al. 2007) are expected to differ between daytime and nighttime hours. Formal tests of odds ratio heterogeneity were performed using the Cochran's Q statistic (Cochrane 1954) to assess the significance of differences in daytime vs. nighttime associations.

In secondary (exploratory) analyses, interaction terms were used to evaluate effect modification by sex, medications (Table 1), and self-reported co-morbidities (diabetes, COPD/asthma, and history of myocardial infarction).

All effect estimates were adjusted for hourly or daily average actigraph-derived physical activity and heart rate, temperature of the same lag average, and day of week. As previously reported, we also adjusted for seasonal (5-day) study phase (via mean centered exposure), and community group (via mean centered exposure) (Delfino et al. 2011). Hour of day also was investigated as a possible confounder, but omitted from final models due to lack of evidence of any confounding. Psychological stress or anxiety (intense, strong or moderate) from the hourly diary reports was not associated with risk of arrhythmias (p = 0.22 and the parameter estimate was negative) and so was not adjusted for in the regression models. Statistical significance was assessed using 1%, 5%, and 10% type I error rates per test, and 95% confidence intervals are reported for effect estimates. Given that we conducted a large number of tests (multiple outcomes, pollutants and pollutant lag times), the percentage of tests yielding significant associations is reported for major categories of comparisons. This is in lieu of advanced corrections for multiple testing bias in order to allow for a more reasoned evaluation of bias.

2.2. RESULTS

2.2.1 Descriptive Data

As expected given the eligibility requirement that subjects have a history of CAD, almost half of the subjects had had a previous myocardial infarction (Table 1). The remaining subjects were eligible based on a history of coronary artery bypass graft or angioplasty, a positive angiogram or stress test, or clinical diagnosis of CAD. Also as expected for this elderly population with CAD, a majority had histories of hypertension (68%) and hypercholesterolemia (76%), and cardiac medications were common (a majority took β-adrenergic blocking or hypocholesterolemic agents). There were more males (62%) than females, and most subjects were overweight, with a mean body mass index of 27. Although all four retirement communities were

non-smoking facilities and none of the participants were current or recent smokers (within 12 months), 44% of the participants were former smokers.

Table 1. Characteristics of participants (N=50).

Characteristic	Mean ±SD or N (%)
Age (years)	83.3 ± 5.95
BMI (kg/m^2)	27.0 ±3.63
Gender	31 (62%) Male 19 (38%) Female
Cardiovascular History	
Confirmation of CAD:[a]	
-Myocardial infarction	23 (46%)
-Coronary artery bypass graft or angioplasty	16 (32%)
-Positive angiogram or stress test	8 (16%)
-Clinical diagnosis[b]	3 (6%)
Current angina pectoris	14 (28%)
Cardiac arrhythmia	10 (20%)
Congestive heart failure	10 (20%)
Hypertension	34 (68%)
Hypercholesterolemia	38 (76%)
Other Medical History:	
Type II Diabetes	7 (14%)
COPD or Asthma	5 (10%)
Stroke or transient ischemic attack	8 (16%)
Medications:	
B-adrenergic blocking agents	30 (60%)
Anti-arrhythmic drugs	4 (8%)
Digitalis	3 (6%)
ACE inhibitors	20 (40%)
HMG-CoA reductase inhibitors (statins)	27 (54%)
Platelet Aggregation Inhibitors or Coumadin	17 (34%)
Calcium Channel Blockers	17 (34%)
Smoking history:	
Never smoker	28 (56%)
Ex-smoker (no smoking last 12 months)	22 (44%)

[a] Each category is hierarchical - excludes being in above diagnostic category

[b] Coronary microvascular disease

ACE: angiotensin I-converting enzyme

HMG-CoA: 3-Hydroxy-3-methylglutaryl coenzyme A

Table 2 presents the summary statistics for outcome variables. We found that pNN50 was similar during day and night, but SDNN and rMSSD were higher during daytime hours than at night.

Table 2. Distribution of outcome variables over all subjects.

VT (ventricular tachycardia) [a]					
	Mean	**Median**	**SD**	**Min**	**Max**
Daily Count	1.73	0	11.14	0	158
SVT (supraventricular tachycardia) [b]					
	Mean	**Median**	**SD**	**Min**	**Max**
Daily Count	1.32	0	3.46	0	36
SDNN (standard deviation of normal R-R intervals in ms)					
	Mean	**Median**	**SD**	**Min**	**Max**
Day & Night	72.91	66	34.98	3	159
Day Time	74.26	68	34.19	6	159
Night Time	69.92	62	36.50	3	159
rMSSD (root mean square of successive differences in ms)					
	Mean	**Median**	**SD**	**Min**	**Max**
Day & Night	49.25	29	45.67	0.06	183
Day Time	50.32	29	46.71	0.06	183
Night Time	46.88	29	43.18	0.11	183
pNN50 (percent of normal R-R interval differences > 50 msec)					
	Mean	**Median**	**SD**	**Min**	**Max**
Day & Night	22.4	5.76	28.7	0	97.3
Day Time	22.5	5.85	28.4	0	91.5
Night Time	22.4	5.65	28.9	0	97.3

[a] VT events were observed in 301 subject-hours among 18 participants.
[b] SVT events were observed in 460 subject-hours among 39 participants.

Outdoor air pollutant measurements sampled at the retirement communities are described in Table 3 and indoor air pollution data are described in Table 4. A more detailed analysis of indoor vs. outdoor exposures and differences by retirement community can be found in our previous publications and is not a part of the current scope (Arhami et al. 2009, 2010; Polidori et al. 2007). There were fewer missing observations for black carbon, $PM_{2.5}$ and the gases because two samplers were operated in parallel at all times. There was more missing primary and secondary OC than total OC because of missing predictor data used to estimate these two OC fractions, including elemental carbon. $PM_{2.5}$ mass was measured hourly with a pair of Beta-Attenuation Mass Monitors resulting in no missing data outdoors and little missing data indoors. The size-fractionated PM mass was measured daily with a single Personal Cascade Impactor Sampler at each indoor and outdoor site, which

resulted in more missing data than the BAM. The distribution of BC and EC were similar with BC>EC mean as expected. There were 23 out of 235 days (10%) when the concentration of $PM_{2.5}$ (by BAM) was > 35 µg/m^3, the current 24-hr NAAQS. Regression effect estimates were standardized to the reported IQR of each pollutant.

For correlations between air pollutants we focus on outdoor exposures. The traffic-related air pollutants were highly positively correlated with each other, with pairwise Spearman correlations ≥0.75, whereas O_3 and secondary OC were not correlated or were negatively correlated with these primary combustion-related pollutants (Table 5). Given the strong correlation among primary pollutants, our regression models test one pollutant at a time.

Table 3. Distribution of outdoor air pollution variables.

Exposure (24-hr averages)[a]	**N**	**N missing** [a]	**Mean**	**SD**	**IQR**	**Min**	**Max**
Continuously-measured PM							
$PM_{2.5}$ (µg/m^3)[b]	235	0	21.1	11.4	16.1	2.3	77.4
Particle number (no./cm^3)	184	51	12,818	5,889	6,351	2,019	30,180
Black carbon (µg/m^3)	235	0	1.7	0.8	1.0	0.3	4.5
Elemental Carbon (µg/m^3)	198	37	1.52	0.6	0.90	0.32	3.34
Organic carbon (µg/m^3)	188	47	7.8	3.7	5.2	2.5	18.7
Primary organic carbon (µg/m^3)	157	78	5.3	2.9	4.4	41.4	12.6
Secondary organic carbon (µg/m^3)	157	78	2.9	1.5	2.1	0.3	7.7
Size-fractionated PM[b]							
$PM_{0.25}$ (µg/m^3)	217	18	9.8	4.1	7.0	2.5	30.1
$PM_{0.25\text{-}2.5}$ (µg/m^3)	226	9	11.4	9.4	10.6	1.0	66.8
$PM_{2.5\text{-}10}$ (µg/m^3)	217	18	9.4	5.0	5.5	0.3	24.6
Air Pollutant Gases							
NO_x (ppb)	235	0	46.6	31.4	42.3	3.2	183.7
CO (ppm)	224	11	0.53	0.30	0.42	0.01	1.68
O_3 (ppb)	232	3	27.1	11.5	17.4	3.8	60.7

[a] The continuously-measured PM variables were averaged hourly and 24-hour averages are presented here.

[b] $PM_{2.5}$ mass was measured hourly with Beta-Attenuation Mass Monitors whereas the size-fractionated PM mass was measured daily with a Personal Cascade Impactor Sampler.

Table 4. Distribution of indoor air pollution variables.

Exposure (24-hr averages)[a]	N	N[a] missing	Mean	SD	IQR	Min	Max
Continuously-measured PM							
$PM_{2.5}$ [b] (μg/m^3)	223	12	12.4	6.68	9.95	2.04	44.4
Particle number, uncharacterized [c] (no./cm^3)	207	28	9,464	7,440	8,654	642	44,261
Particle number, outdoor origin (no./cm^3)	176	59	6,871	4,193	6,191	617	17,768
Elemental Carbon, uncharacterized (μg/m^3)	204	31	1.31	0.46	0.68	0.20	2.87
Elemental Carbon, outdoor origin (μg/m^3)	176	59	1.04	0.40	0.54	0.22	2.58
Organic carbon, uncharacterized [c] (μg/m^3)	206	29	7.53	3.62	5.25	2.32	16.6
Primary organic carbon, outdoor origin (μg/m^3)	162	73	3.65	2.78	2.83	0.01	11.2
Secondary organic carbon, outdoor origin (μg/m^3)	170	65	2.65	1.70	1.89	0.25	11.0
Size-fractionated PM[b]							
$PM_{0.25}$ (μg/m^3)	220	15	8.57	3.34	4.28	1.56	19.4
$PM_{0.25\text{-}2.5}$ (μg/m^3)	223	12	5.98	4.04	5.28	0.81	25.5
$PM_{2.5\text{-}10}$ (μg/m^3)	222	13	3.25	3.99	2.79	0.21	37.6
Air Pollutant Gases							
NO_x (ppb)	224	11	46.1	34.0	38.2	7.77	210.8
CO (ppm)	228	7	0.67	0.28	0.27	0.23	2.01

[a] The continuously-measured PM variables were averaged hourly and 24-hour averages are presented here.

[b] $PM_{2.5}$ mass was measured hourly with Beta-Attenuation Mass Monitors whereas the size-fractionated PM mass was measured daily with a Personal Cascade Impactor Sampler.

[c] Total PN, EC and OC uncharacterized by indoor or outdoor origin, and total OC also uncharacterized by primary or secondary sources.

Table 5. Spearman correlation matrix for outdoor home air pollutant exposures.[a]

	$PM_{2.5}$	Organic carbon	Black carbon	Primary OC	Secondary OC	$PM_{0.25}$	$PM_{0.25\text{-}2.5}$	$PM_{2.5\text{-}10}$	NO_x	CO	O_3
Particle number	-0.13	0.27	0.40	0.47	-0.08	0.36	-0.12	0.06	0.63	0.45	-0.38
$PM_{2.5}$	1.00	0.44	0.58	0.43	0.22	0.20	0.87	0.55	0.14	0.31	0.04
Organic carbon		1.00	0.63	0.65	0.72	0.41	0.33	0.33	0.46	0.59	-0.05
Black carbon			1.00	0.88	0.07	0.52	0.43	0.44	0.83	0.79	-0.38
Primary OC				1.00	0.01	0.55	0.33	0.36	0.79	0.75	-0.36
Secondary OC					1.00	0.09	0.16	0.15	-0.09	0.11	0.26
$PM_{0.25}$						1.00	0.17	0.35	0.51	0.54	0.01
$PM_{0.25\text{-}2.5}$							1.00	0.60	0.01	0.13	0.08
$PM_{2.5\text{-}10}$								1.00	0.18	0.26	0.06
NO_x									1.00	0.82	-0.53
CO										1.00	-0.29

[a] All exposures are 24-hr averages and are mean-centered by retirement community and seasonal phase.

2.2.2 Results of the Regression Analyses

Table 6 shows the results of regression models for the relation of the daily count of VT events and exposure to outdoor air pollutants, adjusting for confounding variables. An IQR increase in 24-hr average $PM_{2.5}$ (same day exposure) was associated with a 50% increase in the daily rate of VT events [95% confidence interval (CI): 2% to 120%]. The estimated effect of 3-day average $PM_{2.5}$ on daily VT was nearly identical, though not statistically significant. There were no significant associations for outdoor particle number. Effect estimates for daily VT in relation to outdoor size-fractionated PM varied by averaging time, ranging from a 0% to 31% increase for $PM_{0.25}$ and $PM_{0.25\text{-}2.5}$, and from a 13% decrease to 20% increase for $PM_{2.5\text{-}10}$. Only one of these models was statistically significant at the 5% level though.

Analysis of $PM_{2.5}$ constituents (Table 6) indicated significant associations of daily VT with the 24-hr average exposures to BC, EC, total OC, and primary OC. The 24-hr average total organic carbon had a notably strong estimated effect on daily VT at a rate ratio of 3.06 (95% CI: 1.82 to 5.17) per IQR of OC ($p < 0.01$). The greatest apparent contribution was from primary as compared with secondary OC, which was of borderline significance. Ozone also had strong effects on daily VT, with the strongest association being with the 3-day O_3 average with a rate ratio of 2.95 (95% CI: 1.29 to 6.74). When the outlying subject (see Methods Analysis section) was included rate ratios for VT events were mostly larger than reported here, often exceeding 5 with wide confidence intervals (data not shown).

Table 6 also shows odds ratios for hourly daytime and nighttime models using the occurrence of one or more VT events as the outcome variable and exposures averaged across the different averaging times up to the hour of the ECG measurement. In these models daytime VT risk was elevated at higher $PM_{2.5}$ concentrations for all averaging times, but only with borderline statistical significance. Nighttime VT risk actually decreased with higher $PM_{2.5}$ concentrations at averaging times shorter than 3 days, but again these effect sizes were not statistically significant. Among PM constituents, BC, EC and OC had the strongest VT effect estimates during the daytime. These PM markers of combustion-related pollutants were significantly elevated across a variety of averaging times. Findings for NO_x (also a marker of primary combustion products), were consistent with the time course of VT associations with the primary PM constituents. However, CO was nonsignificant except for two significant and unexpected inverse associations for nighttime hours (8-hr and 24-hr CO). In contrast, hourly nighttime VT was often inversely associated with PM constituents, to the extent that formal tests of odds ratio (OR) heterogeneity using Cochran's Q statistic often indicated significant daytime-nighttime differences in association for 8- and 24-hr average exposures.

Interestingly, positive associations for outdoor secondary OC were stronger and more significant for short-term (1-8 hour exposures) during the daytime hours than associations for nighttime hours. A mixture of daytime and nighttime positive associations was found for O_3, as well as an unexpected inverse association for 3-day and 5-day average O_3 during the daytime and positive association during the nighttime hours (significant for 24-hour O_3). There were several significant p-values for OR heterogeneity for O_3 day- versus nighttime hour models. This may be a result of lower personal exposure to O_3 during hot days as a result of the use of air

conditioning and limited time outdoors but greater exposure to outdoor air during the cooler nighttime hours.

Table 7 presents associations between daily count of VT events and indoor air pollutants, adjusting for confounding variables (see Table footnote and Analysis Methods). Findings are consistent with results for the outdoor primary pollutants (EC, primary OC and NO_x), but associations for indoor uncharacterized $PM_{2.5}$ were weaker in general and less significant. There were no significant associations for indoor particle number or size-fractionated PM. Of interest is the finding that associations for the daily count of VT was stronger and more significant for exposure to indoor EC of outdoor origin than for uncharacterized indoor EC, suggesting that outdoor combustion sources were important determinants. Relative rate estimates were: 1.41 (95% CI: 0.80, 2.47) for 5-day average uncharacterized EC and 2.14 (95% CI: 1.08, 4.24) for 5-day average indoor EC of outdoor origin. Associations for daily estimated indoor secondary OC of outdoor origin were also consistent with estimates for measured outdoor secondary OC. Positive associations for short-term (1-8 hour) exposures to indoor secondary OC of outdoor origin during the daytime hours were similar to associations for nighttime hours.

Table 6. Associations[a] of ventricular tachycardia with outdoor air pollutants, per IQR.

Exposure	Averaging Time	Daily Rate Ratio (95% CI)	Hourly daytime OR (95% CI)	Hourly nighttime OR (95% CI)	P-value for OR heterogeneity [b]
$PM_{2.5}$	1-hr	--	1.06 (0.83, 1.35)	0.94 (0.60, 1.47)	0.645
	4-hr	--	1.21 (0.95, 1.54)	0.94 (0.56, 1.58)	0.391
	8-hr	--	1.17 (0.95, 1.44)	0.86 (0.51, 1.45)	0.284
	24-hr	1.50 (1.02, 2.20)**	1.23 (0.99, 1.52)*	0.85 (0.64, 1.12)	0.039**
	3-day	1.51 (0.85, 2.70)	1.26 (0.99, 1.61)*	1.19 (0.84, 1.67)	0.778
	5-day	1.16 (0.59, 2.29)	1.30 (0.78, 2.17)	2.15 (0.90, 5.11)*	0.328
Particle number	1-hr	--	1.06 (0.86, 1.30)	0.77 (0.59, 1.01)*	0.071*
	4-hr	--	1.05 (0.84, 1.31)	0.90 (0.62, 1.30)	0.491
	8-hr	--	0.90 (0.64, 1.26)	1.09 (0.70, 1.70)	0.506
	24-hr	0.70 (0.41, 1.20)	0.90 (0.55, 1.46)	0.64 (0.31, 1.31)	0.442
	3-day	0.42 (0.09, 1.94)	1.16 (0.41, 3.26)	0.70 (0.26, 1.92)	0.497
	5-day	0.20 (0.02, 1.67)	2.43 (0.55, 10.7)	0.88 (0.10, 7.89)	0.453
Black carbon	1-hr	--		1.00 (1.00, 1.00)	0.327
	4-hr	--	1.07 (0.91, 1.25)	0.75 (0.44, 1.29)	0.215
	8-hr	--	1.23 (1.06, 1.44)#	0.69 (0.46, 1.03)*	0.008#
	24-hr	1.40 (1.06, 1.84)**	1.30 (0.98, 1.72)*	0.74 (0.54, 1.00)*	0.008#
	3-day	1.59 (0.77, 3.30)	1.88 (1.30, 2.73)#	1.38 (0.78, 2.42)	0.365
	5-day	1.36 (0.64, 2.91)	2.68 (1.32, 5.42)#	2.63 (0.87, 8.00)*	0.978
Elemental carbon	1-hr	--	1.01 (0.92, 1.11)	0.79 (0.56, 1.11)	0.181
	4-hr	--	0.96 (0.85, 1.08)	0.80 (0.47, 1.35)	0.507
	8-hr	--	1.09 (0.92, 1.30)	0.63 (0.38, 1.06)*	0.049**
	24-hr	1.55 (1.09, 2.21)**	1.24 (0.95, 1.60)	0.69 (0.50, 0.96)**	0.006#
	3-day	1.46 (0.65, 3.30)	2.10 (1.08, 4.09)**	1.09 (0.54, 2.22)	0.187
	5-day	1.90 (0.82, 4.37)	3.07 (0.90, 10.5)*	1.57 (0.69, 3.58)	0.375
Organic Carbon (OC)	1-hr	--	1.24 (0.78, 1.97)	0.91 (0.49, 1.68)	0.431
	4-hr	--	2.13 (1.31, 3.49)#	0.84 (0.38, 1.86)	0.051*
	8-hr	--	2.58 (1.47, 4.52)#	0.65 (0.23, 1.81)	0.021**
	24-hr	3.06 (1.82, 5.17)#	1.81 (1.08, 3.06)**	0.57 (0.19, 1.69)	0.060*
	3-day	2.13 (0.60, 7.51)	2.76 (0.40, 19.02)	1.73 (0.65, 4.65)	0.673
	5-day	5.95 (0.79, 44.8)*	4.22 (0.25, 71.7)	3.13 (0.25, 38.6)	0.877
Primary OC	1-hr	--	0.85 (0.65, 1.13)	0.89 (0.54, 1.46)	0.888
	4-hr	--	0.93 (0.62, 1.39)	0.91 (0.44, 1.88)	0.966
	8-hr	--	1.07 (0.54, 2.09)	0.64 (0.30, 1.37)	0.326
	24-hr	2.64 (1.13, 6.21)**	1.90 (0.64, 5.61)	0.99 (0.34, 2.86)	0.400
	3-day	2.16 (0.69, 6.77)	2.07 (0.57, 7.55)	0.78 (0.31, 1.98)	0.231
	5-day	3.15 (0.30, 33.3)	11.2 (0.49, 258)	0.62 (0.07, 5.51)	0.137

Table 6 (cont.)

