Air Pollution and High Density Lipoprotein Structure and Function

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Abstract

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BACKGROUND:

A growing body of evidence suggests that air pollution is an important contributor to risk of cardiovascular disease. Federal regulatory agencies require evidence of the biological mechanisms explaining the relationship between air pollution and cardiovascular disease in order to inform regulatory policy. High density lipoproteins (HDL) are an important cardiovascular risk factor, and air pollution induced changes in HDL may explain some of this relationship. We examined the relationship between air pollution and HDL in a suite of complementary studies.

METHODS:

We first examined the cross-sectional relationship between air pollution and both HDL cholesterol (HDL-C) and HDL particle number (HDL-P) in the Multi-Ethnic Study of Atherosclerosis Air Pollution study (MESA Air), an ethnically diverse cohort of community-dwelling adults. We also examined whether HDL mediated the longitudinal relationship between air pollution and coronary artery calcium (CAC) in MESA. We estimated individual residential ambient fine particulate pollution exposure ($PM_{2.5}$) and black carbon (BC) concentrations using

a fine-scale likelihood-based spatiotemporal model and cohort-specific monitoring. HDL-C and HDL-P were measured using the cholesterol oxidase method and nuclear magnetic resonance spectroscopy, respectively. CAC was measured at multiple exams using computed tomographic imaging with a standardized method. We used multivariable linear regression and linear mixed models to examine the relationship between air pollution exposure, HDL measures, and CAC outcomes.

We also examined the relationship between controlled exposure to air pollution in an experimental setting, using a double-blind crossover design randomized by order of intervention/control status. Diesel-powered motor vehicles are a major source of urban $PM_{2.5}$, and we use well-characterized and tightly controlled diesel exhaust (DE) inhalation as a model of traffic-related air pollution exposure. We examined the effect of traffic-related air pollutants on anti-oxidant function of HDL, on paraoxonase-1 (PON1) activity, and on changes in the HDL proteome. Outcomes were measured before and after exposure to either DE or filtered air and changes in HDL outcomes were examined using linear mixed models.

RESULTS:

In our cross-sectional study, a 0.7 $10^{-6}m^{-1}$ higher exposure to black carbon (a marker of traffic-related pollution) averaged over a one-year period was associated with a lower HDL-C. In the three month averaging time period, a 5 $\mu g/m^3$ higher $PM_{2.5}$ was associated with lower HDL-P, but not HDL-C. In our controlled experimental study, we did not find evidence that exposure to DE was associated with changes in HDL function or measured characteristics. We did not find that HDL mediated the longitudinal relationship between air pollution and CAC. We did, however, find that an averaged one year 5 $\mu g/m^3$ higher $PM_{2.5}$ was associated with lower annual change in HDL-C.

CONCLUSIONS:

These data are consistent with the hypothesis that exposure to ambient air pollution is adversely associated with measures of HDL. Our study used state of the art measurement of air pollution combined with novel measures of HDL to produce new evidence supporting the relationship between air pollution and cardiovascular disease. Future study is needed to explain how these changes occur and if chronic exposure to air pollution may be associated with changes in HDL function over time.

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DEDICATION

For my family and friends.

Chapter 1
INTRODUCTION

AIR POLLUTION AND CARDIOVASCULAR DISEASE

Cardiovascular disease (CVD) is the leading cause of premature morbidity and mortality worldwide(1). Individual level risk factors linked to CVD such as cigarette smoking, hypertension, diabetes, and hypercholesterolemia have been well-studied, but despite successes in prevention and treatment of these early contributors to disease, mortality from these causes remains high. There is growing epidemiologic evidence that long term air pollution exposure is an important contributor to mortality from this disease(2-8). Air pollution is a complex mixture of gases and particles produced by combustion, industrial and agricultural activities, and natural phenomena such as wind-blown dust, sea spray, and volcanic activity. Up to this point, the effects of particulate matter under 2.5 μm in size ($PM_{2.5}$) and other traffic-related air pollutants (TRAP, measured here by nitrogen dioxide (NO_2), oxides of nitrogen (NO_x) and black carbon (BC)) on CVD have been best characterized. Numerous studies in different populations and settings have consistently pointed to a causal link between exposure TRAP and increased risk of CVD. Unlike smoking and some dietary risk factors, individuals (particularly children) are largely unable to choose whether or not to be exposed to air pollution in their daily life, making air pollution regulation a compelling subject of public health research for policy makers. While the global burden of deleterious health effects due to air pollution exposures is increasing, air pollution exposures have generally decreased in the U.S. since the passage of the Clean Air Act. (9) The Environmental Protection Agency (EPA) estimates that health benefits from the reduction of air pollution has resulted in saving billions of dollars per year, making the Clean Air Act one of the most cost-effective pieces of federal regulation ever implemented, a public health success comparable to basic sanitation(10). However, despite improvements in air quality, the health consequences of air pollution continue to be felt even

below current regulations set through the National Ambient Air Quality Standards. Indeed, some estimates of the dose-response curve show further improvements in air quality in the U.S. may have a greater impact than comparable reductions in more polluted regions(11). In order to inform the regulatory policies to protect the health of U.S. citizens, the biological mechanisms explaining the relationship between air pollution and CVD need further elucidation.(2).

AIR POLLUTION EPIDEMIOLOGY

Links between air pollution and human health effects have been the subject of scientific inquiry for decades. Air pollution is generally considered any materials that are suspended in the air, be they particles, fumes, soot, or gases. The most studied air pollutants in the health context are usually the products of incomplete combustion. The earliest studies of air pollution focused on acute air pollution disasters, such as the London Fog incident in 1952. A 1990 analysis in London showed large spikes in mortality during high air pollution days(12). Other analyses of natural experiments in the Utah Valley and Steubenville, OH in the early 1990's showed a similar pattern of acute health effects associated with air pollution(13). Questions about the methods used in these studies led to refinement of time-series analyses of daily mortality and an interest in better measurement of the longer-term effects of air pollution exposure(14). These efforts led to the Six Cities and American Cancer Society (ACS) air pollution studies, which used city-specific mean air pollution concentrations and individual-level measurement of outcomes and covariates(15, 16). The Six Cities and ACS studies found strong and robust associations between higher air pollution exposures and increased risk of mortality from cardiovascular disease due to living in cities with higher levels of air pollution(14). These findings moved the Environmental Protection Agency (EPA) to re-examine air pollution standards. In the open comment on new air quality standards, there were criticisms that there was little evidence of the biological mechanisms supporting the association and that air pollution measurements were

prone to measurement error. It was also argued that there was inadequate adjustment for possible confounding(14). The EPA has since spent over $450 million over the past 15 years to research the mechanisms of air pollution health effects(14). In 2006, they funded the largest, most well-characterized air pollution study to date, the Multi-Ethnic Study of Atherosclerosis Air Pollution Study (MESA Air), which was designed to address some of the criticisms of prior air pollution studies - investigating biological mechanisms, addressing important concerns about air pollution measurement error, and featuring detailed measurement of possible confounding variables(17).

AIR POLLUTION AND BIOLOGICAL MECHANISMS

It is widely accepted that ambient air pollution exposure contributes to CVD, but important questions persist about underlying biological mechanisms (2, 18)**.** Extensive evidence from numerous epidemiologic and experimental studies supports the association between air pollution and CVD(2, 3, 7, 8). Acute, short-term exposure to $PM_{2.5}$ on a time scale of several hours to weeks is associated with CVD-related events such as myocardial infarction and ischemic stroke in time-series studies and natural experiments(19, 20). However, evidence from studies of longer-term exposure to $PM_{2.5}$ on the time scale of months to years indicate there may be an even greater risk of CVD mortality from chronic exposure to air pollution, with no "safe" level below which there are no health effects(2, 21). Long-term exposure to air pollution is also associated with ischemic events, as well as with subclinical changes that can lead to ischemic events, such as thrombosis, atherosclerosis, and alterations in cardiac structure and function. The upstream biological mechanism by which long-term exposure to $PM_{2.5}$ and TRAP may lead to development of atherosclerosis and triggering of CVD events remains uncertain, however there has been much recent work exploring these pathways.(2, 22)

There are many proposed mechanisms by which inhaled air pollutant constituents may promote CVD at a molecular level. Inhaled air pollution particles and gases interact with lung

tissues, translocating into the bloodstream and activating inflammatory cytokine expression(23). Observational studies of long-term exposure to air pollution humans shown associations between air pollution and increased concentrations of inflammatory cytokines, a finding which has been confirmed in experimental studies(23). Air pollution has also been linked with pulmonary and systemic oxidative stress in experimental and observational studies(2). Indeed, in mouse studies, week-long exposures to air pollution cause increased lipid peroxidation and HDL dysfunction(24-26). Increases in systemic inflammation and oxidation are important contributors to endothelial dysfunction as well as metabolic and lipid abonormalities through production of reactive oxygen species (ROS)(23). High levels of ROS can cause vascular dysfunction and atherogenesis through downregulation of nitric oxide, an important vasodilator, as well as through promotion of metabolic dysfunction, and oxidation. Atherogenesis - or the promotion of atherosclerosis, the formations of lipids, immune cells, cellular debris and calcium that build up in the arterial wall - has also been linked to air pollution(27). In studies of ApoE -/- mice, exposure to air pollution cause mice to develop more numerous and complex plaques that are more prone to rupture, causing CVD events(23). In particular, the ability of air pollution to induce inflammation and oxidative stress is a leading hypothesis to explain the association between air pollution and atherosclerosis(2). Pollutant exposure in the lungs may generate oxidized phospholipids through oxidation of lung surfactant, promoting formation of oxidized, dysfunctional lipoproteins, contributing to development of atherosclerosis.(18, 28, 29) Thus, $PM_{2.5}$ and TRAP-associated alterations in lipoproteins, and in particular high-density lipoproteins, are a potential integrating mechanism to explain air pollution effects on CVD.(26, 30)

HIGH DENSITY LIPOPROTEINS

Lipoproteins are complex assemblages of proteins and lipids that circulate in the bloodstream. These particles take on a wide range of sizes that have different functional properties within the

body, from largest being chylomicrons; very low-density lipoproteins (VLDL), low-density lipoproteins (LDL) and high-density lipoproteins (HDL). Total cholesterol (which includes HDL) was recognized as an important risk factor for heart disease in epidemiologic studies in the 1960s. Further study of functional differences in lipids led to theories that higher levels of high-density lipoprotein (HDL) were associated with lower risk of CVD, which were confirmed in longitudinal observational studies like the Framingham Heart Study and the Honolulu Heart study by the 1980s(31). Numerous prospective observational studies have since consistently identified HDL as an independent protective risk factor for CVD, even in the presence of reduced LDL concentrations(31). Human population studies, supported by human and animal mechanistic studies, indicate that HDL plays a key role in a number of well-characterized cardioprotective processes, including mediating transport of macrophage cholesterol efflux, antioxidant capacity, anti-inflammatory properties, nitric oxide (NO) – promoting activities, improved endothelial health and function, and ability to transport proteins with their own intrinsic biological activities, among others(31, 32). Importantly, HDL particles are vital transporters of cholesterol from lipid-carrying macrophages and participate in the maintenance of net cholesterol balance in the arterial wall and in the reduction of proinflammatory responses by arterial cholesterol-carrying macrophages(31, 32).

However, recent clinical trials designed to raise HDL cholesterol (HDL-C) levels have failed to reduce risk of CVD(33-40)**.** The large AIM-HIGH and HPS2-THRIVE trials compared vascular event rates in those assigned to niacin (to raise HDL-C) against those assigned to placebo, reporting no difference in vascular event rates(33, 40). A recent meta-analysis of HDL-C raising trials and a mendelian randomization study support these results.(38, 39) The failure of multiple large, well-designed trials to improve outcomes has led to a growing consensus that measurement of HDL cholesterol is not sufficient to capture the cardioprotective potential of HDL(31, 41-45). Since measurement of cholesterol content of HDLs does not appear to reflect the primary cardioprotective roles of HDL (transporting excess cholesterol from the arterial wall

to the liver, or Reverse Cholesterol Transport), it is plausible that HDL-C, despite its long history and use in clinical practice, may not be the most appropriate or accurate measure of HDL(31, 46). In fact, while high levels of HDL-C may be inversely associated with reduced CVD risk in observational studies, high HDL-C levels are not always protective, and studies of genetic mutations that raise HDL-C do not show them to reduce risk of myocardial infarction(38, 41, 42).

ALTERNATIVE MESAURES OF HDL

Measures of HDL properties other than HDL-C may more accurately reflect HDL's protective qualities(42, 46, 47). A recent consensus document recommends increased use of HDL particle number (HDL-P), proteomic characterization of lipoproteins, and anti-oxidant capacity measures to assess HDL function and structure(31). HDL particles can vary widely in size and concentration in the body and particles that are composed of different proportions of proteins can have vastly different functional implications. While healthy HDLs protect against oxidation, inflammation, and atherosclerosis, HDLs can become dysfunctional, losing their cardioprotective qualities, and even becoming atherogenic(32). Several novel measures of the structure and function of HDL have been proposed to better describe the important and complex roles of HDL in CVD, and we use several promising measures in this dissertation(46).

Animal studies support the thesis that air pollution impacts HDL structure and function(24-26, 30). Several experiments have indicated that exposure to fine or ultrafine PM is capable of impairing several aspects of HDL functionality including anti-inflammatory capacity, anti-oxidant capacity and paraoxonase activity(25, 26, 30). Yin, et al (2013, ATVB), exposed apoE-deficient mice to diesel exhaust at ~250 ug/m^3 and filtered air for two weeks, and found increased peroxidation in brochoalveolar lavage fluid and pro-oxidative changes in HDL, supporting the hypothesis that oxidation in the lung surface can lead to increased oxidation and development of dysfunctional circulating HDL.(26) Li, et al. exposed LDL-receptor null mice to

ultrafine particles for 10 weeks, finding reduced HDL-C, HDL anti-oxidant capacity, and paraoxonase (PON) activity (an important measure of HDL antioxidant activity).(25) In Araujo et al, samples of HDL from mice exposed to fine and ultrafine PM were found to have significantly reduced HDL anti-inflammatory properties compared to unexposed mice, suggesting that PM exposure may reduce the ability of HDL to protect against atherosclerosis(24). In Bass, et al, however, rats exposed to Ozone for up to 13 weeks did not show significant change in HDL-C.(48)

Proteomic approaches to HDL structural analysis are a promising new field of study(49-51). At a molecular level, HDL is an assemblage of over 40 distinct amphipathic proteins that stabilize a lipid cargo(31). While some of these apolipoproteins are involved in cholesterol transport or other cardioprotective processes, others may be pro-inflammatory and atherogenic(31). Proteomic analyses allow for description of the entire complement of proteins in a given biological organism or system at a given time, ie, the protein products of HDL(52). There is compelling evidence that the primary activities of HDL depend on interactions between these associated proteins. A study examining patients with CAD examined their HDL proteomes before and after treatment with statins, and found important differences in proteomic composition of HDL(52). Numerous studies have examined the distribution of HDL proteins from various disease states, but to date no studies have examined the effect of air pollution on the HDL proteome(31). HDL is exchanged by the lungs in numerous processes, including vitamin E transport and surfactant production. Serum amyloid alpha-1 (SAA1) is an important acute phase protein associated with HDL that can displace Apo-A1, the major protein component of HDL, causing altered HDL function. Changes in SAA1 and other proteins carried by HDL represent a potential integrating biological mechanism through which the association of air pollution to inflammation and oxidative stress may be explained. Studies of the HDL proteome have discovered numerous acute phase-response proteins, with are more associated with acute

inflammation than lipid metabolism.(53) Acute effects of air pollution on HDL may be best detected through examination of the effect on acute phase proteins.

AIR POLLUTION AND HDL

There are few observational studies that have examined the association of air pollution with HDL, and none with more novel measures of HDL characteristics**.** To date, we are aware of only two community-dwelling cohort studies investigating the potential relationship between air pollution and HDL in humans. In a population-based survey in Taiwan, Chuang et al. found a decrease in HDL-C per IQR increase in PM_{10} (particulate matter < 10μm in diameter) over a 1-day averaging period before blood draw(54). However, Chuang et al. used a "nearest monitor" method for assigning air pollution exposure, which is unable to detect small-scale differences in spatial air pollution, and is prone to exposure misclassification and measurement error(55). In addition, the study only examined HDL-C, which as previously mentioned may not adequately reflect the atheroprotective qualities of HDL. In a cross-sectional analysis, also in Taiwan, Chuang et al found no association between a 20 ug/m^3 higher one-year averaged $PM_{2.5}$ exposure before blood draw and HDL-C in a cohort of 1023 subjects aged 54 to 90(56). This study also relied on nearest-monitor exposure data and used HDL-C as an outcome. The study also had limited or no data on likely confounding variables such as SES, smoking, alcohol use, physical activity, and use of lipid-lowering medications, which are usually associated with both air pollution and HDL.

The association between $PM_{2.5}$ and HDL-C was also examined in a repeated measures panel study of welders(57). Those exposed to high $PM_{2.5}$ during welding experienced an acute decrease of -2.6 mg/dL (95% CI -5.3, -0.0) in circulating HDL-C levels 18 hours following exposure, compared to their baseline levels(57). Interestingly, in a subgroup analysis of those

who did not weld the previous day, welding was associated with an even greater -4.3 mg/dL (95% CI -8.0, -0.7) decrease in circulating HDL-C.

Evidence from experimental studies of air pollution and HDL is mixed, but suggests an HDL-damaging effect of air pollution(24, 26). Human and animal experimental studies have examined several mechanisms by which air pollutants and mixtures of air pollutants may affect the structural and functional qualities of HDL. While CVD may take many years to develop, recent studies indicate that HDL function and structure can be altered within an hours-to-days timeframe(58-61). Acute inflammation, trauma or infection has shown HDL particles ability to quickly change from a normal to a dysfunctional, pro-oxidative phenotype, possibly playing a role in the instigation of plaque instability and subsequent CV events(24, 58). An insult such as suddenly increased PM exposure could plausibly introduce injury effecting HDL functionality(25).

Controlled exposure studies in humans indicate that acute air pollution can rapidly impact HDL composition and function in brief exposure settings(62). Indeed, in a double-blind trial of 11 smokers, exposure to 2hr of concentrated ambient particles (CAPs) (mean 108.7 ± 24.8 $\mu g/m^3$) was associated with lower HDL-C 22 hours after exposure(62). In an unblinded study of 23 healthy young volunteers exposed to 2hrs of NO_2 and $PM_{2.5}$ CAPs, Huang, et al, found a slight increase in HDL-C after exposure to only NO_2(54). In Rice et al, researchers observed a change in HDL-C after 6-8 hrs of welding(57). These studies confirm the plausibility of rapid changes in HDL structure and composition from acute exposure to CAPs and NO_2. However, a recent exposure study of 32 young, healthy subjects to 2hr of coarse CAP from a rural location found no effect on several markers of HDL function(63). It has also been reported that the HDL functionality can be improved by removal of pro-oxidative chemicals present in cigarette smoke. For example, after a brief smoking cessation program, ex-smokers showed improved HDL function(64).

