Air pollution exposure and novel biomarkers of inflammation and cardiac stress in the Multi-Ethnic Study of Atherosclerosis and Air Pollution (MESA Air)

Marnie Hazlehurst

A thesis

submitted in partial fulfillment of the

requirements for the degree of

Master of Science

University of Washington

2016

Committee:

Joel Kaufman

Anjum Hajat

Program Authorized to Offer Degree:

Epidemiology

University of Washington

Abstract

Air pollution exposure and novel biomarkers of inflammation and cardiac stress in the Multi-Ethnic Study of Atherosclerosis and Air Pollution (MESA Air)

Marnie Hazlehurst

Chair of the Supervisory Committee:
Joel Kaufman, MD, MPH
Department of Environmental and Occupational Health Sciences
Department of Epidemiology
Department of Medicine

Epidemiologic evidence indicates a causal association between long-term air pollution exposure and cardiovascular disease (CVD), but the mechanisms underlying this process are not fully understood. Inflammation in the lungs that spreads to the rest of the body may result in systemic oxidative stress and inflammation. Subsequently, this inflammatory state may lead to endothelial dysfunction, atherosclerosis, increased coagulation, and activation of the renin-angiotensin system. Some studies have utilized measures of subclinical disease or biomarkers to support these potential mechanisms for the effect of air pollution on the development of CVD.

This analysis investigated the effects of long-term exposure to air pollution, specifically four pollutants: fine particulate matter ($PM_{2.5}$), oxides of nitrogen (NO_x), nitrogen dioxide (NO_2), and black carbon (BC). This paper examined the association between long-term exposure and the following novel biomarkers of cardiac stress and inflammation: (1) N-terminal-pro-B-type natriuretic peptide (NT-proBNP), (2) Pentraxin-3 (PTX3), and (3) Serum Amyloid P (SAP). As a

secondary aim, this paper examined the association between short-term exposures to $PM_{2.5}$ and each of the biomarkers of interest.

Data from a prospective cohort study, the Multi-Ethnic Study of Atherosclerosis and Air Pollution (MESA Air) in six US cities, were used for this analysis. Likelihood-based spatiotemporal models were used to calculate air pollution exposures at participant residences. NT-proBNP was measured at baseline (n=5,597) and again at a follow-up exam (n=4,694), which was on average three years after baseline. PTX3 and SAP were both measured at the baseline exam in a subset of participants (n=2,878) selected in equal proportions from each racial/ethnic group. NT-proBNP and PTX3 were log-transformed for analysis; SAP was modeled on the natural scale.

No association was found between air pollution and NT-proBNP in a repeated measures analysis, but an association was observed between air pollution and elevated levels (≥125 pg/mL) of NT-proBNP at Exam 3 among those with a normal NT-proBNP level at baseline (i.e. reflecting incident cases of sub-clinical cardiac stress and/or sub-clinical heart failure). For a 15 ppb increase in NO_2 exposure, the odds ratio was 1.88 (95% CI: 1.20, 2.95; p=0.006) and for a 0.7 10^{-5} m^{-1} increase in BC exposure the odds ratio was 1.72 (95% CI: 1.07, 2.76; p=0.026). Increased exposure to $PM_{2.5}$ was associated with a 2.5 μg/mL increase in SAP (95% CI: 0.3, 4.8; p=0.025), though no associations were found with NO_x, NO_2, or BC. In cross-sectional analyses, no association was observed between air pollution and PTX3. No associations were found between short-term exposure to $PM_{2.5}$ and NT-proBNP, PTX3, or SAP.

INTRODUCTION

The scientific literature supports a causal association between air pollution exposure and cardiovascular disease (CVD),[1,2] with much of this evidence coming from prospective cohort studies including the Harvard Six Cities Study,[3] the American Cancer Society (ACS) study,[4] and the Women's Health Initiative Observational Study (WHI-OS).[5] In the Six Cities study, air pollution was positively associated with death from lung cancer and cardiopulmonary disease, with the strongest associations found for exposure to fine particulate matter ($PM_{2.5}$).[3] In the ACS study, exposure to $PM_{2.5}$ was associated with 4%, 6%, and 8% increases in all-cause, cardiopulmonary, and lung cancer mortality, respectively.[4] In the WHI-OS involving more than 65,000 women in the US, $PM_{2.5}$ exposure was associated with increased risk of cardiovascular events and death from CVD.[5] Additional evidence from epidemiologic studies both in the US and abroad further supports the association between air pollution and CVD.[6–9] However, the mechanisms underlying this process are not fully understood.[1,2]

There are two hypothesized mechanisms by which air pollution causes CVD. First, air pollution inhalation may cause inflammation in the lungs that spreads to the rest of the body Second, exposure may directly affect the autonomic nervous system (ANS).[1] Both of these immediate effects can result in systemic oxidative stress and inflammation through the activation of signaling pathways and interactions with cytokines and innate immunity cells. Subsequent effects of this general inflammatory state include increased activity of the renin-angiotensin system (RAS), endothelial dysfunction, increased coagulation, and atherosclerosis.[10] RAS is responsible for maintaining systemic blood pressure and fluid volumes in the body; abnormal activation of this system can lead to fibrosis, vascular and cardiac hypertrophy, and

vasoconstriction, ultimately resulting in clinical CVD.[11] Endothelial dysfunction and increased coagulation contribute to the development of atherosclerosis and clinical CVD.

Studies of potential mechanisms for air pollution effects include investigation of biomarkers involved in these various pathways for the development of CVD. Commonly studied biomarkers include C-reactive protein (CRP), an acute-phase protein produced in the liver and released into the bloodstream as part of the inflammatory signaling cascade, and fibrinogen, a protein involved in blood coagulation. A population-based, prospective cohort study in Germany, the Heinz Nixdorf Recall Study, found that exposure to $PM_{2.5}$ was associated with both increased CRP and increased fibrinogen among men.[12] A repeated measures analysis in MESA Air, a prospective cohort study in the US, examined several biomarkers related to inflammation and endothelial dysfunction, including CRP, Interleukin 6 (IL-6), D-dimer, fibrinogen, and markers of cellular adhesion. This study found increases in long-term exposure to $PM_{2.5}$ and NO_x to be associated with increased IL-6 and D-dimer, respectively, though these effect were not observed for the other blood markers analyzed.[13]

Other epidemiologic studies of the mechanism of air pollution effects have focused on blood pressure, cardiac remodeling, retinal vasculature, or coronary artery calcium. In the Sister Study, long-term exposures to $PM_{2.5}$ and NO_2 were associated with increased blood pressure.[14] In MESA Air, living close to a roadway was found to be associated with a higher left-ventricular mass index[15] and long-term air pollution exposure was found to be associated with narrower retinal arteriolar diameters, a measure of impaired vasculature.[16] Long-term air pollution exposure was also found to be associated with progression of coronary artery calcium—a measure of atherosclerosis—in the MESA Air cohort.[17]

This paper builds on the prior literature by examining the impact of air pollution on biomarkers both involved in the inflammatory pathway and related to cardiac function that have not yet been investigated, in particular: N-terminal-pro-B-type natriuretic peptide (NT-proBNP), a marker of cardiac stress, and Pentraxin-3 (PTX3) and serum amyloid P (SAP), markers of inflammation. Each of these three proteins are described below.

NT-proBNP

The natriuretic hormone proBNP is released from myocardial cells in the ventricles as demand on the heart increases. The N-terminal of the hormone is cleaved to form two separate peptides: the biologically active peptide BNP and the biologically inactive peptide NT-proBNP, both of which can be measured in blood.[18] These peptides are produced in response to increased blood pressure and volume overload. In addition, increased pressure can result in structural damage to the heart including cardiac hypertrophy, leading to atrial fibrillation (AF) and heart failure (HF).

The prevalence of HF, when the heart can no longer pump enough blood to meet the body's need for blood and oxygen, is estimated to be between 1 and 4% in the general population.[19,20] When weakened contractility of the cardiac muscle prevents the heart from fully emptying the blood from the ventricle, patients experience HF with reduced ejection fraction (HFrEF). Such impairment often occurs as a result of myocardial ischemia. In contrast, thickening and stiffening of the ventricle walls due to hypertension limits the amount of blood required to fill the ventricle and is characteristic of HF with preserved ejection fraction (HFpEF).[20] The prevalence of preserved ejection fraction as opposed to reduced ejection fraction is estimated to be up to 70% of HF cases in screened populations.[21] An increase in release of natriuretic peptides is observed in both forms of HF.

NT-proBNP is typically measured in the blood and analyzed using a commercially available electrochemiluminescent assay.[22] While BNP can also be measured, it has a shorter half-life (18 minutes compared to 118 minutes),[18] and is stable in whole blood for only 24 hours at room temperature with the addition of EDTA, whereas NT-proBNP in whole is stable at room temperature for 72 hours without any additives.[23] BNP is cleared from blood by either binding to natriuretic peptide receptors or neutral endopeptidases, whereas NT-proBNP is cleared almost exclusively through the kidney. Both peptides have been found to predict disease state and prognosis among patients with suspected heart failure.[22] These characteristics make NT-proBNP, as opposed to BNP, favorable for assay in population studies.

Several demographic, physiological, and behavioral characteristics influence NT-proBNP levels. Age and sex are highly important predictors, with 50-70% higher mean NT-proBNP levels reported in females, and mean levels almost doubling with each decade of increase in age in some studies.[24–26] Some studies have suggested that NT-proBNP levels are lower among blacks than among non-Hispanic whites, but the current literature on variation by race/ethnicity is limited.[27–29] Higher NT-proBNP is associated with higher blood pressure and higher left ventricular mass index, but lower cholesterol, lower BMI, and lower creatinine clearance.[25,26] Higher NT-proBNP has also been found among those reporting lack of regular exercise.[25,26]

Concentrations of NT-proBNP in the blood are used clinically as an aid in diagnosing HF by comparison to an age-dependent standard. Previously, 400 pg/mL was used as a common cut-point in diagnosing HF. However, recent work suggests a lower cut-point than previously recommended—125 pg/mL—may be appropriate for identifying those with pre-clinical or potential heart failure, particularly in cases with preserved ejection fraction.[19,21] Even mildly elevated levels of NT-proBNP are associated with an increased risk of AF,[30] HF,[30] stroke,[30] first

cardiovascular event,[30–33] and mortality.[28,30–32] Additionally, given that NT-proBNP is relatively easy to measure in blood, several studies have demonstrated the usefulness of this marker in CVD risk prediction.[32,34]

Effects of air pollution on NT-proBNP have not previously been investigated, though air pollution has been linked to several relevant clinical outcomes. Short-term exposure to air pollution has been found to be associated with hospital admissions for heart failure,[35] AF,[36] and HF,[37,38] and may be a trigger for these conditions. However, long-term exposure to air pollution has also been linked to several of these outcomes, including increased blood pressure[15] and HF,[39] suggesting a role of air pollution in the development of these conditions. Associations between long-term air pollution exposure and NT-proBNP have not previously been studied.

Pentraxin-3

PTX3 is a member of the pentraxin family of proteins, which also includes the short-chain pentraxins CRP and SAP. PTX3 is a prototypic long-chain pentraxin which is differentiated by the length of the amino-terminal domain of the protein.[40] Unlike CRP which is produced in the liver in response to IL-6, PTX3 is produced in a variety of cell types—including endothelial cells, macrophages, fibroblasts, neutrophils, and epithelial cells—in response to proinflammatory signals.[41] After release from these cells, PTX3 binds to the complement component C1q, activating the classical complement pathway as part of the body's innate immune response, which contributes to the creation of a proinflammatory environment.[40,41] PTX3 may be a more useful marker than CRP or SAP as it is produced in cells at the site of inflammation and thus is more specific to endothelial dysfunction and vascular inflammation.[41,42] Specifically, PTX3 is likely related to later stages of atherosclerosis and vascular damage as well as with coronary plaque vulnerability.[43,44]

Several demographic characteristics and disease states are predictive of PTX3 levels. PTX3 has been reported to be higher in females and in older patients.[45,46] PTX3 levels tend to be elevated in patients with impaired kidney function,[46] advanced chronic kidney disease (CKD), and end-stage renal disease.[47] Patients with a variety of infectious disorders including sepsis, tuberculosis, and dengue, as well as patients with some autoimmune disorders also tend to have elevated levels of PTX3.[40] Additionally, lower BMI, lower triglycerides,[45,46] and lower cholesterol[48] have been observed to be associated with higher PTX3 levels.

PTX3 is associated with both subclinical and clinical CVD outcomes independently of CRP. When measured within the first day of onset of symptoms of acute MI, PTX3 is a predictor of mortality.[49] PTX3 has been found to be associated with endothelial dysfunction in subjects with CKD,[46] greater right ventricular mass,[50] higher left ventricular mass index,[46] reduced kidney function,[47] subclinical CVD,[51] CVD events,[46,52] incident HF,[46] CVD-related mortality,[51] and all-cause mortality.[46,51] Though air pollution has been linked to several of these clinical outcomes, no prior studies have investigated the effects of air pollution on PTX3.

Serum Amyloid P

SAP is another member of the pentraxin family, in addition to CRP and PTX3. Similar to CRP, SAP is a short-chain pentraxin and is produced in the liver. However, unlike CRP, acute inflammation does not greatly affect levels of SAP.[40] SAP is found in amyloid deposits in many organs, including the liver, spleen, and kidney, and in amyloid-beta plaques in Alzheimer's disease.[53] In addition to binding to the ligands that are exposed during apoptosis and clearance of dead cells, SAP binds both high density lipoprotein (HDL) and very low density lipoprotein (VLDL) with high affinity.[54] SAP is also involved in regulating inflammatory responses to

immune complexes.[55] SAP has been identified in atherosclerotic lesions and been found to correlate with the severity of those lesions.[56]

Lower levels of SAP have been reported among females.[54] Levels of SAP in the blood have been found to be associated with incident angina and myocardial infarction.[55] SAP may represent different aspects of inflammation and the development of atherosclerosis than CRP, but both the determinants and consequences of elevated SAP levels, particularly as it relates to CVD, have been less frequently studied. No studies to date have investigated the effect of air pollution exposure on SAP levels.

Air pollution

The health effects of several different types of air pollutants have been investigated in epidemiologic studies, including fine particulate matter ($PM_{2.5}$), oxides of nitrogen (NO_x), nitrogen dioxide (NO_2), and black carbon (BC). $PM_{2.5}$ and NO_x are criteria air pollutants, regulated by the US Environmental Protection Agency's (EPA) National Ambient Air Quality Standards (NAAQS).[57] These standards are based on the current scientific evidence for human health and/or environmental effects of the pollutant and are reviewed every five years.

$PM_{2.5}$ includes particles that are less than 2.5 micrometers in diameter. These particles are either directly emitted or formed due to condensation of gaseous emissions in the air. The most common types of compounds comprising particulate matter (PM) vary, with sulfate compounds accounting for a larger proportion of the PM mass in the eastern US compared to organic carbon (OC) in the western part of the country.[58] The most light-absorbent type of fine particulate matter, BC is emitted in the form of soot particles, which also typically contain OC. BC is produced by combustion of fossil fuels, particularly diesel engines, and biomass burning.

Combustion at higher temperatures tends to produce relatively more BC and, due to the strong absorption properties of BC, these emissions exacerbate global warming.[59]

Oxides of nitrogen are gaseous pollutants and NO_2 is analyzed as an indicator for the larger group of nitrogen oxides as well as a marker of traffic-related air pollution. Local reactions with emissions from cars, trucks and buses, power plants, and off-road equipment form NO_2.[58] This gaseous pollutant absorbs short blue and green wavelengths of light and transmits the longer green and red wavelengths, which causes it to appear brown and thus is the pollutant responsible for the brown color on smoggy days.[59] The primary source of NO_2 is the oxidation of nitric oxide (NO) released from combustion, though NO_2 is also produced directly from fossil fuel and biofuel combustion and biomass burning. Near-roadway environments are a major source of exposures to NO_2 and distance from residence to nearest large roadway has been used in previous studies as a proxy for traffic-related pollution exposure.