Exposure	Averaging Time	Daily Rate Ratio (95% CI)	Hourly daytime OR (95% CI)	Hourly nighttime OR (95% CI)	P-value for OR heterogeneity [b]
Secondary OC	1-hr	--	1.18 (0.90, 1.54)	1.12 (0.76, 1.66)	0.833
	4-hr	--	1.68 (1.21, 2.33)#	1.24 (0.76, 2.02)	0.307
	8-hr	--	1.86 (1.06, 3.26)**	1.31 (0.90, 1.90)	0.301
	24-hr	1.43 (0.93, 2.18)*	1.18 (0.69, 2.02)	1.45 (0.70, 3.00)	0.653
	3-day	1.28 (0.47, 3.47)	0.68 (0.20, 2.30)	2.30 (0.41, 13.0)	0.261
	5-day	2.27 (0.79, 6.58)	0.97 (0.22, 4.15)	1.68 (0.54, 5.23)	0.558
$PM_{0.25}$	24-hr	1.04 (0.67, 1.60)	--	--	
	Lag 25-48 hr	1.20 (0.97, 1.47)*	--	--	
	2-day	1.29 (0.73, 2.29)	--	--	
$PM_{0.25-2.5}$	24-hr	1.31 (1.05, 1.64)**	--	--	
	Lag 25-48 hr	1.00 (0.78, 1.28)	--	--	
	2-day	1.30 (0.96, 1.77)*	--	--	
$PM_{2.5-10}$	24-hr	1.20 (0.90, 1.59)	--	--	
	Lag 25-48 hr	0.87 (0.71, 1.06)	--	--	
	2-day	0.97 (0.66, 1.44)	--	--	
O_3	1-hr	--	1.33 (1.09, 1.62)#	0.98 (0.66, 1.47)	0.184
	4-hr	--	1.37 (1.06, 1.77)**	0.99 (0.64, 1.53)	0.213
	8-hr	--	1.37 (0.98, 1.91)*	1.13 (0.74, 1.70)	0.472
	24-hr	1.60 (1.12, 2.30)**	0.66 (0.35, 1.25)	2.68 (1.32, 5.46)#	0.004#
	3-day	2.95 (1.29, 6.74)**	0.30 (0.08, 1.14)*	1.43 (0.45, 4.57)	0.082*
	5-day	0.93 (0.10, 8.16)	0.05 (0.01, 0.53)**	2.05 (0.42, 9.86)	0.010**
NO_x	1-hr	--	0.98 (0.75, 1.29)	1.13 (0.72, 1.78)	0.585
	4-hr	--	0.99 (0.77, 1.27)	0.92 (0.55, 1.54)	0.800
	8-hr	--	1.15 (0.90, 1.47)	0.67 (0.35, 1.27)	0.120
	24-hr	1.37 (0.99, 1.88)*	1.49 (0.85, 2.62)	0.54 (0.28, 1.03)*	0.021**
	3-day	1.80 (0.45, 7.12)	2.90 (0.87, 9.68)*	1.00 (0.30, 3.30)	0.219
	5-day	1.70 (0.54, 5.40)	7.98 (1.48, 43.0)**	1.34 (0.14, 13.1)	0.217
CO	1-hr	--	1.03 (0.82, 1.30)	0.96 (0.66, 1.40)	0.730
	4-hr	--	1.06 (0.84, 1.34)	0.79 (0.55, 1.15)	0.192
	8-hr	--	1.11 (0.88, 1.39)	0.68 (0.47, 0.98)**	0.025**
	24-hr	1.18 (0.84, 1.65)	0.84 (0.45, 1.56)	0.53 (0.32, 0.89)**	0.276
	3-day	2.20 (0.82, 5.94)	1.03 (0.20, 5.35)	1.04 (0.30, 3.63)	0.994
	5-day	0.84 (0.23, 3.03)	1.12 (0.14, 8.66)	1.06 (0.10, 11.0)	0.973

[a] All models are adjusted for aily average actigraph-derived physical activity and heart rate, temperature of the same lag average, day of week, seasonal study phase (mean centered exposure), and community group (mean centered exposure), using generalized estimating equations. Daily RRs use Poisson log-link models with daily VT counts as the outcome, and hourly ORs use binomial logit-link models with hourly absence/presence of any VT as the outcome.

[b] P-values are for heterogeneity of OR by day/night periods.
* $p < 0.1$, ** $p < 0.05$, # $p < 0.01$

Table 7. Associations[a] of ventricular tachycardia with indoor air pollutants, per IQR

Exposure	Averaging Time	Daily RR (95% CI)	Hourly daytime OR (95% CI)	Hourly nighttime OR (95% CI)	P-value for OR heterogeneity [b]
$PM_{2.5}$	1-hr	--	1.16 (0.96, 1.41)	1.08 (0.85, 1.39)	0.976
	4-hr	--	1.32 (0.92, 1.89)	1.10 (0.82, 1.47)	0.660
	8-hr	--	1.23 (0.75, 2.04)	0.99 (0.70, 1.40)	0.300
	24-hr	1.49 (1.01, 2.19)**	1.04 (0.56, 1.93)	0.87 (0.57, 1.34)	0.244
	3-day	1.57 (0.83, 3.00)	0.97 (0.47, 1.98)	0.94 (0.56, 1.59)	0.990
	5-day	1.41 (0.69, 2.88)	1.50 (0.29, 7.64)	1.80 (0.70, 4.61)	0.567
Particle number, uncharacterized	1-hr	--	0.73 (0.38, 1.43)	0.90 (0.76, 1.07)	0.314
	4-hr	--	0.87 (0.63, 1.21)	0.89 (0.75, 1.05)	0.574
	8-hr	--	0.87 (0.48, 1.58)	0.88 (0.69, 1.14)	0.483
	24-hr	1.42 (0.85, 2.35)	0.98 (0.15, 6.19)	1.10 (0.52, 2.33)	0.625
	3-day	1.56 (0.32, 7.60)	0.52 (0.03, 10.3)	0.43 (0.10, 1.85)	0.332
	5-day	1.04 (0.13, 8.19)	2.26 (0.07, 77.5)	0.78 (0.11, 5.33)	0.424
Particle number, outdoor origin	1-hr	--	0.77 (0.37, 1.59)	0.80 (0.57, 1.11)	0.709
	4-hr	--	1.02 (0.50, 2.06)	0.89 (0.65, 1.23)	0.532
	8-hr	--	1.13 (0.64, 1.99)	1.00 (0.76, 1.32)	0.733
	24-hr	0.79 (0.40, 1.58)	1.25 (0.49, 3.17)	1.10 (0.55, 2.19)	0.589
	3-day	0.39 (0.07, 2.30)	0.48 (0.04, 6.42)	0.31 (0.07, 1.39)	0.131
	5-day	0.04 (0.01, 0.74)**	1.49 (0.09, 25.1)	0.44 (0.04, 4.86)	0.622
Elemental carbon, uncharacterized	1-hr	--	1.09 (0.91, 1.30)	0.99 (0.83, 1.18)	0.586
	4-hr	--	0.98 (0.72, 1.35)	0.96 (0.72, 1.28)	0.924
	8-hr	--	1.11 (0.71, 1.73)	0.93 (0.66, 1.30)	0.310
	24-hr	1.30 (0.97, 1.74)*	1.39 (0.67, 2.88)	1.27 (0.76, 2.12)	0.368
	3-day	1.14 (0.65, 1.99)	0.85 (0.23, 3.13)	0.75 (0.33, 1.73)	0.015
	5-day	1.41 (0.80, 2.47)	0.94 (0.11, 8.29)	1.30 (0.61, 2.75)	0.776
Elemental carbon, outdoor origin	1-hr	--	1.01 (0.88, 1.17)	0.98 (0.81, 1.19)	0.894
	4-hr	--	1.06 (0.83, 1.35)	1.03 (0.80, 1.34)	0.880
	8-hr	--	1.25 (0.80, 1.96)	1.01 (0.74, 1.39)	0.212
	24-hr	1.53 (1.00, 2.34)*	1.67 (0.69, 4.02)	1.23 (0.73, 2.07)	0.132
	3-day	1.50 (0.70, 3.23)	1.18 (0.49, 2.84)	0.62 (0.30, 1.25)	0.001
	5-day	2.14 (1.08, 4.24)**	2.00 (0.47, 8.50)	1.21 (0.42, 3.45)	0.455
Organic carbon (OC) , uncharacterized	1-hr	--	1.10 (1.04, 1.17)#	1.08 (0.96, 1.21)	0.850
	4-hr	--	1.25 (1.01, 1.54)**	1.10 (0.88, 1.38)	0.767
	8-hr	--	1.52 (1.17, 1.98)#	1.10 (0.82, 1.47)	0.101
	24-hr	2.05 (0.78, 5.43)	1.55 (0.79, 3.05)	1.12 (0.69, 1.82)	0.149
	3-day	2.36 (0.46, 12.2)	1.08 (0.47, 2.46)	0.83 (0.48, 1.44)	0.941
	5-day	2.09 (0.23, 19.1)	4.97 (1.44, 17.2)**	2.10 (0.80, 5.56)	0.104

Table 7 (cont.) Exposure	Averaging Time	Daily RR (95% CI)	Hourly daytime OR (95% CI)	Hourly nighttime OR (95% CI)	P-value for OR heterogeneity [b]
Primary OC, outdoor origin	1-hr	--	0.94 (0.81, 1.08)	0.93 (0.77, 1.11)	0.997
	4-hr	--	0.99 (0.80, 1.23)	0.94 (0.76, 1.17)	0.842
	8-hr	--	1.12 (0.77, 1.62)	0.95 (0.74, 1.23)	0.465
	24-hr	2.08 (1.41, 3.09)#	1.17 (0.72, 1.93)	1.08 (0.73, 1.62)	0.095
	3-day	1.09 (0.54, 2.20)	1.25 (0.64, 2.42)	0.73 (0.44, 1.23)	0.004
	5-day	1.33 (0.39, 4.55)	2.37 (0.82, 6.84)	1.47 (0.68, 3.18)	0.455
Secondary OC, outdoor origin	1-hr	--	1.21 (0.93, 1.57)	1.23 (1.03, 1.48)**	0.976
	4-hr	--	1.56 (1.18, 2.06)#	1.45 (1.12, 1.86)#	0.359
	8-hr	--	1.78 (1.01, 3.16)**	1.54 (1.14, 2.08)#	0.176
	24-hr	1.38 (0.89, 2.14)	1.10 (0.61, 1.97)	1.16 (0.68, 1.96)	0.344
	3-day	1.58 (0.57, 4.35)	1.24 (0.31, 4.98)	2.11 (1.13, 3.93)**	0.023
	5-day	3.36 (1.26, 8.98)**	2.84 (0.75, 10.8)	2.63 (1.27, 5.45)#	0.357
$PM_{0.25}$	24-hr	1.02 (0.90, 1.15)	--	--	
	Lag 25-48 hr	1.10 (0.93, 1.30)	--	--	
	2-day	1.10 (0.93, 1.29)	--	--	
$PM_{0.25-2.5}$	24-hr	1.02 (0.68, 1.53)	--	--	
	Lag 25-48 hr	1.16 (0.86, 1.58)	--	--	
	2-day	1.27 (0.90, 1.77)	--	--	
$PM_{2.5-10}$	24-hr	0.98 (0.87, 1.11)	--	--	
	Lag 25-48 hr	1.01 (0.91, 1.12)	--	--	
	2-day	0.99 (0.91, 1.08)	--	--	
NO_x	1-hr	--	1.04 (0.93, 1.17)	1.05 (0.96, 1.15)	0.522
	4-hr	--	1.04 (0.91, 1.18)	1.03 (0.93, 1.13)	0.872
	8-hr	--	1.11 (0.99, 1.26)*	1.00 (0.91, 1.11)	0.416
	24-hr	1.09 (0.89 1.35)	1.25 (0.97, 1.59)*	1.07 (0.93, 1.24)	0.193
	3-day	1.29 (0.72 2.31)	1.40 (0.83, 2.37)	1.04 (0.74, 1.48)	0.001
	5-day	1.17 (0.60 2.26)	2.18 (1.11, 4.27)**	1.21 (0.73, 2.01)	0.107
CO	1-hr	--	1.01 (0.75, 1.36)	1.04 (0.80, 1.34)	0.741
	4-hr	--	0.98 (0.69, 1.38)	0.97 (0.75, 1.25)	0.889
	8-hr	--	1.10 (0.69, 1.78)	0.91 (0.68, 1.22)	0.473
	24-hr	1.13 (0.92 1.39)	1.34 (0.76, 2.35)	1.03 (0.74, 1.44)	0.224
	3-day	1.24 (0.66 2.32)	0.71 (0.33, 1.52)	0.92 (0.57, 1.48)	0.774
	5-day	1.00 (0.59 1.68)	1.93 (0.40, 9.29)	1.38 (0.56, 3.40)	0.732

[a] All models are adjusted for adjusted for daily average actigraph-derived physical activity and heart rate, temperature of the same lag average, day of week, seasonal study phase (mean centered exposure), and community group (mean centered exposure), using generalized estimating equations. Daily RRs use Poisson log-link models with daily VT counts as the

outcome, and hourly ORs use binomial logit-link models with hourly absence/presence of any VT as the outcome.

[b] P-values are for heterogeneity of OR by day/night periods.

* $p < 0.1$, ** $p < 0.05$, # $p < 0.01$

In secondary (exploratory) analyses we tested other subject-specific effect measure modifiers but found no consistent differences in VT association with air pollutants by a variety of variables including sex, diabetes, obesity (body mass index ≥ 30), history of myocardial infarction, or medications (anti-arrhythmic medications, beta blockers, digitalis and other inotropic agents, statins, and ACE inhibitors).

Few significant associations were found between supraventricular tachycardia (SVT) and air pollutant variables in either the daily or hourly models of outdoor air pollutants: none of 71 tests were significant at the 1% level, and only 2 tests (2.8%) were significant at the 5% level (Table 8). These significant associations occurred at rates near the expected false positive rates for these tests under a null hypothesis of no associations. Among 27 tests for gaseous pollutants, the only statistically significant increases in SVT were for 4-hr (RR = 1.19; 95% CI: 1.02 to 1.39) and 8-hr ozone (RR = 1.25; 95% CI: 1.00 to 1.56).

Table 8. Associations[a] of supraventricular tachycardia with outdoor air pollutants, per IQR.

Exposure and averaging time	Daily Rate Ratio (95% CI)	Hourly OR (95% CI)
Particle number		
1hr	--	1.02 (0.79, 1.31)
4hr	--	1.00 (0.74, 1.34)
8-hr	--	1.02 (0.74, 1.40)
24-hr	1.14 (0.78, 1.66)	0.96 (0.49, 1.87)
3-day	2.07 (0.50, 8.55)	1.52 (0.42, 5.49)
5-day	0.41 (0.08, 2.22)	0.83 (0.05, 12.76)
Black carbon		
1hr	--	
4hr	--	0.86 (0.73, 1.02)*
8-hr	--	0.92 (0.77, 1.11)
24-hr	0.92 (0.74, 1.13)	0.93 (0.66, 1.32)
3-day	1.15 (0.72, 1.85)	0.83 (0.46, 1.48)
5-day	0.95 (0.42, 2.16)	0.99 (0.49, 1.99)
Elemental carbon		
1hr	--	0.98 (0.88, 1.09)
4hr	--	0.89 (0.76, 1.04)
8-hr	--	0.90 (0.74, 1.09)
24-hr	0.98 (0.80, 1.21)	0.97 (0.75, 1.25)
3-day	1.39 (0.95, 2.03)*	0.93 (0.50, 1.75)
5-day	1.12 (0.54, 2.32)	1.25 (0.55, 2.84)
Organic Carbon (OC)		
1hr	--	0.94 (0.62, 1.41)
4hr	--	0.90 (0.54, 1.51)
8-hr	--	1.00 (0.53, 1.89)
24-hr	0.87 (0.54, 1.42)	0.76 (0.32, 1.82)
3-day	0.83 (0.32, 2.16)	0.51 (0.15, 1.73)
5-day	0.32 (0.04, 2.60)	0.29 (0.07, 1.29)
Primary OC		
1hr	--	0.93 (0.71, 1.22)
4hr	--	0.89 (0.67, 1.20)
8-hr	--	0.87 (0.49, 1.55)
24-hr	1.18 (0.58, 2.40)	0.95 (0.30, 3.02)
3-day	3.57 (0.98, 13.01)*	1.53 (0.31, 7.62)
5-day	8.11 (1.16, 56.66)**	1.22 (0.22, 6.82)

Table 8 (cont.)