CONTRIBUTION OF THIS RESEARCH

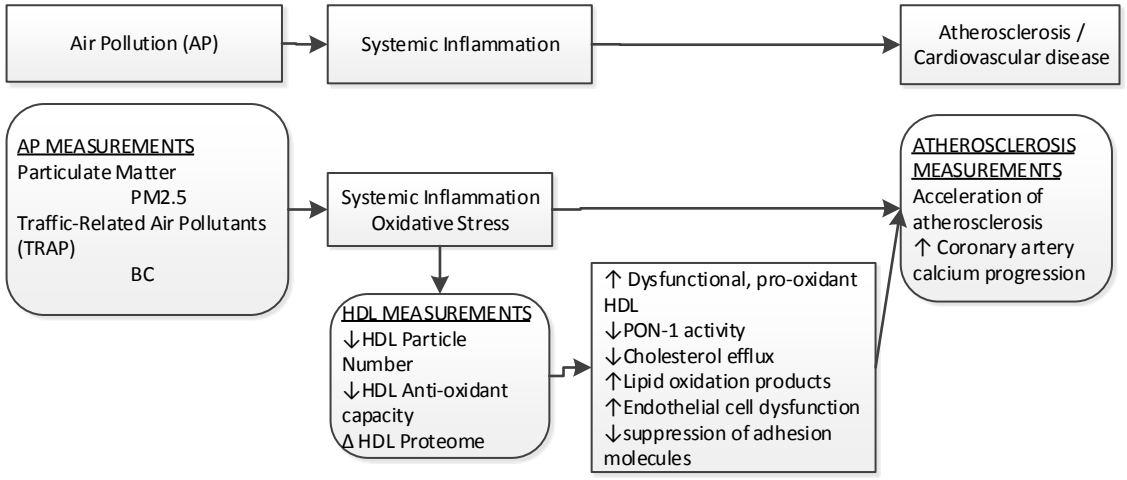
While several observational cohort studies of air pollution and HDL-C have previously been conducted, those epidemiologic studies have had methodological weaknesses (weak control of confounding, less refined measurement of air pollution) that are strengths of the MESA Air cohort. Additionally, prior studies were in ethnically homogenous populations. MESA is an ethnically diverse cohort, and results from the proposed study will be more generalizable to the U.S. population. Preliminary research appears to show a relationship between air pollution and HDL, and mediation analysis should provide insight into the degree to which air pollutions' effect on atherosclerosis could be explained by its effect on HDL. We will use sophisticated air pollution measurements produced by the MESA Air project, combined with high quality data on HDL and longitudinal follow-up of CAC to answer our study questions.

We will also use data from a unique controlled air pollution exposure facility. This controlled exposure study offers a unique chance to examine the biological effects of air pollution without the issues of confounding, measurement error in the exposure, or temporality that can lead to bias in observational studies. Use of experimental measures of HDL function, such as measurement of the capacity of HDL to prevent oxidation and the activity of the HDL-associated anti-inflammatory enzyme paraoxanase-1 (PON1) in this setting help replicate and confirm findings in previous exposure trials. The use of proteomic methods to explore the effects of controlled exposure to air pollution in humans is a further innovation in this dissertation. The diesel exposure center was originally conceived to study the effects of air pollution on the vasculature and blood pressure, and we are able to take advantage of this opportunity to conduct a secondary analysis. The number of subjects recruited for in this study was selected to provide adequate power for these primary outcomes. Given the smaller number of observations available for our,HDL outcomes, our study's strength will be hypothesis-generation rather than confirmation of the specific biological effects of air pollution on HDL.

While several studies have used data from this center, the use of mass spectrometry (MS) to examine the impact of air pollution on the HDL proteome has never been explored either in observational or experimental studies, and represents an innovation of this dissertation. Air pollution and HDL proteins, particularly PON1, have been examined in experimental studies using non-MS methods, however.(25, 26, 63) One of the primary advantages of using MS methods is the capacity of MS to quantify hundreds of proteins in one sample. Shotgun proteomic studies of HDL have discovered a diverse array of more than 89 proteins in HDLs (HDL Proteome Watch - http://homepages.uc.edu/~davidswm/HDLproteome.html). In this work we will examine 42 of the most commonly and abundantly found HDL proteins. The use of MS adds further analytic sensitivity and ability to detect low abundance proteins that might be difficult to detect using other methods, as well as shorter analysis times. This increased sensitivity provides a more finely detailed picture of the biologic effects of air pollution on HDL.(25)

A conceptual model of this dissertation is presented in Figure 1.1. N the figure, we present air pollution (as measured in our study by PM2.5 and Black Carbon, as causing systemic inflammation and oxidative stress in HDL. We measure systemic inflammation and oxidative stress through HDL through a suite of novel measures of HDL, which are associated with a number of adverse biological changes. These changes are hypothesized to contribute to atherosclerosis and cardiovascular disease, measured in our study by coronary artery calcium.

Figure 1.1. Conceptual model of dissertation



Chapter 2

Association of Air Pollution Exposures with High Density Lipoprotein Cholesterol and Particle Number: The Multi-Ethnic Study of Atherosclerosis (MESA)

ABSTRACT

BACKGROUND:

The relationship between air pollution and cardiovascular disease may be explained by changes in high-density lipoprotein (HDL).

METHODS AND RESULTS:

We examined the cross-sectional relationship between air pollution and both HDL cholesterol (HDL-C) and HDL particle number (HDL-P) in the Multi-Ethnic Study of Atherosclerosis Air Pollution study (MESA Air). Study participants were 6,654 white, African-American, Hispanic, and Chinese men and women, 45–84 years of age. We estimated individual residential ambient fine particulate pollution exposure ($PM_{2.5}$) and black carbon (BC) concentrations using a fine-scale likelihood-based spatiotemporal model and cohort-specific monitoring. Exposure periods were averaged to 12 months, 3 months, and two weeks prior to exam. HDL-C and HDL-P were measured using the cholesterol oxidase method and nuclear magnetic resonance spectroscopy, respectively. We used multivariable linear regression to examine the relationship between air pollution exposure and HDL measures. A 0.7 $10^{-6}m^{-1}$ higher exposure to black carbon (a marker of traffic-related pollution) averaged over a one year period was associated with a lower HDL-C (-1.68 mg/dL (95% CI: -2.86, -0.50), and HDL-P (-0.55 mg/dL (95% CI: -1.13, 0.03). The association between black carbon and HDL-C was stronger in women than in men. In the three

month averaging time period, a 5 µg/m^3 higher $PM_{2.5}$ was associated with lower HDL-P (-0.64 µmol/L (95% CI: -1.01, -0.26), but not HDL-C (-0.05 mg/dL (95% CI: -0.82, 0.71).

CONCLUSIONS:

These data are consistent with the hypothesis that exposure to air pollution is adversely associated with measures of HDL. This is the first study to examine the relationship between air pollution and HDL in a multi-ethnic cohort, as well as the first to use NMR-derived measures of HDL particle number and size.

Introduction

High density lipoprotein (HDL) particles possess numerous potentially cardioprotective qualities(31). HDL particles transport cholesterol from lipid-carrying macrophages and are vital in the maintenance of net cholesterol balance in the arterial wall(31). Despite strong epidemiologic evidence that HDL cholesterol (HDL-C) is inversely associated with cardiovascular events, recent clinical trials that raised HDL-C without lowering low density lipoproteins (LDL) have failed to show benefit (33, 35-37). Recent studies suggest measurement of HDL particle number (HDL-P) may better reflect the cardioprotective qualities of HDL than HDL-C(65-67).

Ambient air pollution is associated with atherosclerosis, heart failure, and cardiovascular death(2, 4-9). Air pollution may affect HDL through inflammation and oxidative stress, promoting changes in HDL structure and function that results in proatherogenic or dysfunctional HDL(27, 68). Exposures to both fine and ultrafine particulate matter (PM) have been associated with development of dysfunctional HDL and reduced HDL anti-inflammatory capacity in some (but

not all) experimental studies(25, 26, 30, 63). The association between ambient air pollution and HDL-C, HDL-P and HDL particle size, have not been well-studied.

We examined the relation between long and short-term concentration of air pollutants – $PM_{2.5}$ and black carbon (BC) – and measures of HDL structure in a multiethnic cohort of adults without clinical cardiovascular disease. We hypothesized that exposure to higher levels of air pollution would be associated not only with lower HDL-C, but also with lower HDL-P, which may better reflect HDL function. In a secondary analysis, we examined associations between short-term $PM_{2.5}$ concentrations and HDL. The Multi-Ethnic Study of Atherosclerosis (MESA) - an on-going prospective study of risk factors that predict progression to clinically overt cardiovascular disease or progression of the subclinical disease in a racially diverse population - provides a unique opportunity to combine highly refined measures of air pollution exposure with multiple measures of HDL, including particle number, size, and concentration, in a multi-ethnic population.

Methods

Study Population

MESA is a prospective, population-based, multiethnic cohort study of 6,814 men and women aged 45-84, which has been described in detail elsewhere(17, 69). Briefly, MESA was designed to examine the progression of subclinical atherosclerosis in a racially diverse population of adults. MESA participants were white, African American, Hispanic, and Asian (of Chinese decent) recruited from six communities in the U.S. (Baltimore, MD; Chicago, IL; Forsyth County, NC; Los Angeles County, CA; Manhattan, NY; and St. Paul, MN). Participants were free of self-reported cardiovascular disease (heart attack, stroke, transient ischemic attack, heart failure, angina, current atrial fibrillation, any cardiovascular procedure) at baseline and completed their first (baseline) exam between July 2000 and August 2002. Demographic characteristics, medical history, anthropometry, and laboratory data were collected at the

baseline visit. Institutional review boards at each study site approved the study, and written informed consent was obtained from all participants. In our analysis, we included only participants with complete outcome and covariate information (n=6,042). In a sensitivity analysis, we excluded participants with triglycerides >400 mg/dl or C-reactive protein levels above 10.0mg/L (n=707) as these participants may be in an inflammatory state unrelated to air pollution.

Exposure Assessment

MESA Air is an ancillary study funded by the Environmental Protection Agency (EPA) to combine high quality cohort-specific air pollution monitoring and modeling with MESA's extensive measurement of preclinical cardiovascular disease(17, 70). We estimated individual level $PM_{2.5}$ ($\mu g/m^3$) (incorporating pollutant infiltration into the home, as well as time-location), ambient $PM_{2.5}$ ($\mu g/m^3$) (outdoor at the participant's residence), and light absorption coefficient, a measure of black carbon (BC) computed for each participant based on their residential address. Sources of $PM_{2.5}$ include all types of combustion, while black carbon is considered a marker of traffic-related pollution. If a participant moved during the study period, that information was incorporated into the exposure estimate.

Estimates of air pollution concentrations were calculated using a hierarchical spatio-temporal model with a unified modeling approach for all pollutants, described in detail elsewhere (71, 72). Briefly, data used to produce these estimates came from several sources: the EPA Air Quality System (AQS) regulatory monitoring stations, monitors deployed by MESA Air at fixed sites in all MESA communities, monitors placed at 10% of participant's homes, and monitors specifically located to measure pollutant concentration gradients from roadways(70). Black carbon estimates did not include AQS monitoring data due to lack of comparable BC measurements in regulatory monitoring locations.

Seasonal trends and long-term pollutant averages were modeled with land-use regression using universal kriging. Geographic covariates such as distance to roadway and land use characteristics were used in the universal kriging models to improve prediction. A partial least squares approach (similar to principal component analysis) was used to select the most important geographic covariates from a suite of over 150 geographic elements(72). Separate models were built for each pollutant and each study site. Multiple years of data from AQS and fixed-site monitors were used to assess time trends in pollutant concentrations. The city-specific 10-fold leave-one-out cross-validated R^2 for the $PM_{2.5}$ model was between 0.82 and 0.91 for $PM_{2.5}$, and between 0.79 and 0.99 for BC, depending on city(72).

This modeling approach was used to predict pollutant concentrations at each participant's home location prior to blood draw for each participant. Outdoor $PM_{2.5}$ predictions reflect outdoor air pollution at a participant's home, while the individually time-weighted $PM_{2.5}$ ($PM_{2.5iw}$) predictions integrated data from time activity questions that took the participant's amount of time spent indoors versus outdoors on a typical weekday or weekend day in each season(73, 74). Data about the degree of infiltration of air pollutants into participant's home was derived from questionnaires about construction and characteristics of participant's homes, behavior (i.e., window-opening and air conditioning) and modeled by season based on a residential air pollution infiltration study done in 5% of homes at each MESA city(75). Air pollution concentrations were calculated for each averaging period starting in January of 1999. For our analysis, we used estimated average pollutant concentrations at each participant's home location during the year of their baseline exam, as well as three months and two weeks prior to each participant's baseline exam. Predictions for BC were only available at the one-year averaging time period based on monitoring data from the 2006-2008 period and no comparable agency data from other periods.

We also examined the associations between short-term exposure to $PM_{2.5}$ and HDL measures. Short-term $PM_{2.5}$ concentrations were estimated based on daily observations from

one representative monitor in the region reflecting the temporal variability in short-term air pollution measurements. Short-term averaging periods estimate average $PM_{2.5}$ exposure on the day of blood draw, the day before blood draw, and a moving average of the previous 5 days of $PM_{2.5}$ exposure. $PM_{2.5}$ concentrations were pre-adjusted to control for temporal confounding using splines for calendar time (12 degrees of freedom (df)/year), temperature (6 df/year) and relative humidity (6 df/year) and a day of the week indicator. Use of pre-adjusted exposure estimates for short-term air pollution studies has been shown to efficiently control for temporal confounding while allowing for unbiased health effect estimation in cohort studies(71).

HDL Measures

At their baseline MESA examination, participants gave 12-hour fasting blood samples which were frozen at -70°C(17). Within two weeks after samples were taken, HDL-C was assayed at the Collaborative Studies Clinical Laboratory at Fairview-University Medical Center, Minneapolis, MN, in accordance with Centers for Disease Control/National Heart, Lung and Blood Institute standards, using the cholesterol oxidase method (Roche Diagnostics) following precipitation of non-HDL-C with magnesium/dextran (CV=2.9%)(76). HDL-P was measured at LipoScience Inc. (now part of LabCorp) using nuclear magnetic resonance (NMR) with the LipoProfile-3 algorithm, described in detail elsewhere(77). HDL-P (CV=2%) was calculated as the sum of the particle concentrations of the HDL subclasses (77). These subclasses were quantified according to particle size based on the amplitudes of their lipid methyl group NMR signals. Weighted averages of each HDL subclass were used to calculate mean HDL particle size.(77).

Statistical Analysis

Cross-sectional associations between air pollution and HDL-P were examined using linear regression modeling performed in Stata/IC version 12.1 (StataCorp). In a staged series of

models, we examined potential confounding by age, sex, race/ethnicity (white, African American, Chinese American, Hispanic), and study site. We also examined further demographic and lifestyle factors including smoking (never, former, current), education (<high school, high school or equivalent, some higher education), alcohol consumption (user, nonuser), physical activity in metabolic equivalent (MET)-minutes/week, BMI, history of diabetes mellitus (defined by the 2003 American Diabetes Association fasting criteria algorithm as well as HOMA-IR), C-reactive protein (CRP), HDL-C and low-density lipoprotein cholesterol (LDL-C), triglycerides, history of hypertension (defined as systolic blood pressure >140 mm Hg or diastolic blood pressure >90 mm Hg, or self-reported hypertension and antihypertensive medication use), current use of lipid-lowering drugs, and season. In our final models we adjusted for confounding by meteorology using B-spline fits for city-specific trends in temperature (12 degrees of freedom/yr) and relative humidity (12 degrees of freedom/yr). We checked linearity for all continuous variables using loess smoothing fits, and all continuous variables were modeled linearly. Intervals of 5 ug/m^3 and 0.7 $10^{-6}m^{-1}$ were chosen because they approximated the interquartile range of the pollutants and were comparable to intervals used in prior literature.(78-80)

We explored the possibility of differential susceptibility to air pollution by including interaction terms for age, race/ethnicity, sex, obesity, diabetes, and use of lipid-lowering medications. To examine possible effect modification by site in our models, we also tested for statistically significant interactions by site and investigated results stratified by site. Additionally, we performed sensitivity analysis with adjustment for pack years and secondary smoke, mean HDL particle size, and using alternative models to adjust for site. In a secondary analysis, we also examined air pollution and measures of HDL size (Table 2.5).

Results

Of the 6,814 MESA participants, 160 participants had missing data on HDL, covariates and exposure, leaving 6,654 for analysis. Individually-weighted exposure estimates were available for 5,330 participants and black carbon exposure estimates were available for 6,557 participants. The current study population was comprised of 53% female and 18% with less than a high school education. Participants were 28% African American, 12% Chinese American, 22% Hispanic and 39% White. Sixteen percent of the study population used lipid-lowering drugs, and 45% had hypertension. Predicted outdoor $PM_{2.5}$ concentrations in the year 2000 ranged from 10.7 to 27.9 $\mu g/m^3$, with an IQR of 2.5 $\mu g/m^3$. BC concentrations ranged from 0.28 to 4.77 $10^{-6}m^{-1}$, with an IQR of 0.7 $10^{-6}m^{-1}$. Mean (standard deviation [SD]) HDL-P in our study population was 34.0 (6.6) µmol/L. Mean (SD) HDL-C in our study population was 50.8 (15.5) mg/dL (Table 2.1). Correlations between pollutants can be found in Table 2.5.

HDL Cholesterol

We found a non-significant association between higher $PM_{2.5}$ concentrations and lower HDL-C concentrations (Table 2.2). In the 2-week averaging period, adjusting for age, sex, race/ethnicity and site only, we observed a significant -0.86 mg/dL (95% CI: -1.38, -0.34) difference in HDL-C for a 5 $\mu g/m^3$ higher $PM_{2.5}$, however this association attenuated (and became non–significant) after adjustment for other covariates. We found no significant association between short-term (0 to 5 days prior) $PM_{2.5}$ exposure and HDL-C (Table 2.3).

We found a significant association between higher concentrations of black carbon and lower HDL-C levels. A 0.7 $10^{-6}m^{-1}$ higher BC was associated with a -1.68 mg/dL (95% CI: -2.86 to -0.50; p = 0.001) lower HDL-C when adjusted for traditional cardiovascular risk factors, site and season (Table 2.2). This association remained significant after adjustment for HDL-P, pack-years smoked, income, and niacin use. When using a model with random intercepts and

random slopes to control for site, we observed a 0.7 $10^{-6}m^{-1}$ higher BC was associated with a -0.97 mg/dL (95% CI: -1.97, 0.02; p = 0.055) lower in HDL-C in the 3-month time period. When using a model with a fixed effect for site a 0.7 $10^{-6}m^{-1}$ higher BC was associated with a -1.68 mg/dL (95% CI: -3.04, -0.1; p = 0.025) lower in HDL-C in the 3-month time period.

HDL Particle Number

We found a significant inverse association between medium-term (3-month and 2-week) $PM_{2.5}$ concentrations and HDL-P, but not in the one-year period, although the association in that period was of similar magnitude and direction (Table 2.2). A 5 μg/m^3 higher 3-month average $PM_{2.5}$ concentration was associated with a -0.64 μmol/L (95% CI: -1.02 to -0.26) lower HDL-P, and a 5 μg/m^3 higher 2-week average $PM_{2.5}$ was associated with a -0.29 μmol/L (95% CI: -0.57, -0.01) lower HDL-P in multivariate-adjusted models (Table 2.2). These corresponded to 1.9% and 0.8% lower HDL-P per 5 μg/m^3 higher exposure to $PM_{2.5}$, respectively.

Adjustment for HDL-C did not significantly change the association between $PM_{2.5}$ and HDL-P in the 3-month averaging time period, although the association in the 2-week time was attenuated and not significant (Table 2.5). Further adjustment for pack-years smoked, income, and niacin use did not change the direction and significance of the results (not shown). The findings related to the 3-month exposure period were not affected, in direction or significance, by adjusting for the 3-day average $PM_{2.5}$ concentration. Adjusted for 3-day average $PM_{2.5}$, a 5 μg/m^3 higher $PM_{2.5}$ over the 3-month time period was associated with a -0.50 (95% CI: -0.92, -0.09) change in HDL-P. However, over the 2-week time period adjustment for 3-day average $PM_{2.5}$ attenuated the results, as a 5 μg/m^3 higher $PM_{2.5}$ in the 2-week time period was associated with a -0.14 (95% CI: -0.47, 0.18) difference in HDL-P. When we used a model with random intercepts and random slopes to control for site, we observed a 5 μg/m^3 higher $PM_{2.5}$ was associated with a -0.47 (95% CI: -0.79, -0.14; p = 0.005) difference in HDL-P in the 3-

month time period. A model controlling for site as a fixed effect in the 3-month time period showed a -0.64 μmol/L (95% CI: -1.17, -0.11; p = 0.026) difference in HDL-P per 5 μg/m^3 higher $PM_{2.5}$. In the 2-week period, using a model with random intercepts and random slopes to control for site, we observed that a 5 μg/m^3 higher $PM_{2.5}$ was associated with a -0.22 (95% CI: -0.47, 0.03; p = 0.084) lower HDL-P. A model controlling for site as a fixed effect in the 3-month time period showed a 5 μg/m^3 higher $PM_{2.5}$ was associated with lower HDL-P of -0.30 (95% CI: -0.78, -0.19; p = 0.176).