The primary aim of this analysis was to examine the associations between long-term exposures to several air pollutants and levels of three blood markers. Specifically, this analysis investigated the effects of $PM_{2.5}$, NO_x, NO_2, and light absorption coefficient as a measure of BC, on (1) NT-proBNP as a marker of cardiac stress, (2) PTX3 as a marker related to the atherosclerotic process, and (3) SAP as a marker of vascular inflammation. The secondary aim of this paper was to examine the association between short-term exposures to $PM_{2.5}$ and each of these three biomarkers of interest.

METHODS

Study design and subjects

The Multi-Ethnic Study of Atherosclerosis (MESA) is a large prospective epidemiologic cohort study designed to investigate risk factors for subclinical CVD and progression to clinical CVD.[60,61] Participants in MESA were recruited in six regions in the US between 2000 and 2002: Forsyth County, NC; Northern Manhattan and the Bronx, NY; Baltimore City and Baltimore County, MD; St. Paul, MN; Chicago and the village of Maywood, IL; and Los Angeles County, CA. The field centers in each of these six regions used a variety of recruitment strategies. In NC, Department of Motor Vehicle and Centers for Medicare & Medicaid Services lists were used for random sampling of all eligible residents in the county, stratified by age and sex. In NY, participants were recruited from the members, retirees, and spouses listed in the Local 1199 National Benefit Fund roster who lived within selected zip codes and sampling was stratified by age and sex. In MD, a commercial mailing service's inventory of residences was used to recruit participants from selected census tracts along a rapid transit line. In MN, participants were recruited from four contiguous census tracts in the southern part of the city using the county assessor's list of dwellings within those tracts. In IL, four community areas close to Northwestern University Medical Center or in the village of Maywood provided the source population for recruitment stratified by age and gender using a list of residences provided by the City of Chicago Department of Planning and Development. Lastly, in CA, participants were recruited from within a 15-mile radius from the UCLA medical center using random-digit dialing, with targeted sampling from census tracts with high proportions of Hispanics or Chinese-Americans.

The 6,814 participants enrolled in the core MESA cohort were ages 45 to 84 years old at baseline, from four racial/ethnic groups (white, black, Chinese, and Hispanic), and free of CVD at the time of recruitment into the study. Eligibility requirements also included speaking English, Spanish, Cantonese, or Mandarin. Clinical exams including questionnaires, blood draws, and measurements of subclinical disease were conducted at baseline and at four subsequent time points during follow-up. This analysis used a subset of the participants in the MESA cohort. Specifically, participants were included if they had the biomarker of interest measured at the baseline exam, which occurred between July 2000 and August 2002, and/or at the third exam, which occurred between March 2004 and September 2005, and if they had measures of air pollution predicted at their residential address.

Air pollution exposures

MESA Air is an ancillary study to MESA that has investigated relationships between air pollution exposures and both subclinical atherosclerosis and clinical cardiovascular disease.[62] Estimates of long-term exposures to air pollution were generated for MESA participants from MESA Air likelihood-based spatiotemporal models.[63,64] These models cover regions approximately 75 kilometers from the center of each metropolitan area in MESA for the years 2000 to 2013. They incorporated regulatory network monitoring data of $PM_{2.5}$, NO_x, and NO_2 from the US Environmental Protection Agency (EPA) Air Quality Systems (AQS).

These models also utilized study-specific data from an extensive monitoring campaign conducted between July 2005 and August 2009 at MESA Air community locations and participant homes that was completed in order to better characterize within-city variability. $PM_{2.5}$ was measured with active Harvard PEM gravimetric sampling and light-absorbing carbon was measured on the same filter and analyzed using reflectometry. NO_2 and NO_x were specifically

monitored at varying distances from roadways using passive Ogawa samplers in order to better characterize roadway gradients of these pollutants. Additionally, we generated a large suite of geographic covariates, performed dimension reduction of these geographic covariates using partial least squares (PLS) scores, and used these scores as inputs to the modeling process.

Time-varying predictions of outdoor $PM_{2.5}$, NO_x, and NO_2 from these spatiotemporal models were used to calculate the annual average concentration of each pollutant at each participant's residence over the year prior to the blood draw at which the biomarkers were measured. This is the exposure metric we are primarily interested in. Predictions of BC were not time varying because AQS monitors did not use the same methods as the cohort-specific monitoring and thus this model only characterized spatial variation in exposure. For all pollutants, participants who moved during the year of interest have exposure estimates that time-weight concentrations at all reported addresses.

Sensitivity analyses utilized varying exposure periods, including (1) exposure estimates averaged over the year 2000 for Exam 1 measures and averaged over the year 2004 for Exam 3 measures, (2) year 2000 metrics used for all outcome measures, and (3) individual-level estimates of $PM_{2.5}$ exposure incorporating information about infiltration and individual time-location patterns.[65]

Short-term exposures to $PM_{2.5}$ were calculated for varying periods during the week prior to either Exam 1 or Exam 3 blood draw, including day of blood draw (day 0), prior day (day 1), and 2-, 3-, 4-, and 5-day averages. A central-site AQS monitor was used for these exposures, and thus within-city variability stems from temporal differences rather than spatial ones. To control for confounding, the short-term $PM_{2.5}$ concentrations were regressed against splines for calendar time (12 degrees of freedom [df]/year), temperature (6 df/year), and relative humidity (6 df/year),

and an indicator variable for day of the week. Residuals from this model were used as the exposure variable in the health-effects models. This procedure is known as pre-adjustment or pre-whitening and has been shown to be effective in health effects models.[66]

NT-proBNP

NT-proBNP was measured in 5,597 participants at Exam 1 and 4,694 participants at Exam 3 as part of an ancillary study. Plasma samples from Exam 1 and Exam 3 that were previously unthawed or only thawed once were retrieved and shipped for analysis at the same time. All analyses were performed at a single laboratory (Veteran's Affairs San Diego Healthcare System, La Jolla, CA) using the Elecsys 2010 electrochemiluminescence immunoassay (Roche Diagnostics Corporation, Indianapolis, IN, USA) with an analytic range of 5 to 35,000 pg/mL.[27,28]

Pentraxin-3

PTX3 was measured in 2,838 participants at Exam 1 as part of an ancillary study to MESA. All samples were analyzed at a central laboratory (Laboratory for Clinical Biochemistry Research; University of Vermont) using the PTX3 (human) Detection set (Alexis Biochemicals; San Diego, CA) with an analytic coefficient of variation of 10.2%.[43,50] The limit of detection was 0.075 ng/mL.

Serum Amyloid P

SAP was measured in 2,863 participants at Exam 1 for the same MESA ancillary study at the same central laboratory as PTX3 (Laboratory for Clinical Biochemistry Research; University of Vermont). SAP was measured with an in-house ELISA utilizing a monoclonal antibody to human SAP (Cymbus Biotechnology, Chandlers Ford, UK) as the capture antibody, a rabbit polyclonal antibody to human SAP (Dako Corporation, Carpinteria, CA) as the primary detection

antibody and a horseradish peroxidase-conjugated anti-rabbit antibody as the secondary detection antibody (Jackson Laboratories, West Grove, PA). Purified SAP for a standard was from EMD Biosciences (Calbiochem; San Diego, CA). SAP purity was certified by the manufacturer as ≥99% by immunochemical assay and ≥95% by sodium dodecylsulfate polyacrylamide gel electrophoresis. The analytical CV of the SAP assay was 9%.

Covariates

The additional covariates considered in this analysis were age; race/ethnicity (white, Chinese, black, or Hispanic); sex; individual SES as measured by education (three categories: less than high school or high school graduate, some college, or at least a bachelor's degree) and income (permanent income as the average of the midpoint of reported income category at exams 1 through 3); neighborhood-level SES index;[67] health behaviors (smoking [current, former, or never smoker and number of pack-years], alcohol consumption [yes/no use at baseline], and physical activity [quartiles of intentional exercise in MET-min/wk]); and CVD risk factors (body mass index [BMI], waist-hip ratio, total cholesterol, low-density lipoprotein [LDL], systolic blood pressure, and diabetes [three categories: treated/untreated diabetes, impaired fasting glucose, or normal fasting glucose as defined by the 2003 American Diabetes Association fasting blood glucose criteria algorithm]). In sensitivity analyses, we also adjusted for CRP level, impaired renal function (estimated glomerular filtration rate [eGFR] ≤ 60 mL/min/1.73 m^2 as a binary measure of kidney function), season of biomarker measurement, and CVD-related medication (any of: lipid-lowering medications, hypertension medications, β-blockers, and diuretics).

Data analysis

For the repeated measures analysis of NT-proBNP, a multi-level model with a person-level random intercept was used to account for the within-person correlation of the biomarker. Associations of known CVD risk factors with NT-proBNP were examined in a repeated measures model for comparison. Logistic regression was used to assess elevated NT-proBNP at Exam 3 as a binary outcome among those with a normal NT-proBNP at baseline (i.e. reflecting potential or subclinical incident heart failure cases). Elevated versus normal was defined as ≥125 pg/mL and a sensitivity analysis was conducted with elevated NT-proBNP defined as ≥400 pg/mL. For the cross-sectional analyses of PTX3 and SAP, a linear model was used. Both NT-proBNP and PTX3 have previously been reported to have a highly skewed distribution in the MESA cohort and thus were log-transformed for analysis.[27,52] SAP was modeled on the natural scale. Extreme outliers were identified using histograms and the skewness and kurtosis for each biomarker and were excluded from each analysis: NT-proBNP ≥10,000 pg/mL (n=2), PTX3 ≥10 ng/mL (n=7), and SAP ≥120 µg/mL (n=11).

A staged modeling approach was used with a minimally-adjusted Model 1 including individual demographic variables (age at blood draw, race/ethnicity, and sex), recruitment site, and exam. Model 2 was considered the primary model and included the covariates in Model 1 as well as individual-level SES (education and income), health behaviors (smoking, alcohol, and physical activity), neighborhood-level SES, BMI, and waist-hip ratio. Model 3 was further adjusted for additional potential confounders as well as potential mediators: systolic blood pressure, total cholesterol, LDL, impaired kidney function, CVD-related medication use, and diabetes. The same model-staging approach was used for the analysis of short-term $PM_{2.5}$ exposures. In the repeated measures analysis of NT-proBNP, several covariates were allowed to

vary with time, including age, smoking, physical activity, BMI, and waist-to-hip ratio. In the analysis of elevated NT-proBNP, age was included in the model as age at baseline; the number of days between the baseline and the follow-up exam was also included in these models. Results are presented for increments of increases of exposure that reflect approximately an IQR increase, though these increments have been rounded to commonly used intervals to facilitate comparisons with other studies. Estimates are presented for 5 $\mu g/m^3$ increases in $PM_{2.5}$, 40 ppb increases in NO_x, 15 ppb increases in NO_2, and 0.7 10^{-5} m^{-1} increases in BC.

Effect modification by recruitment site, age at recruitment, race/ethnicity, sex, and BMI category was explored. A number of sensitivity analyses were also conducted, including using varying time periods for exposures, adjusting for additional covariates, and applying additional exclusions for participants included in regression. As stated above, exposures from year 2000 were used for measures at Exam 1 and exposures from year 2004 were used for measures at Exam 3. In a separate analysis, year 2000 exposures were used for all outcome measures. To assess time as a potential confounder, we examined the adjustment for exam as a binary variable. Season of outcome measurement was also added to the model in a sensitivity analysis. Participants diagnosed with AF or HF were excluded in a separate sensitivity analysis. Lastly, cross-sectional analyses at Exam 1 and Exam 3 were conducted, as well as cross-sectional analyses among those less than 65 years old at recruitment and separately among those at least 65 years of age at recruitment. In models of PTX3 and SAP, sensitivity analyses included using year 2000 exposures, removing adjustment for site from the model, and adjusting for CRP.

In all analyses, an alpha of 0.05 was used to determine statistical significance and all confidence intervals shown are 95% confidence intervals. All statistical analyses were done using SAS version 9.4 software (SAS Institute, Inc., Cary, North Carolina).

RESULTS

NT-proBNP

At Exam 1 and Exam 3, 5,597 and 4,694 participants, respectively, had measures of NT-proBNP. Of those with a measure at Exam 1, 1,005 participants (19%) did not have a second measure at Exam 3. Participants without air pollution exposure estimates (n=247 for $PM_{2.5}$, n=168 for NO_x and NO_2, and n=155 for BC, at Exam 1) were excluded because they either lived outside of the modeling areas or did not have a sufficiently accurate geocode (better than zip code centroid) during the time period of interest.

This analysis included 40% white, 13% Chinese, 24% black, and 23% Hispanic participants at baseline; 51% female participants at both exams; and participants were on average 62 years old at Exam 1 and 65 years old at Exam 3 (Table 1). Those included were similar to the entire MESA Air cohort at Exam 1 with regards to sex, race/ethnicity, education, smoking status, and other cardiovascular risk factors such as BMI and cholesterol. There was a slightly higher percentage of participants from the recruitment site in Los Angeles, CA (23%) compared to the entire cohort (19%). The mean time between Exam 1 and Exam 3 was 3.2 years (standard deviation of 4 months). Covariate data was very complete, with less than 1.5% of participants missing data for any given covariate.

Downward trends in air pollution over time are evident across recruitment sites (Figure 1) and in the overall means at Exam 1 and Exam 3 (Table 2). Participants in CA tended to have the highest exposures to all pollutants and participants in NY tended to have high exposures to NO_x, NO_2, and BC relative to participants at other sites. Short-term $PM_{2.5}$ exposures tended to be lower at Exam 3 than Exam 1 and on average lower for multiple-day averaging periods compared to the day-of or day-prior-to the exam. This was likely due to a combination of factors,

including the fact that the longer averaging periods were more likely to include weekend days and that participants in St. Paul, MN were more likely to be missing day-of-exam exposures due to the 1-in-3-day schedule of the AQS monitoring in MN. More low-exposure days and more participants in low exposure areas included in longer averaging periods likely contributes to these observed differences in mean short-term exposures by averaging period.

NT-proBNP was skewed at both exams (Table 2). The geometric mean increased from 49 to 62 pg/mL from Exam 1 to Exam 3. Among participants with repeated outcome measures, NT-proBNP increased by more than 30% in 41% of participants and decreased by more than 30% in 25% of participants, between Exam 1 and Exam 3. The mean NT-proBNP level at Exam 1 was highest among participants recruited in St. Paul, MN and lowest among those recruited at Chicago, IL.

In a minimally-adjusted model, NT-proBNP was found to be positively associated with several demographic characteristics and CVD risk factors, including increased age, being female, increased systolic blood pressure, hypertension, impaired renal function, and CVD-related medication use (Table 3). Increased BMI, increased total cholesterol, and impaired fasting glucose and treated or untreated diabetes were associated with decreased NT-proBNP. Chinese, black, and Hispanic participants had a lower mean NT-proBNP level than white participants. A minimally-adjusted model indicated a 12% higher mean NT-proBNP (95% CI: 9%, 15%) at Exam 3 than at Exam 1. After adjusting for the full suite of other potential confounders, participants had a 15% higher mean NT-proBNP level (95% CI: 12%, 18%) at Exam 3 than at Exam 1.

In the repeated measures analysis after adjustment for the confounders in model 2, a 5 $\mu g/m^3$ increase in long-term $PM_{2.5}$ exposure was associated with a 2% decrease (95% CI: -7%,

4%) in NT-proBNP level (Figure 2). A 40 ppb increase in NO_x exposure was associated with a 1% increase (95% CI: -5%, 9%) and a 15 ppb increase in NO_2 exposure was associated with a 0.04% decrease (95% CI: -9%, 9%), in NT-proBNP. An increase of 0.7 10^{-5} m^{-1} in BC was associated with a 4% increase (95% CI: -6%, 16%) in NT-proBNP, though none of these associations were significant at the 0.05 level.