Exposure and averaging time	Daily Rate Ratio (95% CI)	Hourly OR (95% CI)
Secondary OC		
1hr	--	0.96 (0.75, 1.23)
4hr	--	1.06 (0.75, 1.48)
8-hr	--	1.12 (0.77, 1.62)
24-hr	0.83 (0.57, 1.21)	0.81 (0.37, 1.76)
3-day	0.48 (0.24, 0.94)**	0.65 (0.20, 2.13)
5-day	0.58 (0.29, 1.16)	0.64 (0.31, 1.35)
$PM_{2.5}$		
1hr	--	1.01 (0.76, 1.35)
4hr	--	1.02 (0.75, 1.40)
8-hr	--	0.99 (0.74, 1.34)
24-hr	0.77 (0.54, 1.09)	0.85 (0.62, 1.16)
3-day	0.80 (0.40, 1.61)	0.66 (0.36, 1.18)
5-day	0.87 (0.39, 1.94)	0.45 (0.18, 1.14)*
$PM_{0.25}$		
24-hr	0.94 (0.76, 1.17)	--
Lag 25-48 hr	0.99 (0.79, 1.24)	--
2-day	0.90 (0.67, 1.21)	--
$PM_{0.25-2.5}$		
24-hr	0.89 (0.66, 1.21)	--
Lag 25-48 hr	1.20 (0.98, 1.47)*	--
2-day	1.21 (0.87, 1.68)	--
$PM_{2.5-10}$		
24-hr	0.88 (0.71, 1.10)	--
Lag 25-48 hr	0.97 (0.80, 1.18)	--
2-day	0.91 (0.66, 1.24)	--
O_3		
1hr	--	1.16 (0.99, 1.37)*
4hr	--	1.19 (1.02, 1.39)**
8-hr	--	1.25 (1.00, 1.56)**
24-hr	1.15 (0.80, 1.64)	0.89 (0.50, 1.57)
3-day	0.46 (0.22, 0.95)**	0.33 (0.10, 1.17)*
5-day	0.77 (0.23, 2.64)	0.31 (0.04, 2.54)
NO_x		
1hr	--	0.82 (0.65, 1.02)*
4hr	--	0.77 (0.63, 0.94)**
8-hr	--	0.92 (0.74, 1.15)
24-hr	1.05 (0.81, 1.35)	1.22 (0.74, 2.01)
3-day	1.76 (1.00, 3.13)*	1.51 (0.60, 3.80)
5-day	0.94 (0.37, 2.40)	2.51 (0.55, 11.54)

Table 8 (cont.)

Exposure and averaging time	Daily Rate Ratio (95% CI)	Hourly OR (95% CI)
CO		
1hr	--	0.75 (0.60, 0.93)[#]
4hr	--	0.64 (0.48, 0.87)[#]
8-hr	--	0.79 (0.60, 1.05)*
24-hr	0.92 (0.68, 1.23)	0.89 (0.57, 1.40)
3-day	1.54 (0.92, 2.59)	0.90 (0.41, 1.97)
5-day	1.72 (0.59, 4.98)	1.22 (0.45, 3.29)

[a] All models are adjusted for adjusted for average actigraph-derived physical activity and heart rate, temperature of the same lag average, day of week, seasonal study phase (mean centered exposure), and community group (mean centered exposure).

* $p < 0.1$, ** $p < 0.05$, [#] $p < 0.01$

The above SVT models were then retested using indoor exposures (Table 9). We found four tests (4%) were significant at the 1% level, and seven tests (7%) were significant at the 5% level, but five at either the 1% or 5% level were unexpectedly inverse (protective). As with the outdoor models, these significant associations occurred at rates close to the expected false positive rates for these tests under a null hypothesis of no associations.

Table 9. Associations[a] of supraventricular tachycardia with indoor air pollutants, per IQR.

Exposure	Averaging Time	Daily Rate Ratio (95% CI)	Hourly OR (95% CI)
$PM_{2.5}$	1-hr	--	0.98 (0.81, 1.20)
	4-hr	--	1.00 (0.75, 1.33)
	8-hr	--	1.04 (0.75, 1.42)
	24-hr	0.72 (0.43, 1.21)	0.80 (0.50, 1.28)
	3-day	0.70 (0.31, 1.61)	0.60 (0.37, 0.97)**
	5-day	0.64 (0.34, 1.21)	0.35 (0.18, 0.67)#
Particle number, uncharacterized	1-hr	--	0.74 (0.54, 1.03)*
	4-hr	--	0.81 (0.59, 1.10)
	8-hr	--	0.81 (0.59, 1.12)
	24-hr	1.72 (0.93, 3.18)*	0.89 (0.30, 2.65)
	3-day	6.14 (0.96, 39.2)*	1.97 (0.35, 11.0)
	5-day	7.79 (1.56, 38.9)**	3.62 (0.23, 56.9)
Particle number, outdoor origin	1-hr	--	0.94 (0.67, 1.32)
	4-hr	--	0.99 (0.67, 1.46)
	8-hr	--	1.13 (0.63, 2.01)
	24-hr	0.82 (0.44, 1.52)	1.51 (0.58, 3.92)
	3-day	12.6 (0.43, 367.2)	1.96 (0.50, 7.63)
	5-day	0.39 (0.01, 53.1)	6.63 (0.22, 203.9)
Elemental carbon, uncharacterized	1-hr	--	0.98 (0.85, 1.13)
	4-hr	--	1.01 (0.83, 1.21)
	8-hr	--	0.98 (0.77, 1.27)
	24-hr	1.13 (0.91, 1.40)	0.86 (0.58, 1.29)
	3-day	1.50 (0.96, 2.34)*	0.76 (0.31, 1.85)
	5-day	1.53 (0.86, 2.73)	0.83 (0.39, 1.79)
Elemental carbon, outdoor origin	1-hr	--	1.01 (0.89, 1.14)
	4-hr	--	1.02 (0.87, 1.20)
	8-hr	--	0.97 (0.77, 1.22)
	24-hr	1.10 (0.90, 1.35)	0.84 (0.60, 1.17)
	3-day	1.81 (1.15, 2.84)#	0.79 (0.31, 2.03)
	5-day	1.50 (0.82, 2.75)	0.80 (0.34, 1.87)
Organic carbon (OC), uncharacterized	1-hr	--	1.02 (0.93, 1.12)
	4-hr	--	1.10 (0.94, 1.29)
	8-hr	--	1.23 (1.05, 1.44)#
	24-hr	1.01 (0.45, 2.25)	1.30 (0.90, 1.86)
	3-day	0.83 (0.20, 3.38)	1.23 (0.79, 1.92)
	5-day	0.71 (0.15, 3.39)	0.72 (0.38, 1.34)

Table 9 (cont.)

Exposure	Averaging Time	Daily Rate Ratio (95% CI)	Hourly OR (95% CI)
Primary OC, outdoor origin	1-hr	--	1.05 (0.98, 1.13)
	4-hr	--	1.07 (0.94, 1.21)
	8-hr	--	1.07 (0.86, 1.33)
	24-hr	1.06 (0.70, 1.62)	1.10 (0.81, 1.49)
	3-day	2.42 (1.25, 4.66)**	1.04 (0.56, 1.94)
	5-day	2.04 (0.73, 5.74)	0.92 (0.55, 1.56)
Secondary OC, outdoor origin	1-hr	--	0.97 (0.82, 1.14)
	4-hr	--	1.09 (0.87, 1.36)
	8-hr	--	1.15 (0.89, 1.47)
	24-hr	0.86 (0.61, 1.22)	0.90 (0.55, 1.48)
	3-day	0.54 (0.31, 0.95)**	0.78 (0.40, 1.55)
	5-day	0.64 (0.36, 1.15)	0.82 (0.50, 1.33)
$PM_{0.25}$	24-hr	0.87 (0.74, 1.03)	--
	Lag 25-48 hr	1.23 (1.07, 1.42)#	--
	2-day	1.28 (0.96, 1.70)*	--
$PM_{0.25-2.5}$	24-hr	0.76 (0.56, 1.02)*	--
	Lag 25-48 hr	1.39 (1.04, 1.85)**	--
	2-day	1.42 (0.81, 2.52)	--
$PM_{2.5-10}$	24-hr	0.94 (0.84, 1.05)	--
	Lag 25-48 hr	1.08 (0.91, 1.28)	--
	2-day	1.06 (0.89, 1.27)	--
NO_x	1-hr	--	0.97 (0.93, 1.02)
	4-hr	--	0.98 (0.93, 1.03)
	8-hr	--	1.05 (0.97, 1.14)
	24-hr	1.03 (0.92, 1.16)	1.04 (0.93, 1.17)
	3-day	1.19 (0.89, 1.60)	1.11 (0.85, 1.46)
	5-day	1.27 (0.80, 2.01)	1.33 (0.92, 1.94)
CO	1-hr	--	0.88 (0.78, 0.99)**
	4-hr	--	0.85 (0.75, 0.97)**
	8-hr	--	1.08 (0.86, 1.36)
	24-hr	1.02 (0.85, 1.23)	0.95 (0.73, 1.23)
	3-day	1.05 (0.79, 1.39)	0.84 (0.43, 1.64)
	5-day	0.98 (0.59, 1.62)	0.87 (0.42, 1.80)

[a a] All models are adjusted for adjusted for average actigraph-derived physical activity and heart rate, temperature of the same lag average, day of week, seasonal study phase (mean centered exposure), and community group (mean centered exposure).

* $p < 0.1$, ** $p < 0.05$, [#] $p < 0.01$

We found few significant associations between PM variables and the three hourly HRV outcomes (SDNN, rMSSD, and pNN50). Specifically, none of 123 tests were significant at the 1% level, and only 6 tests (4.9%) were significant at the 5% level (Table 10). In contrast, the 54 tests for outdoor gaseous pollutants (O_3, NO_x, and CO) with hourly HRV yielded 3 significant associations (5.6%) at the 1% level, and 9 significant associations (16.7%) at the 5% level, primarily showing unexpected increased HRV for NO_x and CO exposure at 4- to 8-hr lags. Daytime and nighttime models for the HRV outcomes were similar to 24 hour models in that the numbers of significant models were few and there were unexpected positive associations (in the daytime models) (not shown). We found that 24-hour SDNN was not associated with daily lag 0, 1 and 2-day average size-fractionated (gravimetric) PM mass concentrations (Table 10). Similarly, there were no significant associations of 24-hour SDNN with 0-, 2-, 3-, and 5-day averages of hourly outdoor pollutants (data not shown).

The above models were then retested using indoor exposures (Table 11). A similar lack of significant associations was observed and most models had wide confidence intervals. We did, however, observe significant inverse associations for the lag 0 (last 24 hour) exposures for the two size fractions that make up $PM_{2.5}$ ($PM_{0.25}$ and $PM_{0.25\text{-}2.5}$), but lag 1 day models all had positive, but nonsignificant parameter estimates.

Table 10. Associations of hourly heart rate variability with outdoor air pollutants.

Exposure and averaging time	SDNN Coefficient (95% CI)[a]	rMSSD Coefficient (95% CI)	pNN50 Coefficient (95% CI)
Particle number			
1hr	-0.51 (-1.46, 0.44)	-0.03 (-0.85, 0.80)	-0.010 (-0.052, 0.032)
4hr	-0.40 (-1.71, 0.92)	-0.07 (-1.57, 1.43)	-0.024 (-0.094, 0.047)
8-hr	-1.29 (-2.97, 0.38)	0.55 (-1.69, 2.79)	-0.017 (-0.118, 0.084)
24-hr	-0.92 (-3.79, 1.95)	-0.26 (-4.74, 4.22)	0.011 (-0.182, 0.204)
3-day	1.70 (-4.18, 7.57)	-0.71 (-9.74, 8.32)	0.224 (-0.142, 0.590)
5-day	9.26 (1.23, 17.30)**	2.03 (-10.12, 14.17)	0.192 (-0.347, 0.730)
Black carbon			
1hr			
4hr	0.60 (-0.25, 1.46)	0.55 (-0.51, 1.61)	0.048 (-0.003, 0.100)*
8-hr	0.55 (-0.45, 1.55)	1.20 (-0.20, 2.60)*	0.051 (-0.014, 0.116)
24-hr	0.29 (-1.11, 1.69)	0.03 (-2.16, 2.22)	0.053 (-0.046, 0.153)
3-day	0.45 (-2.03, 2.94)	1.05 (-2.86, 4.97)	0.133 (-0.045, 0.311)
5-day	0.03 (-4.20, 4.26)	5.51 (-1.17, 12.19)	0.159 (-0.132, 0.450)
Elemental carbon			
1hr	0.22 (-0.46, 0.91)	0.29 (-0.31, 0.88)	0.029 (-0.003, 0.061)*
4hr	0.94 (0.02, 1.86)**	0.42 (-0.69, 1.53)	0.044 (-0.012, 0.099)
8-hr	0.78 (-0.30, 1.85)	0.83 (-0.66, 2.33)	0.073 (0.002, 0.143)**
24-hr	0.31 (-1.16, 1.78)	-0.06 (-2.39, 2.27)	0.067 (-0.042, 0.175)
3-day	-0.011 (-2.53, 2.51)	0.82 (-3.00, 4.64)	0.120 (-0.058, 0.299)
5-day	-3.15 (-7.27, 0.97)	3.67 (-2.19, 9.53)	0.203 (-0.074, 0.480)
Organic Carbon (OC)			
1hr	1.16 (-0.72, 3.03)	0.55 (-1.08, 2.19)	0.061 (-0.027, 0.150)
4hr	1.34 (-1.15, 3.84)	0.37 (-2.73, 3.47)	0.081 (-0.075, 0.238)
8-hr	1.16 (-1.68, 4.00)	-0.82 (-4.85, 3.20)	0.075 (-0.120, 0.270)
24-hr	0.68 (-2.64, 3.99)	1.21 (-3.97, 6.39)	0.207 (-0.043, 0.457)
3-day	-1.39 (-6.11, 3.32)	0.28 (-6.93, 7.50)	0.192 (-0.155, 0.540)
5-day	-5.75 (-13.09, 1.58)	-0.39 (-10.88, 10.09)	-0.032 (-0.529, 0.466)
Primary OC			
1hr	0.94 (-1.06, 2.94)	0.73 (-1.02, 2.49)	0.070 (-0.024, 0.163)
4hr	2.62 (-0.06, 5.31)*	1.40 (-1.82, 4.62)	0.036 (-0.124, 0.196)
8-hr	2.06 (-1.12, 5.25)	3.23 (-1.16, 7.62)	0.111 (-0.098, 0.321)
24-hr	-0.21 (-4.98, 4.57)	0.06 (-7.44, 7.57)	0.241 (-0.111, 0.592)
3-day	-0.90 (-8.33, 6.53)	3.78 (-7.28, 14.84)	0.388 (-0.131, 0.906)
5-day	-13.1 (-24.15, -2.11)**	0.64 (-14.33, 15.62)	0.430 (-0.294, 1.154)
Secondary OC			
1hr	-0.06 (-1.14, 1.03)	-0.06 (-0.90, 0.78)	-0.001 (-0.047, 0.044)
4hr	-1.44 (-3.05, 0.17)*	-0.74 (-2.56, 1.07)	-0.033 (-0.126, 0.060)
8-hr	-1.16 (-3.11, 0.79)	-3.10 (-5.62, -0.58)**	-0.133 (-0.257, -0.009)**
24-hr	0.29 (-2.35, 2.93)	1.46 (-2.44, 5.35)	0.076 (-0.112, 0.265)
3-day	-2.17 (-5.94, 1.59)	-0.88 (-6.50, 4.73)	-0.069 (-0.334, 0.196)
5-day	-1.53 (-6.09, 3.03)	-1.61 (-8.07, 4.86)	-0.139 (-0.443, 0.165)

Table 10 (cont.)

Exposure and averaging time	SDNN Coefficient (95% CI)[a]	rMSSD Coefficient (95% CI)	pNN50 Coefficient (95% CI)
BAM $PM_{2.5}$			
1hr	0.28 (-0.65, 1.22)	0.04 (-0.98, 1.06)	0.023 (-0.028, 0.075)
4hr	0.78 (-0.28, 1.85)	0.73 (-0.74, 2.20)	0.013 (-0.056, 0.082)
8-hr	0.33 (-0.84, 1.49)	-0.01 (-1.76, 1.74)	-0.038 (-0.118, 0.041)
24-hr	0.076 (-1.34, 1.49)	-1.23 (-3.48, 1.02)	0.007 (-0.093, 0.107)
3-day	-0.31 (-2.29, 1.66)	-1.04 (-4.25, 2.17)	0.004 (-0.134, 0.143)
5-day	-2.38 (-5.85, 1.09)	0.11 (-5.37, 5.58)	-0.024 (-0.267, 0.219)
Gravimetric PM: [b]			
$PM_{0.25}$			
24-hr Lag	1.06 (-3.20, 5.32)	--	--
25-48 hr	1.64 (-2.58, 5.85)	--	--
2-day	-0.39 (-7.20, 6.42)	--	--
$PM_{0.25-2.5}$			
24-hr Lag	0.80 (-2.53, 4.14)	--	--
25-48 hr	0.03 (-3.77, 3.82)	--	--
2-day	-0.96 (-5.66, 3.74)	--	--
$PM_{2.5-10}$			
24-hr Lag	2.48 (-0.70, 5.64)	--	--
25-48 hr	-1.90 (-5.50, 1.69)	--	--
2-day	-1.30 (-6.88, 4.27)	--	--
O_3			
1hr	-0.19 (-1.12, 0.74)	-0.83 (-1.84, 0.17)	-0.046 (-0.098, 0.006)*
4hr	-0.22 (-1.27, 0.84)	-0.84 (-2.17, 0.49)	-0.019 (-0.084, 0.046)
8-hr	-0.084 (-1.36, 1.20)	-0.59 (-2.33, 1.15)	-0.006 (-0.089, 0.077)
24-hr	2.42 (-0.32, 5.16)*	0.63 (-3.53, 4.79)	0.135 (-0.056, 0.325)
3-day	-0.047 (-4.54, 4.45)	-1.95 (-8.94, 5.04)	-0.076 (-0.392, 0.240)
5-day	-10.68 (-18.32, -3.05)#	-10.48 (-22.31, 1.34)*	-0.490 (-1.029, 0.049)*
NO_x			
1hr	0.29 (-0.64, 1.22)	0.68 (-0.20, 1.55)	0.035 (-0.009, 0.078)
4hr	0.53 (-0.62, 1.68)	1.25 (-0.10, 2.60)*	0.072 (0.009, 0.136)**
8-hr	1.13 (-0.24, 2.50)	2.60 (0.76, 4.43)#	0.113 (0.030, 0.196)#
24-hr	0.67 (-1.58, 2.91)	0.81 (-2.62, 4.24)	0.102 (-0.049, 0.253)
3-day	2.36 (-1.89, 6.61)	3.68 (-2.91, 10.27)	0.305 (0.011, 0.599)**
5-day	5.42 (-1.40, 12.25)	10.19 (-0.50, 20.89)*	0.383 (-0.082, 0.848)
CO			
1hr	-0.30 (-1.30, 0.70)	0.94 (-0.00, 1.89)*	0.043 (-0.005, 0.091)*
4hr	0.18 (-1.03, 1.40)	1.62 (0.22, 3.03)**	0.071 (0.003, 0.139)**
8-hr	0.85 (-0.59, 2.29)	2.37 (0.46, 4.27)**	0.101 (0.013, 0.190)**
24-hr	-0.29 (-2.38, 1.80)	0.02 (-3.06, 3.11)	0.097 (-0.044, 0.239)
3-day	0.45 (-2.88, 3.79)	1.59 (-3.42, 6.61)	0.134 (-0.100, 0.367)
5-day	-0.71 (-6.18, 4.75)	2.45 (-5.79, 10.68)	0.092 (-0.282, 0.466)

[a] All models are adjusted for adjusted for average actigraph-derived physical activity and heart rate for the same hour, temperature of the same lag average, day of week, time of day, seasonal study phase (mean centered exposure), and community group (mean centered exposure).