We did not find a significant association between BC and HDL-P in the one-year averaging period, although it borderline and in the same direction as previous associations. A 0.7 $10^{-6}m^{-1}$ higher BC exposure was associated with -0.55 μmol/L (95% CI: -1.13, 0.03) lower HDL-P. This association did not change with adjustment for mean HDL particle size, HDL-C, pack-years smoked, income, and niacin use. Models using random intercepts and slopes to control for site also showed no change.

In the short-term $PM_{2.5}$ analysis, we found a significant inverse association between higher $PM_{2.5}$ in the 5 days before blood draw and HDL-P (-0.21 μmol/L per 5 μg/m^3 (95% CI: -0.38, -0.04) (Table 2.3). Averaging periods that included fewer days before the blood draw had no association with HDL-P.

We found a significant interaction between sex and the association between air pollution and HDL-C, with the association stronger in women for black carbon and HDL-C (-2.63 mg/dL; 95% CI: -4.46, -0.81) than men (-0.65 mg/dL; 95% CI: -2.14, 0.84). We observed a similarly somewhat stronger relationship in women for 3-month exposure to $PM_{2.5}$ and HDL-P (-0.71 μmol/L; [95% CI: -1.33, -0.09] for women, compared to -0.48 μmol/L; [95% CI: -0.93, -0.02] for men.) The same phenomenon was observed for 2-week exposure to $PM_{2.5}$ and HDL-P (-0.42

μmol/L; [95% CI: -0.89, 0.05] for women, compared to -0.15 μmol/L; [95% CI: -0.49, 0.18] for men.)

Discussion

In a large, multiethnic cohort study of men and women free of prevalent clinical cardiovascular disease, we found that higher concentrations of fine particulate matter and black carbon were associated with lower plasma concentrations of HDL particles and HDL cholesterol in some time periods. Lower HDL particle numbers have been associated with increasing cIMT and cardiovascular events in previous studies, and lower HDL-C is a traditional risk factor for CVD(31, 65, 67, 81).

In MESA participants, a 5 $\mu g/m^3$ higher concentration of $PM_{2.5}$ over a 3 month time period was associated with a -0.64 μmol/L or -1.9% lower HDL-P. This observed lower HDL-P is comparable to other traditional risk factors such as those observed in smoking cessation studies (1.0 μmol/L change).(82). Lower HDL-P levels in MESA participants have been independently associated with carotid atherosclerosis and coronary heart disease. A 0.7 $10^{-6}m^{-1}$ higher exposure to black carbon over a one year period was also associated with a -1.68 mg/dL or -3.2% lower concentration of HDL-C. This lower HDL-C can be compared to the effect of quitting smoking (2.4 mg/dL change) on HDL-C in smoking cessation programs(82). These associations persisted after control for smoking and other risk factors for HDL.

This is the first large observational study to suggest an association between air pollution exposure and HDL particle number and size. Our results build on previous work suggesting an association between air pollution and HDL through the use of additional properties of HDL which may be potentially more clinically relevant, and examining them in a multiethnic cohort with excellent measurement of covariates and cutting edge assessment of air pollution exposure. This study contributes to the hypothesis that air pollution may act through HDL to contribute to

cardiovascular disease at comparably low levels found in developed countries. In examining different pollutant averaging times, our study also adds information suggesting that air pollution may be associated with changes in HDL in both the short and the medium term, and that both time periods may be relevant for examining the effect of air pollution on CVD risk factors.

Two cohort studies, conducted by Chuang et al, in Taiwan have previously investigated the relationship between air pollution and HDL in humans. In a population-based survey, they reported a decrease in HDL-C per IQR increase in PM_{10} over a 1-day averaging period before blood draw(54). In a separate cross-sectional analysis, they found no association between a one IQR increase in one-year averaged air pollution exposure before blood draw and HDL-C in a cohort of 1023 subjects aged 54 to 90 living in Taiwan(56). These studies relied on central-site monitoring data and had limited data on likely confounding variables such as SES, smoking, physical activity, and use of lipid-lowering medications, which are associated with both air pollution and HDL, and may explain why our study found differing results.

Our results are consistent with a prior occupational study of $PM_{2.5}$ and HDL-C in a repeated measures panel study of welders(57). Those exposed to high $PM_{2.5}$ during welding experienced an acute decrease of -2.6 mg/dL (95% CI -5.3, -0.0) in circulating HDL-C levels 18 hours following exposure, compared to their baseline levels(57), significantly higher than effect sizes observed in our study, however welders were exposed to much higher concentrations of pollutants.

Characteristics of HDL beyond total concentration are likely important in the protective effects of the lipoprotein. In our study we focused on particle number as an alternate characteristic, though there is reason to believe that functional characteristics—not assessed here—are also important, and may be affected by air pollutant or other exposures such as tobacco smoke(24-26, 30, 58, 64, 83, 84). For example, in Yin et al, samples of HDL from mice exposed to fine and ultrafine PM were found to have significantly reduced HDL anti-

inflammatory properties compared unexposed mice, suggesting that PM exposure may reduce the ability of HDL to protect against atherosclerosis(26). Experimental study of HDL function and structure is needed to confirm and further characterize the effect of air pollution on HDL.

As a whole, these studies are consistent with the hypothesis that exposure to air pollution increases risk of CVD. Our findings support hypotheses that HDL may play a role in the biological pathway explaining the association between air pollution and CVD(27). PM has been shown to induce creation of dysfunction HDL, which is associated with reduced protection against atherosclerosis through inability to participate in reverse cholesterol transport and antioxidation(26, 27, 85).

Both the relationship between $PM_{2.5}$ and HDL-P, as well the relationship between BC and HDL-C were modified by sex. In both cases, the association between air pollution and HDL was stronger in women, although the association in men was still negative. Women typically have higher levels of both HDL-P and HDL-C than men, which has been attributed to higher estrogen production in women(86). Some research has suggested that air pollutants may induce estrogen-disrupting effects, acting as a potential xenoestrogen involved in generation reactive oxygen species (ROS) and induction of oxidative stress(87, 88). While many of the women in our study were post-menopausal, air pollution-related disruption of the HDL-raising effects of estrogen may explain the stronger results observed in women. Our study found no significant evidence of effect modification by age, race/ethnicity, diabetes, smoking, obesity, or site. Our sensitivity analyses examining adjustment for other aspects of HDL did not strongly change the conclusions of our study. The positive association between 3-month average of $PM_{2.5}$ and HDL-C when controlling for HDL-P is difficult to interpret, however it may be explained by a reduction in the number of small, cholesterol-depleted HDL particles, leaving the average amount of cholesterol in HDL particles higher on a per-particle basis. Smaller HDL particles may play a more important role in cholesterol efflux than larger particles, so a reduction

in the number of smaller HDL particles supports the hypothesis that the relationship between air pollution and cardiovascular disease could be mediated through change in cholesterol efflux.(89)

This study has a number of strengths as a large, population-based, multi-site, multiethnic cohort able to examine the relationship between air pollution and advanced measures of HDL. The MESA study features standardized measurement of numerous covariates, with multiple levels of quality control(17). Great care was taken in producing high-quality air pollution estimates, with cohort-specific monitoring and modeling, and with special attention paid to minimizing measurement error in the estimates(70, 72). This is the first study to examine air pollution and measures of HDL particle number and size in a large, multi-site, multiethnic cohort setting.

However, while our exposure models are more accurate and less susceptible to measurement error than distance to monitor or nearest roadway analyses, we cannot rule out the limitation of measurement error in our models. The measurement error in air pollution estimates is likely to be independent of HDL measurements, so we would generally expect this error to be non-differential and bias estimates towards the null, representing an underestimation of the true measure of effect. Air pollution is a complex mixture of particles and gases, and it is possible that our estimates may be driven by a different, highly correlated pollutant that is unmeasured, or an interaction between the pollutants, rather than $PM_{2.5}$ or BC per se. Further study of multipollutant models and mixtures will be needed to confirm these results. Another limitation of our study is its cross-sectional design. HDL-P was only been measured at one point in time, and a snapshot analysis of air pollution and HDL cannot provide valid inference on the effect of air pollution on HDL over time. Although estimates of air pollution represent participants' exposure in the time period before HDL was measured, associations from this study should be interpreted with caution. Finally, while covariates were measured carefully

during exams, we cannot rule out the possibility that residual confounding exists due to potentially important covariates being unmeasured or measured with error.

In summary, we found evidence that exposure to air pollution was associated with changes in several measures of HDL, and short-term exposure was associated with lower HDL-P in our study of a multi-ethnic population free of cardiovascular disease. These results help strengthen the biological plausibility of the relationship between air pollution and cardiovascular disease.

Table 2.1. Characteristics of MESA Participants in Study

	All Participants (N = 6814)	Participants with complete data (N=6654)	Participants with complete individually-weighted estimates (N=5330)
Age, mean ± SD, y	62.2 ± 10.2	62.2 ± 10.2	61.4 ± 10.0
Male, No. (%)	3213 (47.2)	3134 (47.1)	2527 (47.4)
Race/ethnicity, No. (%)			
non-Hispanic White	2622 (38.5)	2567 (38.6)	2118 (39.7)
Black	1893 (27.8)	1831 (27.5)	1427 (26.8)
Hispanic	1496 (21.9)	1466 (22.0)	1132 (21.2)
Chinese	803 (11.8)	790 (11.9)	653 (12.2)
Smoker, No. (%)			
Never	3417 (50.3)	3344 (50.3)	2716 (51.1)
Former	2487 (36.6)	2448 (36.8)	1957 (36.8)
Current	887 (13.1)	862 (13.0)	644 (12.1)
Current alcohol users, No. (%)	3749 (68.5)	3677 (68.6)	3044 (70.6)
Physical Activity, mean ± SD, hrs/day	12.6 ± 5.9	12.6 ± 5.9	12.8 ± 5.8
Body mass index, mean ± SD, kg/m^2	28.3 ± 5.5	28.3 ± 5.5	28.3 ± 5.4
Diabetes Mellitus, No. (%)			
Normal	4992 (73.5)	4895 (73.6)	3993 (75.1)
Impaired fasting glucose	939 (13.8)	922 (13.9)	725 (13.6)
Nontreated DM	179 (2.6)	177 (2.7)	120 (2.3)
Treated DM	680 (10.0)	660 (9.9)	478 (9.0)
Hypertension, No. (%)	3058 (44.9)	2984 (44.9)	2289 (43.0)
Any lipid-lowering medication, No. (%)	1100 (16.1)	1080 (16.2)	873 (16.4)
Post-menopausal, No. (%)	2949 (82.0)	2884 (82.0)	2259 (80.7)
Systolic blood pressure, mean ± SD, mm Hg	126.6 ± 21.5	126.6 ± 21.5	125.4 ± 20.7
Diastolic blood pressure, mean ± SD, mm Hg	72.0 ± 10.3	72.0 ± 10.3	71.8 ± 10.2
Homeostatic model assessment of insulin resistance (HOMA-IR), mean (SD), mg/dL	2.7 ± 6.4	2.7 ± 6.5	2.6 ± 6.5
C-reactive protein, mean ± SD, (mg/L)	3.8 ± 5.9	3.8 ± 5.8	3.6 ± 5.4
Triglycerides, mean ± SD, mg/dL	131.6 ± 88.8	132.0 ± 89.4	131.0 ± 87.4
Low-density lipoprotein, mean ± SD, mg/dL	117.2 ± 31.5	117.2 ± 31.4	117.1 ± 31.1
Total cholesterol, mean ± SD, mg/dL	194.2 ± 35.7	194.3 ± 35.7	194.1 ± 35.4
HDL Cholesterol, mean ± SD, mg/dL	51.0 ± 14.8	51.0 ± 14.8	51.1 ± 14.8
HDL Particle number, mean ± SD, µmol/L	34.0 ± 6.6	34.1 ± 6.6	34.2 ± 6.7
$PM_{2.5}$ individual year 2000, mean ± SD, $\mu g/m^3$	10.9 ± 3.3	10.9 ± 3.3	10.9 ± 3.3
$PM_{2.5}$ outdoor year 2000, mean ± SD, $\mu g/m^3$	16.7 ± 2.9	16.7 ± 2.9	16.6 ± 2.8
Black carbon year 2000, mean ± SD, $10^{-6}/m^{-1}$	0.9 ± 0.5	0.9 ± 0.5	0.9 ± 0.5

Table 2.2. Associations between long and medium-term air pollutants and HDL - MESA Air

	Individually-weighted $PM_{2.5}$ ($5\mu g/m^3$)	Outdoor $PM_{2.5}$ ($5\mu g/m^3$)	Black carbon ($0.7\ 10^{-6}/m^{-1}$)
Year 2000 average		βeta, (95% CI)	
HDL-C (mg/dL)			
Minimally adjusted Model	-0.13 (-1.24, 0.98)	0.85 (-0.69, 2.40)	-1.40 (-2.58, -0.22)
Final Model	-0.50 (-1.61, 0.61)	0.86 (-0.67, 2.38)	-1.68 (-2.86, -0.50)
HDL-P (µmol/L)			
Minimally adjusted Model	-0.15 (-0.65, 0.34)	0.33 (-0.35, 1.02)	-0.47 (-1.00, 0.05)
Final Model	-0.21 (-0.75, 0.33)	0.42 (-0.32, 1.17)	-0.55 (-1.13, 0.03)
Three Month Average	Individually-weighted $PM_{2.5}$ ($5\mu g/m^3$)	Outdoor $PM_{2.5}$ ($5\mu g/m^3$)	*Black carbon not available at this level
HDL-C (mg/dL)			
Minimally adjusted Model	-0.002 (-0.75, 0.74)	0.88 (0.27, 1.48)	
Final Model	-0.05 (-0.82, 0.71)	0.47 (-0.17, 1.10)	
HDL-P (µmol/L)			
Minimally adjusted Model	-0.64 (-0.97, -0.31)	-0.28 (-0.55, -0.01)	
Final Model	-0.64 (-1.01, -0.26)	-0.27 (-0.58, 0.04)	
Two week Average	Individually-weighted $PM_{2.5}$ ($5\mu g/m^3$)	Outdoor $PM_{2.5}$ ($5\mu g/m^3$)	*Black carbon not available at this level
HDL-C (mg/dL)			
Minimally adjusted Model	-0.86 (-1.38, -0.34)	-0.01 (-0.38, 0.37)	
Final Model	-0.39 (-0.97, 0.18)	0.14 (-0.26, 0.55)	
HDL-P (µmol/L)			
Minimally adjusted Model	-0.35 (-0.58, -0.12)	-0.13 (-0.29, 0.04)	
Final Model	-0.29 (-0.57, -0.01)	-0.07 (-0.27, 0.12)	

Abbreviations: HDL = high-density lipoproteins, PM = particulate matter

Minimally adjusted model is adjusted only for age, site, sex, and race/ethnicity

Final adjusted model is adjusted for the factors in the minimally adjusted model plus BMI, education, physical activity (MET min-wk), smoking (never/former/current), current alcohol use (y/n), diabetes (normal/IFG/Untreated/Treated), hypertension (y/n), use of lipid-lowering drugs (y/n), outdoor temperature, relative humidity, HOMA-IR, log CRP, LDL-C, and triglycerides

Table 2.3. Associations between measures of HDL and short-term exposure to fine particulate air pollution on the day of blood collection and the days prior

Model	Change in HDL per 5 μg/m^3 higher average $PM_{2.5}$ prior to blood draw			
	Day of blood draw	1 day prior	3 days prior	5 days prior
HDL-P (μmol/L)				
Minimally adjusted Model	-0.04 (-0.15, 0.06)	-0.06 (-0.15, 0.04)	-0.11 (-0.23, 0.02)	-0.17 (-0.32, -0.02)
Final model	-0.06 (-0.17, 0.06)	-0.06 (-0.17, 0.04)	-0.13 (-0.27, 0.01)	-0.21 (-0.38, -0.04)
HDL-C (mg/dL)				
Minimally adjusted Model	-0.14 (-0.37, 0.10)	-0.06 (-0.28, 0.16)	-0.06 (-0.34, 0.23)	-0.20 (-0.54, 0.14)
Final model	0.02 (-0.21, 0.26)	0.05 (-0.17, 0.28)	0.01 (-0.28, 0.30)	-0.06 (-0.42, 0.29)

Abbreviations: HDL = high-density lipoproteins, PM = particulate matter

Minimally adjusted model is adjusted only for age, site, sex, and race/ethnicity

Final adjusted model is adjusted for the factors in the minimally adjusted model plus BMI, education, physical activity (MET min-wk), smoking (never/former/current), current alcohol use (y/n) diabetes (normal/IFG/Untreated/Treated), hypertension (y/n), use of lipid-lowering drugs (y/n), HOMA-IR, log CRP, LDL-C, and triglycerides

Table 2.4. Associations between long and medium-term air pollutants and HDL Size - MESA Air

	$PM_{2.5}$ (5μg/m^3)	Outdoor $PM_{2.5}$ (5μg/m^3)	Black Carbon (0.7 10^{-6}/m^{-1})
Year 2000 Averages		βeta, (95% CI)	
Small HDL (μmol/L)			
Minimally adjusted Model	-0.31 (-0.77, 0.15)	-0.56 (-1.19, 0.08)	-0.21 (-0.70, 0.28)
Final Model	-0.29 (-0.77, 0.19)	-0.43 (-1.08, 0.23)	-0.04 (-0.55, 0.47)
Final Model + medium & large HDL	-0.20 (-0.58, 0.17)	-0.05 (-0.56, 0.47)	-0.16 (-0.56, 0.24)
Medium HDL (μmol/L)			
Minimally adjusted Model	0.19 (-0.34, 0.72)	0.69 (-0.04, 1.41)	0.06 (-0.50, 0.61)
Final Model	0.23 (-0.36, 0.82)	0.72 (-0.07, 1.52)	-0.11 (-0.73, 0.51)
Final Model + small & large HDL	0.02 (-0.44, 0.48)	0.40 (-0.23, 1.03)	-0.10 (-0.58, 0.39)
Large HDL (μmol/L)			
Minimally adjusted Model	-0.03 (-0.29, 0.23)	0.20 (-0.16, 0.56)	-0.32 (-0.60, -0.05)
Final Model	-0.15 (-0.41, 0.12)	0.14 (-0.22, 0.50)	-0.40 (-0.67, -0.12)
Final Model + small & medium HDL	-0.17 (-0.43, 0.08)	0.08 (-0.27, 0.43)	-0.39 (-0.67, -0.12)
Mean HDL Size (nm)			
Minimally adjusted Model	0.02 (-0.01, 0.06)	0.05 (0.0003, 0.10)	-0.02 (-0.06, 0.02)
Final Model	0.01 (-0.03, 0.04)	0.03 (-0.02, 0.08)	-0.04 (-0.07, 0.003)
Final Model + HDL-C	0.02 (-0.01, 0.05)	0.01 (-0.03, 0.05)	0.01 (-0.03, 0.05)
Final Model + HDL-P	0.01 (-0.02, 0.05)	0.02 (-0.02, 0.07)	-0.03 (-0.06, 0.01)
HDL-C (mg/dL)			
Final Model + HDL-P	-0.18 (-0.98, 0.63)	0.24 (-0.87, 1.36)	-0.89 (-1.76, -0.03)
Final Model - high TG & CRP*	-0.32 (-1.48, 0.84)	0.91 (-0.67, 2.50)	-1.64 (-2.84, -0.42)
HDL-P (μmol/L)			
Final Model + HDL-C	-0.05 (-0.45, 0.35)	0.15 (-0.39, 0.69)	0.01 (-0.42, 0.43)
Final Model - high TG & CRP*	-0.06 (-0.62, 0.51)	0.52 (-0.24, 1.29)	-0.60 (-1.19, -0.01)
Three Month Averages	$PM_{2.5}$ (5μg/m^3)	Outdoor $PM_{2.5}$ (5μg/m^3)	*Black Carbon not available at this level
Small HDL (μmol/L)			
Minimally adjusted Model	-0.63 (-0.94, -0.32)	-0.69 (-0.94, 0.44)	
Final Model	-0.65 (-0.98, -0.32)	-0.51 (-0.78, -0.24)	
Final Model + medium & large HDL	-0.62 (-0.88, -0.36)	-0.42 (-0.63, -0.20)	
Medium HDL (μmol/L)			
Minimally adjusted Model	0.05 (-0.30, 0.41)	0.28 (-0.008, 0.56)	
Final Model	0.09 (-0.31, 0.50)	0.16 (-0.17, 0.49)	
Final Model + small & large HDL	-0.39 (-0.71, -0.07)	-0.22 (-0.49, 0.04)	
Large HDL (μmol/L)			
Minimally adjusted Model	-0.06 (-0.23, 0.11)	0.13 (-0.01, 0.27)	
Final Model	-0.09 (-0.27, 0.09)	0.08 (-0.07, 0.23)	
Final Model + small & medium HDL	-0.14 (-0.31, 0.04)	0.04 (-0.11, 0.19)	
Mean HDL Size (nm)			
Minimally adjusted Model	0.01 (-0.01, 0.03)	0.03 (0.01, 0.05)	