Effect modification by site, sex, race/ethnicity, age, and BMI was assessed in the repeated measures analysis (Table 4). Estimates tended to be negative for participants in MN, whereas estimates tended to be largest and positive for participants in MD. Effect estimates were positive among females and those less than 65 years of age at enrollment for exposure to $PM_{2.5}$, NO_x, and NO_2, compared to estimates among males and those 65 years or older. Effect estimates tended to be negative among Chinese, but positive among the other racial/ethnic groups with the largest effect estimates for the traffic-related air pollutants observed among black participants. The association between air pollution and NT-proBNP tended to be largest among those in the overweight category, but smaller among those in the obese category.

Sensitivity analyses investigated varying exposure periods in the repeated measures model (Table 5). Using the year 2000 and year 2004 averaging periods for air pollution exposure for Exams 1 and 3, respectively, we estimated increased NT-proBNP with increased exposure across all pollutants. When year 2000 exposures were assigned for all measures, air pollution was associated with increased NT-proBNP and the magnitude of the estimated effect was larger. In model 2 the association with $PM_{2.5}$ was borderline significant (p=0.0499), though for other pollutants the 95% confidence intervals included the null.

Sensitivity analyses for the repeated measures approach also investigated additional potential confounding and exclusionary criteria (Table 5). Adjusting for age at baseline and days

of follow-up, yielded very similar results to account for time, compared to time-varying age and exam number. When exam was removed from the model, effect estimates were highly statistically significant in the unexpected direction. Results were slightly negative but all 95% confidence intervals included zero with the removal of adjustment for recruitment site. Adjustment for the season during which the blood marker was measured did not substantially change the estimates of effect on NT-proBNP. Exclusion of participants with a diagnosis of AF or HF (8% and 4% of participants, respectively) yielded similar results. Results were not statistically significant for effects of $PM_{2.5}$ when exposure estimates were weighted using individual-level infiltration information and time-location patterns. When NT-proBNP was modeled on the natural scale, estimates were not statistically significant after adjustment for confounders (Table 6).

Cross-sectional sensitivity analyses were performed at both Exam 1 and Exam 3 (Table 7). At Exam 1, all pollutants were associated with increased NT-proBNP though the 95% confidence intervals include the null. For all pollutants except $PM_{2.5}$, higher exposures were similarly associated with increased NT-proBNP at Exam 3. For Exam 1, estimates of the increase of NT-proBNP associated with air pollution tended to be larger among participants who were less than 65 years old compared to those who were 65 years old or older, at the time of recruitment into the study. Trends by age at baseline varied across the pollutants at Exam 3.

Air pollution was associated with increased odds of elevated NT-proBNP (Figure 3). There were 509 participants whose NT-proBNP level increased from below to above a cut-point of 125 pg/mL between Exam 1 and Exam 3. Among those 509 participants with an NT-proBNP level greater than 125 pg/mL at Exam 3, the mean NT-proBNP level at Exam 1 was 77 pg/mL (SD 31 pg/mL) and increased on average by 304% to a mean of 224 pg/mL (SD 243 pg/mL) at

Exam 3. In comparison, those participants whose NT-proBNP levels were below 125 pg/mL at Exam 3 had a mean NT-proBNP level of 41 pg/mL (SD 30 pg/mL) at Exam 1 and increased on average by only 79% to a mean of 49 pg/mL (SD 31 pg/mL) at Exam 3. Long-term NO_2 exposure was statistically significantly associated with elevated NT-proBNP (OR=1.88; 95% CI: 1.20, 2.95; p=0.0058). BC was also associated with elevated NT-proBNP (OR=1.72; 95% CI: 1.07, 2.76; p=0.0257). The odds ratios were suggestive of an association for the other pollutants, but 95% confidence intervals were generally wide and included the null.

This analysis generally produced effect estimates in the same direction within subgroups defined by site, sex, race/ethnicity, and age at baseline (Table 8). Larger effect estimates were observed among those in lower BMI categories compared to estimates among obese participants.

Using a cut-point of 400 pg/mL reduced the number of those who experienced an increase from normal to elevated NT-proBNP to 168 participants (Table 9). Estimates using the 400 pg/mL cut-point were of a similar magnitude as the lower cut-point across all pollutants, though 95% confidence intervals included the null.

Short-term exposure to $PM_{2.5}$ was not significantly associated with NT-proBNP for any averaging period (Table 10). Effect estimates were about a 1% increase in NT-proBNP or less across all exposure periods and none were statistically significant at the 0.05 level. Adjustment for additional confounders tended to attenuate these estimates.

Pentraxin-3 and Serum Amyloid P

For the cross-sectional analysis at Exam 1 there were 2,838 participants with a measure of PTX3 and 2,863 participants with a measure of SAP. There were 55 participants with only 1 of either of these biomarkers measured, whereas both biomarkers were measured in the remaining 2,823 participants. Participants in the cross-sectional analysis without air pollution

exposure estimates were excluded because they were either living outside of the air pollution modeling area or did not have a sufficiently accurate geocode (better than zip code centroid) during the time period of interest. Covariate data was very complete, with less than 3% of participants missing data for any given covariate.

In the cross-sectional analyses of PTX3 and SAP, 53% of participants were female, the mean age was 62 years, and the mean BMI was 28 kg/m^2 (Table 1). For the MESA ancillary study which measured PTX3 and SAP at Exam 1, participants were specifically selected based on race/ethnicity and thus there are approximately the same number of participants in each racial/ethnic group. This selection approach resulted in a subset of participants with a lower mean income, slightly lower proportion of former smokers, less intentional exercise, and slightly lower percent with college degree, but similar rates of diabetes, similar mean age, similar mean cholesterol, and similar mean systolic blood pressure. A larger percentage of this subset was recruited from Los Angeles, CA (29%) compared to the overall cohort (19%).

No association was observed between any of the four air pollutants of interest and PTX3 levels in the cross-sectional analysis (Figure 4). Estimates were similarly null with individual $PM_{2.5}$, exposure estimates from year 2000, and adjustment for CRP level (Table 11). However, when recruitment site was not included in the model, positive associations were observed between NO_x, NO_2, and BC, and PTX3.

A 5 $\mu g/m^3$ increase in $PM_{2.5}$ was associated with a 2.5 µg/mL increase (95% CI: 0.3, 4.8; p=0.0246) in SAP after adjusting for the confounders in model 2 (Figure 5). This association remained statistically significant after adjustment for CRP level (Table 12). While a consistent small, but statistically significant association with $PM_{2.5}$ was observed across the varying staged

models, estimates for the other pollutants are not statistically significant at the 0.05 level. No associations were observed with individual $PM_{2.5}$ exposures or year 2000 exposures.

No associations were observed between short-term exposure to $PM_{2.5}$ and either PTX3 or SAP (Table 13).

DISCUSSION

A repeated measures analysis found no association between air pollution and NT-proBNP. Additional adjustment for potential confounders or mediators tended to attenuate the effect estimates while sensitivity analyses using varying exposure timeframes suggested a positive association between air pollution and NT-proBNP. Long-term air pollution exposure was associated with elevated NT-proBNP at follow-up among those with a normal level at baseline. In cross-sectional analyses, no association was observed between air pollution and PTX3. Increased exposure to $PM_{2.5}$ was associated with an increase in SAP. No associations were found between short-term exposure to $PM_{2.5}$ and NT-proBNP, PTX3, or SAP.

NT-proBNP serves as a marker of cardiac stress resulting from systemic inflammation, hypertension, and cardiac remodeling. In this study population, baseline NT-proBNP levels were associated with incident coronary heart disease (CHD),[28] incident AF,[68] and incident HF.[27] Furthermore, racial/ethnic differences in NT-proBNP levels have previously been described in this population.

Prior literature on determinants of NT-proBNP is relatively scarce. Few studies have assessed the effect of environmental factors on NT-proBNP with prior work primarily focused on short-term exposures.[36] The role of air pollution as a trigger for acute events is supported by the close temporal association observed in a meta-analysis of short-term air pollution and heart

failure.[37] The analysis presented builds upon this previous literature by examining the association between long-term exposure to air pollution and these biomarkers.

In this study population, associations between known CVD risk factors such as BMI and NT-proBNP were similar in magnitude and exhibited similar trends as those previously reported in the literature.[18,24] It has been hypothesized that the substantial difference in NT-proBNP levels between men and women is related to differences in metabolism, whereas differences in NT-proBNP by age are likely related to subclinical changes in myocardial structure and/or function.[69] Though no statistically significant associations between air pollution and NT-proBNP were observed in the repeated measures analysis of this blood marker, using an incidence approach did find a statistically significant association, and thus there are several important considerations in the interpretation of these results.

Unlike many studies of NT-proBNP, the MESA cohort is a relatively healthy adult population with all participants free of CVD at enrollment in the study. Furthermore, there are likely important differences between those who are free of clinical CVD at enrollment at age 45 compared to those who are free of clinical CVD and enrolled at age 75. Selection bias due to differential selection of healthy older adults may impact this repeated measures analysis. Sensitivity analyses investigating the potential presence of such bias found larger increases in NT-proBNP among those younger than 65 years old at enrollment, both in a cross-sectional analysis at Exam 1 and in the repeated measures analysis, compared to those who were at least 65 years old at enrollment.

A second consideration and potential limitation of this analysis is that the year prior to blood marker measurement may not be the appropriate exposure period for air pollution effects on NT-proBNP. The year prior to the blood draw was chosen to reflect a long-term air pollution

exposure, but it may be that the most relevant exposure window to impact cardiac stress and NT-proBNP levels is longer than or occurs earlier than the year prior to the blood draw. A limitation of the current study is that the spatiotemporal model predictions do not extend back in time prior to the year 2000 and thus we cannot average exposure over a longer period prior to Exam 1. However, using only year 2000 exposures for all outcome measures is suggestive of a positive relationship between each of the four air pollutants and NT-proBNP.

The relevant exposure window was further explored in an analysis of elevated NT-proBNP at follow-up, given a normal NT-proBNP at baseline. Year 2000 exposures to NO_2 and BC are estimated to be associated with statistically significantly increased odds of experiencing an elevation of NT-proBNP level from less than 125 pg/mL at Exam 1 to greater than 125 pg/mL at Exam 3. Some increase in NT-proBNP might be expected as a result of regression to the mean after selecting those with low levels at baseline. However, it is unlikely that regression to the mean is responsible for all increases in NT-proBNP given that the mean increase in the elevated group was much greater than that in the normal group.

In contrast to the results using a cut-point of 125 pg/mL, estimates of the odds ratio defining NT-proBNP with a higher a cut-point of 400 pg/mL were not significant. Recent work has suggested that the common clinical cut-point of 400 pg/mL of NT-proBNP may be too strict and that a cut-point of 125 pg/mL is likely more appropriate to capture potential cases of HF, particularly HF with preserved ejection fraction.[19] This lower cut-point likely includes a population with less severe disease who may be less likely to have clinical symptoms of heart failure. This population is of particular interest as these levels of NT-proBNP at the lower end of the non-normal spectrum may reflect pre-clinical increases in cardiac stress. These pre-clinical changes are of particular interest for investigating mechanistic pathways. The strong association

observed in this population may also be indicative of the mechanism by which these pollutants influence the disease pathway. With a lower cut-point, the group likely includes more participants with HF with preserved ejection fraction, which suggests an effect of air pollution on those pathways, including hypertension and cardiac remodeling.

The strong secular downward trends in air pollution may confound this analysis if time is not accounted for in the model. Without accounting for time (either by adjustment for age at baseline and days of follow-up or by adjustment for exam and age at exam), the repeated measures model produced effect estimates in the unexpected direction. There is a strong association between time and the exposure, an association between time and the outcome even after adjustment for all of the other covariates, and a large difference in effect estimates when adjusting for exam. Time, as reflected in a binary variable for follow-up versus baseline exam, fulfills the definition of a confounder.

A final consideration in interpretation of this analysis is the large within-person variability reported in this biomarker. NT-proBNP may vary within an individual over even as short a period of time as one week, with prior studies indicating up to 25% intra-individual variation.[70,71] This large variability may make it more difficult to detect associations with air pollution.

Prior literature investigating effects of long-term air pollution exposures on blood markers has focused on other markers, primarily CRP and fibrinogen.[12,13] While both PTX3 and SAP are proteins in the same family as CRP and are hypothesized to play a causal role in the inflammatory pathway causing CVD, they may each reflect slightly different components of this process. SAP has not been well-characterized with regards to CVD and the association between $PM_{2.5}$ and SAP is a novel finding that requires further confirmation.

A major strength of this analysis was the use of a sophisticated and well-validated spatiotemporal air pollution models to calculate long-term exposures. The prospective cohort study design and collection of extensive covariate information on participants from several racial/ethnic groups at baseline and follow-up are also strengths of this analysis, as is the large sample size. Additionally, all samples for a given biomarker were measured at the same laboratory.

This study also had several limitations. Generalizability of this study may be limited as participants were required to be free of clinical CVD at recruitment into the study. The association between air pollution and these biomarkers may vary among those with a history of heart disease. Large within-person variability in biomarkers such as NT-proBNP may influence the results or the interpretability of the results. Measures of PTX3 and SAP were only available at the baseline exam and thus were limited to cross-sectional analyses.

This analysis contributes to the literature investigating the potential mechanism by which air pollution causes CVD and could be expanded with future analyses. It should be noted that this is the first study to examine the association between long-term exposure and these biomarkers. Within the MESA Air cohort, planned future research may expand upon the results here. The planned addition of NT-proBNP measures at third and fourth follow-up time-points may allow for additional investigation of trends over time in NT-proBNP and the effect of air pollution on trajectories of this blood marker. Other future research may include examining the effects of pollutant mixtures on these biomarkers, assessing longer-term exposure windows, and replicating these findings in another cohort.

ACKNOWLEDGEMENTS

This publication was developed under a STAR research assistance agreement, No. RD831697 (MESA Air), awarded by the U.S. Environmental Protection Agency. It has not been formally reviewed by the EPA. The views expressed in this document are solely those of the authors and the EPA does not endorse any products or commercial services mentioned in this publication. Support for the main MESA study includes contracts NO1-HC-95159, NO1-HC-95160, NO1-HC-95161, NO1-HC-95162, NO1-HC-95163, NO1-HC-95164, NO1-HC-95165, and NO1-HC-95166 from the National Heart, Lung, and Blood Institute. Biomarker measurement was funded by Roche Diagnostics (for NT-proBNP) and by the American Heart Association AHA 0430032N (for PTX3 and SAP). We thank the other investigators, the staff, and the participants of the MESA study for their valuable contributions. A full list of participating MESA investigators and institutions can be found at http://www.mesa-nhlbi.org.

TABLES & FIGURES

Table 1. Demographics and CVD risk factors for participants with measures of PTX3 or SAP at Exam 1 or with measures of NT-proBNP at Exams 1 and/or 3, compared to the entire MESA Air cohort at Exam 1.

	PTX3 and/or SAP		**NT-proBNP**				**MESA Air cohort**	
	Exam 1 (n=2,878)		**Exam 1** (n=5,597)		**Exam 3** (n=4,694)		**Exam 1** (n=6,814)	
	n	*(%)*	*n*	*(%)*	*n*	*(%)*	*n*	*(%)*
Female	1,539	(53)	2,882	(51)	2,404	(51)	3,601	(53)
Race/Ethnicity								
White	728	(25)	2,217	(40)	1,939	(41)	2,622	(38)
Chinese	720	(25)	742	(13)	597	(13)	803	(12)
Black	713	(25)	1,353	(24)	1,112	(24)	1,893	(28)
Hispanic	717	(25)	1,285	(23)	1,046	(22)	1,496	(22)
Education[1]								
Some college	800	(28)	1,581	(28)	1,351	(29)	1,937	(28)
College graduate	926	(32)	1,971	(35)	1,743	(37)	2,393	(35)
Smoking[2]								
Current	392	(14)	700	(13)	464	(10)	887	(13)
Former	918	(32)	2,055	(37)	2,089	(45)	2,487	(36)
Diabetes[3]								
Impaired fasting glucose	417	(15)	799	(14)	727	(15)	939	(14)
Untreated/treated diabetes	379	(13)	697	(12)	712	(15)	859	(13)
Alcohol use[4]	1,436	(50)	3,094	(55)	2,685	(57)	3,749	(55)
	mean	*(SD)*	*mean*	*(SD)*	*mean*	*(SD)*	*mean*	*(SD)*
Age (yrs)	62	(10)	62	(10)	65	(10)	62	(10)
Income ($1,000/yr)	47	(37)	50	(37)	52	(37)	51	(37)
Pack-years of smoking	10	(20)	11	(21)	11	(21)	11	(21)
Intentional exercise[5]	1,454	(2,143)	1,548	(2,277)	1,485	(2,061)	1,553	(2,340)
BMI (kg/m^2)	27.9	(5.5)	28.2	(5.5)	28.3	(5.5)	28.3	(5.5)
Waist-to-hip ratio	0.93	(0.08)	0.93	(0.08)	0.94	(0.08)	0.93	(0.08)
Total cholesterol (mg/dL)	194	(35)	194	(36)	188	(36)	194	(36)
LDL (mg/dL)	117	(31)	117	(31)	111	(32)	117	(31)
SBP (mmHg)	126	(21)	126	(21)	123	(20)	127	(21)

[1]Group not shown is high school or less.
[2]Group not shown is never smokers.
[3]Group not shown is normal fasting glucose.
[4]Yes or no at baseline.
[5]Measured in MET-in/wk.
MET=metabolic equivalent. LDL= low-density lipoprotein. SBP=systolic blood pressure.