[b] In analyses of daily gravimetric PM filter data, we focused only on 24-hour SDNN spanning the gravimetric PM mass sampling periods.

* $p < 0.1$, ** $p < 0.05$, # $p < 0.01$

Table 11. Associations of hourly heart rate variability with indoor air pollutants.

Exposure and averaging time	SDNN Coefficient (95% CI)[a]	rMSSD Coefficient (95% CI)	pNN50 Coefficient (95% CI)
Particle number, uncharacterized			
1hr	-0.57 (-1.07, -0.07)**	-0.35 (-0.71, 0.01)*	-0.003 (-0.021, 0.015)
4hr	-0.42 (-1.16, 0.32)	-0.05 (-0.74, 0.63)	0.015 (-0.019, 0.048)
8-hr	-0.48 (-1.48, 0.53)	0.15 (-1.01, 1.31)	0.029 (-0.028, 0.086)
24-hr	0.34 (-2.73, 3.40)	-0.36 (-4.75, 4.02)	-0.015 (-0.196, 0.166)
3-day	-0.33 (-6.63, 5.97)	3.93 (-5.26, 13.1)	-0.011 (-0.387, 0.365)
5-day	-1.74 (-10.3, 6.79)	0.55 (-12.2, 13.3)	-0.002 (-0.547, 0.542)
Particle number, outdoor origin			
1hr	-0.58 (-2.68, 1.51)	1.23 (-0.66, 3.11)	0.059 (-0.033, 0.151)
4hr	-0.96 (-3.64, 1.73)	0.79 (-2.35, 3.94)	0.029 (-0.116, 0.174)
8-hr	-3.63 (-6.95, -0.32)**	-0.17 (-4.68, 4.34)	-0.047 (-0.250, 0.156)
24-hr	-3.60 (-9.26, 2.07)	2.39 (-6.54, 11.3)	0.061 (-0.318, 0.439)
3-day	0.55 (-11.4, 12.4)	13.8 (-3.93, 31.5)	0.638 (-0.103, 1.378)*
5-day	11.2 (-5.02, 27.4)	15.5 (-8.82, 39.7)	-0.071 (-1.132, 0.990)
Elemental carbon, uncharacterized			
1hr	-0.29 (-0.97, 0.39)	-0.22 (-0.85, 0.41)	0.007 (-0.026, 0.040)
4hr	-0.38 (-1.24, 0.48)	0.21 (-0.85, 1.28)	0.011 (-0.040, 0.062)
8-hr	-0.51 (-1.54, 0.52)	0.34 (-1.08, 1.76)	-0.001 (-0.067, 0.066)
24-hr	-0.64 (-2.35, 1.07)	-0.28 (-2.92, 2.35)	0.037 (-0.081, 0.154)
3-day	-1.81 (-5.00, 1.38)	-0.63 (-5.47, 4.21)	0.064 (-0.155, 0.282)
5-day	-0.28 (-4.36, 3.80)	5.12 (-1.09, 11.3)	0.182 (-0.101, 0.464)
Elemental carbon, outdoor origin			
1hr	-0.14 (-0.85, 0.57)	0.09 (-0.57, 0.74)	0.022 (-0.011, 0.055)
4hr	0.09 (-0.82, 0.99)	0.04 (-1.08, 1.16)	0.016 (-0.037, 0.069)
8-hr	-0.25 (-1.32, 0.81)	0.05 (-1.46, 1.56)	0.023 (-0.045, 0.091)
24-hr	-0.55 (-2.11, 1.01)	-0.22 (-2.67, 2.23)	0.050 (-0.060, 0.159)
3-day	-0.64 (-3.84, 2.56)	2.03 (-2.65, 6.70)	0.167 (-0.041, 0.374)
5-day	-1.59 (-5.33, 2.16)	6.08 (0.63, 11.5)**	0.314 (0.069, 0.559)**
Organic Carbon (OC), uncharacterized			
1hr	-0.33 (-1.97, 1.31)	-0.46 (-1.80, 0.88)	0.023 (-0.039, 0.084)
4hr	-0.81 (-3.16, 1.55)	1.00 (-1.56, 3.57)	0.018 (-0.102, 0.138)
8-hr	-1.51 (-4.35, 1.34)	2.20 (-1.50, 5.90)	0.080 (-0.092, 0.251)
24-hr	0.55 (-4.29, 5.39)	0.11 (-7.27, 7.50)	0.143 (-0.189, 0.475)
3-day	-1.17 (-8.64, 6.31)	-3.35 (-14.8, 8.12)	0.085 (-0.430, 0.601)
5-day	-4.79 (-14.6, 5.00)	-3.22 (-18.4, 12.0)	0.055 (-0.617, 0.727)

Table 11 (cont.)

Exposure and averaging time	SDNN Coefficient (95% CI)[a]	rMSSD Coefficient (95% CI)	pNN50 Coefficient (95% CI)
Primary OC, outdoor origin			
1hr	0.24 (-0.93, 1.41)	0.22 (-0.81, 1.25)	0.038 (-0.017, 0.093)
4hr	1.25 (-0.28, 2.77)	0.51 (-1.35, 2.38)	0.022 (-0.071, 0.114)
8-hr	0.93 (-0.87, 2.74)	2.30 (-0.24, 4.85)*	0.092 (-0.027, 0.212)
24-hr	-0.01 (-2.54, 2.52)	0.19 (-3.90, 4.28)	0.090 (-0.099, 0.280)
3-day	1.28 (-3.31, 5.87)	1.44 (-5.61, 8.49)	0.254 (-0.069, 0.578)
5-day	-0.16 (-5.35, 5.03)	9.02 (1.47, 16.6)**	0.588 (0.129, 1.048)**
Secondary OC, outdoor origin			
1hr	-0.02 (-1.04, 1.00)	-0.01 (-0.82, 0.81)	0.018 (-0.026, 0.063)
4hr	-1.40 (-2.92, 0.13)*	-0.38 (-2.14, 1.38)	0.006 (-0.086, 0.098)
8-hr	-1.37 (-3.19, 0.45)	-3.03 (-5.48, -0.59)**	-0.081 (-0.206, 0.044)
24-hr	-0.09 (-2.40, 2.22)	0.30 (-3.20, 3.81)	0.098 (-0.082, 0.278)
3-day	-2.33 (-5.68, 1.03)	-1.30 (-6.35, 3.76)	-0.015 (-0.268, 0.238)
5-day	-1.75 (-4.69, 1.19)	-3.88 (-8.08, 0.32)*	-0.071 (-0.369, 0.227)
BAM $PM_{2.5}$			
1hr	-0.15 (-1.04, 0.74)	0.26 (-0.54, 1.07)	0.028 (-0.014, 0.071)
4hr	-0.32 (-1.44, 0.81)	0.30 (-1.12, 1.72)	0.026 (-0.044, 0.095)
8-hr	-0.66 (-1.98, 0.65)	-0.67 (-2.53, 1.20)	-0.018 (-0.107, 0.070)
24-hr	-0.07 (-1.75, 1.61)	-1.85 (-4.48, 0.78)	-0.011 (-0.134, 0.112)
3-day	-0.60 (-2.78, 1.59)	-0.64 (-4.11, 2.83)	0.028 (-0.125, 0.180)
5-day	-2.59 (-6.00, 0.83)	0.28 (-5.04, 5.60)	-0.001 (-0.242, 0.242)
Gravimetric PM: [b]			
$PM_{0.25}$			
24-hr Lag	-2.60 (-4.92, -0.27)**	--	--
25-48 hr	0.93 (-1.60, 3.46)	--	--
2-day	-1.08 (-4.07, 1.90)	--	--
$PM_{0.25-2.5}$			
24-hr Lag	-4.00 (-7.82, -0.19)**	--	--
25-48 hr	4.04 (0.003, 8.08)*	--	--
2-day	-1.32 (-7.23, 4.58)	--	--
$PM_{2.5-10}$			
24-hr Lag	-0.25 (-2.31, 1.82)	--	--
25-48 hr	0.11 (-2.67, 2.89)	--	--
2-day	-0.12 (-2.89, 2.65)	--	--
NO_x			
1hr	0.29 (-0.16, 0.73)	0.50 (0.05, 0.95)**	0.034 (0.012, 0.056)#
4hr	0.35 (-0.17, 0.87)	1.03 (0.40, 1.66)#	0.060 (0.031, 0.090)#
8-hr	0.52 (-0.09, 1.13)*	1.18 (0.36, 2.00)#	0.072 (0.034, 0.109)#
24-hr	0.09 (-0.86, 1.04)	0.02 (-1.44, 1.48)	0.044 (-0.021, 0.108)
3-day	0.73 (-1.09, 2.55)	0.45 (-2.34, 3.23)	0.081 (-0.042, 0.205)
5-day	2.26 (-0.60, 5.12)	3.02 (-1.38, 7.41)	0.081 (-0.115, 0.278)

Table 11 (cont.)

Exposure and averaging time	SDNN Coefficient (95% CI)[a]	rMSSD Coefficient (95% CI)	pNN50 Coefficient (95% CI)
CO			
1hr	0.27 (-0.39, 0.93)	0.58 (-0.04, 1.19)*	0.005 (-0.026, 0.037)
4hr	0.36 (-0.45, 1.18)	0.97 (-0.01, 1.94)*	0.040 (-0.006, 0.087)*
8-hr	0.75 (-0.24, 1.73)	1.32 (-0.01, 2.64)*	0.046 (-0.015, 0.107)
24-hr	-0.44 (-1.86, 0.99)	-0.68 (-2.88, 1.52)	0.025 (-0.073, 0.124)
3-day	-0.16 (-2.54, 2.22)	-1.71 (-5.41, 1.99)	0.035 (-0.130, 0.200)
5-day	-0.49 (-4.12, 3.15)	0.82 (-4.83, 6.47)	-0.045 (-0.294, 0.204)

[a] All models are adjusted for adjusted for average actigraph-derived physical activity and heart rate for the same hour, temperature of the same lag average, day of week, time of day, seasonal study phase (mean centered exposure), and community group (mean centered exposure).

[b] In analyses of daily gravimetric PM filter data, we focused only on 24-hour SDNN spanning the gravimetric PM mass sampling periods.

* $p < 0.1$, ** $p < 0.05$, # $p < 0.01$

2.2.3 Effect modification of air pollutant effects on HRV by medication use

Although there were no main effects detected in the overall analysis, there may be a susceptible subgroup or a protected subgroup as a result of the medication they are taking. This type exploratory analysis of medication effect modification is routinely done in the present type of epidemiologic analysis (see Analysis section). We tested potential effect measure modification of outdoor air pollutant effects on HRV by medication use (Table 1) and found no significant differences except for ACE inhibitors. Outdoor $PM_{2.5}$, EC, BC, OC (both primary and secondary fractions), and ozone were significantly and inversely associated with hourly SDNN only among the 20 participants taking ACE inhibitors (Figure 2). Many interaction term tests for effect measure modification by ACE inhibitor use on these air pollutants were significant in the hourly SDNN models mainly for 4-hr and 8-hr exposures (suggesting very acute effects). There were no differences in associations by ACE inhibitor use for particle number or NO_x (Figure 2), or CO (not shown) We did not observe many differences by ACE medication use in longer-term daily average SDNN models except for lag 0 (1 d) of the photochemically-related air pollutants (secondary organic carbon and ozone). Models testing effect measure modification of indoor air pollutant effects on HRV by medication use also showed no significant differences except for ACE inhibitors (Figure 3). The indoor air pollutant models were consistent with findings for the outdoor air pollutants. The effect modification was greatest for indoor OC showing the largest decrease in HRV per interquartile increase in the air pollutant among subjects taking ACE medications.

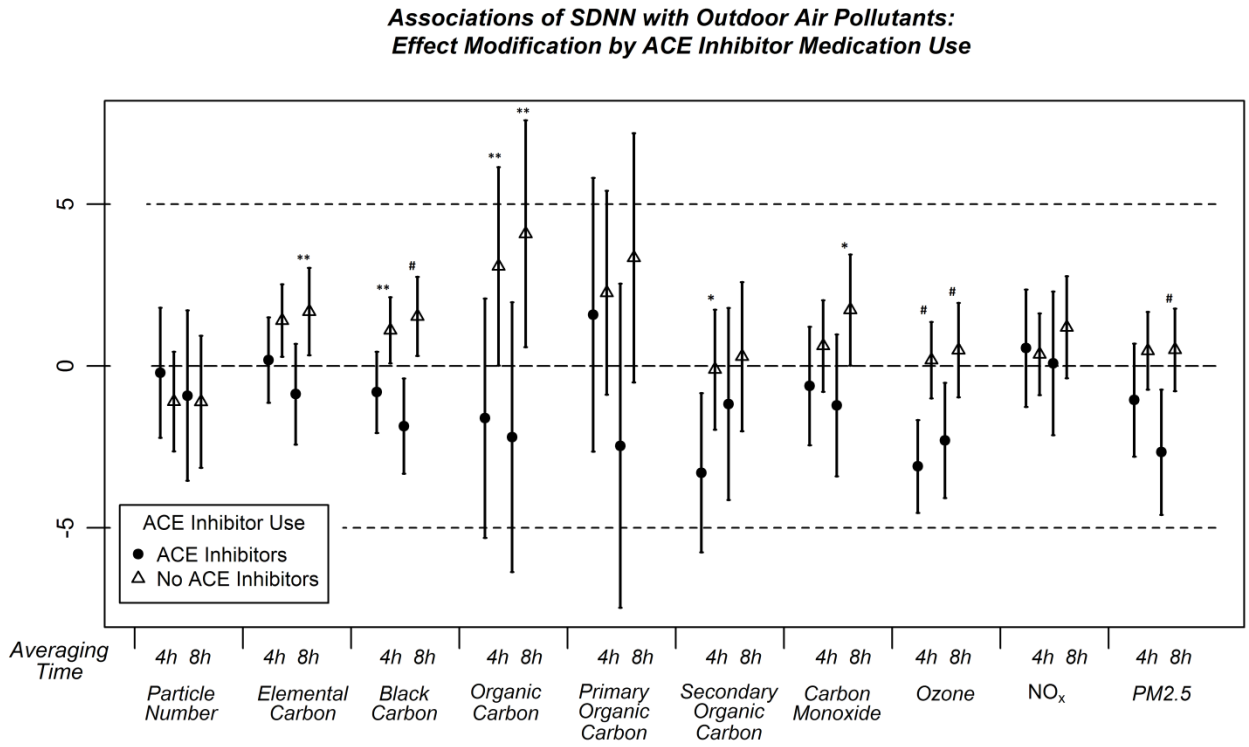

Figure 2. **Associations of SDNN with outdoor air pollutants: effect modification by ACE inhibitor medication use.** Product term p-values are indicated: * <0.1, ** 0.05, and # 0.01.

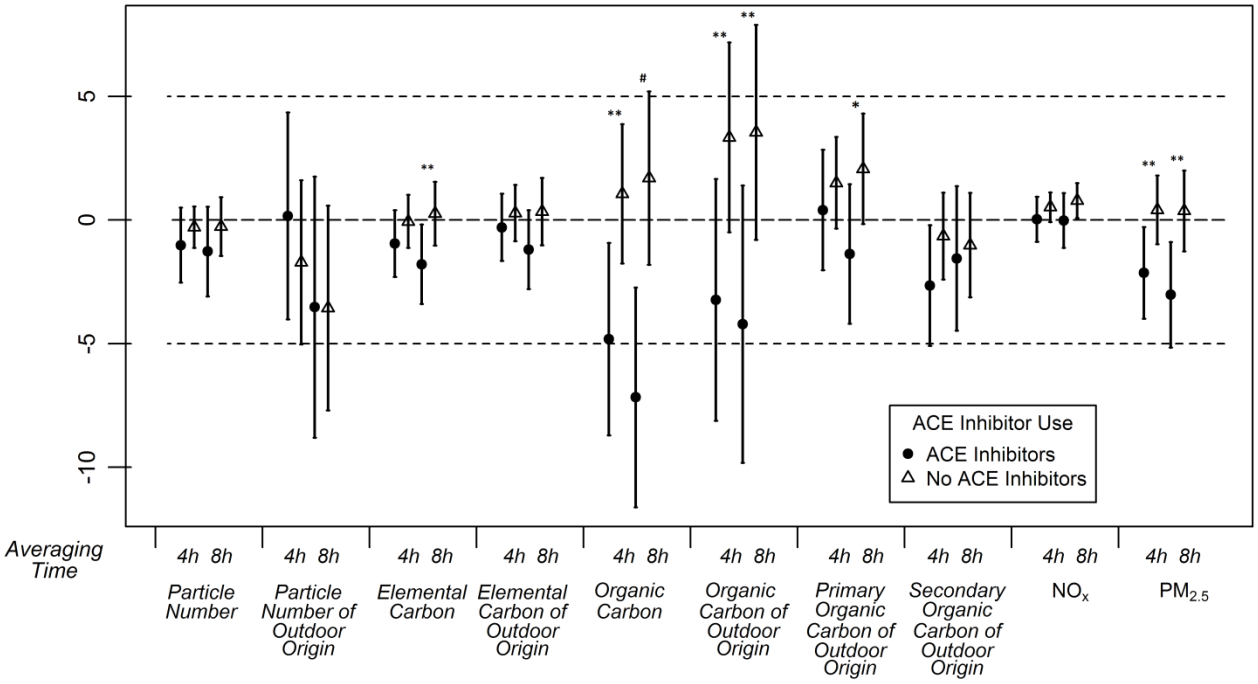

Figure 3. **Associations of SDNN with indoor air pollutants: effect modification by ACE inhibitor medication use.** Product term p-values are indicated: * <0.1, ** 0.05, and # 0.01.