Final Model	0.01 (-0.01, 0.04)	0.02 (0.002, 0.04)
Final Model + HDL-C	0.01 (-0.01, 0.03)	0.01 (-0.003, 0.03)
Final Model + HDL-P	0.02 (-0.001, 0.05)	0.03 (0.01, 0.05)
HDL-C (mg/dL)		
Final Model + HDL-P	0.86 (0.30, 1.41)	0.83 (0.37, 1.29)
Final Model - high TG & CRP*	0.01 (-0.79, 0.81)	0.44 (-0.22, 1.11)
HDL-P (µmol/L)		
Final Model + HDL-C	-0.62 (-0.90, -0.35)	-0.42 (-0.64, -0.19)
Final Model - high TG & CRP*	-0.57 (-0.96, -0.18)	-0.26 (-0.58, 0.06)

Two week Averages	$PM_{2.5}$ (5µg/m^3)	Outdoor $PM_{2.5}$ (5µg/m^3)
Small HDL (µmol/L)		
Minimally adjusted Model	0.05 (-0.17, 0.26)	-0.11 (-0.26, 0.05)
Final Model	-0.17 (-0.42, 0.08)	-0.15 (-0.32, 0.03)
Final Model + medium & large HDL	-0.20 (-0.39, -0.003)	-0.12 (-0.26, 0.02)
Medium HDL (µmol/L)		
Minimally adjusted Model	-0.22 (-0.47, 0.03)	-0.01 (-0.18, 0.17)
Final Model	-0.03 (-0.33, 0.27)	0.05 (-0.16, 0.26)
Final Model + small & large HDL	-0.14 (-0.38, 0.09)	-0.06 (-0.23, 0.10)
Large HDL (µmol/L)		
Minimally adjusted Model	-0.18 (-0.30, -0.06)	-0.01 (-0.10, 0.07)
Final Model	-0.10 (-0.24, 0.03)	0.02 (-0.07, 0.12)
Final Model + small & medium HDL	-0.11 (-0.25, 0.02)	0.01 (-0.08, 0.10)
Mean HDL Size (nm)		
Minimally adjusted Model	0.01 (-0.03, 0.004)	0.004 (-0.01, 0.02)
Final Model	0.0003 (-0.02, 0.02)	0.01 (-0.005, 0.02)
Final Model + HDL-C	0.01 (-0.01, 0.02)	0.01 (-0.004, 0.02)
Final Model + HDL-P	0.01 (-0.01, 0.02)	0.01 (-0.003, 0.02)
HDL-C (mg/dL)		
Final Model + HDL-P	0.02 (-0.40, 0.44)	0.25 (-0.05, 0.54)
Final Model - high TG & CRP*	-0.27 (-0.87, 0.33)	0.23 (-0.19, 0.65)
HDL-P (µmol/L)		
Final Model + HDL-C	-0.16 (-0.36, 0.05)	-0.12 (-0.26, 0.02)
Final Model - high TG & CRP*	-0.23 (-0.53, 0.06)	-0.04 (-0.24, 0.16)

* Black Carbon not available at this level

Abbreviations: HDL-C = high-density lipoprotein cholesterol, HDL-P = high-density lipoprotein particle number, Small HDL = 7.3-8.3 nanometers, Medium HDL = 8.2-9.4 nanometers, Large HDL = 9.4-14 nanometers, PM = particulate matter, TG = triglycerides, CRP = C-reactive protein

Minimally adjusted model is adjusted only for age, site, sex, and race/ethnicity

Final adjusted model is adjusted for the factors in the minimally adjusted model plus BMI, education, physical activity (MET min-wk), smoking (never/former/current), current alcohol use (y/n), diabetes (normal/IFG/Untreated/Treated), hypertension (y/n), use of lipid-lowering drugs (y/n), outdoor temperature and relative humidity, HOMA-IR, log CRP, LDL-C, and triglycerides

* Models excluding those with triglycerides >400 and CRP >3

Table 2.5. Correlations within exposures and HDL measures

Variable	Large HDL	Medium HDL	Small HDL	Mean HDL Size	HDL-C	HDL-P
Large HDL	1					
Medium HDL	0.2774	1				
Small HDL	-0.2782	-0.6323	1			
Mean HDL Size	0.872	0.2148	-0.3395	1		
HDL-C	0.9086	0.4502	-0.2832	0.7244	1	
HDL-P	0.5658	0.6286	0.0672	0.3819	0.6919	1

Variable	Individually weighted $PM_{2.5}$, 2000	Individually weighted $PM_{2.5}$, 3 month avg	Individually weighted $PM_{2.5}$, 2 week avg	Outdoor $PM_{2.5}$, 2000	Outdoor $PM_{2.5}$, 3 month avg	Outdoor $PM_{2.5}$, 2 week avg	Black Carbon, 2000
Individually weighted $PM_{2.5}$, 2000	1						
Individually weighted $PM_{2.5}$, 3 month avg	0.8923	1					
Individually weighted $PM_{2.5}$, 2 week avg	0.759	0.8279	1				

Outdoor $PM_{2.5}$, 2000	0.8366	0.7453	0.6345	1			
Outdoor $PM_{2.5}$, 3 month avg	0.7092	0.8304	0.6608	0.788	1		
Outdoor $PM_{2.5}$, 2 week avg	0.5418	0.6365	0.8525	0.6088	0.7236	1	
Black Carbon, 2000	0.5771	0.5367	0.4477	0.4064	0.3894	0.291	1

Chapter 3

Diesel Exhaust Inhalation and Changes in HDL Function and Structure

ABSTRACT

BACKGROUND

Experimental studies of $PM_{2.5}$ in humans and animals have identified molecular and cellular changes that are associated with cardiovascular disease: changes in lipid oxidation and metabolism, systemic oxidative stress and inflammation, pulmonary oxidative stress and inflammation. High-density lipoprotein (HDL) modifications may mediate or be affected by $PM_{2.5}$-associated molecular effects. We examined whether controlled exposure to diesel exhaust changed several measures of HDL in two separate experiments.

METHODS AND RESULTS

Twenty-four adults in one randomized, double-blind, multi-factorial, crossover exposure study were exposed to diesel exhaust (~200 ug/m^3 $PM_{2.5}$) and filtered air (FA) for 2 hours, and an anti-oxidant or placebo in four different experimental arms. Ten adults in a second randomized, double-blind, multi-factorial, crossover exposure study were exposed to diesel exhaust with a higher concentration of $PM_{2.5}$ (~300 ug/m^3 $PM_{2.5}$) and filtered air (FA) for 2 hours, and terazosin or placebo in four different experimental arms. We measured aspects of HDL taken from fasted blood samples before and after exposure to DE and FA. We used linear mixed models to compare difference in differences in HDL measures. We did not find significant differences in HDL oxidant index: 0.01 (-0.06, 0.07), or PON activity: 0.05 (-0.02, 0.12) in the DE group compared to the FA group. We also used linear mixed models to examine difference in

differences in HDL proteins, and found differences in complement C3, serum amyloid A1 peptide GPGG, and cholesteryl ester transport protein, however these were not significant after accounting for multiple comparisons.

CONCLUSIONS

We did not find significant evidence of association comparing differences between exposure to DE and changes in the HDL proteome or HDL oxidant index before and after exposure. This result is consistent with a previous study examining the effects of exposure to concentrated coarse ambient particles. More research is needed into different measurements of HDL function, exposure periods, and in different populations who may be at more risk from the deleterious health effects of DE.

Introduction

Increasing evidence suggests a causal relationship between traffic-related fine particulate matter ($PM_{2.5}$) and cardiovascular disease (2, 3). The relationship between long-term exposure to traffic-related air pollution and cardiovascular disease has been documented in numerous observational studies, but biological mechanisms explaining this association are not fully understood(23).

Experimental studies of $PM_{2.5}$ in humans and animals have identified molecular and cellular changes related to $PM_{2.5}$ exposure that are also associated with cardiovascular disease: changes in lipid oxidation and metabolism, systemic oxidative stress and inflammation, pulmonary oxidative stress and inflammation(23, 27). High-density lipoprotein (HDL)

modifications may mediate $PM_{2.5}$-associated molecular effects. While HDL cholesterol is a traditional cardiovascular risk factor associated with reduced risk of cardiovascular events, many of HDL's protective qualities are not well measured by the amount of cholesterol in the particles, and may be better measured by directly assessing characteristics of the HDL(46). HDLs protect against cardiovascular disease through several mechanisms - exerting anti-inflammatory effects, inhibiting lipid oxidation, preserving endothelial function, and transporting excess cholesterol from vascular walls (also called Reverse Cholesterol Transport, or RCT). HDL can become dysfunctional as it takes on oxidized phospholipids and lose its protective effects, in some cases becoming pro-inflammatory(90). Prior work suggests that HDL can protect against $PM_{2.5}$-induced damage to endothelial cells and macrophages, and that air pollutants can make HDLs become dysfunctional(25, 26, 30, 68).

Examination of the HDL proteome can provide insight into the functional qualities of HDL molecules(91). In recent years, mass spectrometry has allowed researchers to find and measure the abundance of proteins in HDL, revealing an exceedingly diverse collection of apolipoproteins, enzymes, co-factors for enzymes and many other proteins(92). During conditions of infection, inflammation or trauma, the body undergoes a number of proteomic and metabolomic changes called the acute phase response(APR). In the context of APR, HDL undergo a number of changes to their levels and composition. Some proteins tend to be reduced, such as Apo-A1, transtherytin, and retinol-binding protein(93). This stimulates release of free ligands and 'acute-booster reactants' needed for repair processes, such as vitamin A and metal ions(93). These proteins can be replaced in HDL by serum amyloid A1 (SAA1), which is produced by hepatocytes stimulated by circulating cytokines upregulated during the acute phase(94). In some animal models, the amount of SAA1 expressed in HDL can increase 1000-fold with 24 hours of APR(93, 95). SAA1-enriched HDL particles impair the anti-inflammatory properties of HDL and are cleared from circulation faster, leading to lower levels of HDL during APR(93, 96). In addition to SAA1, HDL contains numerous other proteins associated with APR

that may undergo remodeling during inflammation. Air pollution is associated with higher levels of inflammatory markers(80).

Diesel-powered motor vehicles are a major source of urban $PM_{2.5}$. We use well-characterized, tightly controlled, freshly generated, diluted, and aged diesel exhaust (DE) inhalation as a model exposure to determine the effect of urban $PM_{2.5}$, and traffic-related air pollutants, on anti-oxidant function of HDL. In our study we examine the hypotheses that exposure to $PM_{2.5}$ in the form of DE results in dysfunctional HDL, as measured by decreased antioxidant capacity of HDL and paraoxanase-1 (PON1) activity, in a trial of anti-oxidant administration. An increase in HDL dysfunction due to $PM_{2.5}$ exposure could reveal an important pathway by which ambient air pollution effects CVD and mortality. We were able to take advantage of existing data from exposure studies that were originally designed and powered to examine the effect of exposure to air pollution on blood pressure and the vasculature as a primary outcome.

Methods

Both study designs employed a double-blind, 4-arm, crossover exposure trial design with subjects randomized to the order of exposures (diesel or filtered air) and drug (anti-oxidant/placebo in study 1, and terazosin/placebo in study 2), with a minimum two-week washout period to ensure no carryover effects of either DE exposure or drug. For Study 1, data is available on 18 of 22 young healthy adult subjects, who received either an antioxidant/placebo and either DE/filtered air (FA) at each study visit for four exposure situations. In study 2, a subset of 10 healthy subjects (out of 23) have HDL proteome data. For both studies, data was collected on age, sex, race/ethnicity, smoking, and medication use via

interview by study staff. Smoking status was confirmed by urinary cotinine at the baseline study visit, and self-reported smokers and smokers identified from cotinine were excluded from this study. Using questionnaire, spirometry, fasting glucose, lipid panel and ECG data, subjects were excluded if there was evidence or history of hypertension, asthma, diabetes mellitus, hypercholesterolemia, cardiovascular illness, or other existing chronic medical conditions. In order to participate, subjects were also required to have body mass index below 30 kg/m2 and fasting blood sugar (glucose) below 126 mg/dL. Subjects were also excluded if they were taking blood thinning medications, statins, or antioxidants. Subjects capable of bearing children were enjoined to use contraception during the study period, and were given a urine pregnancy test before at the enrollment study visit and at each subsequent exposure visit. No subjects had a positive pregnancy test at any time through the duration of the study.

Trial Protocol

In order to limit variation between sessions, all exposures and assessments were conducted at the same time of day. Fasting blood samples were taken at 7:30am at patients' arrival to the facility. At 8:30 am on separate days, participants were exposed for 120 minutes to either DE or filtered air (FA). Measurements were taken before, during and after the exposure at the same time for each participant at each study visit. Blood samples used in this analysis were taken before exposure (time 0), 8 hours after time 0, and 24 hours after time 0. Participants, nurses, and researchers were blinded as to which exposure was received. Following each exposure, participants rested at the Clinical Research Center, where they received the same standardized meal 4 hours after the start of the exposure. Participants returned the following morning for follow-up measurements 24 hours after the exposure start. All researchers, nurses, and laboratory technicians in contact with the participant or involved in the study procedures were blinded to exposure and medication treatments.

Diesel Exhaust Exposures

The exposures have previously been described(97). Briefly, fresh diesel exhaust was generated, carefully diluted and aged to duplicate, in inhalational models, ambient air pollution exposures to which humans would be exposed in urban and workplace environments. Separate exposure rooms were developed for humans or animals to be exposed to DE with excellent control over the concentration of air pollutants and duration of time exposed(97). Numerous projects have used this facility to successfully study biological responses to short-term DE exposures(98-105). In Study 1, as described in detail elsewhere, the exposure facility used a 2002 model turbocharged direct-injection 5.9-L Cummins B-series engine in a 100-kW generator set, running at steady state before subject arrival, to generate DE (6BT5.9G6; Cummins, Inc, Columbus, IN) in a 116 m^3 room(97). In Study 2, the facility used a 5.5-kW, single-cylinder generator (2010 Yanmar model YDG5500EV-6EI) to produce the exposure mixture(106). Particle composition was similar to the U.S. Environmental Protection Agency (EPA) light-duty DE profile for both studies. $PM_{2.5}$ concentration was maintained at 200 ug/m^3 in the breathing zone for Study 1, and 300 ug/m^3 in the breathing zone for Study 2. Nephelometers were incorporated into a tapered element oscillating microbalance (1400a $PM_{2.5}$; Rupprecht and Patashnick Co, Albany, NY) which was used with a feedback control system to continuously adjust exhaust levels over the course of the experiment was used to ensure that $PM_{2.5}$ concentrations remained stable. A carbon matrix filter and HEPA filter (99.99% efficient) were used to clean ambient air, and were identically conditioned for temperature and humidity for FA exposures.

Terazosin and anti-oxidant regimes

The Investigational Drug Service (IDS) at the University of Washington Medical Center (UWMC) and prepared the 500 mg vitamin C (ascorbate; AA) and 600 mg N-acetylcysteine (NAC) capsules and matched placebos using pharmaceutical grade materials. Starting seven days before each controlled exposure, subjects took 500mg AA capsules every 12 hours until the controlled exposure day. On the exposure day, subjects were administered 1000 mg AA and 600 mg NAC or placebos 2 hours before the exposure began. The IDS also prepared and administered either 1 mg or 2 mg terazosin and placebo for Study 2. A pre-study run-in visit prior to exposure sessions was run to ensure that subjects were able to tolerate the drug. Subjects were assigned 2 mg terazosin if they were able to tolerate the dose without dizziness at their run-in visit, and 1 mg otherwise. On the exposure day, subjects were administered 1 or 2 mg terazosin or placebos 2 hours before the exposure began. Differences in terazosin dose were due to a protocol modification to improve agent tolerability. We do not hypothesize that terazosin would affect the HDL proteome, regardless of dose.

The study protocol and informed consent forms were approved by the University of Washington Human Subjects review division and Internal Review Board. All potential study subjects were required to give full written informed consent prior to screening.

Outcomes

HDL oxidant index

We assessed HDL anti-oxidant capacity using a cell-free fluorescence assay, as detailed previously(25, 26, 30, 63). We used dichlorofluorescein (DCF) fluorescence to measure the ability of HDL to prevent LDL oxidation. To do this, we first thawed plasma samples and isolated HDL cholesterol using dextran-sulphate precipitation. This involved intubating 50mL samples of plasma with 10 mL of LipiDirect Magnetic HDL cholesterol precipitating reagent (Reference

Diagnostics, Inc, Bedford, MA) at room temperature for 10 minutes. We then centrifuged this mixture for 5 min at 12,000 rpm at 4 C, and collected the HDL supernatant.

As LDL oxidation causes dihydrodichlorofluorescein (DCFH) to change to the fluorescent DCF via free radicals generated in the LDL oxidation process, we measured the intensity of fluorescence of DCF during the air oxidation of LDL both with and without HDL supernatant in order to test the HDL's ability to reduce oxidation(26). In order to measure the oxidation through the amount of fluorescence, we mixed 12.5 µl of human LDL (50µg cholesterol/ml) with 12.5 µl of the HDL supernatant and 75 µl of Tris-HCL buffer (pH7.4) and incubated them at 37°C for 60 min. We then added 25 µl of DCFH solution (50µg/ml) to each well, mixed, and incubated at 37°C for 2h. We measured the intensity of fluorescence using a plate reader (SynergyMx, BioTek, Vermont, USA) set at an excitation wavelength of 485 nm and emission wavelength of 530 nm. HDL oxidation index (HOI) was derived by dividing the amount of DCF fluorescence in the presence of HDL by the amount of DCF fluorescence in the absence of HDL. An HOI below 1.0 indicates the HDL is protective HDL with anti-oxidant capacity, while an HOI over 1.0 indicates pro-oxidant HDL. Samples were analyzed for possible hemolysis during processing using spectrophotometry. As hemolysis can interfere with the accuracy of the assay, samples found to be hemolyzed with above 0.6 optical density of 410nm (OD410) were excluded from analysis. We also present an adjusted HOI which has been adjusted using the variability shown by the control sample on each plate to correct for possible hemolysis.

Paraoxonase-1 Activity

Paraxonase-1 (PON1), a protein found in HDL that exerts anti-oxidant effects, was examined in our sample. As described in detail elsewhere, PON1 activity was assessed using 5 µL of plasma, which was mixed with paraoxon substrate containing 2mM calcium chloride, 2.0 M sodium chloride in 100mM Tris–HCl buffer (pH 8.5) (26, 30, 107). Spectrophotometry was

used to quantify the hydrolysis of paraoxon substrate (diethyl-p-nitrophenyl phosphate) to p-nitrophenol by PON1. Kinetics of p-nitrophenol formation was monitored by logging absorbance at 405 nm every 15 seconds for 4 minutes, and PON1 activity is reported as umol of p-nitrophenol produced per 1 mL of plasma per minute. All assays were performed at room temperature.

HDL Proteome measures

Targeted mass spectrometry was used to quantify associated HDL proteins using protein internal standards and stable isotope-labeled peptides and described in detail elsewhere(108). Briefly, using thawed EDTA plasma, sequential potassium bromide density gradient ultracentrifugation was used to isolate the HDL (ρ = 1.063-1.210 g/mL) fraction. HDL protein concentration was measured using the Bradford assay. The HDL proteins were then reduced, alkylated, and digested with trypsin before being analyzed using nanoflow liquid chromatography-tandem mass spectrometry (LC/MS). Peptides from the target proteins were separated by peak area and identified by their retention time on the chromatographic column, isolating their precursor mass, fragmenting it, and then analyzing the resulting fragment ions as the mass-to-charge ratio as measured by the mass spectrometer. Trypsin digestion variability, matrix effects, and fluctuations in instrument performance are controlled for by use of stable isotope labelled internal standard Apo-A1 peptides. Skyline software was used to match chromatographic peak areas to the corresponding HDL protein peptides. The relative abundance of each HDL protein was calculated as the sum of the peak area of between two and six of the strongest precursor transitions of the endogenous peptide. These peak area ratios were then normalized by the peak area of the stable isotope-labelled VQPY internal standard protein in order to control for trypsin digestion variability, matrix effects, and instrument error(108).