Table 2. Number of participants and mean (standard deviation) of short- and long-term air pollution concentrations and blood markers for participants with measures of PTX3 or SAP at Exam 1 or with measures of NT-proBNP at Exams 1 and/or 3.

	PTX3 and/or SAP			**NT-proBNP**					
	Exam 1 (n=2,878)			**Exam 1** (n=5,597)			**Exam 3** (n=4,694)		
Long-term air pollution concentrations [a]	*n*	*mean*	*(SD)*	*n*	*mean*	*(SD)*	*n*	*mean*	*(SD)*
$PM_{2.5}$ (μg/m^3)	2,750	17.3	(3.7)	5,350	16.8	(3.6)	4,532	14.7	(3.2)
NO_x (ppb)	2,787	54.2	(27.7)	5,429	50.8	(27.2)	4,529	41.0	(22.1)
NO_2 (ppb)	2,787	23.1	(9.3)	5,429	21.8	(9.1)	4,529	19.5	(8.3)
BC (10^{-5} m^{-1})	2,795	0.88	(0.37)	5,442	0.83	(0.37)	4,541	0.81	(0.37)
Pre-adjusted short-term $PM_{2.5}$ (μg/m^3) [b]	*n*	*mean*	*(SD)*	*n*	*mean*	*(SD)*	*n*	*mean*	*(SD)*
Day of exam	2,232	17.9	(9.2)	4,219	17.7	(9.4)	3,593	15.4	(7.6)
Day prior to exam	2,159	17.9	(8.5)	4,076	17.6	(8.6)	3,495	15.3	(6.9)
2-day average	2,159	18.0	(8.2)	4,076	17.7	(8.3)	3,495	15.3	(6.6)
3-day average	2,159	18.0	(7.9)	4,076	17.7	(8.0)	3,495	15.3	(6.4)
4-day average	2,683	10.9	(8.4)	5,251	10.3	(8.6)	4,094	9.7	(7.0)
5-day average	2,730	8.5	(7.6)	5,342	8.0	(7.6)	4,345	7.2	(6.6)
Blood markers [c]	*n*	*mean*	*(SD)*	*n*	*mean*	*(SD)*	*n*	*mean*	*(SD)*
NT-proBNP (pg/mL)	-	-	-	5,597	102.5	(250.0)	4,694	137.3	(469.8)
NT-proBNP (GM) [d]	-	-	-	5,597	49.4	(3.1)	4,694	62.1	(2.9)
PTX3 (ng/mL)	2,838	2.2	(1.4)	-	-	-	-	-	-
PTX3 (GM) [d]	2,838	1.9	(1.6)	-	-	-	-	-	-
SAP (μg/mL)	2,863	47.0	(18.6)	-	-	-	-	-	-
SAP (GM) [d]	2,863	43.8	(1.5)	-	-	-	-	-	-

[a]Annual average for the year prior to the exam.
[b]Short-term $PM_{2.5}$ concentrations have been pre-adjusted for calendar time, temperature, relative humidity, and day of the week to account for temporal confounding.
[c]High outliers were excluded: NT-proBNP ≥10,000 pg/mL (n=2), PTX3 ≥10 ng/mL (n=7), or SAP ≥120 μg/mL (n=11).
[d]GM = geometric mean.

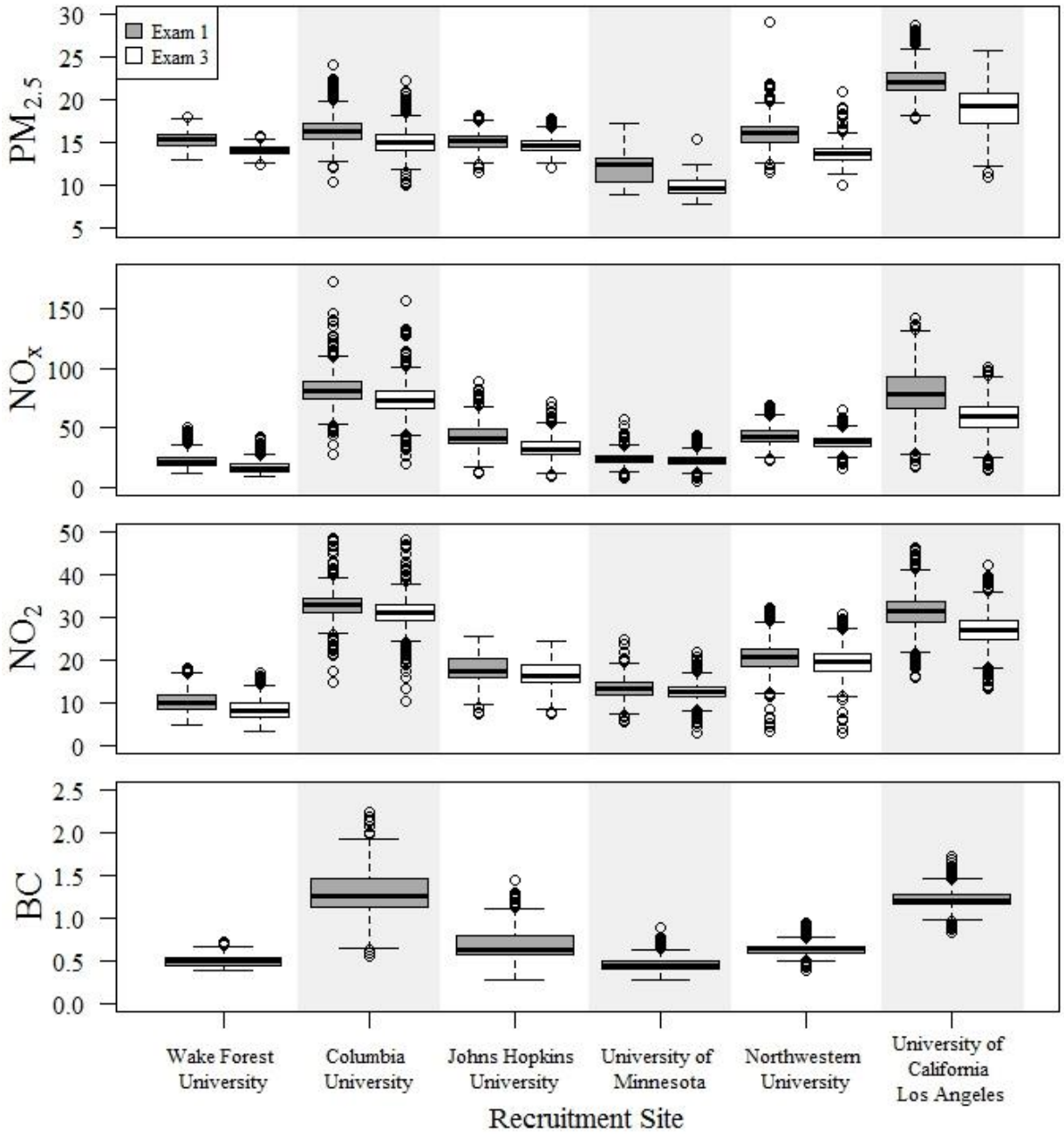

Figure 1. Boxplots of air pollution exposures for the repeated measures analysis of NT-proBNP by site and by exam. Exposures for each pollutant—$PM_{2.5}$ in $\mu g/m^3$, NO_x in ppb, NO_2 in ppb, and BC in 10^{-5} m^{-1} —were calculated for the year prior to the date of the clinical exam at which the blood draw was done. Note that BC exposures are derived from a spatial-only model and thus exposure concentrations are shown for Exam 1 only. Boxes extend from the 25th to the 75th percentile, horizontal bars show the median, whiskers show 1.5 times the interquartile range below and above the 25th and 75th percentiles, respectively, and outliers are shown as points.

Table 3. Percent difference (95% confidence interval) in NT-proBNP associated with demographic characteristics and established cardiovascular disease risk factors in a repeated measures analysis using a minimally-adjusted model.[a]

CVD Risk Factor	NT-proBNP % Difference (95% CI)	
Age[b]	30.7	(29.2, 32.2)
Female	72.1	(64.2, 80.3)
Race/ethnicity[c]		
Chinese	-35.2	(-40.6, -29.3)
Black	-33.5	(-37.6, -29.3)
Hispanic	-19.0	(-24.7, -13.0)
BMI (kg/m^2)[d]		
25.0-29.9	-15.5	(-19.6, -11.2)
≥ 30.0	-21.9	(-26.3, -17.3)
Systolic blood pressure[e]	25.8	(22.3, 29.4)
Hypertension	25.2	(20.1, 30.6)
Total cholesterol[f]	-13.6	(-15.6, -11.6)
LDL[g]	-13.0	(-15.0, -11.0)
Diabetes		
Impaired fasting glucose	-15.1	(-19.2, -10.8)
Treated/Untreated diabetes	-6.2	(-11.9, -0.1)
Impaired kidney function[h]	23.2	(16.6, 30.0)
CVD-related medication use[i]	11.7	(7.4, 16.3)
Exam[j]	11.8	(9.0, 14.6)
Exam[k]	14.7	(11.8, 17.7)

[a]Model adjusted for age at exam, race/ethnicity, sex, site, and exam.
[b]For a 5-year increase in age.
[c]Reference group is white participants
[d]Reference group is 18.5–25.0 kg/m^2. Results not shown for participants <18.5 kg/m^2.
[e]For an increase of 30 mmHg.
[f]For an increase of 45 mg/dL.
[g]For an increase of 40 mg/dL.
[h]Defined as having an eGFR <60 mL/min.
[i]CVD-related medications, including lipid-lowering medications, hypertension medications, β-blockers, or diuretics.
[j]Referent group is exam 1.
[k]Fully-adjusted model included age at exam, race/ethnicity, sex, site, education, income, smoking, alcohol consumption, physical activity, neighborhood SES, BMI, waist-to-hip ratio, total cholesterol, LDL, diabetes, systolic blood pressure, impaired kidney function, and CVD-related medication use.

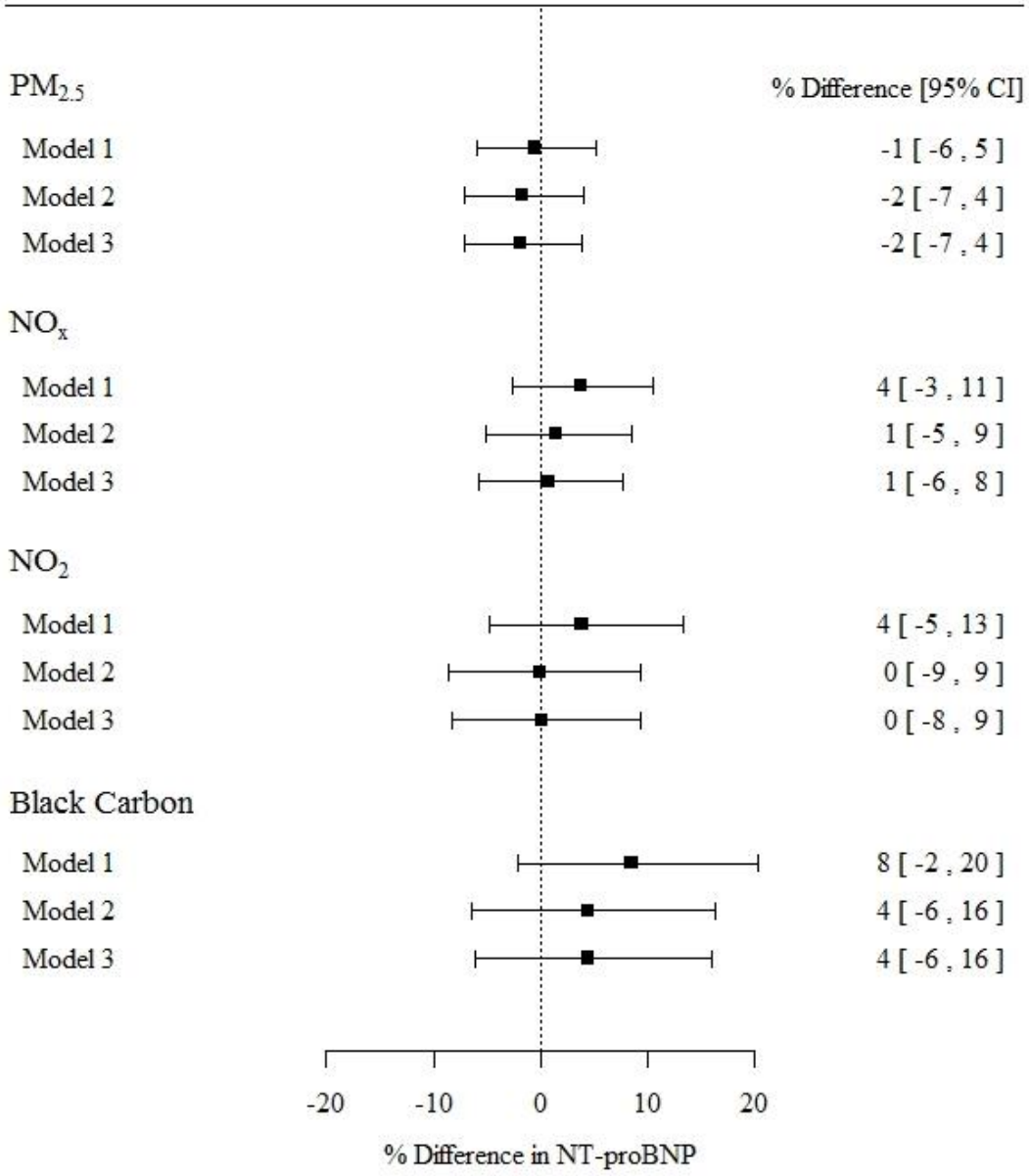

Figure 2. Percent difference (■) in NT-proBNP level associated with increases in air pollution. Whiskers show 95% confidence intervals. Estimates are shown for a 5 $\mu g/m^3$ increase in $PM_{2.5}$, a 40 ppb increase in NO_x, a 15 ppb increase in NO_2, and a 0.7 10^{-5} m^{-1} increase in BC. Air pollution exposures are for the year prior to the exam at which the blood was drawn. Model 1 is adjusted for age at exam, race/ethnicity, sex, site, and exam. Model 2 includes the covariates in model 1 as well as education, income, smoking, alcohol use, physical activity, neighborhood SES, BMI, and waist-hip ratio. Model 3 was further adjusted for SBP, total cholesterol, LDL, diabetes, impaired kidney function, and CVD-related medication use.

Table 4. Percent difference (95% confidence interval) in NT-proBNP associated with $PM_{2.5}$, NO_x, NO_2, and BC, by recruitment site, sex, race/ethnicity, age at study enrollment, or BMI.