2.3 DISCUSSION

Earlier studies of HRV and air pollution before 2005 recorded short ECG strips at in-clinic visits at rest to control for effects of physical activity on respiratory sinus arrhythmia (Delfino et al. 2005). Since then, several studies have used ambulatory ECG to produce continuous datasets. The present study employed ambulatory ECG adjusted for physical activity in subjects at potentially high risk of adverse cardiovascular outcomes (elderly with CAD). Data were more extensive compared to many previous studies given that we collected Holters for up to 10 days in each subject. This was combined with detailed air pollutant monitoring in the subject's retirement community that included measurements of PM mass fractions and PM constituents and analyses of their relation to both HRV and risk of cardiac arrhythmias over prolonged time periods.

A recent meta-analysis was conducted to evaluate associations between $PM_{2.5}$ and HRV, and was based on a concern over the variable results in the literature (Pieters et al. 2012). Their findings supported the hypothesis that short-term increases in PM air pollution are associated with decreased HRV. This included findings for SDNN (-0.12%, 95% CI: -0.22% to -0.03%) and for rMSSD (-2.18%, 95% CI: -3.33% to -1.03%) in relation to an increase of 10 $\mu g/m^3$ in $PM_{2.5}$, mostly from ambient monitoring stations. However, the present results do not support the hypothesis that elevations in outdoor pollution are associated with decreased HRV, adjusted for likely confounding variables. The exception is that subjects taking ACE inhibitors did show the hypothesized inverse association (discussed below). There was also no evidence of an association of SVT with air pollution exposure. Our confounding controls are as good as or better than those used in previous investigations using Holters that often lacked actigraph data.

Nevertheless, the lack of association for PM with HRV or SVT in our overall panel population was unlikely to be due to exposure error since we used home-based exposure measurements considered to be more representative of personal exposure than ambient monitoring at central sites. Outdoor and indoor air pollutant exposures measured at home may still not completely represent personal exposure because air pollutant exposures during times spent away from the home would not be accurately represented. Our study subjects spent relatively little time away from their retirement community. For a sensitivity analysis we restricted the ventricular tachycardia models to the nighttime hours plus the 91% of daytime hours for which subjects reported in diaries that they were at home. Results of this analysis were similar to the results including all times in terms of significance and magnitude of effects (not shown).

The absence of effects of ambient PM on HRV in other studies have been previously discussed by Barclay et al. (2009) who suggested that beta-blockers and other cardiac vagotonic medications like digitalis may modulate effects of PM on autonomic changes that would otherwise result in decreased HRV. Consistent with this possibility, de Hartog et al. (2009) observed an inverse association between SDNN and PM exposure only among CAD subjects who were not taking beta-blockers. However, we did not find any evidence effect modification of pollutant associations with HRV by beta-blockers or other cardiac medications aside from ACE inhibitors.

Although the overall results were null, we did find a decrease in SDNN with higher air pollutant exposures among those taking ACE inhibitors. This is a

preliminary finding given the number of interaction tests of medications by air pollution (the rest of which were nonsignificant) and the fact that these tests were exploratory. Interestingly, a recent cross-sectional study using the SAPALDIA general population cohort found that 10-year average PM_{10} was associated with decreased HRV only among 97 subjects taking ACE inhibitors out of 1607 subjects 50-72 years of age (Adam et al. 2012). Subjects were followed with ambulatory 24-hr electrocardiograms as in the present study, but for only one day.

The effect modification by ACE inhibitors may be related in some way to the well-known side effect of ACE inhibitor-induced cough. Several mechanisms for this side effect of ACE inhibitors have been proposed. This includes an accumulation in the airways of protussive mediators (bradykinin and substance P) that are degraded by ACE, and/or activation by ACE inhibitors of bradykinin receptors on rapidly adapting pulmonary vagal afferents (Dicpinigaitis et al. 2006; Hargreaves et al. 1992). Therefore, ACE inhibitors might enhance the respiratory irritant response to pollutants, and ACE inhibitor modification of SDNN response to air pollutant exposure may result from stimulation of pulmonary vagal afferents by bradykinin.

The most notable finding in this study is the strong association between risk of VT and PM ($PM_{2.5}$, BC, EC, OC) and ozone exposures. Except for indoor $PM_{2.5}$, findings for indoor exposures were consistent with outdoor exposures. Interestingly, associations for the daily count of VT was stronger and more significant for exposure to indoor EC of outdoor origin than for uncharacterized indoor EC, suggesting that outdoor combustion sources were important determinants of risk. We did not find that quasi-ultrafine particle mass ($PM_{0.25}$) was more strongly associated with VT risk than accumulation mode particle mass ($PM_{0.25\text{-}2.5}$) suggesting that components in both of these size fractions comprising $PM_{2.5}$ were important. The lack of association with particle number concentration also suggests that ultrafine particles (that dominate particle numbers) were not any more important in relation to VT risk than larger particles.

Although it is conceivable that an effect of air pollution on HRV could affect arrhythmia risk, we could not find any evidence of that in the present study since we found no consistent effects of air pollutants on HRV overall. PM-arrhythmia associations could be mediated by an autonomic mechanism, but this may or may not be captured by ambulatory HRV data. Similarly, Folino et al. (2009) found strong associations between PM and VT without HRV associations in CAD patients in their Holter study. One other recent Holter study of CAD patients found associations of PM with VT but did not report any findings on HRV (Berger et al. 2009). One study of patients with implantable cardiac defibrillators found no association of VT with ambient air pollution (Metzger et al. 2007) whereas two others did (Dockery et al. 2005; Ljungman et al. 2008). The use of ambient air pollution data may have resulted in exposure error and bias toward the null hypothesis of no effect.

$PM_{2.5}$, BC, EC, OC and NO_x had the strongest VT effect estimates during the daytime across a variety of averaging times as compared with nighttime estimates. The day/night effect modification for VT with many pollutants might be a result of contrasting indoor infiltration conditions during day and night periods as well as differences in air pollutant composition in the daytime versus nighttime. For example, personal exposure to O_3 might differ substantially in by day and night as a result of air conditioning use combined with limited time outdoors during hotter daytime hours with more exposure to outdoor air during cooler nighttime hours. It might also indicate a difference in cardiac response to air pollutants during waking

versus sleeping hours, although we are unaware of a specific mechanism to support this speculation.

The present findings of an adverse effect of air pollution exposure on risk of VT are consistent with our findings for other clinically important cardiovascular outcomes in the same panel. We previously reported that several outcomes were positively associated with PM exposure, including systolic and diastolic blood pressure, ischemic ST segment depression, and circulating markers of systemic inflammation and platelet activation (Delfino et al. 2009, 2010a, 2010b, 2011). These integrated findings suggest that there may be shared mechanisms for PM-related cardiac electrical effects.

It is possible that unmeasured confounders could have sufficiently strong proarrhythmic actions to explain the observed associations with VT. Another limitation includes the fact that results are limited to 50 subjects that may not be representative of the target population of elderly subjects with coronary artery disease. VT events were also relatively rare, N=305 out of 8,952 hours of ambulatory Holter data, and this may explain the wide confidence intervals for many models.

2.4 SUMMARY AND CONCLUSIONS

Risk of ventricular tachycardia was significantly increased with higher exposure to markers of traffic-related particles, secondary organic carbon, and ozone. Consistent associations were not observed for supraventricular tachycardia. Ozone and PM exposure ($PM_{2.5}$, EC, BC, and OC) were significantly associated with decreased heart rate variability only in the 20 participants using ACE inhibitors.

In conclusion, the present study supports the hypothesis that exposure to particulate air pollution linked to fossil fuel combustion sources increases the risk of ventricular tachycardia in a population of elderly people with coronary artery disease. We also found that risk of ventricular tachycardia was also increased with ozone exposure, possibly via its effects on the lungs. An increased rate of arrhythmia at higher PM concentrations has direct medical relevance and strong support in the present study. Ventricular arrhythmia in particular is the most common cause of sudden cardiac arrest and usually seen most often in people with CAD and VT increases risk (John et al. 2012). These results are consistent with previous findings in this cohort for adverse effects of air pollution on systemic inflammation, blood pressure and electrocardiographic evidence of ischemia. However, autonomic dysfunction as measured by decreased heart rate variability was not associated with air pollutants except in subjects on ACE inhibitors. Future panel studies on cardiac effects of air pollution exposure might benefit from assessments of other possible mechanisms in addition to heart rate variability.

References

Adam M, Felber Dietrich D, Schaffner E, Carballo D, Barthélémy JC, Gaspoz JM, Tsai MY, Rapp R, Phuleria HC, Schindler C, Schwartz J, Künzli N, Probst-Hensch NM. Long-term exposure to traffic-related PM(10) and decreased heart rate variability: is the association restricted to subjects taking ACE inhibitors? Environ Int. 2012;48:9-16.

Albrecht P, Cohen RJ. 1988. Estimation of heart rate power spectrum bands from real-world data: Dealing with ectopic beats and noisy data. Comput Cardiol 15:311-314.
Arhami M, Kuhn T, Fine PM, Delfino RJ, Sioutas C. 2006. Effects of sampling artifacts and operating parameters on the performance of a semi-continuous particulate EC/OC monitor. Environ Sci Technol 40:945-953.
Arhami M, Minguillón MC, Polidori A, Schauer JJ, Delfino RJ, Sioutas C. Organic compound characterization and source apportionment of indoor and outdoor quasi-ultrafine PM in retirement homes of the Los Angeles basin. Indoor Air, 2010;20:17-30.
Arhami M, Polidori A, Delfino RJ, Tjoa T, Sioutas C. Associations between personal, indoor, and residential outdoor pollutant concentrations: Implications for exposure assessment to size fractionated PM. J Air Waste Manage Assoc, 2009;59:392–404.
Ayres JG, Borm P, Cassee FR, Castranova V, Donaldson K, Ghio A, et al. 2008. Evaluating the toxicity of airborne particulate matter and nanoparticles by measuring oxidative stress potential--a workshop report and consensus statement. Inhal Toxicol 20:75-99.
Barclay JL, Miller BG, Dick S, Dennekamp M, Ford I, Hillis GS, et al. 2009. A panel study of air pollution in subjects with heart failure: negative results in treated patients. Occup Environ Med 66:325–334.
Berger A, Zareba W, Schneider A, Rückerl R, Ibald-Mulli A, Cyrys J, et al. 2006. Runs of ventricular and supraventricular tachycardia triggered by air pollution in patients with coronary heart disease. J Occup Environ Med 48:1149-1158.
Brook RD, Rajagopalan S, Pope CA 3rd, Brook JR, Bhatnagar A, Diez-Roux AV, et al. 2010. Particulate matter air pollution and cardiovascular disease: An update to the scientific statement from the American Heart Association. Circulation 121:2331-78.
Cabada J.C; Pandis S.N.; Subramanian R.; Robinson A.L.; Polidori A.; Turpin B. 2004. Estimating the Secondary Organic Aerosol Contribution to PM2.5 Using the EC Tracer Method. Aerosol Sci. Technol 38:140-155.
Cochran WG. The Combination of Estimates from Different Experiments. Biometrics. 1954;10(1):101–129.
Cornbleet, P.J.; Gochman, N. 1979. Incorrect Least-Squares Regression Coefficients in Method-Comparison Analysis; Clin. Chem 25:432-438.
de Hartog JJ, Lanki T, Timonen KL, Hoek G, Janssen NA, Ibald-Mulli A, Peters A, Heinrich J, Tarkiainen TH, van Grieken R, van Wijnen JH, Brunekreef B, Pekkanen J. 2009. Associations between PM2.5 and heart rate variability are modified by particle composition and beta-blocker use in patients with coronary heart disease. Environ Health Perspect 117:105-11.
Delfino RJ, Gillen DL, Tjoa T, Staimer N, Polidori A, Arhami M, et al. 2011. Electrocardiographic ST-Segment Depression and Exposure to Traffic-Related Aerosols in Elderly Subjects with Coronary Artery Disease. Environ Health Perspect 119:196-202.
Delfino RJ, Sioutas C, Malik S. 2005. Potential Role of Ultrafine Particles in Associations between Airborne Particle Mass and Cardiovascular Health. Environ Health Perspect 113: 934–946.
Delfino RJ, Staimer N, Tjoa T, Arhami M, Polidori A, George SC, et al. 2010a. Associations of primary and secondary organic aerosols with airway and systemic inflammation in an elderly panel cohort. Epidemiology 21:892-902.

Delfino RJ, Staimer N, Tjoa T, Gillen D, Polidori A, Arhami M, et al. 2009. Air pollution exposures and circulating biomarkers of effect in a susceptible population: clues to potential causal component mixtures and mechanisms. Environ Health Perspect 117:1232–1238.

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

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

Dicpinigaitis PV. 2006. Potential future therapies for the management of cough: ACCP evidence-based clinical practice guidelines. Chest 129(1 Suppl):284S-286S.

Dockery DW, Luttmann-Gibson H, Rich DQ, Link MS, Mittleman MA, Gold DR, et al. 2005. Association of air pollution with increased incidence of ventricular tachyarrhythmias recorded by implanted cardioverter defibrillators. Environ Health Perspect 113:670-4.

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

Griendling KK, FitzGerald GA. 2003. Oxidative Stress and Cardiovascular Injury Part II: Animal and Human Studies. Circulation 108: 2034-2040.

Grossman P, Taylor EW. 2007. Toward understanding respiratory sinus arrhythmia: relations to cardiac vagal tone, evolution and biobehavioral functions. Biol Psychol 74:263-85.

Grossman P, Wilhelm FH, Spoerle M. 2004. Respiratory sinus arrhythmia, cardiac vagal control, and daily activity. Am J Physiol Heart Circ Physiol 287:H728-34.

Hampel R, Schneider A, Bruske I, Zareba W, Cyrys J, Rückerl R, et al. 2010. Altered Cardiac Repolarization in Association with Air Pollution and Air Temperature among Myocardial Infarction Survivors. Environ Health Perspect 118:1755–1761.

Hargreaves M, Ravi K, Senaratne MP, Kappagoda CT. 1992. Responses of airway rapidly adapting receptors to bradykinin before and after administration of enalapril in rabbits. Clin Sci (Lond) 83:399-407.

John RM, Tedrow UB, Koplan BA, Albert CM, Epstein LM, Sweeney MO, Miller AL, Michaud GF, Stevenson WG. Ventricular arrhythmias and sudden cardiac death. Lancet 2012;380:1520-9.

Kleiger RE, Stein PK, Bigger JT Jr. 2005. Heart rate variability: measurement and clinical utility. Ann Noninvasive Electrocardiol 10:88-101.

Lampert R, Rosenfeld L, Batsford W, Lee F, McPherson C. 1994. Circadian variation of sustained ventricular tachycardia in patients with coronary artery disease and implantable cardioverter-defibrillators. Circulation 90:241-247.

Lim, H.J.; Turpin, B.J.; Russell, L.M.; Bates, T.S. 2003.Organic and Elemental Carbon Measurements during ACE-Asia Suggest a Longer Atmospheric Lifetime for Elemental Carbon; Environ. Sci. Technol 37:3055-3061.

Link MS, Dockery DW. 2010. Air pollution and the triggering of cardiac arrhythmias. Curr Opin Cardiol 25:16–22.

Ljungman PLS, Berglind N, Holmgren C, Gadler F, Edvardsson N, Pershagen G, et

al. 2008. Rapid effects of air pollution on ventricular arrhythmias. Eur Heart J 29: 2894–2901.
Lunden, M.M.; Revzan, K.L.; Fischer, M.L.; Thatcher, T.L.; Littlejohn, D, et al. 2003. The transformation of outdoor ammonium nitrate aerosols in the indoor environment. Atmos. Environ 37:5633-5644.
Meng QY, Turpin BJ, Polidori A, Lee JH, Weisel C, Morandi M, et al. 2005. PM2.5 of ambient origin: estimates and exposure errors relevant to PM epidemiology. Environ Sci Technol 39:5105-112.
Metzger KB, Klein M, Flanders WD, Peel JL, Mulholland JA, Langberg JJ, et al. 2007. Ambient Air Pollution and Cardiac Arrhythmias in Patients With Implantable Defibrillators. Epidemiology 18: 585–592.
Misra C, Singh M, Hall P, Sioutas C. 2002. Development and evaluation of a personal cascade impactor sampler (PCIS). J Aerosol Science 33:1027-1047.
Pieters N, Plusquin M, Cox B, Kicinski M, Vangronsveld J, Nawrot TS. 2012. An epidemiological appraisal of the association between heart rate variability and particulate air pollution: a meta-analysis. Heart 98:1127-35.
Polidori A, Arhami M, Delfino RJ, Allen R, Sioutas C. 2007. Indoor-outdoor relationships, trends and carbonaceous content of fine particulate matter in retirement communities of the Los Angeles basin. J Air Waste Manage Assoc 57:366-379.
Sarnat, S.E.; Coull, B.A.; Ruiz, P.A.; Koutrakis, P.; Suh, H.H. 2006. The Influences of Ambient Particle Composition and Size on Particle Infiltration in Los Angeles, CA, Residences, J. Air & Waste Manage. Assoc 56:186-196**.**
Schneider A, Hampel R, Ibald-Mulli A, Zareba W, Schmidt G, Schneider R, et al. 2010. Changes in deceleration capacity of heart rate and heart rate variability induced by ambient air pollution in individuals with coronary artery disease. Particle and Fibre Toxicology 7:29.
Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. 1996. Heart rate variability: Standards of measurement, physiological interpretation, and clinical use. Circulation 93:1043-1065.
Turpin, B.J.; Huntzicker, J.J. 1995. Identification of Secondary Organic Aerosol Episodes and Quantitation of Primary and Secondary Organic Aerosol Concentrations during SCAQS; Atmos. Environ 29:3527-3544.
Vardas, PE, Kochiadakis GE, Manios EG, Kanoupakis EM, Zouridakis EG, Chlouverakis GI. 1996. Spectral analysis of heart rate variability before and during episodes of nocturnal ischaemia in patients with extensive coronary artery disease. Eur Heart J 17:388-393.
Verma V, Polidori A, Schauer JJ, Shafer MM, Cassee FR, Sioutas C. 2009. Redox activity of urban quasi-ultrafine particles from primary and secondary sources. Atmos Environ 43:6360-6368.
Wallace L. 1996 . Indoor particles: a review. J. Air Waste Manag. Assoc 46: 98, 126.
Zanobetti A, Gold DR, Stone PH, Suh HH, Schwartz J, Coull BA, et al. 2010. Reduction in Heart Rate Variability with Traffic and Air Pollution in Patients with Coronary Artery Disease. Environ Health Perspect 118:324–330.
Ziegelstein RC. 2007. Acute emotional stress and cardiac arrhythmias. JAMA 298:324-329.