Statistical Methods

Study 1

We examined outcomes at all three time points, and used mixed effects linear regression models with random effects for subject to examine differences in trajectory of the outcome over time between the DE exposed group and FA group. Our models were adjusted for age, sex, race, BMI, glucose, triglycerides, LDL, and hemolysis. Our model also included an interaction term for exposure - antioxidant treatment. This modelling approach allowed us to use data from subjects who were missing one or more measurements, and to increase statistical power for our measures of effect. We created an interaction term between exposure and an indicator for pre or post exposure to compare the changes in outcome in DE group to the changes in the outcome in the FA group. We also conducted sensitivity analyses to exclude period or carry-over effects. Stata 12.1 was used to perform all statistical analyses (Stata Corp, College Station TX).

Study 2

We generated descriptive statistics to compare variables in crude analyses. Protein abundance measures were log transformed for analysis to address right skewness in the data. We examined outcomes at both time points, and used mixed effects linear regression models with random intercepts for subject to examine differences in trajectory of the outcome over time between the DE exposed group and FA group. Stata 12.1 was used to perform all statistical analyses (Stata Corp, College Station TX).

Results

Study 1

Descriptive statistics for the 18 enrolled study subjects who met inclusion criteria for Study 1 are presented in Table 3.1. Mean and median HDL oxidant index, adjusted HDL oxidant index, and PON activity for each time period and study arm are presented in Tables 3.2 and 3.3. Unadjusted differences between DE and FA are presented in Table 3.4, with adjusted differences in Table 3.5. Mixed effects models for our primary analysis are presented in Table 6. Log-transformed HOI was increased in the DE group over time compared to the FA group, however the increase was not significant. There were no significant differences in HOI or PON activity between diesel exhaust and filtered air. There was a non-significant first order trend towards higher logHOI and adjusted logHOI (indicating reduced HDL anti-oxidant capacity) at 8h post exposure in those exposed to diesel exhaust, which remained elevated but still not significant the following morning (Table 3.6). Results were robust to consideration of total HDL-C concentrations, and there was no evidence of period or carryover effects.

Study 2

Descriptive statistics for the 10 enrolled study subjects who met inclusion criteria for Study 2 are presented in Table 3.1. Median and interquartile ranges for the mass to charge ratio of each HDL protein are presented in Tables 3.6 and 3.7. Results of mixed effects models examining changes in HDL proteins comparing DE and FA exposed participants are presented in Figure 3.1. DE was associated with changes in three HDL proteins: Serum Amyloid Alpha peptide GPGG (SAA_GPGG), Cholesteryl Ester Transport Protein (CETP), and Complement C3 protein, however none were significant at the Benjamini-Hochberg false discovery rate-corrected level. SAA1 had the greatest increase in abundance compared to the FA group,

although this large magnitude change fell short of significance. Results did not change with adjustment for batch, order of exposure or season of exposure.

Discussion

We did not find strong evidence to reject the null hypothesis that there is no association between brief exposure to diesel exhaust for 2 hours and changes in HDL anti-oxidant function or PON1 activity 8 or 24 hours later. Diesel exhaust was associated with changes in three HDL proteins, however these associations were not significant after correction for multiple comparisons. SAA1, an important protein involved in acute phase response, had the largest change in abundance. Although HOI increased following diesel exposure compared to those exposed to filtered air, indicating greater oxidation, the differences were not significant. This result is consistent with a previous experimental study of a different experimental pollution model (concentrated ambient particles, or CAPs) on the same measures of human HDL function, and does not provide strong evidence to support the hypothesis that acute changes in HDL function are an important mechanism to explain the relationship between short-term increases in air pollution and increased risk of cardiovascular events(63). This is the first study to examine the relationship between acute exposure to DE and the HDL proteome. Our results are similar to some other experimental studies examining the effect of air pollution on HDL function. In a similar experimental exposure design in 32 humans, Maiseyeu et al. reported no effect of 2-hour exposure to coarse (particulate matter between 2.5 and 10 μm in diameter) concentrated ambient particles (CAPs) (mean $PM_{10-2.5}$ = 76.2 ± 51.5 μg/m^3) from a rural location on HDL anti-oxidant capacity or paraoxonase activity(63). The Maiseyeu study not only used a different exposure system, but also used only post-exposure measurements of HDL function; our study used both pre and post exposure measurements and was able to better account for

between-person variability by analyzing change in HDL function from baseline due to the exposure(63).

While this is the second study to report no statistically significant effect of air pollution on HDL function, questions about the relationship remain. Other exposure studies in humans have reported that acute exposure to air pollution can rapidly impact HDL composition in brief exposure settings(62). In a double-blind trial of 11 smokers, exposure to 2 hours of coarse CAPs (mean $PM_{10-2.5}$ = 108.7 ± 24.8 μg/m^3) was associated with lower HDL-C 22 hours after exposure(62). In an unblinded study of 23 healthy young volunteers exposed to 2hrs of NO_2 and $PM_{2.5}$ CAPs (mean $PM_{2.5}$ 89.5 ± 10.7 μg/m^3), Chuang, et al, found a slight increase in HDL-C after exposure(54). In Rice et al, researchers observed a strong decrease in HDL-C after 6-8 hrs of welding in welders who did not weld the previous day (-4.3 mg/dL)(57). While this was an observational rather than a controlled experimental study, the large decrease in HDL-C after longer exposures to welding imply that specific chemical mixtures of PM and durations of exposure may play an important role in HDL response and these factors are worthy of future study. HDL is also a highly complex molecule involved in numerous biological processes and other structural and functional aspects of HDL, such as their proteomic and lipidomic content should be examined over longer time periods with a variety of different pollutant mixtures(2, 31, 46).

Animal studies have been more consistent in supporting the hypothesis that air pollution impacts HDL structure and function(24-26, 30). Several experiments have indicated that exposure to fine or ultrafine PM is capable of impairing several aspects of HDL functionality including anti-inflammatory capacity, anti-oxidant capacity and paraoxonase activity(25, 26, 30). Yin, et al (2013, ATVB), exposed ApoE-deficient mice to DE at ~250 ug/m^3 and filtered air for two weeks, and found increased peroxidation in bronchoalveolar lavage fluid and pro-oxidative changes in HDL, supporting the hypothesis that oxidation in the lung surface can lead to

increased oxidation and development of dysfunctional circulating HDL(26). Li, et al. exposed LDL-receptor null mice to ultrafine particles for 10 weeks, finding reduced HDL-C, HDL anti-oxidant capacity, and paraoxonase (PON) activity (an important measure of HDL antioxidant activity)(25). In Araujo et al, samples of HDL from mice exposed to fine and ultrafine PM were found to have significantly reduced HDL anti-inflammatory properties compared unexposed mice, suggesting that PM exposure may reduce the ability of HDL to protect against atherosclerosis(24). In Bass, et al, rats exposed to O_3 for up to 13 weeks did not show significant change in HDL-C, although exposure to O_3 was not assessed in our study(48).

Some explanation for the differences between animal and human studies may be explained by the length of exposure time. Mice in air pollution exposure studies are generally exposed to pollution for a period of days or weeks, while humans were generally exposed for 2 hours or less. In addition, due to their smaller size, the effective dose of the same concentration of air pollution is much greater than humans. This much longer exposure period is compounded by the shorter lifespan of mice, resulting in much higher effective air pollution dosage for a much longer proportion of the mice' lifespan. We hypothesize that if exposed for comparable amounts of time, humans might show responses more consistent with animal studies.

Apo-E -/- mice of the type used in most previous exposure studies are used to model at an accelerated pace, developing detectable signs of atherosclerosis as early as 3 months of age(109). Healthy young subjects are generally at very low risk of development of atherosclerosis compared to Apo-E -/- mice, which reflect a model of atherosclerotic processes more comparable to that of older adults(109). In order to detect whether air pollution effects HDL function, studies in populations more generalizable to the broader adult population more susceptible to risk of atherosclerosis may be required.

The biological mechanisms by which air pollution caused dysfunctional changes in HDL in previous animal studies remain unclear. Numerous studies of air pollution exposure have

shown that air pollution causes pro-inflammatory and oxidative effects(2, 27, 80). These processes can lead to activation of cytokines and hepatocytes which express acute phase proteins such as SAA1, which remodel the HDL proteome, causing the HDL to become dysfunctional and pro-inflammatory(90, 94). We observed increased SAA1 in the DE exposed group compared to the FA group, however the study may have been underpowered to detect a significant effect. These acute phase proteins may also cause dysfunctional HDL through oxidative protein modification mediated by the enzyme myeloperoxidase, which can bind and modify apo-A1, causing HDL to become dysfunctional and pro-inflammatory(61, 90). Complement C3 is an important activating protein in the complement system, involved in chronic inflammation and implicated in diabetes and hypertension. PM activates C3 in mice, and air pollution has been associated with circulating C3 in several human studies(110-113). CETP has been an area of very active research as a potential drug target due to its central role in the transport of triglycerides and cholesterol esters between lipoproteins, however no published studies to date have explored the relationship between air pollution and CETP levels. While there has been little study of SAA_GPGG, the peptide has been identified as a potential calcium binding site(114). Although changes in these proteins were observed, however, none were significant at the FDR. The process of HDL remodeling can take place over a time period of hours or days, and our measurements may have missed critical time windows during which an inflammatory response took place and may have been observable.

Strengths and limitations

Our study had a number of strengths. This was the first controlled experimental study to examine acute effects of DE on HDL function and proteome in humans. Our study also took advantage of a unique exposure facility designed to examine the biological effects of air pollution without the issues of residual confounding or temporality that can lead to bias in

observational studies. HDL function was measured before and after exposure, allowing us to examine changes in the outcome due to the exposure in our analysis.

Our study also had several limitations. Our study examined physiological responses to an acute exposure to DE, while typical exposure to DE is chronic and repeated. This difference in quality of exposure may not provoke the same responses as long term exposure. A three-week washout period was implemented to ensure no carryover effects from prior exposures, and adjustment for treatment order showed no difference, but it is possible that there may have been some effects. The timing of the measurement of HDL function may have not captured the effects of DE on HDL function. HDL dysfunction can occur over a period of hours to days, but HDL dysfunction may have occurred after exposure to DE but then resolved through homeostasis and turnover of oxidized HDL before it could be measured, or it could have occurred primarily after the 24-hour measurement period. The cell-free DCF assay itself has been criticized for being non-specific to ROS, as numerous one-electron-oxidizing species can oxidize DCFH to DCF(115). Additionally, $DCF^{\bullet-}$, the intermediate radical, can react with O_2 to form superoxide, which yields products that can artificially amplify the fluorescence intensity(115). These measurement errors (due to inaccuracies inherent in the fluorescence assay) are likely to be non-differential, as they should be unassociated with either the exposure or outcome, which in most cases would bias results towards the null. Measurement error may also be present in the HDL proteome results. The protein digestion process using trypsin may vary by sample, adding non-differential error(116). There was high between-person variability in the HDL proteome than expected, which would reduce the power of our study, making it more difficult to detect an association in a small sample which was a secondary analysis not well-powered to detect small associations. Our study used a young, healthy population, which is less susceptible to inflammation, oxidative stress, and HDL dysfunction than an older population(117). Older individuals' immune systems respond differently to challenge and may

have a different immune response to air pollution. This may make the results of our study less generalizable to the older adult population most at risk for air pollution-related health effects.

In conclusion, we found no association between exposure to DE and changes in the HDL proteome or HDL oxidant index 24 hours after exposure. This result is consistent with a previous study examining the effects of exposure to coarse CAPs. More research is needed into different measurements of HDL function, exposure periods, and in different populations who may be at more risk from the deleterious health effects of DE.

Table 3.1. Descriptive statistics for study participants in both diesel exposure studies

Characteristics	Study 1 (n=18)	Study 2 (n=10)
Age, mean (SD)	28.6 (9.5)	27.8 (8.8)
Gender, n (%)		
Female	7 (41.2)	5 (50.0)
Male	10 (58.8)	5 (50.0)
Race n (%)		
White	15 (88.2)	8 (80.0)
Non-white	2 (11.8)	2 (20.0)
BMI, mean (sd)	22.4 (1.9)	23.9 (2.6)
HDL (mg/dL)	48.1 (12.7)	50.2 (9.0)
LDL mg/dL	100.4 (19.7)	106.2 (19.2)
Triglycerides (mg/dL)	69.7 (40.3)	80.2 (37.8)
Glucose (mg/dL)	89.2 (4.5)	N/A

Table 3.2. Median and IQR HDL Measures at Each Time Point

	N	Median	IQR	N	Median	IQR	N	Median	IQR
	Pre-exposure			8 hours post exposure			22 hours post exposure		
HDL Oxidant Index									
Filtered Air	25	0.20	0.08	24	0.22	0.09	27	0.18	0.13
Placebo	12	0.21	0.10	12	0.23	0.09	16	0.19	0.09
Antioxidant	13	0.18	0.08	12	0.19	0.15	11	0.17	0.08
Diesel	27	0.18	0.08	20	0.21	0.07	32	0.20	0.09
Placebo	13	0.23	0.10	11	0.23	0.10	14	0.21	0.14
Antioxidant	14	0.18	0.05	9	0.18	0.06	18	0.20	0.09
Adjusted HOI									
Filtered Air	25	0.20	0.10	24	0.22	0.12	27	0.20	0.09
Placebo	12	0.22	0.10	12	0.23	0.11	16	0.21	0.08
Antioxidant	13	0.18	0.10	12	0.17	0.15	11	0.17	0.13
Diesel	27	0.19	0.09	20	0.18	0.06	32	0.20	0.07
Placebo	13	0.20	0.14	11	0.22	0.09	14	0.20	0.08
Antioxidant	14	0.18	0.04	9	0.18	0.03	18	0.20	0.07
PON Activity (mmol min^{-1} · ml $plasma^{-1}$)									
Filtered Air	33	0.44	0.59	33	0.43	0.61	33	0.46	0.55
Placebo	16	0.42	0.55	16	0.43	0.57	16	0.46	0.53
Antioxidant	17	0.45	0.76	17	0.43	0.78	17	0.46	0.74
Diesel	34	0.45	0.46	31	0.45	0.58	33	0.45	0.60
Placebo	16	0.43	0.35	16	0.44	0.52	16	0.43	0.63
Antioxidant	18	0.46	0.47	15	0.46	0.65	17	0.45	0.63

Table 3.3. Comparing unadjusted trajectories of change in diesel exhaust to filtered air exposure settings

	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)
	Baseline differences	8 hours post exposure	22 hours post exposure
HOI	0.02 (-0.03, 0.06)	-0.03 (-0.10, 0.03)	0.0003 (-0.06, 0.06)
Placebo	0.04 (-0.02, 0.10)	-0.08 (-0.17, 0.002)	-0.04 (-0.12, 0.04)
Antioxidant	-0.01 (-0.07, 0.05)	0.01 (-0.08, 0.10)	0.04 (-0.04, 0.12)
Adjusted HOI	0.01 (-0.03, 0.06)	-0.03 (-0.09, 0.03)	0.003 (-0.06, 0.06)
Placebo	0.05 (-0.01, 0.10)	-0.08 (-0.16, 0.01)	-0.04 (-0.11, 0.04)
Antioxidant	-0.02 (-0.07, 0.04)	0.01 (-0.07, 0.10)	0.05 (-0.03, 0.12)
PON Activity (mmol min^{-1} · ml $plasma^{-1}$)	-0.06 (-0.10, 0.01)	0.02 (-0.05, 0.09)	0.06 (-0.005, 0.13)
Placebo	-0.04 (-0.10, 0.03)	0.04 (-0.06, 0.13)	0.09 (-0.01, 0.18)
Antioxidant	-0.08 (-0.14, -0.01)	-0.001 (-0.09, 0.09)	0.04 (-0.05, 0.13)

Unadjusted values

Table 3.4. Comparing trajectories of change in diesel to filtered air exposure settings

	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)
	Baseline differences	8 hours post exposure	22 hours post exposure
HOI	0.003 (-0.04, 0.05)	-0.03 (-0.10, 0.04)	0.01 (-0.06, 0.07)
Placebo	0.03 (-0.03, 0.10)	-0.07 (-0.16, 0.02)	-0.03 (-0.11, 0.06)
Antioxidant	-0.02 (-0.08, 0.04)	0.01 (-0.09, 0.10)	0.04 (-0.05, 0.12)
Adjusted HOI	0.001 (-0.04, 0.04)	-0.03 (-0.09, 0.04)	0.01 (-0.05, 0.07)
Placebo	0.03 (-0.03, 0.09)	-0.07 (-0.15, 0.02)	-0.03 (-0.11, 0.05)
Antioxidant	-0.02 (-0.08, 0.03)	0.01 (-0.08, 0.10)	0.04 (-0.04, 0.13)
PON Activity (mmol min^{-1} · ml $plasma^{-1}$)	-0.05 (-0.11, 0.002)	0.02 (-0.05, 0.09)	0.05 (-0.02, 0.12)
Placebo	-0.04 (-0.11, 0.04)	0.04 (-0.07, 0.14)	0.09 (-0.02, 0.19)
Antioxidant	-0.07 (-0.14, 0.006)	-0.003 (-0.12, 0.11)	0.02 (-0.07, 0.12)

Adjusted for age, sex, race, BMI, glucose, triglycerides, LDL, and hemolysis

Table 3.5. Comparing trajectories of change in diesel to filtered air exposure settings, log-transformed variables

	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)
	Baseline differences	8 hours post exposure	22 hours post exposure
logHOI	-0.07 (-0.23, 0.09)	-0.01 (-0.25, 0.23)	0.13 (-0.10, 0.35)
Placebo	-0.04 (-0.26, 0.18)	-0.11 (-0.42, 0.20)	0.05 (-0.25, 0.35)
Antioxidant	-0.19 (-0.56, 0.18)	0.07 (-0.26, 0.40)	0.19 (-0.12, 0.50)
Adjusted logHOI	-0.07 (-0.23, 0.08)	-0.01 (-0.25, 0.22)	0.12 (-0.10, 0.33)
Placebo	-0.01 (-0.22, 0.19)	-0.12 (-0.42, 0.17)	0.03 (-0.26, 0.31)
Antioxidant	-0.16 (-0.52, 0.19)	0.08 (-0.23, 0.40)	0.21 (-0.08, 0.51)
logPON Activity	-0.05 (-0.11, 0.01)	0.06 (-0.03, 0.15)	0.04 (-0.05, 0.13)
Placebo	-0.05 (-0.13, 0.04)	0.05 (-0.07, 0.18)	0.09 (-0.04, 0.21)
Antioxidant	-0.06 (-0.15, 0.03)	0.07 (-0.06, 0.19)	0.001 (-0.12, 0.12)

Adjusted for age, sex, race, BMI, glucose, triglycerides, LDL, and hemolysis

Figure 3.1. Adjusted changes in logHOI comparing diesel exhaust exposure and filtered air exposure