	% Difference (95% CI) in NT-proBNP							
	$PM_{2.5}$ (per 5 μg/m^3)		**NO_x** (per 40 ppb)		**NO_2** (per 15 ppb)		**BC** (per 0.7 10^{-5} m^{-1})	
Recruitment site[a,b]								
WFU	5.4	(-14.6, 30.0)	1.1	(-27.6, 41.1)	-8.0	(-32.8, 26.0)	-30.1	(-64.3, 37.1)
COL	1.0	(-13.4, 17.7)	3.4	(-10.2, 19.0)	-5.3	(-23.1, 16.6)	-3.5	(-17.4, 12.8)
JHU	1.3	(-18.8, 26.3)	17.1	(-1.3, 38.8)	43.6	(11.6, 84.8)	20.3	(-1.2, 46.4)
UMN	-5.2	(-16.4, 7.5)	-10.3	(-39.1, 32.1)	-11.7	(-35.7, 21.2)	-20.3	(-50.5, 28.5)
NWU	4.0	(-6.6, 15.8)	7.5	(-14.8, 35.7)	5.4	(-13.8, 28.9)	-9.3	(-37.3, 31.2)
UCLA	-3.0	(-9.2, 3.6)	-1.2	(-8.7, 6.9)	-5.1	(-16.2, 7.4)	32.3	(-1.4, 77.4)
Sex[a]								
Male	-5.3	(-11.1, 0.8)	-0.3	(-7.7, 7.6)	-1.4	(-10.6, 8.7)	6.9	(-5.0, 20.4)
Female	2.5	(-3.8, 9.2)	3.2	(-4.3, 11.2)	1.4	(-8.1, 11.8)	2.3	(-9.0, 14.9)
Race/ethnicity[a]								
White	1.1	(-6.9, 9.7)	5.4	(-4.5, 16.4)	4.3	(-7.1, 17.1)	2.5	(-10.0, 16.6)
Chinese	-6.1	(-13.1, 1.4)	-13.1	(-22.8, -2.2)	-12.2	(-23.5, 0.8)	-13.8	(-28.9, 4.4)
Black	-0.1	(-9.4, 10.1)	10.1	(-0.3, 21.5)	10.7	(-3.0, 26.4)	10.8	(-4.6, 28.8)
Hispanic	0.8	(-6.2, 8.2)	2.6	(-5.9, 11.9)	4.6	(-7.5, 18.3)	11.4	(-2.8, 27.8)
Age[a]								
<65 years	-1.3	(-7.0, 4.6)	2.3	(-4.7, 9.9)	0.2	(-8.7, 9.9)	4.7	(-6.4, 17.1)
≥65 years	-1.8	(-7.3, 4.0)	0.5	(-6.6, 8.1)	-0.2	(-9.1, 9.5)	4.2	(-6.9, 16.7)
BMI (kg/m^2)[a]								
<18.5	-4.9	(-25.0, 20.6)	1.2	(-29.5, 45.2)	-4.7	(-34.0, 37.5)	-9.4	(-39.8, 36.4)
18.5-24.9	-0.2	(-6.8, 7.0)	1.3	(-6.9, 10.1)	-1.8	(-11.4, 8.9)	0.6	(-11.3, 14.0)
25.0-29.9	-0.6	(-6.8, 6.1)	3.6	(-4.1, 12.0)	3.1	(-6.6, 13.8)	9.3	(-2.8, 22.8)
>30.0	-5.0	(-11.5, 2.0)	-2.0	(-9.6, 6.2)	-4.0	(-13.6, 6.7)	0.4	(-11.3, 13.7)

[a]All models were adjusted for age, race/ethnicity, sex, recruitment site, exam, education, income, smoking, alcohol consumption, physical activity, neighborhood SES, BMI, and waist-to-hip ratio.
[b]WFU=Wake Forest University. COL=Columbia University. JHU=John's Hopkins University. UMN=University of Minnesota. NWU=Northwestern University. UCLA=University of California Los Angeles.

Table 5. Percent difference (95% confidence interval) in NT-proBNP associated with increases in $PM_{2.5}$, NO_x, NO_2, and BC in repeated measures sensitivity analyses.

	% Difference (95% confidence interval) in NT-proBNP							
	$PM_{2.5}$ (per 5 μg/m^3)[a]		**NO_x** (per 40 ppb)[a]		**NO_2** (per 15 ppb)[a]		**BC** (per 0.7 10^{-5} m^{-1})[a]	
Year 2000 and 2004 exposures[b]								
Model 1[c]	6.6	(-1.7, 15.6)	3.3	(-2.9, 9.8)	5.5	(-3.3, 15.2)	9.0	(-1.7, 20.7)
Model 2[d]	3.6	(-4.6, 12.4)	1.2	(-5.2, 7.9)	1.3	(-7.4, 10.9)	4.8	(-6.0, 16.8)
Model 3[e]	5.8	(-2.3, 14.6)	0.5	(-5.6, 7.1)	1.8	(-6.8, 11.2)	4.8	(-5.7, 16.5)
Year 2000 exposures[f]								
Model 1	14.9	(3.0, 28.1)	8.6	(1.3, 16.4)	12.1	(1.3, 24.1)	10.8	(-0.6, 23.6)
Model 2	11.6	(0.0, 24.6)	5.9	(-1.8, 14.3)	7.4	(-3.5, 19.4)	7.3	(-4.5, 20.6)
Model 3	9.3	(-1.8, 21.6)	4.8	(-2.6, 12.8)	7.6	(-3.0, 19.3)	6.5	(-4.9, 19.2)
Adjusting for age at baseline and days of follow-up								
Model 1	-0.6	(-6.0, 5.1)	3.6	(-2.8, 10.5)	3.9	(-4.8, 13.3)	9.0	(-1.6, 20.8)
Model 2	-1.6	(-7.0, 4.1)	1.8	(-4.9, 8.9)	0.1	(-8.5, 9.5)	4.4	(-6.3, 16.4)
Model 3	-1.6	(-6.9, 4.0)	1.2	(-5.3, 8.1)	0.4	(-8.0, 9.6)	4.4	(-6.0, 16.1)
Without adjustment for exam								
Model 1	-12.0	(-15.7, -8.2)	-8.9	(-13.9, -3.7)	-10.9	(-17.7, -3.5)	7.1	(-3.4, 18.7)
Model 2	-13.2	(-16.9, -9.3)	-11.8	(-16.8, -6.4)	-14.6	(-21.3, -7.3)	3.4	(-7.3, 15.4)
Model 3	-14.2	(-17.8, -10.5)	-13.0	(-17.9, -7.7)	-15.3	(-21.8, -8.2)	3.0	(-7.4, 14.5)
Without adjustment for recruitment site								
Model 1	-1.1	(-4.4, 2.3)	-0.6	(-4.5, 3.4)	-1.5	(-5.8, 3.0)	-0.4	(-5.2, 4.8)
Model 2	-1.8	(-5.1, 1.6)	-1.8	(-5.7, 2.3)	-2.6	(-6.9, 1.9)	-2.1	(-7.0, 3.0)
Model 3	-2.0	(-5.2, 1.3)	-0.2	(-4.0, 3.8)	0.1	(-4.2, 4.6)	-0.5	(-5.3, 4.6)
Adjusting for season of blood draw[g]								
Model 1	-1.0	(-6.4, 4.7)	3.2	(-3.2, 10.0)	3.8	(-4.8, 13.2)	9.1	(-1.5, 20.9)
Model 2	-2.1	(-7.5, 3.6)	0.8	(-5.8, 7.8)	-0.1	(-8.7, 9.2)	4.9	(-5.9, 16.9)
Model 3	-2.5	(-7.7, 3.1)	0.0	(-6.4, 6.8)	0.0	(-8.3, 9.1)	4.9	(-5.6, 16.6)
Excluding participants with AF or HF[h]								
Model 1	-0.1	(-5.6, 5.6)	2.1	(-4.2, 8.8)	2.0	(-6.5, 11.1)	8.5	(-2.0, 20.2)
Model 2	-1.1	(-6.6, 4.6)	0.1	(-6.4, 7.0)	-1.6	(-9.9, 7.6)	4.9	(-5.8, 16.7)
Model 3	-1.8	(-7.1, 3.8)	-0.5	(-6.8, 6.3)	-1.3	(-9.5, 7.7)	4.6	(-5.8, 16.1)
Exposures individually weighted using infiltration and time-location patterns								
Model 1	-1.9	(-7.8, 4.3)	-		-		-	
Model 2	-4.2	(-10.1, 2.0)	-		-		-	
Model 3	-3.9	(-9.7, 2.2)	-		-		-	

[a]Unless otherwise specified, air pollution exposures are annual averages for the year prior to the exam at which NT-proBNP was measured, weighted by residential history.
[b]Annual averages of air pollution for the year 2000 were used as the exposure for measures at Exam 1 and annual averages of air pollution for the year 2004 were used as the exposure for measures at Exam 3.
[c]Model 1 was adjusted for age at exam, sex, race/ethnicity, site, and exam.
[d]Model 2 was adjusted for the covariates in model 1, as well as education, income, smoking, alcohol consumption, physical activity, neighborhood SES, BMI, and waist-to-hip ratio.
[e]Model 3 was adjusted for all of the covariates in models 1 and 2, as well as total cholesterol, LDL, diabetes, systolic blood pressure, impaired kidney function, and CVD-related medication use.
[f]Annual averages of air pollution for the year 2000 were used as the exposure for participants both at Exam 1 and Exam 3.
[g]Season was defined as four categories: January – March, April – June, July – September, and October – December.
[h]8% of participants were excluded due to a diagnosis of AF and 4% of participants were excluded due to a diagnosis of HF.

Table 6. Absolute difference (95% confidence interval) in NT-proBNP (in pg/mL) associated with increased $PM_{2.5}$, NO_x, NO_2, and BC, in a repeated measures analysis.

	Absolute Difference (95% CI) in NT-proBNP (in pg/mL)							
	$PM_{2.5}$ (per 5 $\mu g/m^3$)[a]		**NO_x** (per 40 ppb)[a]		**NO_2** (per 15 ppb)[a]		**BC** (per 0.7 10^{-5} m^{-1})[a]	
NT-proBNP modeled on the natural scale								
Model 1[b]	10.7	(-14.2, 35.5)	19.7	(-4.8, 44.2)	16.8	(-16.3, 49.8)	4.7	(-32.2, 41.6)
Model 2[c]	4.0	(-21.2, 29.3)	16.2	(-10.2, 42.6)	8.8	(-25.7, 43.3)	-5.6	(-44.9, 33.8)
Model 3[d]	4.4	(-21.0, 29.7)	18.3	(-8.2, 44.8)	11.1	(-23.4, 45.5)	-2.9	(-42.0, 36.2)

[a]Air pollution exposures are annual averages for the year prior to the exam at which NT-proBNP was measured, weighted by residential history.
[b]Model 1 was adjusted for age at exam, sex, race/ethnicity, site, and exam.
[c]Model 2 was adjusted for the covariates in model 1, as well as education, income, smoking, alcohol consumption, physical activity, neighborhood SES, BMI, and waist-to-hip ratio.
[d]Model 3 was adjusted for all of the covariates in models 1 and 2, as well as total cholesterol, LDL, diabetes, systolic blood pressure, impaired kidney function, and CVD-related medication use.

Table 7. Percent difference (95% confidence interval) in NT-proBNP associated with increased $PM_{2.5}$, NO_x, NO_2, and BC, in cross-sectional analyses at Exam 1 and Exam 3.

	% Difference (95% CI) in NT-proBNP							
	$PM_{2.5}$ (per 5 µg/m^3)[a]		**NO_x** (per 40 ppb)[a]		**NO_2** (per 15 ppb)[a]		**BC** (per 0.7 10^{-5} m^{-1})[a]	
Exam 1[b]								
Model 1[d]	10.4	(0.4, 21.4)	7.8	(-0.4, 16.6)	8.8	(-2.6, 21.6)	10.2	(-2.4, 24.5)
Model 2[e]	7.7	(-2.2, 18.5)	5.1	(-3.7, 14.6)	3.9	(-7.6, 16.7)	6.4	(-6.6, 21.2)
Model 3[f]	6.2	(-3.4, 16.7)	4.2	(-4.3, 13.5)	6.2	(-5.3, 19.0)	5.9	(-6.7, 20.3)
<65 years old at recruitment [b]								
Model 1	17.7	(2.4, 35.3)	15.2	(3.7, 27.9)	12.2	(-3.5, 30.5)	12.9	(-4.9, 34.0)
Model 2	12.3	(-2.6, 29.6)	11.1	(-1.1, 24.9)	3.3	(-12.1, 21.4)	6.1	(-11.8, 27.7)
Model 3	11.0	(-3.6, 27.9)	10.4	(-1.6, 23.9)	7.3	(-8.5, 25.8)	5.5	(-12.0, 26.6)
≥65 years old at recruitment [b]								
Model 1	3.3	(-9.9, 18.4)	-1.8	(-13.0, 10.8)	3.9	(-11.8, 22.5)	6.0	(-10.8, 25.9)
Model 2	3.6	(-9.7, 18.8)	-2.6	(-14.5, 11.0)	4.7	(-11.5, 24.1)	4.3	(-13.1, 25.2)
Model 3	2.4	(-10.4, 17.1)	-4.0	(-15.5, 9.1)	5.0	(-11.0, 23.9)	3.1	(-13.7, 23.2)
Not lost to follow-up [b]								
Model 1	12.5	(1.7, 24.6)	7.7	(-1.2, 17.4)	8.5	(-3.7, 22.2)	11.4	(-2.4, 27.2)
Model 2	9.6	(-1.1, 21.4)	4.2	(-5.2, 14.4)	3.0	(-9.1, 16.7)	8.1	(-6.0, 24.4)
Model 3	8.4	(-2.0, 19.9)	4.3	(-5.0, 14.4)	5.0	(-7.1, 18.8)	8.0	(-5.9, 23.9)
Exam 3 [c]								
Model 1[d]	-0.7	(-10.7, 10.4)	7.4	(-3.6, 19.5)	11.1	(-2.2, 26.1)	10.2	(-3.4, 25.7)
Model 2[e]	-2.2	(-12.1, 8.7)	2.5	(-8.6, 14.8)	4.7	(-8.2, 19.4)	4.8	(-8.7, 20.4)
Model 3[f]	-4.2	(-13.7, 6.4)	0.7	(-10.0, 12.6)	4.2	(-8.4, 18.5)	4.1	(-9.2, 19.2)
<65 years old at recruitment [c]								
Model 1	-0.1	(-13.4, 15.3)	10.7	(-3.5, 27.1)	6.4	(-9.7, 25.3)	10.6	(-7.1, 31.7)
Model 2	-2.7	(-15.7, 12.4)	3.9	(-10.4, 20.6)	-2.3	(-17.6, 15.8)	2.6	(-14.7, 23.4)
Model 3	-5.0	(-17.7, 9.7)	3.3	(-10.8, 19.7)	-1.1	(-16.5, 17.0)	3.9	(-13.4, 24.7)
≥65 years old at recruitment [c]								
Model 1	-4.5	(-19.4, 13.1)	2.4	(-13.7, 21.5)	16.5	(-4.8, 42.6)	9.6	(-10.5, 34.1)
Model 2	-4.8	(-19.7, 12.8)	0.1	(-16.3, 19.8)	14.7	(-6.7, 41.1)	7.5	(-12.9, 32.7)
Model 3	-7.0	(-21.1, 9.8)	-3.7	(-19.1, 14.6)	11.9	(-8.4, 36.7)	3.7	(-15.5, 27.2)

[a]Air pollution exposures are annual averages for the year prior to the exam at which NT-proBNP was measured.
[b]There were 5,597 participants included in the cross-sectional analyses at Exam 1: 3,109 participants younger than 65 years of age and 2,488 participants 65 years old or older. 4,592 of the participants at Exam 1 (82%) had a second measurement of NT-proBNP at follow-up.
[c]There were 4,694 participants included in the cross-sectional analyses at Exam 3: 2,709 participants who were younger than 65 years of age at baseline and 1,985 participants who were 65 years old or older at baseline.
[d]Model 1 was adjusted for age at follow-up exam, sex, race/ethnicity, site.
[e]Model 2 was adjusted for the covariates in model 1, as well as education, income, smoking, alcohol consumption, physical activity, neighborhood SES, BMI, and waist-to-hip ratio.
[f]Model 3 was adjusted for all of the covariates in models 1 and 2, as well as total cholesterol, LDL, diabetes, systolic blood pressure, impaired kidney function, and CVD-related medication use.