3. CHAPTER THREE: TASK 2

3.0 INTRODUCTION

We hypothesized that there would be stronger associations between air pollution exposures and both decreased HRV and increased risk of arrhythmia among subjects with null genotypes for the glutathione S-transferase M1 (GSTM1) gene or the glutathione S-transferase T1 (GSTT1) gene. This is supported by the fact that the homozygous null genotype leads to an absence of the glutathione S-transferase enzymes that protect cells by detoxification of electrophiles or reactive intermediates (e.g, polycyclic aromatic hydrocarbon epoxides) by conjugation with glutathione (GSH). However, there was no evidence of any consistent main effect of air pollution on HRV outcome, making it less likely that we would observe effect modification by GST genotypes. However, it is possible that a subgroup oif genetically susceptible individuals is masked by the overall results. The opposite is true for VT given the informative nature of those findings. Nevertheless, as will be discussed, there were a limited number of subjects with any VT events making further stratification a major limitation in this exploratory aim.

3.1 METHODS

3.1.1 Genotyping

The GSTM1 gene is located at chromosome1p13.3. The homologous recombination between two almost identical 4.2 Kb regions flanking the GSTM1 gene results in a 16Kb deletion containing the entire gene. It is referred to as a null allele of the GSTM1 gene. Similarly, a null allele occurs in the GSTT1 gene.

A triplex polymerase chain reaction (PCR) was used to amplify the wild-type allele of the GSTM1 gene, the wild-type allele of the GSTT1 gene, and the interleukin 5 receptor-alpha (IL5RA) gene that served as the internal control to monitor the DNA quality of each sample. PCR primers are listed in the Table 12.

Table 12. Primer sequences for the triplex PCR.

Primers*	primer sequence	expected size of PCR product
GSTM1-1F:	CTGCCCTACTTGATTGATGGG	
GSTM1-1R:	CTGGATTGTAGCAGATCATGC	273 bp
GSTT1-1F	TTCCTTACTGGTCCTCACATCTC	
GSTT1-1R	TCACCGGATCATGGCCAGCA	480 bp
IL5RA-3602F	TTCACCGCACATTGCACATTAGGG	
IL5RA-3936R	AGTGGAAATGGTGTTGCCACCTTG	334 bp

*Primer sequences for the amplification of GSTT1 gene and GSTM1 gene are from Bailey et al. (1998).

If good DNA quality in that sample can be confirmed by the success amplification of 334 bp PCR product in the IL5RA gene, the absence of 273-bp PCR product in a DNA sample indicates the presence of homozygous null alleles of GSTM1 gene. On the other hand, the absence of the 480-bp PCR product indicates the presence of homozygous null alleles of GSTT1 gene (Figure 4). For example, the sample in lane four in Figure 4 has homozygous null alleles in both the GSTT1 gene and the GSTM1 gene; samples in lane 8 to lane 12 show homozygous null alleles in the GSTM1 gene.

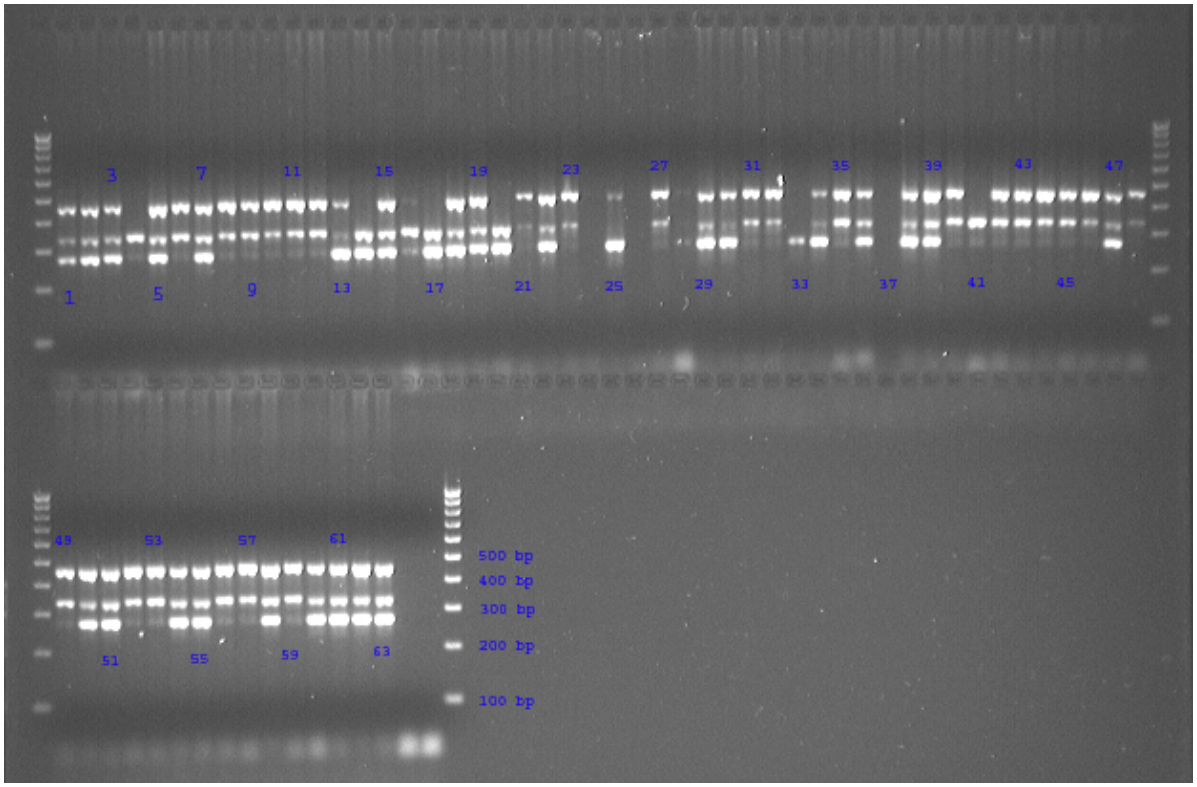

Figure 4. PCR amplification of the GSTT1 gene (480 bp), the IL5RA gene (334bp) and the GSTM1 (273bp) gene for 63 DNA samples.

For samples in lane 24, 26, 28, 37 in Figure 4, PCR amplification failed for all of the three genes, which indicate that DNA quality for those samples was likely poor. After being purified by Zymo-clean column, those samples worked well in the following PCR (Figure 5), except one sample with sample ID of A015. A015 showed very weak PCR product for both the IL5RA control gene and the GSTT1 gene. This sample also showed a very low DNA concentration. Therefore, the PCR product for the GSTM1 gene may be unreliable. For this sample, DNA was re-extracted from 0.5 ml of another sample of whole blood and PCR reactions were redone. PCR bands were then observed for both the GSTM1 and the GSTT1 gene.

For samples in lane 13, 21, 25, 30, 47, 48 in Figure 4, the PCR product for the IL5RA gene was weak, so PCR was repeated for those samples (Figure 6). The PCR products of the IL5RA gene were present although they were much weaker than the PCR product of the GSTM1 gene.

The amplification of 273 bp PCR product of GSTM1 can be used to identify the presence of GSTM1 allele. However, it cannot distinguish heterozygous individuals carrying one copy of null allele and one copy of wild type allele from homozygous individuals carrying two copies of wild type allele (in both cases, the protein product will be produced). Due to the high sequence identity between the two 4.2-Kb repeats, it is not possible to identify the exact breakpoint of recombination. Therefore, we did not amplify the small PCR product to directly identify the null allele.

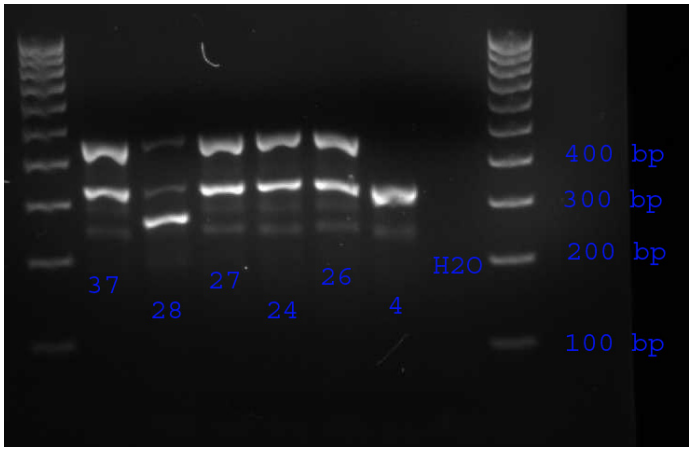

Figure 5. Re-PCR of samples that failed in the first batch of PCR (lane 37, 28, 24, 26 in Figure 4). Samples in lane 4 and lane 27 from Figure 4 served as the positive controls since they worked well in the first batch of PCR.

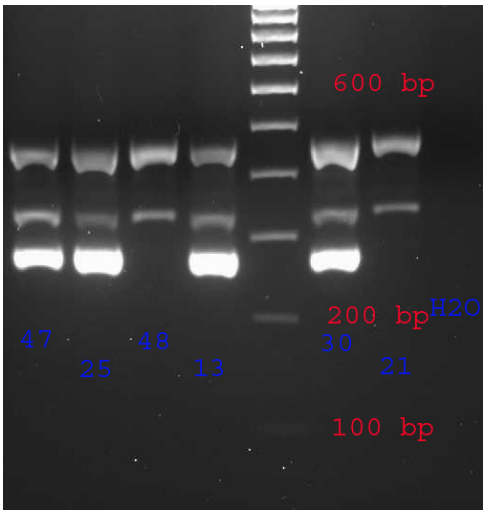

Figure 6. PCR repeated for six samples showing weak PCR product of the IL5RA gene in Figure 4.

3.1.2 Analysis

Dependent and independent variables were described above for Task 1 except we add the subject genotypes for GSTM1 and GSTT1. We evaluated the potential of the present design to detect effect modification of relationships in Task 1 by the the null genotype for GSTM1 and GSTT1 that are likely involved in susceptibility to oxidative stress. In this exploratory aim, we assessed the genetic effects by looking at all the pairwise interactions between exposure conditions and null allele in relation

to subject health outcomes. Significant interaction (product) terms are considered at the nominal p-value < 0.1. Other genes will be evaluated later in consort with other sequencing work that is planned in other funded projects.

Regression models as described above (generalized estimating equations) were used to evaluate interaction between air pollutant exposures and genotype in relation to HRV and to arrhythmia. Analysis were performed by including genotypes for each polymorphism, as variables modifying the intercept parameters (e.g. inclusion of genes as modifiers of the slope parameters, by including interaction variables between genotype and air pollutant variables into the time-varying covariate vectors). A vector of the genotype variables specifies GST-M1 (or T1) null genotype vs. non-null genotype to test the hypothesis that there will be a stronger associations of air pollutants with the outcomes among subjects with the null genotype.

3.2 RESULTS

Of the 50 subjects with HRV data, 22 subjects (44%) had at least one wild-type GSTM1 allele (GSTM1=1) and 28 subjects (56%) were homozygous for null alleles (GSTM1=0, i.e., no functional enzyme is produced). For GSTT1, 46 subjects (92%) had at least one wild-type allele (GSTT1=1) and 4 subjects (8%) were homozygous for null alleles (GSTT1=0).

In hourly SDNN models we found product terms of GSTM1 with 2-day averages (not shown) and/or 3-day averages of primary OC, EC, NO_2 and NOx were significant at the nominal product term p-value < 0.1, but not in the direction anticipated (Table 12). Subjects with GSTM1 non-null showed decreased SDNN with exposure to air pollution (mostly nonsignificant) in contrast to subjects with the null alleles (the higher risk alleles since no functional GST enzyme is produced). On the other hand, some of the SDNN models for testing interaction between air pollutants and GSTT1 genotype were significant (p <0.1, see Methods) in the direction expected (Table 13). Subjects with the GSTT1 null genotype showed decreased SDNN with 1-day to 3-day average exposures to several traffic-related air pollutants, including BC, EC, primary OC, and NO_x, as well as quasi-ultrafine $PM_{0.25}$ (lag 1 day and nominally 2-day average). The models for rMSSD testing interaction between air pollutants and either GSTM1 or GSTT1 genotype were largely nonsignificant at the nominal product term p-value < 0.1 (Tables 12-13).

In contrast, we found stronger associations of ventricular tachycardia with OC and both primary and secondary OC fractions among subjects who were GSTM1 null (Table 14). The *p*-value for interaction from the product term of GSTM1 by air pollutant was significant at p < 0.07 for half of these OC models (mostly air pollutant averages of 2-day through 5-day averages). However, there were no consistent differences in association between genotypes for the primary pollutant markers EC, NO_x or CO and no clear difference by particle size fraction. Furthermore, out of the 18 subjects with any VT episodes (Table 2) there were only 9 subjects with GSTM1 null alleles and 9 subjects with GSTM1 non-null alleles who had any ventricular tachycardia. Among the subjects with the null alleles, only 37 daily ECG records had any ventricular tachycardias and 227 records had no ventricular tachycardias. This limited the ability to obtain stable estimates of association for a few of the models, which had implausibly extreme estimates and very wide confidence limits. For GSTT1 only 3 subjects were homozygous for null alleles and had any ventricular tachycardia (36 hours out of almost 9,000 hours of ECG data) making it not possible to perform a valid analysis.

3.3 DISCUSSION

The lack of GSTM1 or GSTT1 enzymes, which is the case among subjects with null alleles, may enhance oxidative stress-induced autonomic effects under the assumption that this would lead to decreased phase II conjugation of glutathione to xenobiotic electrophiles and elimination of these chemicals. We did not find much supportive evidence for that. We found that subjects with the more common GSTM1 null genotype actually showed some positive associations between HRV (SDNN) and air pollution. The less common GSTT1 null genotype did show the anticipated inverse association of decreased SDNN with air pollutant exposure, primarily traffic-related pollutants. However, only 4 subjects had the risk allele limiting the external validity of this finding. The reason for why GSTT1 and not GSTM1 would show this interaction is unclear although differences in function and tissue localization may play a role (Eaton and Bammler 1999). There were no differences in the null associations we observed overall for the rMSSD models stratified by either GSTM1 or GSTT1 genotype. In a differently designed study, Schwartz et al. (2005) examined HRV in a single 7-min ECG strip in 497 men in relation to ambient $PM_{2.5}$ exposures in Boston MA. They found a significant 34% decrease in the high frequency component of HRV (similar to rMSSD or PNN50) in relation to interquartile range increases in 2-day average $PM_{2.5}$ (16.1 $\mu g.m^3$) only among subjects with GSTM1 null genotype. Furthermore, this association in GSTM1 null subjects was not seen among those taking statins. They did not report effects on the low frequency component. However, in a follow-up of this analysis, ambient $PM_{2.5}$ was associated with decreased SDNN, high frequency and low frequency HRV among subjects with both GSTM1 null and HO-1 long repeat polymorphisms (Chahine 2007). Probst-Hensch et al. (2008) in a differently-designed (cross-sectional) analysis observed decreased HRV (total power) among subjects with more than 2 hours per day of environmental tobacco smoke exposure who also had the GSTM1 null genotype.

The GSTM1 null genotype strengthened associations of VT with exposure to OC and both the primary and secondary fractions of OC. However, there was no other consistent evidence of effect modification of associations by GSTM1 genotypes for other primary pollutant markers as has been observed in this study panel when primary OC was associated with of other cardiovascular outcomes (ambulatory blood pressure and electrocardiographic ischemic ST segment depression) or with circulating biomarkers of inflammation (Delfino et al. 2009, 2010a, 2010b, 2011). The limited number of days with any VT events may have diminished the power to detect differences in associations in the genotype strata. Nevertheless, it is possible that an increased propensity to oxidative stress in GSTM1 null individuals may have enhance vulnerability to pollutant-induced VT.

3.4 SUMMARY AND CONCLUSIONS

We found limited evidence for effect modification by the GSTM1 null genes that would be expected to increase risk of a decrease in HRV in relation to increased air pollution exposures. The expectation is based on there being a lack of the protective enzyme product of GST genes. We observed contradictory findings for GSTM1 while we found the expected inverse associations between SDNN and traffic-related air pollutants for subject with the GSTT1 null genotype. However, only

4 subjects had the risk allele making interpretation of this finding unclear. Therefore, overall, these finding are not supportive of the hypothesis that GSTM1 or T1 are important effect modifiers. However, we did find some limited evidence that GSTM1 null increased the risk of ventricular tachycardia from exposure to organic carbon, but not the other exposures. There are many other GST enzymes in this super-family of enzymes and the associated genes are in a supergene family located on at least seven chromosomes (Strange et al. 2001). Therefore, it is conceivable that adaptive mechanisms in other GST genes or other genes override a lack of these specific GST members. We conclude that further work with a broad array of genes involved in phase I and phase II metabolism is needed to determine if genetic variability in these important biological pathways is a determinant of cardiovascular susceptibility to air pollution.

Table 12. Associations[a] of hourly heart rate variability with outdoor air pollutants: effect modification by the GSTM1 null genotype.