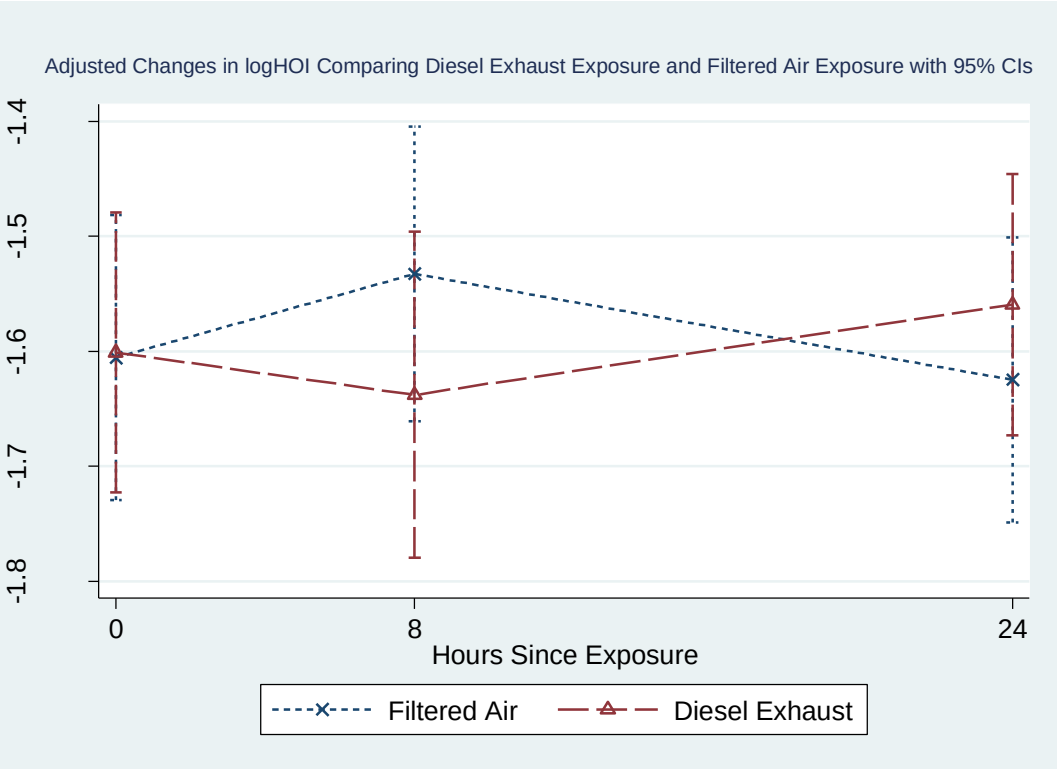


Figure 3.2. Adjusted changes in logPON1 activity comparing diesel exhaust exposure and filtered air exposure

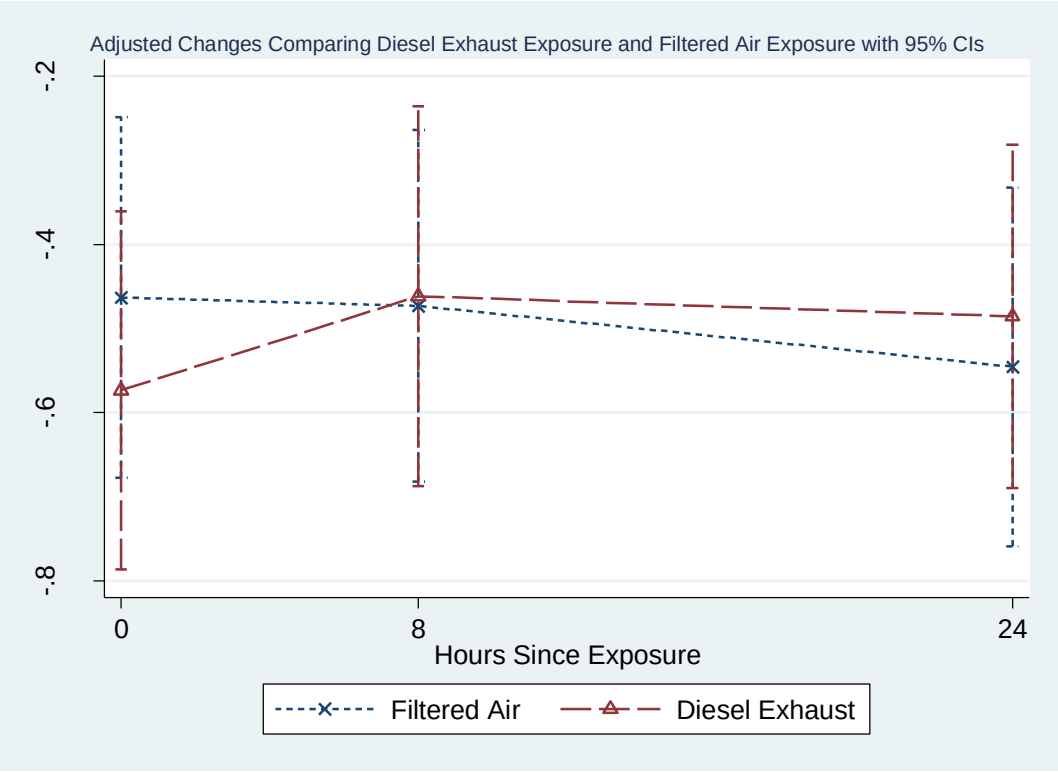


Table 3.6. Median HDL Protein Abundance Before and After Diesel Exhaust and Filtered Air Exposures

HDL Protein	Filtered Air		Diesel Exhaust	
	Pre-exposure	Post-exposure	Pre-exposure	Post-exposure
ALB	0.1188727	0.1931395	0.2345737	0.1958616
APOA1	41641760	39738230	32201039	31136231
APOA2	0.3528850	0.2977593	0.2804487	0.2893887
APOA4	0.0005863	0.0006795	0.0007515	0.0006484
APOA5	0.0000324	0.0000489	0.0001259	0.0000637
APOB	0.0310392	0.0503954	0.0561522	0.0511026
APOC1	0.0193295	0.0242640	0.0238048	0.0172783
APOC2	0.0182970	0.0169174	0.0211852	0.0167921
APOC3	0.0059897	0.0060682	0.0082199	0.0049183
APOC4	0.0008929	0.0007115	0.0009489	0.0005942
APOD	1.8350540	2.6989030	2.8889680	2.2541040
APOE	0.1552945	0.2398983	0.2497566	0.2102641
APOF	0.0031467	0.0036993	0.0035431	0.0026877
APOH	0.0013173	0.0025973	0.0021295	0.0015754
APOL1	0.0557864	0.0633201	0.0716049	0.0659022
APOM	1.4912390	2.1200960	2.6430160	2.1299000
C3	0.0079214	0.0114101	0.0085647	0.0074294
C4A	0.0021816	0.0037266	0.0036940	0.0029014
CETP	0.0020281	0.0033509	0.0032636	0.0028115
CLU	0.0145502	0.0177019	0.0147674	0.0131721
HBB	0.0028201	0.0027194	0.0038462	0.0031379
HP	0.0120007	0.0141061	0.0157126	0.0150566
HPR	0.0121629	0.0173261	0.0187131	0.0158919
IHH	0.0019702	0.0030989	0.0034352	0.0029768
LCAT	0.0311277	0.0415043	0.0439024	0.0333720
LPA	0.0018183	0.0037072	0.0042557	0.0026078
LpPLA2	0.0011688	0.0009614	0.0011761	0.0008911
PCYOX1	0.0018798	0.0018520	0.0017750	0.0019024
PLTP	0.0314061	0.0394504	0.0412157	0.0323535
PON1	0.8657785	1.4710430	1.3062270	1.3076900
PON3	0.0024130	0.0021248	0.0020609	0.0022955
RBP4	0.0144444	0.0201356	0.0178425	0.0178607
SAA1	0.0022050	0.0022696	0.0026528	0.0038081
SAA1_2_DPNH	0.0055251	0.0040201	0.0049875	0.0033314
SAA1alpha	0.0020509	0.0027280	0.0017443	0.0021600
SAA2alpha	0.0001332	0.0001561	0.0000865	0.0001531

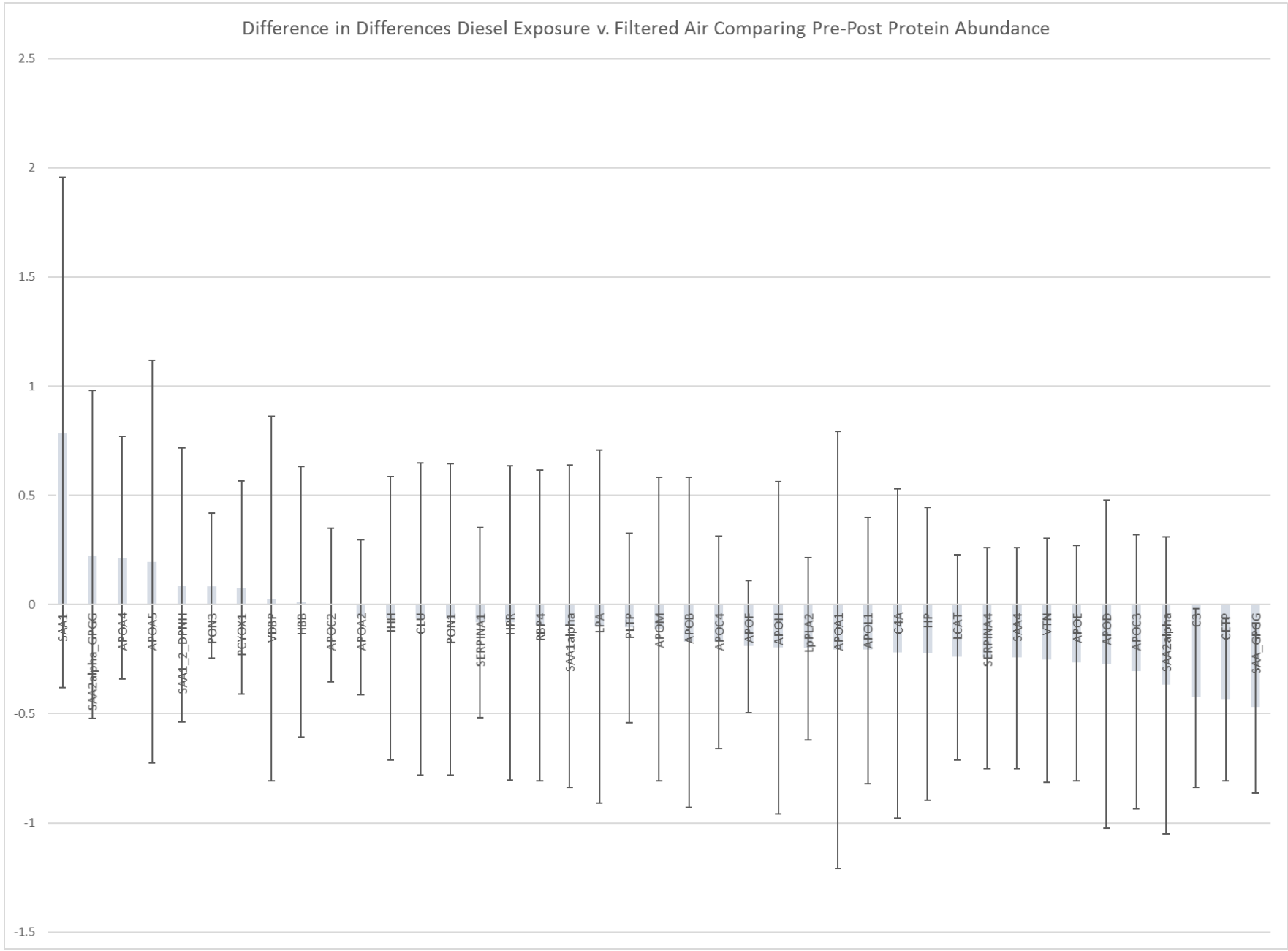
SAA2alpha_GPGG	0.0000790	0.0000740	0.0000760	0.0000685
SAA4	0.0593523	0.0587323	0.0697869	0.0671773
SAA_GPGG	0.9273863	1.6759460	1.5396010	1.1076970
SERPINA1	0.0259582	0.0199790	0.0281130	0.0173139
SERPINA4	0.0594888	0.0587112	0.0698674	0.0672256
VDBP	0.0006370	0.0014862	0.0013695	0.0012963
VTN	0.0064081	0.0075043	0.0089194	0.0059701

Table 3.7. Interquartile ranges of HDL Protein Abundance Before and After Diesel Exhaust and Filtered Air Exposures

HDL Protein	Filtered Air		Diesel Exhaust	
	Pre-exposure	Post-exposure	Pre-exposure	Post-exposure
ALB	0.3105459	0.4152404	0.3550928	0.4726938
APOA1	54730693	47245838	39272329	36883610
APOA2	0.2329197	0.1027453	0.1763765	0.127538
APOA4	0.0004708	0.0004599	0.0006775	0.0006191
APOA5	0.0000899	0.0001107	0.000141	0.0001405
APOB	0.0348959	0.0673699	0.0603649	0.0796689
APOC1	0.0076451	0.0124781	0.0106606	0.0079905
APOC2	0.0146041	0.0128797	0.0161658	0.0076334
APOC3	0.00956	0.0046327	0.0088179	0.0029578
APOC4	0.001174	0.0011663	0.0013419	0.0008161
APOD	1.015905	1.8873	3.177036	2.914748
APOE	0.1934734	0.2905482	0.3090006	0.2199041
APOF	0.0013073	0.0022433	0.0030351	0.0024222
APOH	0.001897	0.0025583	0.0026179	0.0042691
APOL1	0.0697233	0.1362701	0.1409528	0.0994276
APOM	1.191018	2.114333	2.903119	2.749455
C3	0.0066158	0.0109925	0.0099344	0.0059713
C4A	0.0049799	0.007444	0.0069991	0.0054061
CETP	0.0019867	0.0029528	0.0026914	0.0023451
CLU	0.0116928	0.009532	0.0082685	0.005874
HBB	0.0021161	0.0031063	0.0046948	0.0034117
HP	0.0107982	0.0129152	0.0205025	0.0161036
HPR	0.0112412	0.0143203	0.0221314	0.0185725
IHH	0.0020141	0.0024909	0.002351	0.0027701
LCAT	0.0178826	0.0285632	0.0418423	0.0332706
LPA	0.0038599	0.0068361	0.0085422	0.0060991
LpPLA2	0.0009151	0.0008416	0.0008551	0.0005907
PCYOX1	0.0013794	0.0012238	0.0012078	0.0013516
PLTP	0.0274947	0.0340488	0.0310967	0.0161966
PON1	1.250026	1.632367	2.598962	1.193081
PON3	0.0020407	0.0011167	0.0014074	0.0016619
RBP4	0.0085398	0.0157885	0.029574	0.0259785
SAA1	0.002145	0.0037003	0.0048926	0.0043559
SAA1_2_DPNH	0.0102107	0.0119059	0.0181986	0.0160452
SAA1alpha	0.0035153	0.0033134	0.0086924	0.0059317
SAA2alpha	0.00145	0.0030399	0.0027976	0.002819
SAA2alpha_GPGG	0.0000605	0.0001006	0.0000885	0.000083
SAA4	0.0888975	0.1218351	0.1205317	0.1300799

SAA_GPGG	1.143679	1.69806	1.689212	1.67571
SERPINA1	0.0205798	0.0142083	0.0268915	0.0150737
SERPINA4	0.0889829	0.1217835	0.1206391	0.129906
VDBP	0.0014274	0.0017747	0.0019573	0.0031683
VTN	0.0063849	0.0050251	0.0112816	0.0071956

Figure 3.3. Difference in Differences Comparing Diesel Exhaust and Filtered Air Pre-Post Exposure in HDL Proteins


Difference in Differences Diesel Exposure v. Filtered Air Comparing Pre-Post Protein Abundance
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
SAA1
SAA2alpha_GPGG
APOA4
APOA5
SAA1_2_DPNH
PON3
PCYOX1
VDBP
HBB
APOC2
APOA2
HHT
CLU
PON1
SERPINA1
HPR
RBP4
SAA1alpha
LPA
PLTP
APOM
APOB
APOC4
APOF
APOH
LpPLA2
APOA1
APOL1
C4A
HP
LCAT
SERPINA4
SAA4
VTN
APOE
APOD
APOC3
SAA2alpha
C3
CETP
SAA_GPGG

Chapter 4

Air Pollution, Longitudinal HDL Cholesterol and CAC: The Multi-Ethnic Study of Atherosclerosis (MESA)

ABSTRACT

BACKGROUND

One hypothesized mechanism of action explaining the association between air pollution and cardiovascular disease is that air pollution is associated with increased atherosclerosis. This finding has been reported in the Multi-Ethnic Study of Atherosclerosis, however further research into biological pathways explaining this relationship would strengthen the finding. High-density lipoproteins (HDLs) are associated with lower risk of cardiovascular disease, and have important anti-atherogenic effects. We proposed to examine the extent to which there is an HDL lowering effect of air pollution over time, and if that may be on the pathway between air pollution and atherosclerosis, as measured by coronary artery calcium.

METHODS AND RESULTS

Our analysis was conducted with the Multi-Ethnic Study of Atherosclerosis and Air Pollution (MESA Air), a prospective, ethnically diverse cohort study of 6,814 U.S. men and women with up to five study exams from 2000 – 2011. HDL cholesterol was measured at each visit for most subjects and CAC was measured in most participants at baseline and subsets of all participants at follow-up exams. $PM_{2.5}$ exposure was assessed using validated spatiotemporal models which utilized air quality monitoring data, cohort-specific air pollution measurements, and land-use data to generate participant-specific estimates of air pollution for the year prior to each exam.

We used time-varying linear mixed effects models adjusted for covariates of interest to model the relationship between $PM_{2.5}$ and HDL and used the product method to determine the extent to which HDL mediated the relationship between $PM_{2.5}$ and longitudinal CAC. We found a 5 ug/m^3 higher $PM_{2.5}$ was associated with -0.77 (-1.10, -0.44) lower HDL overall and -0.18 (-0.23, -0.13) lower HDL per year in minimally adjusted models (adjusted for age, sex, race/ethnicity, and site). A 5 ug/m^3 higher $PM_{2.5}$ was associated with -0.38 (-0.66, -0.11) mg/dl overall and -0.18 (-0.22, -0.14) mg/dl lower HDL per year in fully adjusted models. In the mediation models, a 5 ug/m^3 higher $PM_{2.5}$ was associated with a 3.41 (1.99, 4.85) higher Agatston score per year, with a change of 1 mg/dl HDL explaining 0.025 (-0.155, 0.003) of the association. We observed no significant mediation of the relationship between air pollution and CAC by HDL.

CONCLUSIONS

We found that exposure to one-year averaged $PM_{2.5}$ was associated with lower concentrations of HDL cholesterol, however this association did not explain a significant amount of the relationship between $PM_{2.5}$ and CAC progression in our prospective multi-ethnic cohort study of community-dwelling men and women, perhaps due to the small magnitude of the association between air pollution and HDL. This study provides further evidence for the biological plausibility of the relationship between air pollution and CVD, however further research is needed into longitudinal measures of HDL function, as well as mechanisms explaining the relationship between air pollution and progression of CAC.

Introduction

Chronic exposure to air pollution is an accepted risk factor for morbidity and mortality in the United States as well as the developing world(1). Much of the burden of mortality attributed to air pollution is believed to be through increases in the risk of cardiovascular disease, although the mechanisms that underlie this relationship are not well characterized(11). Atherosclerosis is an important cause of cardiovascular disease, characterized by the build-up of plaques in the arteries, which can harden and narrow, leading to increased blood pressure and increased risk of myocardial infarction and stroke. Growing evidence suggests that air pollution may effect cardiovascular disease risk through numerous biological effects at the molecular level, including lipid oxidation, systemic oxidative stress, and inflammation(2).

High density lipoprotein (HDL) is an independent predictor of cardiovascular disease in epidemiologic studies(31). HDL is thought to play an important role in the transport of cholesterol from plaques in the vascular walls (a process described as Reverse Cholesterol Transport, or RCT), which is associated with lower levels of atherosclerosis(31). In addition to RCT, HDL has numerous other atheroprotective qualities, including anti-inflammatory, anti-oxidative, beneficial endothelial function effects, among others(31). Observational studies have shown that higher levels of air pollution are associated with lower levels of HDL, and experimental trials have shown that exposure to air pollution can cause HDL to become lose their protective qualities and become dysfunctional and pro-atherogenic(25-27, 30).

Atherosclerotic plaques can develop in the arteries as a person ages, forming complex lesions consisting of lipids, dead cells, immune cells, and calcium. Calcium appears in more developed plaques, and can be measured using computed tomographic (CT) imaging. Calcium measured in the coronary artery (coronary artery calcium, or CAC), is frequently described as a measure of atherosclerosis in the body, and the presence of CAC and increases in CAC over

time are strongly associated with cardiovascular disease(118). Some studies show that HDL may be a predictor of CAC(119, 120). A recent study by Kaufman et al, published in The Lancet, examined the longitudinal association between air pollution and CAC, finding that higher levels of air pollution were also associated with increased CAC progression over time(121)]. Air pollution's effect on HDL may explain some of this relationship. Characterizing mechanisms of action by which air pollution may effect CAC helps clarify the scientific understanding of human biological response to air pollution, as well as identifying targets for intervention to protect those more susceptible to its deleterious effects.