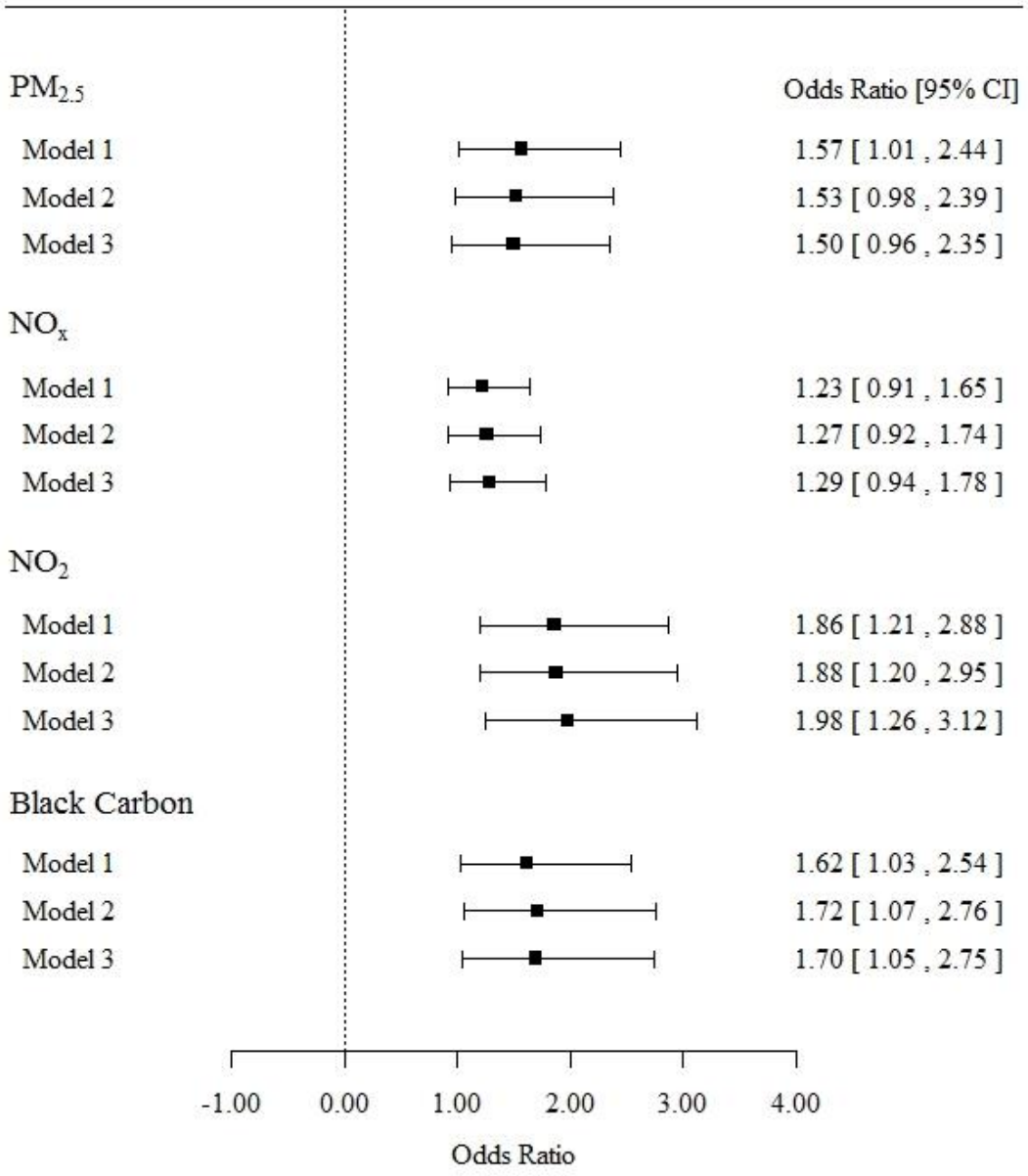

Figure 3. Odds ratios (■) for association of elevated NT-proBNP level with increases in air pollution. Whiskers show 95% confidence intervals. Estimates are shown for a 5 μg/m^3 increase in $PM_{2.5}$, a 40 ppb increase in NO_x, a 15 ppb increase in NO_2, and a 0.7 10^{-5} m^{-1} increase in BC. Air pollution exposures are for the year 2000. Model 1 is adjusted for age at baseline, time between baseline and the follow-up exam, race/ethnicity, sex, and site. Model 2 includes the covariates in model 1 as well as education, income, smoking, alcohol use, physical activity, neighborhood SES, BMI, and waist-hip ratio. Model 3 was further adjusted for SBP, total cholesterol, LDL, diabetes, impaired kidney function, and CVD-related medication use. This analysis included 3,652 participants with an NT-proBNP level lower than 125 pg/mL at baseline and of these participants, 509 had an elevated NT-proBNP at follow-up.

Table 8. Odds ratios (95% confidence interval) for elevated (defined here as ≥125 pg/mL) NT-proBNP associated with $PM_{2.5}$, NO_x, NO_2, and BC, by recruitment site, sex, race/ethnicity, age at study enrollment, or BMI.

	OR (95% CI) for elevated NT-proBNP							
	$PM_{2.5}$ (per 5 μg/m^3)		**NO_x** (per 40 ppb)		**NO_2** (per 15 ppb)		**BC** (per 0.7 10^{-5} m^{-1})	
Recruitment site[a,b]								
WFU	1.07	(0.13, 8.80)	1.68	(0.29, 9.57)	1.29	(0.29, 5.72)	2.32	(0.18, 30.1)
COL	1.14	(0.52, 2.50)	1.35	(0.72, 2.53)	1.58	(0.54, 4.65)	1.31	(0.66, 2.59)
JHU	10.9	(2.06, 57.3)	2.97	(1.22, 7.25)	4.29	(1.19, 15.5)	2.59	(1.08, 6.21)
UMN	1.29	(0.19, 8.61)	2.37	(0.39, 14.4)	2.68	(0.48, 14.9)	0.64	(0.06, 6.52)
NWU	1.82	(0.73, 4.51)	1.45	(0.43, 4.92)	1.88	(0.69, 5.07)	1.80	(0.33, 9.90)
UCLA	1.26	(0.52, 3.06)	0.99	(0.66, 1.50)	1.64	(0.80, 3.38)	2.29	(0.68, 7.75)
Sex[a]								
Female	1.48	(0.92, 2.38)	1.26	(0.90, 1.78)	1.86	(1.16, 2.99)	1.62	(0.98, 2.70)
Male	1.58	(0.98, 2.54)	1.28	(0.89, 1.82)	1.91	(1.18, 3.09)	1.83	(1.09, 3.06)
Race/ethnicity[a]								
White	1.46	(0.85, 2.53)	1.28	(0.84, 1.96)	1.62	(0.95, 2.77)	1.50	(0.86, 2.62)
Chinese	1.89	(0.95, 3.75)	1.54	(0.81, 2.94)	2.65	(1.25, 5.65)	2.57	(1.01, 6.55)
Black	1.45	(0.70, 2.99)	1.18	(0.76, 1.81)	1.67	(0.90, 3.11)	1.74	(0.92, 3.29)
Hispanic	1.42	(0.84, 2.40)	1.25	(0.83, 1.88)	1.85	(1.01, 3.38)	1.80	(1.00, 3.25)
Age[a]								
<65 years	1.48	(0.94, 2.32)	1.16	(0.82, 1.63)	1.74	(1.09, 2.77)	1.58	(0.96, 2.59)
≥65 years	1.55	(0.99, 2.42)	1.34	(0.97, 1.86)	1.99	(1.26, 3.14)	1.82	(1.12, 2.96)
BMI (kg/m^2)[a,c]								
<18.5	2.11	(1.22, 3.65)	2.83	(1.26, 6.33)	3.59	(1.62, 7.95)	3.75	(1.47, 9.53)
18.5-24.9	1.63	(1.03, 2.56)	1.43	(1.01, 2.03)	2.06	(1.29, 3.27)	1.89	(1.15, 3.13)
25.0-29.9	1.56	(1.00, 2.45)	1.35	(0.97, 1.88)	1.97	(1.24, 3.13)	1.83	(1.13, 2.98)
>30.0	1.39	(0.88, 2.19)	0.99	(0.68, 1.43)	1.46	(0.89, 2.40)	1.36	(0.81, 2.29)

[a]All models were adjusted for age at baseline, time between baseline and follow-up exam, race/ethnicity, sex, recruitment site, education, income, smoking, alcohol consumption, physical activity, neighborhood SES, BMI, and waist-to-hip ratio.

[b]WFU=Wake Forest University. COL=Columbia University. JHU=John's Hopkins University. UMN=University of Minnesota. NWU=Northwestern University. UCLA=University of California Los Angeles.

[c]P-values for the interaction between air pollution and BMI were 0.065, 0.013, 0.026, and 0.057 for $PM_{2.5}$, NO_x, NO_2, and BC, respectively.

Table 9. Odds ratios (95% confidence interval) for elevated (defined here as ≥400 pg/mL) NT-proBNP at Exam 3 associated with increases in $PM_{2.5}$, NO_x, NO_2, or BC, among those with an NT-proBNP level within the normal range at Exam 1.

	OR (95% CI) for elevated NT-proBNP							
	$PM_{2.5}$ (per 5 μg/m^3) [a]		**NO_x** (per 40 ppb) [a]		**NO_2** (per 15 ppb) [a]		**BC** (per 0.7 10^{-5} m^{-1}) [a]	
Elevated NT-proBNP defined as ≥400 pg/mL [b]								
Model 1[c]	1.85	(0.90, 3.78)	1.33	(0.80, 2.21)	1.49	(0.72, 3.11)	1.65	(0.82, 3.32)
Model 2[d]	2.04	(0.97, 4.31)	1.36	(0.79, 2.33)	1.47	(0.69, 3.13)	1.69	(0.81, 3.52)
Model 3[e]	1.90	(0.89, 4.08)	1.34	(0.78, 2.32)	1.52	(0.71, 3.30)	1.64	(0.77, 3.48)

[a]Air pollution exposures are for the year 2000.

[b]Elevated NT-proBNP defined as NT-proBNP ≥400 pg/mL. 4,484 participants with an NT-proBNP level lower than 400 pg/mL at baseline were included in this analysis; 168 of those participants had an elevated NT-proBNP level at follow-up.

[c]Model 1 was adjusted for age at baseline, time between baseline and the follow-up exam, sex, race/ethnicity, and site.

[d]Model 2 was adjusted for the covariates in model 1, as well as education, income, smoking, alcohol use, physical activity, neighborhood-SES index, BMI, and waist-to-hip ratio.

[e]Model 3 was adjusted for all of the covariates in models 1 and 2, as well as total cholesterol, LDL, diabetes, systolic blood pressure, impaired renal function, and CVD-related medication use.

Table 10. Percent difference (95% confidence interval) in NT-proBNP associated with 5 μg/m^3 increases in short-term $PM_{2.5}$ exposure.

	% Difference (95% CI) in NT-proBNP	
Day of exam		
Model 1[b]	0.4	(-0.6, 1.5)
Model 2[c]	0.2	(-0.8, 1.3)
Model 3[d]	0.2	(-0.8, 1.3)
Day prior to exam		
Model 1	0.8	(-0.3, 2.0)
Model 2	0.7	(-0.5, 1.8)
Model 3	0.5	(-0.6, 1.7)
2-day average		
Model 1	1.0	(-0.2, 2.2)
Model 2	0.9	(-0.4, 2.1)
Model 3	0.7	(-0.5, 1.9)
3-day average		
Model 1	1.2	(-0.1, 2.4)
Model 2	1.0	(-0.2, 2.3)
Model 3	0.8	(-0.4, 2.1)
4-day average		
Model 1	0.8	(-0.4, 2.0)
Model 2	0.6	(-0.5, 1.8)
Model 3	0.4	(-0.7, 1.6)
5-day average		
Model 1	0.6	(-0.6, 1.9)
Model 2	0.5	(-0.8, 1.7)
Model 3	0.3	(-0.9, 1.5)

[a]Short-term $PM_{2.5}$ concentrations have been pre-adjusted for calendar time, temperature, relative humidity, and day of the week to account for confounding.
[b]Model 1 was adjusted for age at exam, sex, race/ethnicity, site, and exam.
[c]Model 2 was adjusted for the covariates in model 1, as well as education, income, smoking, alcohol consumption, physical activity, neighborhood SES, BMI, and waist-to-hip ratio.
[d]Model 3 was adjusted for all of the covariates in models 1 and 2, as well as total cholesterol, LDL, diabetes, systolic blood pressure, impaired kidney function, and CVD-related medication use.

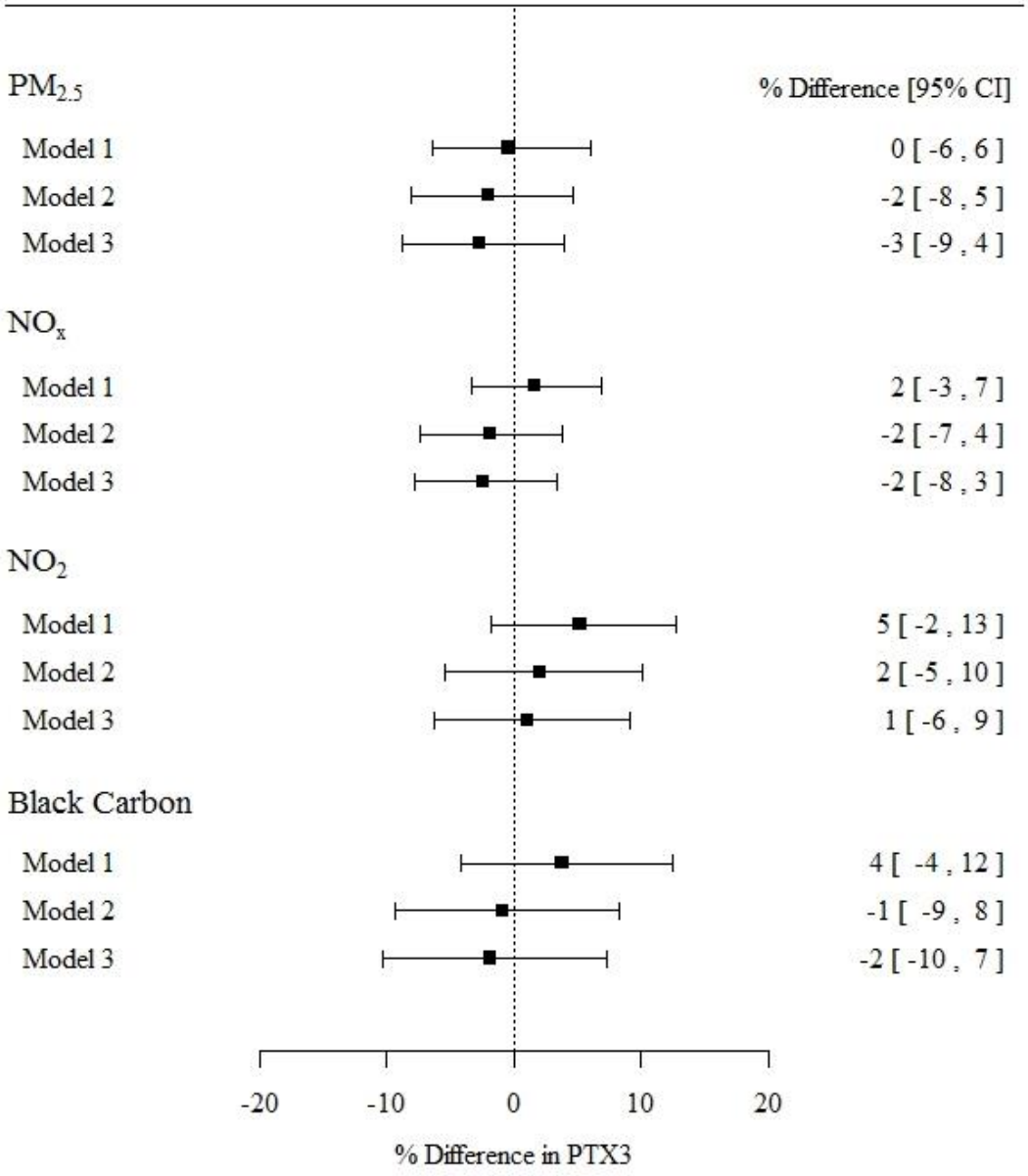

Figure 4. Percent difference (■) in PTX3 levels associated with long-term air pollution exposure in a cross-sectional analysis. Whiskers show 95% confidence intervals. Results are shown for a 5 $\mu g/m^3$ increase in $PM_{2.5}$, a 40 ppb increase in NO_x, a 15 ppb increase in NO_2, and a 0.7 10^{-5} m^{-1} increase in black carbon (BC). Air pollution exposures are for the year prior to the exam at which the blood was drawn. Model 1 is adjusted for age at exam, race/ethnicity, sex, and site. Model 2 includes the covariates in model 1 as well as education, income, smoking, alcohol use, physical activity, neighborhood-level SES, BMI, and waist-hip ratio. Model 3 was further adjusted for SBP, total cholesterol, LDL, and diabetes.