Exposure and averaging time	SDNN Coefficient (95% CI)[a]		P-value for interaction[c]	rMSSD Coefficient (95% CI)		P-value for interaction
	GSTM1 Non-null	GSTM1 Null		GSTM1 Non-null	GSTM1 Null	
Particle number						
1hr	-0.72 (-2.15, 0.70)	-0.47 (-1.68, 0.74)	0.790	0.69 (-0.59, 1.98)	-0.42 (-1.46, 0.63)	0.188
4hr	-1.52 (-3.47, 0.43)	0.40 (-1.28, 2.09)	0.142	0.31 (-1.94, 2.56)	-0.31 (-2.19, 1.57)	0.678
8-hr	-2.28 (-4.78, 0.23)	0.20 (-2.07, 2.48)	0.147	-0.43 (-3.78, 2.93)	1.85 (-1.09, 4.78)	0.315
24-hr	-0.81 (-4.73, 3.12)	-0.59 (-4.94, 3.76)	0.942	1.43 (-4.65, 7.52)	-1.98 (-8.58, 4.63)	0.449
3-day	-0.51 (-8.05, 7.02)	4.71 (-4.64, 14.1)	0.368	0.16 (-11.4, 11.7)	-2.37 (-16.0,11.2)	0.772
Black carbon						
1hr	0.15 (-0.80, 1.10)	0.34 (-0.57, 1.25)	0.775	-0.11 (-1.02, 0.79)	0.07 (-0.77, 0.92)	0.763
4hr	0.20 (-0.99, 1.40)	0.59 (-0.57, 1.76)	0.638	0.17 (-1.30, 1.64)	0.60 (-0.83, 2.03)	0.679
8-hr	0.29 (-1.11, 1.69)	0.59 (-0.82, 2.00)	0.765	0.93 (-1.01, 2.87)	1.33 (-0.62, 3.27)	0.771
24-hr	-0.03 (-2.01, 1.95)	0.07 (-1.95, 2.08)	0.948	-0.61 (-3.66, 2.44)	0.71 (-2.41, 3.84)	0.549
3-day	-1.35 (-4.68, 1.97)	1.85 (-1.64, 5.34)	0.169	-0.05 (-5.14, 5.05)	2.61 (-2.83, 8.06)	0.463
Elemental carbon						
1hr	-0.08 (-1.00, 0.84)	0.51 (-0.45, 1.48)	0.376	-0.11 (-0.92, 0.70)	0.64 (-0.20, 1.49)	0.208
4hr	0.68 (-0.57, 1.93)	1.00 (-0.30, 2.31)	0.720	0.32 (-1.18, 1.81)	0.05 (-1.50, 1.61)	0.807
8-hr	0.60 (-0.86, 2.06)	0.81 (-0.79, 2.41)	0.845	1.11 (-0.89, 3.11)	-0.09 (-2.27, 2.09)	0.422
24-hr	-0.42 (-2.46, 1.63)	0.42 (-1.82, 2.67)	0.586	-.002 (-3.16,3.15)	-0.52 (-3.97, 2.93)	0.828
3-day	-2.51 (-5.73, 0.70)	3.22 (-0.93, 7.38)	0.029**	1.03 (-3.69, 5.76)	-0.02 (-6.20, 6.16)	0.787
Organic Carbon (OC)						
1hr	0.60 (-2.03, 3.24)	1.64 (-1.04, 4.32)	0.585	0.71 (-1.56, 2.99)	0.84 (-1.49, 3.17)	0.938
4hr	1.77 (-1.81, 5.35)	1.21 (-2.34, 4.76)	0.825	3.14 (-1.22, 7.50)	-1.15 (-5.42, 3.13)	0.161
8-hr	1.21 (-2.86, 5.28)	2.14 (-1.97, 6.24)	0.749	1.88 (-3.71, 7.47)	-0.93 (-6.52, 4.67)	0.478
24-hr	0.44 (-4.19, 5.07)	1.03 (-3.94, 6.00)	0.863	2.30 (-4.68, 9.29)	-0.35 (-7.86, 7.16)	0.609
3-day	-1.82 (-8.08, 4.45)	1.06 (-6.65, 8.78)	0.564	0.78 (-8.28, 9.85)	-0.98 (-12.5, 10.5)	0.811
Primary OC						
1hr	-1.14 (-3.92, 1.63)	2.10 (-0.71, 4.91)	0.104	-0.33 (-2.79, 2.12)	1.56 (-0.89, 4.00)	0.283
4hr	0.14 (-3.63, 3.91)	3.50 (-0.25, 7.25)	0.207	1.18 (-3.29, 5.65)	0.25 (-4.13, 4.63)	0.768
8-hr	-0.15 (-4.63, 4.33)	3.28 (-1.36, 7.91)	0.290	2.40 (-3.63, 8.42)	1.67 (-4.53, 7.87)	0.867
24-hr	-3.68 (-10.5, 3.13)	-0.21 (-7.32, 6.91)	0.478	1.21 (-9.06,11.5)	-3.20 (-13.9, 7.49)	0.551
3-day	-8.51 (-17.8, 0.75)	7.97 (-4.74,20.7)	0.033**	5.71 (-7.73,19.2)	0.27 (-17.3, 17.9)	0.620
Secondary OC						
1hr	0.40 (-1.14, 1.94)	-0.06 (-1.50, 1.38)	0.658	0.57 (-0.65, 1.80)	-0.27 (-1.41, 0.87)	0.316
4hr	-0.40 (-2.74, 1.94)	-1.40 (-3.53, 0.73)	0.503	1.10 (-1.54, 3.73)	-0.64 (-3.02, 1.75)	0.312
8-hr	-0.93 (-3.82, 1.96)	-0.19 (-2.81, 2.43)	0.684	-1.25 (-4.96, 2.46)	-1.43 (-4.74, 1.88)	0.939
24-hr	1.29 (-2.70, 5.29)	0.59 (-2.86, 4.03)	0.776	3.93 (-1.79, 9.64)	0.33 (-4.70, 5.36)	0.327
3-day	0.77 (-4.77, 6.31)	-2.33 (-7.34, 2.68)	0.378	-1.71 (-9.55, 6.13)	-0.24 (-7.53, 7.04)	0.776

Table 12 (cont.)

Exposure and averaging time	SDNN Coefficient (95% CI)[a]		P-value for interaction	rMSSD Coefficient (95% CI)		P-value for interaction
	GSTM1 Non-null	GSTM1 Null		GSTM1 Non-null	GSTM1 Null	
BAM $PM_{2.5}$						
1hr	1.07 (-0.34, 2.48)	-0.72 (-1.96, 0.52)	0.060*	0.21 (-1.28, 1.69)	-0.31 (-1.69, 1.06)	0.615
4hr	1.64 (-0.01, 3.28)	-0.32 (-1.73, 1.08)	0.073*	0.67 (-1.50, 2.85)	0.24 (-1.71, 2.19)	0.771
8-hr	1.16 (-0.66, 2.98)	-0.67 (-2.18, 0.84)	0.125	-0.08 (-2.72, 2.56)	-0.32 (-2.59, 1.96)	0.893
24-hr	0.01 (-2.19, 2.21)	-0.29 (-2.18, 1.59)	0.833	-2.59 (-5.99, 0.80)	-0.52 (-3.53, 2.50)	0.354
3-day	-0.71 (-3.81, 2.40)	0.14 (-2.50, 2.79)	0.678	-2.21 (-7.01, 2.58)	0.29 (-4.07, 4.66)	0.440
Gravimetric PM: [b]						
$PM_{0.25}$				--	--	
24-hr Lag	2.59 (-3.34, 8.52)	-0.63 (-6.74, 5.47)	0.456	--	--	
25-48 hr	1.58 (-4.34, 7.50)	1.61 (-4.45, 7.67)	0.994	--	--	
2-day	0.11 (-9.09, 9.30)	-0.93 (-11.2, 9.32)	0.882			
$PM_{0.25-2.5}$				--	--	
24-hr Lag	0.26 (-4.51, 5.02)	1.30 (-3.25, 5.85)	0.753	--	--	
25-48 hr	-0.60 (-5.93, 4.74)	0.60 (-4.66, 5.86)	0.749	--	--	
2-day	-2.42 (-9.13, 4.28)	0.39 (-6.11, 6.88)	0.550			
$PM_{2.5-10}$				--	--	
24-hr Lag	4.80 (0.20, 9.40)	0.43 (-3.87, 4.72)	0.170	--	--	
25-48 hr	-3.80 (-9.00, 1.41)	-0.39 (-5.26, 4.48)	0.342	--	--	
2-day	-1.53 (-9.60, 6.54)	-1.25 (-8.92, 6.42)	0.960	--	--	
O_3						
1hr	-0.15 (-1.33, 1.02)	-0.02 (-1.18, 1.14)	0.828	0.18 (-1.13, 1.49)	-0.69 (-1.96, 0.57)	0.235
4hr	-0.28 (-1.64, 1.08)	0.08 (-1.27, 1.43)	0.591	0.74 (-1.01, 2.49)	-0.24 (-1.96, 1.49)	0.266
8-hr	0.28 (-1.39, 1.95)	1.00 (-0.70, 2.69)	0.378	1.53 (-0.74, 3.79)	0.90 (-1.39, 3.18)	0.560
24-hr	0.86 (-2.95, 4.67)	4.75 (0.90, 8.60)	0.141	-0.14 (-5.85, 5.57)	1.05 (-4.70, 6.81)	0.765
3-day	0.34 (-6.09, 6.77)	3.05 (-2.71, 8.81)	0.462	-4.40 (-14.1, 5.29)	2.31 (-6.44,11.1)	0.242
NO_x						
1hr	-0.81 (-2.08, 0.46)	0.93 (-0.28, 2.14)	0.046**	-0.05 (-1.28, 1.17)	1.11 (-0.05, 2.27)	0.168
4hr	-1.04 (-2.60, 0.53)	1.14 (-0.36, 2.64)	0.042**	0.10 (-1.75, 1.94)	1.44 (-0.32, 3.20)	0.287
8-hr	0.14 (-1.74, 2.03)	1.52 (-0.39, 3.43)	0.298	1.55 (-0.98, 4.09)	2.88 (0.36, 5.39)	0.455
24-hr	0.17 (-2.91, 3.25)	0.70 (-2.49, 3.88)	0.809	0.45 (-4.22, 5.12)	1.52 (-3.29, 6.32)	0.748
3-day	-1.50 (-6.87, 3.86)	4.72 (-0.91,10.4)	0.070*	1.51 (-6.65, 9.68)	5.09 (-3.43, 13.6)	0.501
CO						
1hr	-0.68 (-2.06, 0.69)	-0.05 (-1.39, 1.30)	0.508	0.36 (-0.97, 1.69)	1.25 (-0.02, 2.52)	0.331
4hr	-0.55 (-2.19, 1.10)	0.45 (-1.23, 2.12)	0.395	0.56 (-1.34, 2.47)	1.66 (-0.23, 3.55)	0.412
8-hr	0.32 (-1.61, 2.25)	1.17 (-0.94, 3.28)	0.550	0.98 (-1.57, 3.53)	3.23 (0.53, 5.93)	0.227
24-hr	-0.48 (-3.24, 2.29)	-0.50 (-3.85, 2.85)	0.991	-0.29 (-4.30, 3.72)	0.45 (-4.42, 5.32)	0.815
3-day	-1.62 (-6.23, 2.99)	2.28 (-2.78, 7.33)	0.259	-0.22 (-6.97, 6.54)	2.57 (-4.93, 10.1)	0.584

[a] All models are adjusted for adjusted for hourly average actigraph-derived physical activity and heart rate temperature of the same lag average, day of week, time of day, seasonal study phase (mean centered exposure), and community group (mean centered exposure). Stratified results are from product term models of air pollutant by gene variant.

[b] In analyses of daily gravimetric PM filter data, we focused only on 24-hour SDNN spanning the gravimetric PM mass sampling periods.

[c] P-values are for the product term of GSTM1 by air pollutant.

* $p < 0.1$, ** $p < 0.05$, # $p < 0.01$

Table 13. Associations[a] of hourly heart rate variability with outdoor air pollutants: effect modification by the GSTT1 null genotype.

Exposure and averaging time	SDNN Coefficient (95% CI)[a]		P-value for interaction[c]	rMSSD Coefficient (95% CI)		P-value for interaction
	GSTT1 Non-null	GSTT1 Null		GSTT1 Non-null	GSTT1 Null	
Particle number						
1hr	-0.44 (-1.43, 0.56)	-1.42 (-3.85, 1.01)	0.464	0.07 (-0.81, 0.94)	-0.22 (-2.30, 1.86)	0.806
4hr	-0.21 (-1.59, 1.17)	-1.66 (-5.06, 1.74)	0.439	0.13 (-1.43, 1.69)	-1.16 (-4.97, 2.65)	0.538
8-hr	-0.77 (-2.60, 1.06)	-1.84 (-6.47, 2.78)	0.672	0.82 (-1.57, 3.21)	1.16 (-4.87, 7.18)	0.919
24-hr	-0.29 (-3.57, 2.99)	-2.56 (-9.67, 4.56)	0.573	0.23 (-4.78, 5.24)	-1.66 (-12.7, 9.38)	0.761
3-day	2.36 (-3.92, 8.64)	-12.6 (-35.1, 10.0)	0.202	-0.71 (-10.1, 8.68)	-0.97 (-35.9, 33.9)	0.988
Black carbon						
1hr	0.23 (-0.46, 0.93)	0.40 (-1.93, 2.74)	0.890	0.04 (-0.60, 0.69)	-0.66 (-2.80, 1.48)	0.536
4hr	0.43 (-0.46, 1.31)	0.11 (-2.83, 3.06)	0.841	0.44 (-0.64, 1.52)	-0.15 (-3.59, 3.30)	0.750
8-hr	0.52 (-0.52, 1.57)	-0.74 (-4.39, 2.91)	0.511	1.16 (-0.29, 2.60)	0.76 (-4.09, 5.61)	0.878
24-hr	0.26 (-1.19, 1.71)	-5.40 (-12.0, 1.20)	0.100*	0.23 (-2.02, 2.47)	-3.87 (-13.6, 5.88)	0.421
3-day	0.48 (-2.06, 3.02)	-12.4 (-24.2, -0.67)	0.032**	1.06 (-2.86, 4.98)	4.94 (-13.1, 23.0)	0.675
Elemental carbon						
1hr	0.34 (-0.36, 1.05)	-1.20 (-3.39, 1.00)	0.190	0.34 (-0.28, 0.95)	-0.64 (-2.61, 1.32)	0.351
4hr	1.02 (0.05, 1.99)	-0.81 (-3.62, 2.01)	0.226	0.39 (-0.77, 1.54)	-1.45 (-4.72, 1.82)	0.297
8-hr	0.91 (-0.24, 2.06)	-1.38 (-4.86, 2.09)	0.216	0.61 (-0.96, 2.18)	0.14 (-4.45, 4.72)	0.848
24-hr	0.27 (-1.28, 1.83)	-5.13 (-11.1, 0.88)	0.086*	0.02 (-2.40, 2.43)	-4.08 (-12.9, 4.78)	0.380
3-day	0.04 (-2.57, 2.66)	-13.1 (-25.2, -1.08)	0.034**	0.62 (-3.25, 4.49)	0.78 (-17.0, 18.6)	0.987
Organic Carbon (OC)						
1hr	1.09 (-0.88, 3.06)	1.37 (-5.59, 8.33)	0.940	0.97 (-0.74, 2.68)	-1.32 (-6.89, 4.25)	0.441
4hr	1.38 (-1.28, 4.04)	2.98 (-6.47, 12.4)	0.748	1.09 (-2.14, 4.32)	-0.57 (-11.2, 10.0)	0.769
8-hr	1.36 (-1.68, 4.40)	6.25 (-5.13, 17.6)	0.414	0.91 (-3.26, 5.07)	-5.07 (-19.8, 9.72)	0.444
24-hr	0.77 (-2.72, 4.27)	-1.06 (-18.8, 16.7)	0.843	1.74 (-3.52, 7.00)	-15.8 (-41.8, 10.3)	0.197
3-day	-0.69 (-5.71, 4.33)	-0.53 (-23.4, 22.3)	0.989	0.63 (-6.70, 7.96)	-12.2 (-45.1, 20.8)	0.454
Primary OC						
1hr	0.59 (-1.52, 2.69)	-0.66 (-6.77, 5.45)	0.705	0.91 (-0.93, 2.75)	-2.01 (-7.43, 3.40)	0.315
4hr	2.02 (-0.85, 4.89)	0.36 (-7.42, 8.14)	0.693	1.39 (-2.00, 4.77)	-4.06 (-12.9, 4.77)	0.257
8-hr	1.94 (-1.52, 5.39)	-2.01 (-11.7, 7.65)	0.449	1.99 (-2.65, 6.63)	2.43 (-10.2, 15.0)	0.948
24-hr	-0.92 (-6.11, 4.28)	-16.9 (-34.7, 0.93)	0.089*	0.23 (-7.61, 8.07)	-15.0 (-41.0, 11.1)	0.269
3-day	-2.41 (-10.4, 5.58)	-12.4 (-40.6, 15.9)	0.502	2.01 (-9.34, 13.4)	26.1 (-13.4, 65.6)	0.248
Secondary OC						
1hr	-0.04 (-1.17, 1.10)	2.14 (-1.38, 5.66)	0.244	0.12 (-0.77, 1.00)	0.12 (-2.73, 2.96)	0.998
4hr	-1.32 (-3.08, 0.44)	2.45 (-2.44, 7.35)	0.146	-0.20 (-2.15, 1.74)	3.45 (-2.17, 9.07)	0.220
8-hr	-1.27 (-3.45, 0.91)	6.49 (0.52,12.5)	0.014**	-1.05 (-3.78, 1.68)	-4.38 (-12.3, 3.51)	0.424
24-hr	0.22 (-2.69, 3.13)	7.40 (-0.98,15.8)	0.106	2.18 (-1.96, 6.31)	-1.28 (-14.1, 11.5)	0.609
3-day	-1.75 (-5.87, 2.38)	5.76 (-4.50, 16.0)	0.164	-0.43 (-6.27, 5.41)	-5.17 (-20.5, 10.1)	0.556

Table 13 (cont.)