Despite the association of HDL with air pollution and atherosclerosis, few studies have examined the longitudinal relationship between air pollution and HDL in multi-ethnic cohort. Bind et al, found an association between air pollution and lower levels of HDL in a cohort of primarily non-Hispanic White men in the Boston area(122). Other studies in Taiwan and in occupational settings have examined the cross-sectional association between air pollution and HDL, but our study is the first to use sophisticated air pollution exposure measurement combined with detailed measurement of covariates and multiple measures of HDL in a diverse prospective cohort(54, 56).

Our study examined whether exposure to $PM_{2.5}$ was associated with lower levels of HDL cholesterol over time in a prospective cohort of men and women living in six cities across the United States. Our longitudinal study uses multiple blood measurements of HDL over a 10-year period of follow-up as well as state of the art exposure assessment and detailed measurement of covariates(17). To explore mechanisms underlying the relationship between air pollution and CAC, we also examined whether HDL was a mediator of this association.

Methods

Study Population

Our study was conducted within the MESA cohort. MESA is a longitudinal study of 6814 multi-ethnic men and women aged 45-84 and free from heart disease at baseline, living at six sites across the U.S. (Baltimore, MD; Chicago, Illinois; Los Angeles, CA; New York, NY; St. Paul, MN, and Winston-Salem, NC). MESA began enrolling participants in 2000, following participants for cardiovascular outcomes in five clinical examinations over an average of 8.3 years of follow-up for each participant. The study made special efforts to enroll ethnically diverse participants in order to be powered to study differences in cardiovascular risk by race/ethnicity(17). Participants were non-Hispanic White, African American, Hispanic, and of Chinese decent. This study was approved by human subjects committees at each field center and at the University of Washington, and subjects' written informed consent was obtained prior to participation in the present study.

Eligibility criteria required participation at least one MESA study visit, and having exposure and outcome data measured during the study time period. 6814 participants had at least one HDL measurement and exposure measurement. 195 were excluded for not having exposure data or for having incomplete covariate information. 6619 participants contributed a total of 29076 visits for this study.

Exposure assessment

Air pollution exposure was measured using $PM_{2.5}$ concentrations at each participant's home averaged to a year prior to blood draw (in the longitudinal HDL analysis) and CT scan (in the mediation analysis). Pollutant concentrations were estimated using spatiotemporal models based on long-term regulatory monitoring networks, cohort-specific measurements, and a suite of over 800 land use covariates(17, 72). $PM_{2.5}$ was selected for this analysis a priori due to its

association with cardiovascular disease and HDL in previous studies and its association with longitudinal CAC in MESA(121). Further description of air pollution exposure models can be found in Chapter 2.

Outcome Assessment

HDL cholesterol

All HDL-C measurements were performed at the central Collaborative Studies Clinical Laboratory at Fairview-University Medical Center in Minneapolis, MN. Blood samples were drawn after a 12 hour fast, and were stored at -70 C until they were ready for processing. Samples were assayed with two weeks of collection using CDC/NHLBI standards from thawed EDTA plasma. After precipitation of non-HDL-C with magnesium/dextran, HDL-C was determined using the cholesterol oxidase method (Roche Diagnostics) (CV=2.9%)(76).

Coronary artery calcium

Our study used electrocardiogram (ECG)-gated electron-beam computed tomography (CT) measured CAC at three field centers, and multidetector computed tomography at the remaining three field centers from exams 1 to 3. Multidetector CT scanners were used in all centers by exam 5. CAC was quantified using the Agatston scoring method and calibrated to adjust for phantoms. Two CT images were taken at each visit and averaged together to improve accuracy. CT images were independently analyzed at a central reading center. Although different CT scanners were used over the course of the study, electron-beam and multidetector computed tomography scanners had high re-scan agreement(118, 119). Both interobserver and intraobserver agreement were high (Kappa = 0.93 and 0.90)(119).

Covariate assessment

Covariate information was collected at each of the five study examinations through physical examination, guided interview, and measurement of biomarkers from blood samples. At each study visit, participant's medical histories were updated they and were asked to bring current prescriptions and medication containers. The following covariates were included in the analysis: age, race/ethnicity (white, black, Hispanic, or Chinese), gender, study exam (one through five), site, body mass index (weight in kilograms/height in meters squared), individual socioeconomic status (SES) defined as education (less than or equal to high school, some college, greater than or equal to college graduate), income (specified continuously as permanent income, which is the average of all reported income over all exams), smoking status (current, former, never smoker), second-hand smoke exposure (yes or no), current alcohol consumption (yes or no), diabetes (normal, borderline, or treated/untreated as defined by the 2003 American Diabetes Association fasting blood glucose criteria algorithm), and use of lipid-lowering medications (yes or no).

Statistical analysis

Exposures, outcomes, and most covariates in the analyses were time-varying. Non time-varying covariates included race/ethnicity, sex, education, and income. For our longitudinal analysis of HDL we used time-varying linear mixed effects models with random intercepts to address correlation of repeated measures of HDL. In order to evaluate whether $PM_{2.5}$ was associated with annual change in HDL, we added an interaction term between 1-year PM 2.5 and years since first visit. In our analyses, we fit two separate models. Model 1 minimally adjusted for age, sex, race/ethnicity, and site, while Model 2 additionally adjusted for BMI (kg/m^2), low-density lipoproteins (mg/dl), diabetes, education, smoking status (current, former, never as reference), cumulative pack years smoked, alcohol use, physical activity, use of statins, temperature and relative humidity. Normality of the residuals in the linear mixed-effects

model was confirmed by examination of qq plots. We also evaluated sex, race, smoking, and diabetes status as potential effect modifiers.

Given the interest in exploring the biological mechanisms which explain the relationship between air pollution and atherosclerosis as measured by CAC, we hypothesized that air pollution's association with lower HDL may mediate this relationship. In Chapter 2, we describe air pollution's association with lower concentrations of HDL in MESA. Here we conducted a mediation analysis to calculate the amount of the association between air pollution and longitudinal CAC that is mediated through longitudinal HDL. Using methods proposed by Krull and McKinnon and adapted by Bind, et al (2014, 2016), we simultaneously fit two time-varying linear effects models (123, 124):

$M_{ij} = (\gamma_0+u_i)+ \gamma_1 X_{ij} +\Sigma_k \gamma_{2k} C_{kij} + \varepsilon_{ij}$ with $\varepsilon_{ij} \sim N(0, \sigma^2)$ and $u_i \sim N(0,\sigma_u^2)$

$Y_{ij} = (\beta_0+g_i) + \beta_1 X_{ij}+ \beta_2 M_{ij} +\Sigma_k \beta_{3k} C_{kij}+\Sigma_k \beta_{4k}+\eta_{ij}$ with $\eta_{ij} \sim N(0, \sigma^2)$, and $g_i \sim N(0,\sigma_g^2)$

In these models, i, j, k, are defined as individuals, visits, and covariates; M represents HDL; X represents $PM_{2.5}$; Y represents CAC; C represents covariates of interest; and g represents the random intercept for HDL. The product formula $\gamma_1\beta_2$ estimates the mediated effect. We are able to approximate the variance of the estimated mediated effect by $Var(\beta_2)\gamma_1^2 + 2\ Cov(\gamma_1,\beta_2)\gamma_1\beta_2 + Var(\gamma_1)\beta_2^2$ using the delta method with bootstrapped standard errors. Covariates of interest in the mediation analysis were baseline age, sex, race/ethnicity, site, CT scanner, BMI, physical activity, smoking status, pack years, family history of CVD, total cholesterol, triglycerides, statin use, education, income, and the interactions between these variables and study time. Most variables were time-varying.

Assuming there is no time-varying confounding of the relationship between $PM_{2.5}$ and HDL, our models must meet four identification assumptions in order to be valid: 1) that there is no unmeasured confounding of the association between $PM_{2.5}$ and HDL conditional on covariates and random effects, 2) that there are no unmeasured confounders of the relationship

between HDL and CAC, 3) there are no unmeasured confounders of the relationship between $PM_{2.5}$ and HDL, and 4) that there are no confounders of the relationship between HDL and CAC that are associated with $PM_{2.5}$(123).

Results

Descriptive statistics for patient characteristics at visits one, five and all visits are summarized in Table 1. Observations were excluded if there was missing data on exposure, outcome, or covariates of interest. At baseline, the mean age of participants was 62.2, and overall the mean age was 65.1. Mean HDL was higher among participants at each visit. This was true among those with complete data, as well as those under 55y at baseline. As expected, the prevalence of other cardiovascular risk factors such as diabetes, hypertension, and cholesterol were higher in exam 5. $PM_{2.5}$ decreased over time, with the one-year average $PM_{2.5}$ 16.1 ug/m^3 at exam 1, and 11.0 ug/m^3 at exam 5.

The associations between $PM_{2.5}$ and HDL are presented in Table 4.3. A 5 ug/m^3 higher $PM_{2.5}$ was associated with -0.77 (-1.10, -0.44) lower HDL overall and -0.18 (-0.23, -0.13) lower HDL per year in minimally adjusted models (adjusted for age, sex, race/ethnicity, and site). A 5 ug/m^3 higher PM2.5 was associated with -0.38 (-0.66, -0.11) mg/dl overall and -0.18 (-0.22, -0.14) mg/dl lower HDL per year in fully adjusted models. The association between one-year average $PM_{2.5}$ was robust to adjustment for multiple confounders. There was no significant effect modification by sex, race, diabetes status, or smoking. While the interaction was not significant, we observed a larger decline in HDL per year associated with $PM_{2.5}$ in women -0.23 (-0.28, -0.17) mg/dl per year, compared to men -0.10 (-0.16, -0.05) mg/dl per year. Our mediation analysis is presented in Table 4.3. We observed no significant mediation of the relationship between air pollution and CAC by HDL. A 5 ug/m^3 higher $PM_{2.5}$ was associated with a 3.41 (1.99, 4.85) higher Agatston score per year through a 0.025 (-0.155, 0.003) lower HDL

per 1 mg/dl, assuming mediation assumptions are met. Sensitivity analysis are presented in Table 4.4. The between persons analysis (not accounting for within-persons correlation) shows 5 ug/m^3 higher $PM_{2.5}$ was associated with a 0.08 (-0.43, 0.59) higher HDL-C.

Discussion

In this prospective, multiethnic cohort study of adult men and women, we found evidence of an association between higher long-term $PM_{2.5}$ with lower HDL over time. The magnitude of the association between $PM_{2.5}$ and HDL was stronger in women than men. In a mediation analysis of a previously reported association between $PM_{2.5}$ and longitudinal CAC, we found no significant evidence that this relationship was mediated by HDL, however we cannot rule out that we did not meet several unverifiable assumptions required for this analysis to be valid. The effect size for the association between $PM_{2.5}$ and HDL can be compared to that of smoking. While a 5 ug/m^3 higher $PM_{2.5}$ exposure was associated with a -0.38 lower HDL, being a current smoker was associated with a -0.63 mg/dl lower HDL, and taking a statin was associated with a 1.06 mg/dl higher HDL in our study. These are almost 2 and 3 times stronger than the estimated effects of a one-year averaged $PM_{2.5}$ exposure per year.

Our finding that air pollution is associated with HDL over time is consistent with prior studies of air pollution and HDL. While there have been few studies that have examined air pollution and HDL in a prospective cohort over time, the Veteran's Administration Normative Aging Study (a prospective cohort study of elderly, predominantly non-Hispanic White men) found that higher $PM_{2.5}$ was associated with lower HDL, however this varied depending on baseline HDL, and they performed a quantile analysis reporting scaled differences which are not directly comparable to our results(122). To our knowledge, this is the first reporting of the association between air pollution and HDL in a longitudinal cohort. Several studies have

examined the association between air pollution and HDL in cross sectional studies, as discussed in Chapter 2.

The biological mechanisms that may explain the relationship between chronic air pollution and lower HDL concentrations are not clear. Evidence suggests that exposure to $PM_{2.5}$ may result in pulmonary oxidative stress and inflammation due to changes in cell signaling pathways as a result of interaction between toxic PM components and lung tissues(23, 27). This response may generate ROS such as OH· and HOO·, and induce inflammatory cytokine expression both within the lung and in the bloodstream(23, 29). HDL is susceptible to oxidation from ROS, and oxidative modification of HDL the impairs anti-inflammatory function and the ability to transport excess cholesterol to the liver to for excretion(31, 90, 117, 125). Responses to $PM_{2.5}$ -induced oxidation and inflammation may cause HDL to be cleared more quickly from the bloodstream, resulting in lower circulating HDL levels consistent with results seen in our study(96, 126).

Our hypothesis was that longitudinal HDL mediated the relationship between air pollution and CAC, however we did not find significant evidence for this in our mediation analysis. The small magnitude of the longitudinal association between air pollution and HDL meant that it would be difficult for HDL to be a meaningful mediator of the association between air pollution and CAC unless HDL were a very strong predictor of CAC progression. Previous studies of predictors of CAC progression show HDL is not a particularly strong a predictor of CAC, and only large changes in HDL seem to make a meaningful difference in CAC progression(120). In Gassett et al, a 10 mg/dl higher HDL was associated with -1.8 Agatston units of CAC per year in MESA(120). Higher HDL was associated with lower CAC volume progression in Wong et al, who found those with HDL >60mg/dl had less progression of CAC volume compared to those with HDL <40mg/dl(127). In Kronmal et al, HDL was not associated with reduced CAC progression among those with prevalent CAC(119). The discrepancy could be explained by the

longer amount of follow up time in Gassett, et al – as accumulation of CAC is a relatively slow process, more follow-up time may allow the progression to be determined more accurately(120).

We also found that average HDL was progressively higher at each exam. Although all HDL assays were performed at the same central facility using the same technique, they were performed at different times and we cannot rule out the possibility of assay drift over time. The trajectory of increasing HDL over time was seen even when the analysis was restricted to participants with data at exam 5, to participants with complete data, to those <55y at baseline, and to those who never took statins. The robustness of this finding suggests that the apparent increase in HDL over time was not likely to be due to loss to follow-up, the effect of treatment, or selection bias due to age. Selection bias due to age can occur in cohorts who select participants conditional on their having no prior history of cardiovascular disease. Since older individuals are more likely to have cardiovascular disease than younger individuals, older individuals enrolled in a cohort are likely to less representative of the underlying population and perhaps less susceptible to cardiovascular risk factors than the underlying population from which they are drawn. This increase in HDL over time was not expected, but is not out of line with previous studies, which have shown inconsistent results(128). Studies done in different populations have found increasing HDL with age, no change in HDL with age, and decreasing HDL with age(129-134). Research into the causes for the change in HDL in MESA and other studies over time is a potential area of future research. To explore why our repeated measures analysis appeared to differ from the baseline association reported in Table 2.2, we conducted several sensitivity analyses in Table 4.4. When the repeated measures analysis was performed without accounting for correlation between individuals, the association disappeared and changed direction from -0.38 mg/dl per 5 ug/m^3 higher $PM_{2.5}$, to 0.08 mg/dl per 5 ug/m^3 higher $PM_{2.5.}$ This suggests that the association in the repeated measures analysis was due to within individual effects of $PM_{2.5}$ rather than between individual effects. We also examined the potential that secular trends of

decreasing $PM_{2.5}$ and increasing HDL over time may have explained the association. We examined the relationship between $PM_{2.5}$ and HDL without adjustment for years since baseline, and found a much stronger relationship between air pollution and HDL from -0.38 mg/dl per 5 ug/m^3 higher $PM_{2.5}$, to -2.94 mg/dl per 5 ug/m^3 higher $PM_{2.5}$. This indicates that our adjustment for years since baseline, as well as exam, did remove a large effect of secular trends in our exposure and outcome. We also explored whether the association between air pollution and HDL was effected by selection effects through age and statin use, and found no indication that younger, less selected participants or healthier participants who never took a statin were different from our main results.

Our study had a number of strengths. We used well-validated and state-of-the-art spatio-temporal models to estimate exposure to $PM_{2.5}$. The prospective study design allowed us to examine the effects of $PM_{2.5}$ on a group of individuals through time rather than cross-sectionally, as was done in prior studies. The cohort also had a large sample size of a multi-ethnic group of adults, making it more generalizable to an increasingly diverse U.S. population. Our study did have several limitations. HDL increased over time - possibly due to assay drift - however lab measurement error in HDL would likely be non-differential, which in most cases would bias results towards the null. As this was an observational study, there is the potential for residual confounding by unmeasured factors associated with air pollution and HDL or CAC. Our mediation analysis requires a number of strong no-confounding assumptions, which may not have been met in our study. HDL cholesterol is an intermediate marker of CVD risk, and while it is higher in those with CVD, HDL cholesterol does directly reflect HDL's functional capacities regarding RCT, or its anti-inflammatory and anti-inflammatory properties. It is unclear the degree to which HDL may be an independent causal factor in the development of CVD or a correlated factor(135).

We found that exposure to one-year averaged $PM_{2.5}$ was associated with lower concentrations of HDL cholesterol, however this association did not explain a significant amount of the relationship between $PM_{2.5}$ and CAC progression in our prospective multi-ethnic cohort study of community-dwelling men and women. This study provides further evidence for the biological plausibility of the relationship between air pollution and CVD, however further research is needed into longitudinal measures of HDL function, as well as mechanisms explaining the relationship between air pollution and progression of CAC.