Table 11. Percent difference (95% confidence interval) in PTX3 associated with long-term $PM_{2.5}$, NO_x, NO_2, and BC exposure in cross-sectional sensitivity analyses.

	% Difference (95% CI) in PTX3							
	$PM_{2.5}$ (per 5 μg/m^3) [a]		**NO_x** (per 40 ppb) [a]		**NO_2** (per 15 ppb) [a]		**BC** (per 0.7 10^{-5} m^{-1}) [a]	
Year 2000 exposures[b]								
Model 1[c]	1.6	(-6.2, 10.0)	0.3	(-4.5, 5.4)	4.1	(-3.0, 11.8)	2.8	(-5.2, 11.4)
Model 2[d]	-1.1	(-9.0, 7.4)	-3.1	(-8.4, 2.4)	0.8	(-6.7, 8.9)	-1.6	(-10.0, 7.5)
Model 3[e]	-2.1	(-9.9, 6.4)	-3.6	(-8.8, 2.0)	-0.3	(-7.8, 7.7)	-2.6	(-11.0, 6.5)
Adjusted for C-reactive protein (CRP) level								
Model 1	-0.6	(-6.6, 5.8)	1.2	(-3.7, 6.4)	4.7	(-2.3, 12.2)	3.4	(-4.6, 12.0)
Model 2	-1.9	(-8.0, 4.7)	-2.2	(-7.6, 3.5)	1.9	(-5.5, 9.9)	-0.9	(-9.3, 8.2)
Model 3	-2.6	(-8.8, 3.9)	-2.8	(-8.3, 2.9)	0.9	(-6.5, 8.9)	-1.9	(-10.2, 7.2)
Without adjustment for site								
Model 1	-1.1	(-3.7, 1.5)	6.4	(3.5, 9.3)	9.4	(6.1, 12.9)	9.1	(5.2, 13.0)
Model 2	-1.9	(-4.6, 0.8)	5.5	(2.4, 8.6)	8.6	(5.1, 12.2)	7.9	(3.9, 12.1)
Model 3	-1.9	(-4.5, 0.9)	5.7	(2.6, 8.9)	8.9	(5.3, 12.6)	8.1	(4.0, 12.3)
Exposures individually weighted using infiltration and time-location patterns								
Model 1	0.4	(-1.5, 2.3)	--		--		--	
Model 2	-0.2	(-2.2, 1.8)	--		--		--	
Model 3	-0.4	(-2.4, 1.6)	--		--		--	

[a]Exposures are annual averages of air pollution over the year prior to the date of the exam, unless otherwise specified.
[b]Exposures are annual averages of air pollution during the year 2000.
[c]Model 1 was adjusted for age at exam, sex, race/ethnicity, site.
[d]Model 2 was adjusted for the covariates in model 1, as well as education, income, smoking, alcohol consumption, physical activity, neighborhood SES, BMI, and waist-to-hip ratio.
[e]Model 3 was adjusted for all of the covariates in models 1 and 2, as well as total cholesterol, LDL, diabetes, and systolic blood pressure.

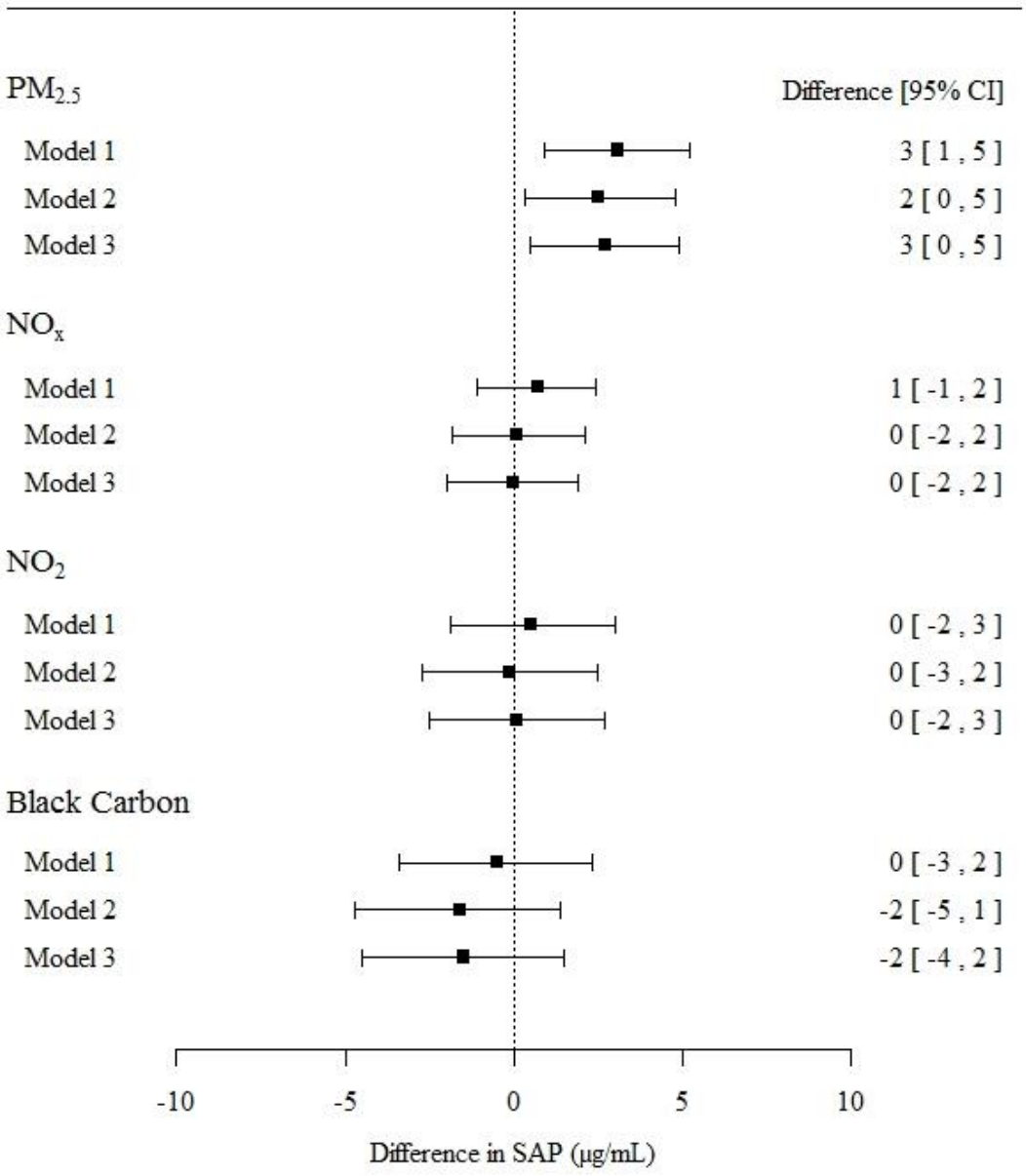

Figure 5. Absolute difference (■) in SAP levels (in µg/mL) associated increased annual average air pollution concentrations. Whiskers show 95% confidence intervals. Results are shown for a 5 $\mu g/m^3$ increase in $PM_{2.5}$, a 40 ppb increase in NO_x, a 15 ppb increase in NO_2, and a 0.7 10^{-5} m^{-1} increase in black carbon (BC). Air pollution exposures are for the year prior to the exam at which the blood was drawn. Model 1 is adjusted for age at exam, race/ethnicity, sex, and site. Model 2 includes the covariates in model 1 as well as education, income, smoking, alcohol use, physical activity, neighborhood-level SES, BMI, and waist-hip ratio. Model 3 was further adjusted for SBP, total cholesterol, LDL, and diabetes.

Table 12. Absolute difference (95% confidence interval) in µg/mL of SAP associated with long-term exposure to $PM_{2.5}$, NO_x, NO_2, and BC in cross-sectional sensitivity analyses.

	Difference (95% CI) in SAP							
	$PM_{2.5}$ (per 5 µg/m^3) [a]		**NO_x** (per 40 ppb) [a]		**NO_2** (per 15 ppb) [a]		**BC** (per 0.7 10^{-5} m^{-1}) [a]	
Year 2000 exposure estimates[b]								
Model 1[c]	-0.3	(-3.1, 2.5)	-0.5	(-2.2, 1.2)	-0.3	(-2.8, 2.2)	-0.5	(-3.4, 2.3)
Model 2[d]	-1.2	(-4.1, 1.6)	-1.3	(-3.2, 0.6)	-1.2	(-3.9, 1.4)	-1.7	(-4.7, 1.4)
Model 3[e]	-0.7	(-3.5, 2.2)	-1.3	(-3.2, 0.6)	-0.9	(-3.5, 1.7)	-1.5	(-4.6, 1.5)
Adjusted for C-reactive protein (CRP) level								
Model 1	3.2	(1.0, 5.3)	0.2	(-1.5, 1.9)	0.2	(-2.2, 2.5)	-1.0	(-3.8, 1.7)
Model 2	2.9	(0.7, 5.1)	-0.1	(-2.1, 1.8)	-0.2	(-2.8, 2.3)	-1.7	(-4.7, 1.2)
Model 3	2.9	(0.8, 5.1)	-0.3	(-2.2, 1.6)	0.0	(-2.5, 2.5)	-1.6	(-4.6, 1.3)
Without adjustment for site								
Model 1	0.9	(-0.1, 1.9)	0.4	(-0.6, 1.4)	0.4	(-0.7, 1.6)	0.2	(-1.0, 1.5)
Model 2	1.1	(0.2, 2.0)	0.8	(-0.2, 1.8)	0.9	(-0.2, 2.0)	0.6	(-0.7, 1.9)
Model 3	1.0	(0.0, 1.9)	0.7	(-0.3, 1.7)	0.9	(-0.2, 2.0)	0.6	(-0.7, 1.9)
Exposures individually weighted using infiltration and time-location patterns								
Model 1	0.4	(-1.5, 2.3)	--		--		--	
Model 2	-0.2	(-2.2, 1.8)	--		--		--	
Model 3	-0.4	(-2.4, 1.6)	--		--		--	

[a]Exposure estimates are annual averages for the year prior to the exam, unless otherwise specified.
[b]Exposures are annual averages of air pollution during the year 2000.
[c]Model 1 was adjusted for age at exam, sex, race/ethnicity, site.
[d]Model 2 was adjusted for the covariates in model 1, as well as education, income, smoking, alcohol consumption, physical activity, neighborhood SES, BMI, and waist-to-hip ratio.
[e]Model 3 was adjusted for all of the covariates in models 1 and 2, as well as total cholesterol, LDL, diabetes, and systolic blood pressure.

Table 13. Percent difference in PTX3 and absolute difference (in µg/mL) in SAP and 95% confidence intervals, associated with a 5 µg/m^3 increase in pre-adjusted short-term $PM_{2.5}$ exposure.[a]

	PTX3		SAP	
	% Difference (95% CI)		Absolute difference (95% CI)	
Day of exam				
Model 1[b]	-0.8	(-1.8, 0.3)	0.0	(-0.4, 0.4)
Model 2[c]	-0.8	(-1.8, 0.3)	0.1	(-0.3, 0.4)
Model 3[d]	-0.8	(-1.9, 0.3)	0.1	(-0.3, 0.4)
Day prior to exam				
Model 1	-0.6	(-1.8, 0.6)	0.0	(-0.5, 0.4)
Model 2	-0.6	(-1.8, 0.6)	0.0	(-0.4, 0.4)
Model 3	-0.7	(-1.9, 0.6)	0.1	(-0.3, 0.5)
2-day average				
Model 1	-0.5	(-1.8, 0.7)	0.0	(-0.4, 0.4)
Model 2	-0.5	(-1.8, 0.8)	0.1	(-0.3, 0.5)
Model 3	-0.6	(-1.9, 0.7)	0.1	(-0.3, 0.5)
3-day average				
Model 1	-0.5	(-1.8, 0.8)	0.0	(-0.4, 0.5)
Model 2	-0.5	(-1.8, 0.8)	0.1	(-0.3, 0.6)
Model 3	-0.6	(-1.9, 0.7)	0.1	(-0.3, 0.6)
4-day average				
Model 1	-0.3	(-1.5, 1.0)	-0.1	(-0.5, 0.3)
Model 2	-0.3	(-1.6, 0.9)	-0.1	(-0.5, 0.4)
Model 3	-0.4	(-1.7, 0.9)	-0.1	(-0.5, 0.3)
5-day average				
Model 1	-0.7	(-2.0, 0.6)	-0.3	(-0.7, 0.2)
Model 2	-0.7	(-2.1, 0.6)	-0.2	(-0.7, 0.2)
Model 3	-0.8	(-2.2, 0.5)	-0.3	(-0.7, 0.2)

[a]Short-term $PM_{2.5}$ concentrations have been pre-adjusted for calendar time, temperature, relative humidity, and day of the week to account for temporal confounding.
[b]Model 1 was adjusted for age at exam, sex, race/ethnicity, site.
[c]Model 2 was adjusted for the covariates in model 1, as well as education, income, smoking, alcohol consumption, physical activity, neighborhood SES, BMI, and waist-to-hip ratio.
[d]Model 3 was adjusted for all of the covariates in models 1 and 2, as well as total cholesterol, LDL, diabetes, and systolic blood pressure.

REFERENCES

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

2 Pope CA 3rd, Burnett RT, Thurston GD, Thun MJ, Calle EE, Krewski JJ. Cardiovascular mortality and long-term exposure to particulate air pollution: Epidemiological evidence of general pathophysiological pathways of disease. *Circulation* 2003; **109**: 71–77.

3 Dockery DW, Pope CA 3rd, Xu X, Spengler JD, Ware JH, Fay ME *et al.* An association between air pollution and mortality in six U.S. cities. *N Engl J Med* 1993; **329**: 1753–1759.

4 Pope CA 3rd, Burnett RT, Thun MJ, Calle EE, Krewski D, Thurston GD *et al.* Lung cancer, cardiopulmonary mortality, and long-term exposure to fine particulate air pollution. *JAMA* 2002; **287**: 1132–41.

5 Miller KA, Siscovick DS, Sheppard L, Shepherd K, Sullivan JH, Anderson GL *et al.* Long-term exposure to air pollution and incidence of cardiovascular events in women. *N Engl J Med* 2007; **356**: 447–458.

6 Puett RC, Hart JE, Yanosky JD, Paciorek C, Schwartz J, Suh H *et al.* Chronic fine and coarse particulate exposure, mortality, and coronary heart disease in the Nurses' Health Study. *Environ Health Perspect* 2009; **117**: 1697–1701.