Exposure and averaging time	SDNN Coefficient (95% CI)[a]		P-value for interaction	rMSSD Coefficient (95% CI)		P-value for interaction
	GSTT1 Non-null	GSTT1 Null		GSTT1 Non-null	GSTT1 Null	
BAM $PM_{2.5}$						
1hr	0.11 (-0.85, 1.08)	-0.88 (-4.74, 2.98)	0.624	-0.10 (-1.15, 0.95)	0.21 (-3.51, 3.93)	0.876
4hr	0.52 (-0.58, 1.63)	0.08 (-4.57, 4.73)	0.856	0.43 (-1.08, 1.93)	0.51 (-5.38, 6.40)	0.979
8-hr	0.10 (-1.10, 1.30)	-0.65 (-5.99, 4.69)	0.788	-0.15 (-1.93, 1.62)	-1.55 (-9.00, 5.90)	0.719
24-hr	-0.02 (-1.52, 1.49)	-4.42 (-12.1, 3.21)	0.267	-1.33 (-3.71, 1.04)	-4.14 (-15.6, 7.35)	0.639
3-day	-0.02 (-2.15, 2.11)	-2.80 (-10.3, 4.75)	0.488	-0.92 (-4.36, 2.52)	-0.24 (-11.8, 11.3)	0.913
Gravimetric PM: [b]						
$PM_{0.25}$						
24-hr Lag	0.45 (-3.90, 4.79)	13.6 (-4.64, 31.9)	0.165	--	--	
25-48 hr	2.71 (-1.59, 7.00)	-19.6 (-39.2, 0.12)*	0.031**	--	--	
2-day	0.83 (-6.16, 7.81)	-24.6 (-57.0, 7.77)	0.133	--	--	
$PM_{0.25-2.5}$						
24-hr Lag	0.37 (-3.08, 3.83)	6.20 (-5.87, 18.3)	0.360	--	--	
25-48 hr	0.33 (-3.62, 4.29)	-3.43 (-16.6, 9.75)	0.589	--	--	
2-day	-0.71 (-5.60, 4.18)	-3.88 (-21.3, 13.5)	0.730	--	--	
$PM_{2.5-10}$						
24-hr Lag	2.85 (-0.37, 6.08)*	-8.33 (-25.6, 8.94)	0.211	--	--	
25-48 hr	-2.00 (-5.63, 1.64)	1.36 (-24.3, 27.0)	0.798	--	--	
2-day	-0.92 (-6.64, 4.80)	-10.1 (-38.5, 18.3)	0.536	--	--	
O_3						
1hr	-0.004 (-1.02, 1.01)	-0.76 (-2.75, 1.24)	0.446	-0.31 (-1.41, 0.78)	0.03 (-2.27, 2.33)	0.768
4hr	-0.08 (-1.29, 1.12)	-0.21 (-2.44, 2.01)	0.904	0.15 (-1.38, 1.69)	0.95 (-1.93, 3.82)	0.570
8-hr	0.64 (-0.86, 2.15)	0.48 (-2.22, 3.19)	0.901	1.28 (-0.77, 3.33)	0.81 (-2.78, 4.41)	0.786
24-hr	2.83 (-0.10, 5.76)	2.28 (-5.65, 10.2)	0.896	0.59 (-3.76, 4.95)	-1.00 (-13.0, 11.0)	0.801
3-day	2.02 (-2.93, 6.98)	-0.18 (-11.6, 11.3)	0.699	-0.26 (-7.68, 7.15)	-5.36 (-23.0, 12.3)	0.566
NO_x						
1hr	0.08 (-0.86, 1.03)	0.30 (-2.44, 3.05)	0.881	0.71 (-0.20, 1.61)	-0.67 (-3.26, 1.91)	0.319
4hr	0.13 (-1.05, 1.30)	-0.14 (-3.50, 3.21)	0.879	0.94 (-0.44, 2.32)	-0.36 (-4.25, 3.53)	0.535
8-hr	0.96 (-0.49, 2.41)	-0.49 (-4.68, 3.69)	0.515	2.30 (0.37, 4.22)	1.50 (-4.05, 7.06)	0.789
24-hr	0.63 (-1.74, 3.01)	-1.79 (-9.28, 5.70)	0.543	1.39 (-2.21, 4.98)	-3.31 (-14.5, 7.85)	0.429
3-day	1.72 (-2.63, 6.08)	-15.0 (-33.3, 3.27)	0.070*	3.13 (-3.43, 9.68)	7.34 (-20.4, 35.1)	0.765
CO						
1hr	-0.44 (-1.46, 0.58)	0.49 (-2.79, 3.78)	0.594	0.91 (-0.06, 1.89)	-0.03 (-3.04, 2.97)	0.556
4hr	-0.09 (-1.33, 1.16)	0.26 (-3.81, 4.32)	0.873	1.22 (-0.21, 2.64)	0.02 (-4.52, 4.55)	0.618
8-hr	0.70 (-0.80, 2.20)	0.81 (-4.30, 5.91)	0.968	2.01 (0.06, 3.97)	2.32 (-4.12, 8.76)	0.928
24-hr	-0.38 (-2.58, 1.82)	-2.46 (-11.7, 6.78)	0.666	0.26 (-2.95, 3.46)	-4.48 (-17.7, 8.71)	0.492
3-day	0.40 (-3.10, 3.89)	-6.65 (-23.3, 10.0)	0.415	0.88 (-4.28, 6.04)	4.49 (-19.6, 28.6)	0.772

[a] All models are adjusted for adjusted for hourly average actigraph-derived physical activity and heart rate temperature of the same lag average, day of week, time of day, seasonal study phase (mean centered exposure), and community group (mean centered exposure). Stratified results are from product term models of air pollutant by gene variant.

[b] In analyses of daily gravimetric PM filter data, we focused only on 24-hour SDNN spanning the gravimetric PM mass sampling periods.

[c] P-values are for the product term of GSTM1 by air pollutant.

* $p < 0.1$, ** $p < 0.05$, # $p < 0.01$

Table 14. Associations of ventricular tachycardia with outdoor community air pollutants: effect modification by the GSTM1 genotype.

Exposure and averaging time[a]	All subjects RR (95% CI)[b]	GSTM1 Null RR (95% CI)	GSTM1 Non-null RR (95% CI)	P-value for interaction [c]
Particle number				
24-hr	0.70 (0.41, 1.20)	0.55 (0.18, 1.73)	0.85 (0.50, 1.42)	0.505
2-day	0.97 (0.41, 2.33)	1.66 (0.49, 5.68)	0.51 (0.27, 0.98)**	0.096
3-day	0.42 (0.09, 1.94)	0.83 (0.11, 6.06)	0.31 (0.12, 0.78)**	0.379
5-day	0.20 (0.02, 1.67)	0.02 (0.00, 0.72)**	1.28 (0.11, 14.5)	0.059
Black carbon				
24-hr	1.40 (1.06, 1.84)**	1.56 (0.95, 2.54)*	1.15 (0.60, 2.22)	0.473
2-day	1.73 (1.03, 2.92)**	1.42 (0.80, 2.51)	1.73 (0.70, 4.29)	0.715
3-day	1.59 (0.77, 3.30)	1.26 (0.40, 3.99)	1.85 (0.91, 3.76)*	0.582
5-day	1.36 (0.64, 2.91)	1.49 (0.28, 8.07)	1.17 (0.57, 2.42)	0.799
Elemental carbon				
24-hr	1.55 (1.09, 2.21)**	1.62 (1.13, 2.32)#	1.49 (0.76, 2.93)	0.826
2-day	2.76 (1.34, 5.68)#	1.78 (1.26, 2.51)#	2.80 (1.55, 5.07)#	0.194
3-day	1.46 (0.65, 3.30)	1.41 (0.56, 3.55)	2.05 (0.98, 4.29)*	0.534
5-day	1.90 (0.82, 4.37)	2.96 (0.85, 10.3)*	1.57 (0.61, 4.02)	0.426
Organic Carbon (OC)				
24-hr	3.06 (1.82, 5.17)#	5.34 (1.79, 15.9)#	2.21 (0.43, 11.3)	0.379
2-day	1.70 (0.60, 4.83)	6.54 (2.30, 18.6)#	0.46 (0.02, 10.2)	0.112
3-day	2.13 (0.60, 7.51)	15.5 (1.63, 149)**	0.52 (0.05, 5.01)	0.037
5-day	5.95 (0.79, 44.9)*	99.2 (5.8, 1687)#	2.25 (0.14, 37.0)	0.062
Primary OC				
24-hr	2.64 (1.13, 6.21)**	4.17 (1.03, 16.8)**	2.30 (0.15, 34.5)	0.701
2-day	6.01 (1.28, 28.3)**	3.72 (0.11, 126)		
3-day	2.16 (0.69, 6.77)	6.92 (0.15, 315)	0.71 (0.11, 4.80)	0.296
5-day	3.15 (0.30, 33.3)	89.8 (1.2, 6877)**	0.20 (0.001, 21.4)	0.061
Secondary OC				
24-hr	1.43 (0.93, 2.18)*	1.72 (1.10, 2.69)**	0.93 (0.31, 2.77)	0.305
2-day	0.39 (0.07, 2.05)	2.04 (1.11, 3.74)**	0.18 (0.03, 1.32)*	0.022
3-day	1.28 (0.47, 3.47)	4.83 (2.35, 9.93)#	0.25 (0.03, 1.83)	0.006
5-day	2.27 (0.79, 6.58)	11.9 (1.88, 75.2)#	1.14 (0.30, 4.35)	0.043
$PM_{2.5}$				
24-hr	1.50 (1.02, 2.20)**	1.45 (0.74, 2.85)	1.55 (0.71, 3.35)	0.907
2-day	1.31 (0.84, 2.03)	0.84 (0.31, 2.26)	1.59 (0.68, 3.71)	0.335
3-day	1.51 (0.85, 2.70)	0.65 (0.13, 3.28)	1.88 (0.67, 5.23)	0.278
5-day	1.16 (0.59, 2.29)	0.99 (0.04, 23.2)	1.19 (0.37, 3.89)	0.916
$PM_{0.25}$				
24-hr	1.04 (0.67, 1.60)	1.30 (0.66, 2.54)	0.92 (0.49, 1.75)	0.472
Lag 25-48 hr	1.20 (0.97, 1.47)*	1.32 (1.02, 1.72)**	1.10 (0.64, 1.89)	0.545
2-day	1.29 (0.73, 2.29)	2.10 (0.80, 5.47)	0.95 (0.35, 2.58)	0.262
$PM_{0.25-2.5}$				
24-hr	1.31 (1.05, 1.64)**	1.17 (0.60, 2.27)	1.31 (0.97, 1.78)*	0.752
Lag 25-48 hr	1.00 (0.78, 1.28)	0.65 (0.22, 1.88)	1.75 (1.19, 2.56)#	0.086
2-day	1.30 (0.96, 1.77)*	0.94 (0.24, 3.74)	1.79 (1.11, 2.86)**	0.390
$PM_{2.5-10}$				
24-hr	1.20 (0.90, 1.59)	1.23 (0.79, 1.93)	1.13 (0.79, 1.62)	0.766
Lag 25-48 hr	0.87 (0.71, 1.06)	1.15 (0.60, 2.23)	0.87 (0.62, 1.21)	0.447
2-day	0.97 (0.66, 1.44)	1.34 (0.51, 3.54)	1.08 (0.64, 1.80)	0.691

Table 14 (cont)

Exposure and averaging time[a]	All subjects RR (95% CI)[b]	GSTM1 Null RR (95% CI)	GSTM1 Non-null RR (95% CI)	P-value for interaction [c]
O_3				
24-hr	1.60 (1.12, 2.30)**	2.70 (1.39, 5.23)#	1.27 (0.69, 2.35)	0.103
2-day	0.75 (0.19, 2.92)	0.72 (0.31, 1.66)	0.67 (0.13, 3.44)	0.931
3-day	2.95 (1.29, 6.74)**	0.70 (0.15, 3.29)	2.14 (0.49, 9.25)	0.303
5-day	0.93 (0.10, 8.16)	0.10 (0.0018, 5.90)	1.83 (0.10, 32.6)	0.255
NO_x				
24-hr	1.37 (0.99, 1.88)*	1.27 (0.82, 1.97)	1.41 (0.71, 2.78)	0.809
2-day	2.19 (1.17, 4.11)**	1.54 (0.82, 2.89)	3.07 (1.22, 7.73)**	0.224
3-day	1.80 (0.45, 7.12)	1.62 (0.23, 11.2)	2.81 (0.58, 13.7)	0.667
5-day	1.70 (0.54, 5.40)	1.46 (0.30, 6.98)	2.45 (0.67, 9.02)	0.616
CO				
24-hr	1.18 (0.84, 1.65)	1.07 (0.65, 1.77)	1.15 (0.57, 2.32)	0.869
2-day	1.57 (0.85, 2.90)	1.11 (0.56, 2.18)	2.39 (0.75, 7.56)	0.260
3-day	2.20 (0.82, 5.94)	1.43 (0.51, 3.99)	4.30 (1.57, 11.8)#	0.134
5-day	0.84 (0.23, 3.03)	0.60 (0.11, 3.38)	3.16 (1.46, 6.85)#	0.085

[a] Pollutant averages include current day 24-hr average, and 2-day through 5-day average of the current day and lag day averages.

[b] Rate ratios and 95% confidence intervals are for the expected change in daily ECG observations of ventricular tachycardia counts associated with an interquartile range increase in the air pollutant (see Table 2). All models are adjusted for adjusted for daily average actigraph-derived physical activity and heart rate, temperature of the same lag average, day of week, seasonal study phase (mean centered exposure), and community group (mean centered exposure), using generalized estimating equations. Daily RRs use Poisson log-link models with daily VT counts as the outcome. Stratified results are from product term models of the air pollutant by gene variant.

[c] P-values are for the product term of GSTM1 by air pollutant.

* $p < 0.1$, ** $p < 0.05$, # $p < 0.01$

References

Bailey LR, Roodi N, Verrier CS, Yee CJ, Dupont WD, Parl FF. Breast cancer and CYPIA1, GSTM1, and GSTT1 polymorphisms: evidence of a lack of association in Caucasians and African Americans. Cancer Res. 1998;58(1):65-70.

Chahine T, Baccarelli A, Litonjua A, Wright RO, Suh H, Gold DR, Sparrow D, Vokonas P, Schwartz J. Particulate Air Pollution, Oxidative Stress Genes, and Heart Rate Variability in an Elderly Cohort. Environ Health Perspect 2007;115:1617-22.

Eaton DL, Bammler TK. Concise review of the glutathione S-transferases and their significance to toxicology. Toxicol Sci 1999;49:156-64.

Schwartz J, Park SK, O'Neill MS, Vokonas PS, Sparrow D, Weiss S, Kelsey K. Glutathione-S-transferase M1, obesity, statins, and autonomic effects of particles: gene-by-drug-by-environment interaction. Am J Respir Crit Care Med 2005;172:1529-33.

Strange RC, Spiteri MA, Ramachandran S, Fryer AA. Glutathione-S-transferase family of enzymes. Mutat Res 2001;482:21-6.

4. Chapter 4. Overall Summary and Conclusions

The finding of associations between risk of ventricular tachycardia and air pollution supports the current limited epidemiological evidence (Brook et al. 2010; Link and Dockery 2010). The risk of ventricular tachycardia was significantly increased in particular with higher exposure to markers of traffic-related particles, secondary organic carbon, and ozone. Consistent associations were not observed for supraventricular tachycardia. Ozone and PM exposure ($PM_{2.5}$, EC, BC, and OC) were also significantly associated with decreased heart rate variability only in the 20 participants using ACE inhibitors. This may be a particularly susceptible population, but other data supportive of this finding are limited to a cross-sectional study (Adam et al. 2012).

Except for indoor $PM_{2.5}$, findings for indoor exposures were consistent with outdoor exposures. Exposure to indoor EC of outdoor origin was more strongly and more significantly associated with the daily count of VT than was uncharacterized indoor EC, suggesting that outdoor combustion sources were important determinants of risk. We did not find that quasi-ultrafine particle mass ($PM_{0.25}$) was more strongly associated with VT risk than accumulation mode particle mass ($PM_{0.25\text{-}2.5}$) suggesting that components in both of these size fractions comprising $PM_{2.5}$ were important. The lack of association with particle number concentration also suggests that ultrafine particles (that dominate particle numbers) were not any more important in relation to VT risk than larger particles.

The results for VT are biologically coherent with previous findings in this cohort for adverse effects of air pollution on systemic inflammation, blood pressure and electrocardiographic evidence of ischemia (Delfino et al. 2009, 2010a, 2010b, 2011). However, autonomic dysfunction as measured by decreased heart rate variability was not associated with air pollutants except in subjects on ACE inhibitors. Acute cardiac effects of air pollution exposure should continue to be evaluated in the future using cohort panel and controlled exposure studies. These studies could benefit from the measurement of other markers of altered autonomic balance other than heart rate variability, as well as other possible mechanisms.

With respect to the findings in the Task 2 analysis of gene-environment interactions we found limited evidence for effect modification, primarily observing increased risk of ventricular tachycardia among those with the GSTM1 null genotype. Further work is needed to determine if genetic variability in phase I and phase II metabolic pathways is a determinant of susceptibility to air pollution exposures.

5. Chapter 5. Recommendations

The present findings add to the growing literature on adverse cardiovascular effects of traffic-related air pollutants that has had a primary focus on PM. Components that may be of importance in adverse effects include primary and secondary particle-bound chemicals with oxidative and/or electrophilic potential, although data on such characterization in human population research is limited. Vapor-phase chemicals, namely semivolatile organic compounds, may also be important but there is even less work in this area. More research in human subjects targeting these areas is recommended. The present findings of positive relations between ventricular tachycardia and markers of primary combustion-related air

pollutants point to the need for additional research to determine the importance other non-residential sources of exposure to key chemical components in pollutants from traffic emissions, including exposures at other non-residential fixed locations like the workplace, and exposures while traveling in vehicles. These exposures to mobile sources air pollution are a major concern in California. Any efforts to decrease traffic emissions via increased fuel efficiency or the use of zero emission vehicles is likely to have a major impact on adverse cardiovascular events, including ventricular arrhythmias that have been linked to sudden cardiac death.

6. Publication Produced

Bartell S, Tjoa T, Longhurst J, Sioutas C, Delfino RJ. Particulate air pollution, ambulatory heart rate variability and arrhythmia in elderly subjects with coronary artery disease. Submitted to *Environ Health Perspect* 8-2012 (revision submitted).

7. Abbreviations

ACE: angiotensin-converting-enzyme
AER: air exchange rates
BAM: Beta-Attenuation Mass
BC: black carbon
CAD: coronary artery disease
CHAPS: Cardiovascular Health and Air Pollution Study
CI: confidence interval
CO: carbon monoxide
EC: elemental carbon
ECG: electrocardiograph/electrocardiogram
F_{inf}: infiltration factors
GSTM1: glutathione S-transferase M1
GSTT1: glutathione S-transferase T1
HF: high frequency power spectra of heart rate
HRV: heart rate variability
I/O: indoor/outdoor ratios
ICD: implantable cardioverter defibrillator
IQR: interquartile range
IL5RA: interleukin 5 receptor-alpha
LF: low frequency power spectra of heart rate
$NC_{0.02\text{-}1}$: particle number concentrations for particles 0.02-1.0 μm in diameter
NN: normal-to-normal heart beat interval
NO_2: nitrogen dioxide
NO_x: nitrogen oxides
O_3: ozone
OC: organic carbon

OC_{pri}: organic carbon attributed to primary organic carbon
PAH: polycyclic aromatic hydrocarbons
PCR: polymerase chain reaction
PM: particulate matter
$PM_{0.25}$: quasi-ultrafine particulate matter < 0.25 micrometers in aerodynamic diameter
$PM_{0.25\text{-}2.5}$: accumulation particulate matter 0.25-2.5 micrometers in aerodynamic diameter
$PM_{2.5\text{-}10}$: coarse mode particulate matter 0.25-2.5 micrometers in aerodynamic diameter
PM_{10}: particulate matter < 10 µm in aerodynamic diameter
$PM_{2.5}$: particulate matter < 2.5 µm in aerodynamic diameter
PN: particle number
pNN50: percent of adjacent normal R-R interval differences greater than 50 msec
rMSSD: root mean square of successive R-R differences in ms
RR: rate ratio
SDNN: standard deviation of normal R-R intervals in ms
SOC: organic carbon attributed to secondary organic carbon
SVT: supraventricular tachycardia
UFP: ultrafine particles, PM < 0.1 µm in aerodynamic diameter
VT: ventricular tachycardia