Table 4.1. Characteristics of MESA participants at first, fifth, and all exams

Characteristics	First exam n=6814	Fifth Exam n=4581	All Exams n=29076
Age, years	62.2 (10.2)	69.9 (9.5)	65.1 (10.3)
Gender			
Male	3213 (47.2)	2150 (46.9)	13767 (47.4)
Female	3601 (52.9)	2431 (53.1)	15309 (52.7)
Race/ethnicity			
Caucasian	2622 (38.5)	1862 (40.7)	11558 (39.8)
African-American	1892 (27.8)	1203 (26.3)	7835 (27.0)
Hispanic	1496 (22.0)	991 (21.6)	6304 (21.7)
Chinese-American	804 (11.8)	525 (11.5)	3379 (11.6)
HDL Cholesterol, mg/dl	51.0 (14.8)	56.0 (16.8)	52.4 (15.5)
LDL Cholesterol, mg/dl	117.2 (31.5)	105.4 (32.6)	112.1 (32.6)
Body mass index, kg/m^2	28.3 (5.5)	28.5 (5.7)	28.4 (5.5)
Total cholesterol, mg/dl	194.2 (35.7)	183.0 (37.4)	189.4 (36.7)
$PM_{2.5}$ (year before exam average), ug/m^3	16.5 (3.4)	11.0 (1.4)	14.6 (3.4)
Smoking status			
Never	3404 (50.3)	2042 (45.0)	13444 (46.5)
Former	2480 (36.7)	2134 (47.1)	12378 (42.8)
Current	882 (13.0)	359 (7.9)	3083 (10.7)
Pack years	11.3 (20.9)	10.9 (20.5)	11.2 (20.8)
Current alcohol use, y/n	3737 (68.5)	1974 (43.5)	14325 (51.8)
Diabetes Mellitus			
Diagnosed	857 (12.6)	912 (20.0)	4510 (15.5)
Impaired fasting glucose	939 (13.8)	946 (20.7)	4981 (17.1)
Normal	4978 (73.5)	2722 (59.4)	19565 (67.3)
Hypertension, y/n	3047 (44.9)	2730 (59.6)	14329 (49.6)
Use of lipid-lowering medications, y/n	1008 (14.9)	1707 (37.3)	6839 (24.0)

Table 4.2. HDL and Agatston score by exam

HDL, mg/dl

	Exam 1	**Exam 2**	**Exam 3**	**Exam 4**	**Exam 5**
Full Data	51.0 (14.8)	51.9 (15.0)	51.7 (15.1)	52.7 (15.6)	56.1 (16.8)
w/ Exam 5 data	51.2 (14.9)	52.0 (15.1)	51.9 (15.1)	52.9 (15.6)	56.1 (16.8)
Complete data	51.3 (15.1)	52.1 (15.1)	51.9 (15.1)	52.9 (15.6)	56.0 (16.9)
If <55y at enrollment	49.5 (14.1)	50.7 (14.9)	50.3 (14.9)	51.1 (15.1)	54.0 (16.0)
Not currently taking a statin	51.0 (15.0)	52.1 (15.4)	51.9 (15.6)	53.1 (16.1)	56.6 (17.6)
Agatston score	145.9 (417.3)	169.9 (463.2)	217.3 (534.9)	257.0 (620.2)	285.4 (590.8)

Table 4.3. Adjusted differences in HDL per 5 ug/m^3 higher $PM_{2.5}$ and HDL

	Beta, (95% CI)
Model 1	-0.77 (-1.10, -0.44)
Model 2	-0.38 (-0.66, -0.11)

Adjusted differences in annual change in HDL since first visit per 5 ug/m^3 higher $PM_{2.5}$ and HDL

	Beta, (95% CI)
Model 1	-0.18 (-0.23, -0.13)
Model 2	-0.18 (-0.22, -0.14)

Model 1 adjusted for age, sex, race/ethnicity, and site

Model 2 additionally adjusted for age, sex, race/ethnicity, site, education, smoking, alcohol consumption, BMI, physical activity, diabetes, statin use, LDL cholesterol

Table 4.4 Sensitivity analyses of repeated measures analysis

	Beta, (95% CI)
Full model	-0.38 (-0.66, -0.11)
-without accounting for correlation within subject	0.08 (-0.43, 0.59)
-without adjustment for years since baseline or exam	-2.94 (-3.14, -2.74)
-without adjustment for years since baseline, with adjustment for exam	-0.64 (-0.97, -0.30)
Full model, using baseline PM2.5 for all time periods	-0.36 (-1.65, 0.93)
Full model with only those >75yo at baseline	-1.05 (-2.12, 0.02)
Full model with only those <55yo at baseline	-0.40 (-0.99, 0.18)
Full model with those who never took statins	-0.64 (-1.06, -0.23)

Full model adjusted for age, sex, race/ethnicity, site, education, smoking, alcohol consumption, BMI, physical activity, diabetes, statin use, LDL cholesterol

Chapter 5. Conclusions and Future Research

Consistent and increasingly strong evidence suggests that exposure to air pollution has adverse health effects in humans, but the full extent of these effects and the mechanisms through which these effects are manifested are not fully understood(2). In this dissertation we examined the relationship between long and short-term air pollution exposure and an important cardiovascular risk factor, HDL, which had not been well studied prior to our work. This dissertation used state of the art air pollution exposure models and a unique experimental trial as well as several new and experimental measures of HDL to add innovative new data to the field. We found an association between air pollution and a novel measure of HDL, HDL particle number, as well as an association between air pollution and HDL cholesterol at baseline in a multi-ethnic cohort. We also found an association between air pollution and lower HDL cholesterol in a longitudinal analysis. Our results support the plausibility of the relationship between air pollution and cardiovascular disease, and provide information to policy makers about potentially vulnerable populations.

Our findings may help inform regulatory action under the Clean Air Act as legislated through sections 108 and 109 of Title 42 of the U.S. Code, 2003. These sections direct the EPA to set primary and secondary air quality standards (National Ambient Air Quality Standards (NAAQS)) based on scientific review at 5 year-intervals. These standards direct the EPA to regulate criteria air pollutants (specified in Section 108) that “may reasonably be anticipated to endanger public health and welfare” and whose “presence … in the ambient air results from numerous or diverse mobile or stationary sources.” (42 U.S.C. 7408(b)). The standards are set by the EPA after thorough reviews of all recent scientific

research relevant to the health effects of air quality standards. These reviews are ordered to “accurately reflect the latest scientific knowledge useful in indicating the kind and extent of identifiable effects on public health or welfare which may be expected from the presence of [a] pollutant in ambient air…” (42 U.S.C. 7408(b)). In setting national clean air standards, the EPA administrator is directed to include an adequate margin of safety in order to address uncertainties associated with inconclusive scientific information. It has been argued that uncertainties in the biological processes explaining the relationship between air pollution and CVD add doubt as to a potential causal relationship. Our findings strengthen the biological understanding of the relationship between air pollution and CVD by exploring a well-known CVD risk factor.

Our studies are generally consistent with the findings of prior studies of the relationship between air pollution and HDL. Our findings that air pollution is associated with measures of HDL in observational studies are supported by some cohort studies of air pollution, but not with others(54, 56, 122, 136). Some other cohort studies relied on central-site monitoring data and had limited data on likely confounding variables such as SES, smoking, physical activity, and use of lipid-lowering medications, which may be associated with both air pollution and HDL, and may explain why our study found differing results. Our study is also consistent with previous occupational studies(57). Our study is the first to examine this association in a multi-ethnic cohort using advanced air pollutant modelling of exposures, and the first to report an association between air pollution and HDL particle number, an experimental new measure of the concentration of HDL particles in the blood.

Studies of HDL in controlled exposure to air pollution have had mixed results. Several (although not all) experimental studies of air pollution in animals have found biological effects in HDL(25, 26, 30). Yin, et al (2013, ATVB), exposed ApoE-deficient mice to DE at ~250 ug/m^3 and filtered air for two weeks, and found increased peroxidation in brochoalveolar lavage fluid and pro-oxidative changes in HDL, supporting the hypothesis

that oxidation in the lung surface can lead to increased oxidation and development of dysfunctional circulating HDL(26). Li, et al. exposed LDL-receptor null mice to ultrafine particles for 10 weeks, finding reduced HDL-C, HDL anti-oxidant capacity, and paraoxonase (PON) activity (an important measure of HDL antioxidant activity)(25). In Araujo et al, samples of HDL from mice exposed to fine and ultrafine PM were found to have significantly reduced HDL anti-inflammatory properties compared unexposed mice, suggesting that PM exposure may reduce the ability of HDL to protect against atherosclerosis(24). However, studies in humans have had more mixed results. Maiseyeu et al found no significant difference in HDL from humans exposed to 2 hours of coarse particles, however another controlled exposure study did find lower HDL cholesterol a group of humans exposed to air pollution(62, 63). There may be several reasons why we do not see a significant change in measures of HDL function in our study. Our study may not have had the statistical power to detect a small effect of air pollution on HOI. HOI and SAA did increase after DE exposure compared to FA exposure, but the trend was not significant, possibly due to the amount of variability in the measurement and the relatively small sample size. Our power was also compromised by accounting for multiple comparisons. It is also possible that changes in HDL may occur over a longer (or shorter) time frame than was measured by our study. The effects of air pollution in a brief exposure setting in young, healthy subjects may be different biologically than a lower level, chronic exposure to aging adults. HDL may be effected by air pollution in ways not reflected in the measurements used in our study. In future controlled studies of air pollution and HDL, larger sample sizes should be used, with careful attention to the variability in measurements. It is also important to consider the time frame during which biological changes in humans might occur. If possible, studies of effects of longer exposures may provide insight into biological effects, particularly those that may occur in some developing countries where comparably high levels of air pollution are typical.

We also found that those exposed to higher concentrations of air pollution had lower HDL cholesterol longitudinally, but that the magnitude of this association is small. This is a novel finding that has not been published previously. In a mediation analysis, we did not find significant evidence that HDL mediated the relationship between air pollution and CAC. This may be in part due to the weakness of HDL as a predictor of CAC and the small magnitude of change in HDL associated with air pollution. In Gassett et al, a 10 mg/dl higher HDL was associated with 1.8 Agatston units lower CAC progression, an association which disappeared after adjustment for other cardiovascular risk factors(120).

While CAC correlates with atherosclerotic plaque burden and has been described as a measure of atherosclerosis, CAC represents just one aspect of atherosclerotic plaques(137). Atherosclerotic plaques are heterogenous structures that first develop as thickening of the intima, or inner portion of the blood vessel wall. These collections of smooth muscle cells in the intima can begin to accumulate lipids, as well as macrophages engorged with lipids (called foam cells), forming fatty streaks(138). All fatty streaks do not become plaques, however, and many fatty streaks can simply disappear(139). As the lesions grow, accumulating lipids and foam cells, smooth muscle cells and immune cells that move into the streaks begin to die, which leads to greater macrophage accumulation and lipid deposit into a growing lipid core, and development of a fibrous cap and microvasculature within the plaque(140). At this stage some advanced lesions can develop a microvasculature from both the luminal and medial sides of the vessel wall, and the lipid rich core may eventually become calcified(140). Lesions can be classified into a number of different types and subtypes based on development of various features, but calcification comes somewhat later in the development of atherosclerotic lesions(141).

While the exact molecular biology underlying vascular calcification remains unclear, there appear to be two categories of mechanisms under which the mechanisms may be grouped: induction of *osteogenesis* (generation of bone material) and *loss of inhibitors of mineralization*. The osteogenesis hypothesis suggests that vascular calcification is a result of the presence osteoblast cells of unknown origin in the blood vessel wall(142). Several bone proteins such as osteopontin, osteocalcin, and bone mineralization protein 2 (BMP2), matrix vesicles, as well as bone and cartilage formation in calcified vascular lesions may suggest that osteogenic mechanisms play a role in vascular calcification(143). Demer argues that smooth muscle cells in the vascular wall may undergo various changes induced by BMP2 (produced by endothelial cells exposed to hypoxia, reactive oxygen species (ROS), high pressure and inflammation) and BMP4 (produced by foam cells when stimulated by oxidized LDLs) that induce them to become osteoblast-type cells(139). Pericyte cells (which wrap around endothelial cells) in the vascular wall also appear to have potential to act as osteoblast-like cells when stimulated by BMP. Generation of new blood vessels, or angiogenesis, may also be necessary for calcification to occur(139). Cytokines such as BMP and angiogenic vascular endothelial growth factor (VEGF), enable migration and differentiation to osteoblasts, further allowing potential ossifying factors to travel to the vascular wall(139).

Calcification in the vascular wall is not a passive process – there appears to be constant regulation of mineralization in the vascular tissues. Several proteins in the vascular wall normally inhibit mineralization. Important inhibitors include matrix *γ*-carboxyglutamic acid protein (MGP), which prevents BMP2/BMP-receptor-2 interactions; OPG, which inhibits osteoblastic phenotype in VSMCs; OPN and fetuin, which both inhibit hydroxyapatite formation; and BMP7 and Smad 6, which both antagonize BMP osteogenic signaling(139). These mineralization inhibitors keep circulating calcium and phosphate in the tissues from reacting to create calcium structures in the intima, but in the presence of oxidized lipids, inflammation, and macrophage

products, smooth muscle cells appear to express fewer of these inhibitory proteins, leading to calcification of atherosclerotic plaques(139).

However, the extent of calcification may not necessary reflect cardiovascular risk. Microcalcifications and small calcific nodules in the fibrous caps of plaques may make the plaques more likely to rupture, leading to thrombosis(144). Air pollution has been associated with plaque instability in several studies(84, 145). Plaques that are more completely calcified with less lipid content can be more stable and less prone to rupture, perhaps leading to reduced CVD risk(146). Statins have a number of effects which reduce risk of CVD, one of which may be promotion of plaque stabilization through increased calcification, suggesting that increased calcification could be an indicator of healing(147). These features of CAC and development of atherosclerosis add complexity to interpretation of CAC progression.

Future studies of air pollution and HDL are needed in order to replicate these results. Future studies could also contribute by examination of longitudinal effects of air pollution on measurements of HDL function, such as HDL cholesterol efflux capacity, that measure important aspects of HDL function not reflected by HDL cholesterol.

Measurement error can lead to bias in health studies, and measurement error in assessment of exposures is a common challenge in studies of environmental epidemiology. In many large-scale epidemiologic cohort studies, everyday exposures such as diet, pollution, or other ubiquitous exposures cannot be directly measured due to cost or feasibility. Outside of experimental studies, where air pollution exposures can be assigned and controlled for intensity, composition, and duration, exposures must generally be estimated using surrogate or approximate measurements. The “gold standard” for an ideal air pollution measurement could be a measurement of the exact amount of air pollution inhaled by the subject in their breathing zone over a given period of time, corresponding to

their breathing rate and depth of inhalation. Because air pollution measurement devices tend to be large, inconvenient and expensive, even short-term gold standard measurement of air pollution in an observational setting can be impractical. Prior epidemiologic studies of air pollution assigned exposures to study subjects using data from the nearest air pollution monitoring station. The use of central monitors to assign air pollution exposures to large numbers of study subjects for which individual level monitoring would be prohibitively expensive or technically impossible allows investigators to do large-scale studies of air pollution, but at the cost of measurement error.

There are several sources of error in air pollution measurement using a nearest-monitor approach. The first may be described as classical measurement error, and has to do with a subject's actual personal exposure versus their estimated ambient exposure. Some researchers consider this to be the most important source of exposure measurement error in air pollution epidemiology(148). These earlier epidemiologic studies often used a measure of ambient air pollution that estimates the concentration of outdoor air pollution at a subject's residence, without regard for the subject's location, be it indoors or outdoors, or their personal location, be it at home or other location throughout the day. Since many older study subjects spend the majority of their time indoors, this estimate will contain some error, as only a fraction of outdoor air pollution penetrates into homes. Subjects also spend time away from their homes, and may also spend time in traffic on roadways, which can be significant source of air pollution exposure itself, further exposing them to air pollution that is generally not taken into account by outdoor air pollution exposure estimates. While this personal exposure may be the most relevant exposure for estimating the health effects of exposure to air pollution, it can be difficult to interpret given that it is the sum of all of a subject's exposures to air pollution. Outdoor ambient measures at a subject's home may be more relevant for interventions by air quality regulatory agencies such as the EPA, as an outdoor ambient measure is much less dependent on an individual's behavior and the sources of those pollutants can be more clearly regulated.

A second type of error arises from the process of estimating a subject's ambient air pollution exposure. This type of error is described as Berkson or Berkson-like error, and it arises when a group's average is assigned to each individual suiting the group's characteristics. This is in contrast with classical or classical-like measurement error, in which a feature is measured repeatedly and those measurements would yield different results varying around the true value. With Berkson error, the group's observation is "measured value" which becomes part of the analysis, and the individual value is the "true value," and differ within the group(149). In this way, classical-like measurement error is independent of the true variable, while Berkson-like error is independent of the observed variable. Air pollution prediction models, being based on central monitors, can induce this Berkson-like error component due to spatial correlation between study subjects(150). Random and nonrandom between-person variability in behavior may produce Berkson-like error in personal exposure estimates. The effects of spatial smoothing in air pollution prediction models can also convert classical measurement error to Berkson error(150). Both error types can be present simultaneously in air pollution estimation, although they have different effects. Berkson error in air pollution predictions is typically thought to lead to negligible or small amounts of bias in the health effect estimates (in linear models), but higher standard errors. Classical measurement error will typically bias health effect estimates towards the null (assuming the error is non-differential) and lead to more or less variation, depending on the nature of the error.

The manner in which exposures are measured in air pollution epidemiology can lead to different sources of measurement error, with exposures that occur daily and can vary from day to day (random within-person error) and person to person (between-person error). Both are subject to measurement error in the exposure as an important source of bias in health effect studies. Studies are also subject to random error between persons, which may occur as a result of using not using enough measurements per subject in the presence of random within-person error, as well as systematic error between persons, which can occur if there is within-person

error that affects subjects non-randomly(151). The effects of these errors would usually bias the results of health effects studies towards the null. The MESA study uses high-quality measurement of covariates in a large number of participants followed over time in order to address error from these sources.

The MESA Air study addressed the complexities of measurement error in air pollution through a collection of measurements in order to build predictive models accounting for numerous sources and types of error(71, 72). These models estimated air pollution exposures for $PM_{2.5}$, NO, and NO_2 based on publicly available EPA AQS monitoring networks based in urban areas; (which produce excellent quality daily air pollution measurements and provide information on time-varying trends in air pollution); individual-level air pollution monitoring of participants; and a suite of over 800 covariates (including important contributors to air pollution variability such as roadway density, meteorological data, emission data, and land use variables) in order to make accurate air pollution exposure predictions at a very fine-scale resolution(72). These models perform very well (10-fold cross-validated R^2 >0.8 for most sites and pollutants) leveraging a large number of measurements in order to reduce measurement error(72). Air pollution predictions have the advantage that they do not depend on subject self-report, and that they are in a sense exogenous: characteristics within an individual such as age or do not cause ambient air pollution, which is caused by external factors such as meteorology and combustion.

These studies had a number of strengths. Our work helps fill important gaps in the current body of evidence explaining the relationship between air pollution and CVD. The Multi-Ethnic Study of Atherosclerosis is a unique, racially diverse longitudinal cohort with excellent measurement of cardiovascular risk factors and subclinical atherosclerosis. The MESA Air initiative added new air pollution measurements using state of the art modelling techniques with special attention given to reducing measurement error. A novel measurement of HDL was available for most patients at the baseline visit, offering an opportunity to further explore the

relationship between air pollution and HDL beyond HDL cholesterol. Longitudinal measurement of CAC was a key feature in this study, and great care was taken in creating standardized protocols for its scanners and readers to ensure comparability between centers and across exams. Linear mixed effects model allowed for analysis of multiple measurements over time while using all available data. In the controlled exposure studies - in contrast to epidemiologic studies where measurement error in the exposure is a concern, exposures in the experimental studies were blinded, tightly controlled and randomized. We had before and after exposure measurements that allowed us to compare changes in the outcome in time between the diesel exhaust and exposure groups, removing baseline effects and controlling for any effect of sitting in the exposure chamber to allow any effect to be purely that of the diesel exhaust. This allowed for a stronger causal interpretation of results. Linear mixed effects models allowed for use of all available data.

Our studies also had several limitations. Air pollution exposure measurements in observational studies were generated from prediction models and not directly measured, so the potential for measurement error in the exposure cannot be ruled out. However, significant effort was made to ensure air pollution exposure data used in MESA are of the highest quality currently available for epidemiologic studies. Assuming errors in air pollution measurement were not associated with HDL or CAC, these errors usually bias effect estimates towards the null, representing an underestimation of the true measure of effect. Although it has been associated with CVD in observational studies, the NMR-derived measurement of HDL particle number used in our study has not been fully validated and remains an experimental measure(46). Further validation of this technique is required to draw stronger inference about the relationship between air pollution and HDL-P. Measurement of CAC does not fully capture the complexity of atherosclerotic burden(140, 146). Atherosclerotic plaques can be composed of lipids, necrosed cells, and calcium, and interactions between these factors within a plaque can make a plaque more or less prone to rupture(141). The biological processes underlying incident CAC may be

different than those that associated with CAC progression. Despite significant study of CAC, understanding of CAC and HDL's role in the pathogenesis of CAC is limited. While CAC is correlated with atherosclerotic burden, it is an imperfect measure of the risk of CVD due to atherosclerosis. While it appears there is an association between air pollution and some measures of HDL, further research into measures of HDL functionality that better capture the important atheroprotective qualities of HDL could be a useful step towards understanding the mechanisms that explain the relationship between air pollution and CVD, and offer some possible targets for intervention to help prevent its harmful effects. Our experimental studies of controlled exposure to air pollution were secondary analyses that were not pre-specified for our outcomes. A larger sample size may have improved our ability to detect significant results

Summary

In summation, our findings help fill important gaps in existing literature on air pollution and atherosclerosis, and point to possible mechanisms contributing to increased CVD risk. Results from experimental studies may have been underpowered to find strong evidence of the molecular mechanisms explaining this relationship, but provide exploratory analyses which suggest possible hypotheses explaining the association. While the magnitude of the association between air pollution and measures of HDL is small, the association is robust to adjustment for confounding variables. Larger longitudinal studies are needed to confirm biological effects of air pollution on HDL and other lipids. There may also be genetic subtypes that are more susceptible to the HDL-lowering effects of air pollution that could be explored. Additional studies examining other functional qualities of HDL are also needed. Studies examining the relationship between chronic exposure to air pollution and cholesterol efflux capacity of HDL could help explain how air pollution contributes to development of atherosclerotic plaques. It may also be useful to study the relationship between air pollution and the lipid content of HDL. A developing field, HDL lipidomics, studies the lipid cargo of HDL molecules, and could reveal useful insights

into the effects of air pollution on HDL at the molecular level. Larger studies of the way air pollution remodels HDL molecular structure could potentially provide important information into the precise mechanisms that explain the relationship between air pollution and cardiovascular disease.

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