7 Hoek G, Brunekreef B, Goldbohm S, Fischer P, Van Den Brandt PA. Association between mortality and indicators of traffic-related air pollution in the Netherlands: A cohort study. *Lancet* 2002; **360**: 1203–1209.

8 Gehring U, Heinrich J, Kramer U, Grote V, Hochadel M, Sugiri D *et al.* Long-term exposure to ambient air pollution and cardiopulmonary mortality in women. *Epidemiology* 2006; **17**: 545–51.

9 Ostro B, Lipsett M, Reynolds P, Goldberg D, Hertz A, Garcia C *et al.* Long-term exposure to constituents of fine particulate air pollution and mortality: Results from the California Teachers Study. *Environ Health Perspect* 2010; **118**: 363–369.

10 Forastiere F, Agabiti N. Assessing the link between air pollution and heart failure. *Lancet* 2013; **382**: 1008–1010.

11 Sparks MA, Crowley SD, Gurley SB, Mirotsou M, Coffman TM. Classical Renin-Angiotensin system in kidney physiology. *Compr Physiol* 2014; **4**: 1201–1228.

12 Hoffmann B, Moebus S, Dragano N, Stang A, Möhlenkamp S, Schmermund A *et al.* Chronic residential exposure to particulate matter air pollution and systemic inflammatory markers. *Environ Health Perspect* 2009; **117**: 1302–1308.

13 Hajat A, Allison M, Diez-Roux AV, Jenny NS, Jorgensen NW, Szpiro AA *et al.* Long-term exposure to air pollution and markers of inflammation, coagulation, and endothelial activation: A repeat-measures analysis in the Multi-Ethnic Study of Atherosclerosis (MESA). *Epidemiology* 2015; **26**: 310–320.

14 Chan SH, Van Hee VC, Bergen S, Szpiro AA, DeRoo LA, London SJ *et al.* Long-term air pollution exposure and blood pressure in the Sister Study. *Environ Health Perspect* 2015; **123**: 951–958.

15 Van Hee VC, Adar SD, Szpiro AA, Barr RG, Bluemke DA, Diez-Roux AV *et al.* Exposure to traffic and left ventricular mass and function the Multi-Ethnic Study of Atherosclerosis. *Am J Respir Crit Care Med* 2009; **179**: 827–834.

16 Adar SD, Klein R, Klein BEK, Szpiro AA, Cotch MF, Wong TY *et al.* Air pollution and the microvasculature: A cross-sectional assessment of in vivo retinal images in the population-based Multi-Ethnic Study of Atherosclerosis (MESA). *PLoS Med* 2010; **7**: e1000372.

17 Kaufman JD, Adar SD, Barr RG, Budoff M, Burke GL, Curl CL *et al.* Association between air pollution and coronary artery calcification within six metropolitan areas in the USA (the Multi-Ethnic Study of Atherosclerosis and Air Pollution): a longitudinal cohort study. *Lancet* 2016; **6736**: 1–9.

18 Bettencourt PM. Clinical usefulness of B-type natriuretic peptide measurement: present and future perspectives. *Heart* 2005; **91**: 1489–94.

19 Rutten FH, Clark AL, Hoes AW. How big a problem is heart failure with a normal ejection fraction? *BMJ* 2016; **352**: i1706.

20 Borlaug BA. The pathophysiology of heart failure with preserved ejection fraction. *Nat Rev Cardiol* 2014; **11**: 507–515.

21 Taylor CJ, Roalfe AK, Tait L, Davis RC, Iles R, Derit M *et al.* Observational longitudinal cohort study to determine progression to heart failure in a screened community population: the Echocardiographic Heart of England Screening Extension (ECHOES-X) study. *BMJ Open* 2014; **4**: e005256.

22 De Lemos JA, McGuire DK, Drazner MH. B-type natriuretic peptide in cardiovascular disease. *Lancet* 2003; **362**: 316–322.

23 Weber M, Hamm C. Role of B-type natriuretic peptide (BNP) and NT-proBNP in clinical routine. *Heart* 2006; **92**: 843–849.

24 Raymond I, Groenning BA, Hildebrandt PR, Nilsson JC, Baumann M, Trawinski J *et al.* The influence of age, sex and other variables on the plasma level of N-terminal pro brain natriuretic peptide in a large sample of the general population. *Heart* 2003; **89**: 745–751.

25 Olsen MH, Hansen TW, Christensen MK, Gustafsson F, Rasmussen S, Wachtell K *et al.* N-terminal pro brain natriuretic peptide is inversely related to metabolic cardiovascular risk factors and the metabolic syndrome. *Hypertension* 2005; **46**: 660–666.

26 Daniels LB, Laughlin GA, Clopton P, Maisel AS, Barrett-Connor E. Minimally elevated cardiac troponin T and elevated N-terminal pro-B-type natriuretic peptide predict mortality in older adults: results from the Rancho Bernardo Study. *J Am Coll Cardiol* 2008; **52**: 450–9.

27 Choi E-Y, Bahrami H, Wu CO, Greenland P, Cushman M, Daniels LB *et al.* N-terminal pro-B-type natriuretic peptide, left ventricular mass, and incident heart failure: the Multi-Ethnic Study of Atherosclerosis. *Circ Hear Fail* 2012; **5**: 727–734.

28 Daniels LB, Clopton P, DeFilippi CR, Sanchez OA, Bahrami H, Lima JAC *et al.* Serial measurement of N-terminal pro–B-type natriuretic peptide and cardiac troponin T for cardiovascular disease risk assessment in the Multi-Ethnic Study of Atherosclerosis (MESA). *Am Heart J* 2015; **170**: 1170–1183.

29 Kapuku GK, Davis HC, Thomas P, Januzzi J, Harshfield GA. Does the relationship between natriuretic hormones and diastolic function differ by race? *Am J Med Sci* 2012; **344**: 96–99.

30 Wang TJ, Larson MG, Levy D, Benjamin EJ, Leip EP, Omland T *et al.* Plasma natriuretic peptide levels and the risk of cardiovascular events and death. *N Engl J Med* 2004; **350**: 655–663.

31 Kistorp C, Faber J. N-terminal pro-brain natriuretic peptide, C-reactive protein, and urinary albumin levels as predictors of mortality and cardiovascular events in older adults. *JAMA* 2005; **293**: 1609–1616.

32 Olsen MH, Hansen TW, Christensen MK, Gustafsson F, Rasmussen S, Wachtell K *et al.* N-terminal pro-brain natriuretic peptide, but not high sensitivity C-reactive protein, improves cardiovascular risk prediction in the general population. *Eur Heart J* 2007; **28**: 1374–81.

33 Di Angelantonio E, Chowdhury R, Sarwar N, Ray K, Gobin R, Saleheen D *et al.* B-type natriuretic peptides and cardiovascular risk. Systematic review and meta-analysis of 40 prospective studies. *Circulation* 2009; **120**: 2177–2187.

34 Nambi V, Liu X, Chambless LE, de Lemos JA, Virani SS, Agarwal S *et al.* Troponin T and N-terminal pro-B-type natriuretic peptide: a biomarker approach to predict heart

failure risk--the Atherosclerosis Risk in Communities study. *Clin Chem* 2013; **59**: 1802–10.

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

36 Link MS, Luttmann-Gibson H, Schwartz J, Mittleman MA, Wessler B, Gold DR *et al.* Acute exposure to air pollution triggers atrial fibrillation. *J Am Coll Cardiol* 2013; **62**: 816–825.

37 Shah AS V, Langrish JP, Nair H, McAllister DA, Hunter AL, Donaldson K *et al.* Global association of air pollution and heart failure: a systematic review and meta-analysis. *Lancet* 2013; **382**: 1039–48.

38 Pope CA 3rd, Renlund DG, Kfoury AG, May HT, Horne BD. Relation of heart failure hospitalization to exposure to fine particulate air pollution. *Am J Cardiol* 2008; **102**: 1230–1234.

39 Atkinson RW, Carey IM, Kent AJ, van Staa TP, Anderson HR, Cook DG. Long-term exposure to outdoor air pollution and incidence of cardiovascular diseases. *Epidemiology* 2013; **24**: 44–53.

40 Mantovani A, Garlanda C, Bottazzi B, Peri G, Doni A, Martinez de la Torre Y *et al.* The long pentraxin PTX3 in vascular pathology. *Vascul Pharmacol* 2006; **45**: 326–330.

41 Presta M, Camozzi M, Salvatori G, Rusnati M. Role of the soluble pattern recognition receptor PTX3 in vascular biology. *J Cell Mol Med* 2007; **11**: 723–38.

42 Peri G, Introna M, Corradi D, Iacuitti G, Signorini S, Avanzini F *et al.* PTX3, a prototypical long pentraxin, is an early indicator of acute myocardial infarction in humans. *Circulation* 2000; **102**: 636–641.

43 Jenny NS, Blumenthal RS, Kronmal RA, Rotter JI, Siscovick DS, Psaty BM. Associations of pentraxin 3 with cardiovascular disease: the Multi-Ethnic Study of Atherosclerosis. *J Thromb Haemost* 2014; **12**: 999–1005.

44 Soeki T, Niki T, Kusunose K, Bando S, Hirata Y, Tomita N *et al.* Elevated concentrations of pentraxin 3 are associated with coronary plaque vulnerability. *J Cardiol* 2011; **58**: 151–157.

45 Latini R, Gullestad L, Masson S, Nymo SH, Ueland T, Cuccovillo I *et al.* Pentraxin-3 in chronic heart failure: The CORONA and GISSI-HF trials. *Eur J Heart Fail* 2012; **14**: 992–999.

46 Dubin R, Li Y, Ix JH, Shlipak MG, Whooley M, Peralta CA. Associations of pentraxin-3 with cardiovascular events, incident heart failure, and mortality among persons with coronary heart disease: data from the Heart and Soul Study. *Am Heart J* 2012; **163**: 274–9.

47 Dubin R, Shlipak M, Li Y, Ix J, De Boer IH, Jenny N *et al.* Racial differences in the association of pentraxin-3 with kidney dysfunction: The Multi-Ethnic Study of Atherosclerosis. *Nephrol Dial Transplant* 2011; **26**: 1903–1908.

48 Jylhävä J, Haarala A, Kähönen M, Lehtimäki T, Jula A, Moilanen L *et al.* Pentraxin 3 (PTX3) is associated with cardiovascular risk factors: The Health 2000 Survey. *Clin Exp Immunol* 2011; **164**: 211–217.

49 Latini R, Maggioni AP, Peri G, Gonzini L, Lucci D, Mocarelli P *et al.* Prognostic significance of the long pentraxin PTX3 in acute myocardial infarction. *Circulation* 2004; **110**: 2349–54.

50 Leary PJ, Jenny NS, Barr RG, Bluemke DA, Harhay MO, Heckbert SR *et al.* Pentraxin-3 and the right ventricle: the Multi-Ethnic Study of Atherosclerosis–Right Ventricle Study. *Pulm Circ* 2014; **4**: 250–259.

51 Jenny NS, Arnold AM, Kuller LH, Tracy RP, Psaty BM. Associations of pentraxin 3 with cardiovascular disease and all-cause death: the Cardiovascular Health Study. *Arter Thromb Vasc Biol* 2009; **29**: 594–599.

52 Jenny NS, Blumenthal RS, Kronmal RA, Rotter JI, Siscovick DS, Psaty BM. Associations of pentraxin 3 with cardiovascular disease: The Multi-Ethnic Study of Atherosclerosis. *J Thromb Haemost* 2014; **12**: 999–1005.

53 Verwey NA, Schuitemaker A, Van Der Flier WM, Mulder SD, Mulder C, Hack CE *et al.* Serum amyloid P component as a biomarker in mild cognitive impairment and Alzheimer's disease. *Dement Geriatr Cogn Disord* 2008; **26**: 522–527.

54 Xi D, Luo T, Xiong H, Liu J, Lu H, Li M *et al.* SAP: Structure, function, and its roles in immune-related diseases. *Int J Cardiol* 2015; **187**: 20–26.

55 Jenny NS, Arnold AM, Kuller LH, Tracy RP, Psaty BM. Serum amyloid P and cardiovascular disease in older men and women: Results from the Cardiovascular Health Study. *Arterioscler Thromb Vasc Biol* 2007; **27**: 352–358.

56 Song Z, Cai L, Guo L, Tsukamoto Y, Yutani C, Li XA. Accumulation and expression of serum amyloid P component in human atherosclerotic lesions. *Atherosclerosis* 2010; **211**: 90–95.

57 US Environmental Protection Agency. Criteria Air Pollutants. https://www.epa.gov/criteria-air-pollutants (accessed 19 May2016).

58 Godish T. *Air Quality*. 4th ed. Lewis Publishers, 2004.

59 Jacobson MZ. *Air Pollution and Global Warming: History, Science and Solution.* 2nd ed. 2012.

60 Bild DE, Bluemke DA, Burke GL, Detrano R, Diez Roux AV, Folsom AR *et al.* Multi-Ethnic Study of Atherosclerosis: Objectives and design. *Am J Epidemiol* 2002; **156**: 871–881.

61 MESA Coordinating Center. MESA Website. https://www.mesa-nhlbi.org/ (accessed 30 Apr2016).

62 Kaufman JD, Adar SD, Allen RW, Barr RG, Budoff MJ, Burke GL *et al.* Prospective study of particulate air pollution exposures, subclinical atherosclerosis, and clinical cardiovascular disease: The Multi-Ethnic Study of Atherosclerosis and Air Pollution (MESA Air). *Am J Epidemiol* 2012; **176**: 825–37.

63 Keller JP, Olives C, Kim S-Y, Sheppard L, Sampson PD, Szpiro AA *et al.* A unified spatiotemporal modeling approach for predicting concentrations of multiple air pollutants in the Multi-Ethnic Study of Atherosclerosis and Air Pollution. *Environ Health Perspect* 2015; **123**: 301–309.

64 Cohen MA, Adar SD, Allen RW, Avol E, Curl CL, Gould T *et al.* Approach to estimating participant pollutant exposures in the Multi-Ethnic Study of Atherosclerosis and Air Pollution (MESA Air). *Environ Sci Technol* 2009; **43**: 4687–4693.

65 Allen RW, Adar SD, Avol E, Cohen M, Curl CL, Larson T *et al.* Modeling the residential infiltration of outdoor $PM_{2.5}$ in the Multi-Ethnic Study of Atherosclerosis and Air Pollution (MESA Air). *Environ Health Perspect* 2012; **120**: 824–830.

66 Szpiro AA, Sheppard L, Adar SD, Kaufman JD. Estimating acute air pollution health effects from cohort study data. *Biometrics* 2014; **70**: 164–174.

67 Hajat A, Diez-Roux AV, Adar SD, Auchincloss AH, Lovasi GS, O'Neill M *et al.* Air pollution and individual and neighborhood socioeconomic status: Evidence from the Multi-Ethnic Study of Atherosclerosis (MESA). *Environ Health Perspect* 2013; **121**: 1325–1333.

68 Patton KK, Heckbert SR, Alonso A, Bahrami H, Lima JAC, Burke G *et al.* N-terminal pro-B-type natriuretic peptide as a predictor of incident atrial fibrillation in the Multi-Ethnic Study of Atherosclerosis: the effects of age, sex and ethnicity. *Heart* 2013; **99**: 1832–6.

69 Weber M, Hamm C. Role of B-type natriuretic peptide (BNP) and NT-proBNP in clinical routine. *Heart* 2006; **92**: 843–849.

70 Schou M, Gustafsson F, Nielsen PH, Madsen LH, Kjaer A, Hildebrandt PR. Unexplained week-to-week variation in BNP and NT-proBNP is low in chronic heart failure patients during steady state. *Eur J Heart Fail* 2007; **9**: 68–74.

71 DeFilippi CR, Christenson RH, Gottdiener JS, Kop WJ, Seliger SL. Dynamic cardiovascular risk assessment in elderly people. The role of repeated N-terminal pro-B-type natriuretic peptide testing. *J Am Coll Cardiol* 2010; **55**: 441–450.