Materials and Methods

Study Population

MESA is a population-based, multiethnic prospective cohort study of 6,814 men and women aged 45-84, which has been described in detail elsewhere[1, 2]. 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; Northern Manhattan, NY; and St. Paul, MN). Participants were free of clinical cardiovascular disease (physician-diagnosed 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[1, 3]. 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 [4, 5]. 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[3]. 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[5]. Multiple years of data from AQS and fixed-site monitors were used to assess time trends in pollutant concentrations. Separate models were built for each pollutant and each study site. 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[5].

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[6, 7]. 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[8]. 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 our 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 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. 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. $PM_{2.5}$ 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[4].

HDL Measures

At their baseline MESA examination, participants gave 12-hour fasting blood samples which were frozen at -70°C[1]. 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%)[9]. 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[10]. HDL-P (CV=2%) was calculated as the sum of the particle concentrations of the HDL subclasses [10]. 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.[10].

Data 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 our statistical 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-minutes/week, BMI, history of diabetes mellitus (defined by the 2003 American Diabetes Association fasting criteria algorithm), C-reactive protein, 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. Normailty of HDL measures was confirmed through examination of histogram plots. Intervals of 5 ug/m^3 and 0.7 $10^{-6}m^{-1}$ were chosen because they were comparable to intervals used in prior literature.[11-13]

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. 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 (Supplementary Tables 1-3).

Supplementary Table 1. Associations between 1-year averaged air pollutants and HDL - 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 - excluding those with TG >400mg/dL and CRP >3mg/L	-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 - excluding those with TG >400mg/dL and CRP >3mg/L	-0.06 (-0.62, 0.51)	0.52 (-0.24, 1.29)	-0.60 (-1.19, -0.01)
Ratio of HDL-C/HDL-P			
Minimally adjusted Model	0.01 (-0.02, 0.03)	0.02 (-0.02, 0.05)	-0.02 (-0.04, 0.01)
Final Model	-0.001 (-0.02, 0.02)	0.02 (-0.01, 0.05)	-0.02 (-0.04, .003)

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

Supplementary Table 2. Associations between 3-month averaged air pollutants and HDL - MESA Air

Three Month Averages	$PM_{2.5}$ (5µg/m^3)	Outdoor $PM_{2.5}$ (5µg/m^3)
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 - excluding those with TG >400mg/dL and CRP >3mg/L	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 - excluding those with TG >400mg/dL and CRP >3mg/L	-0.57 (-0.96, -0.18)	-0.26 (-0.58, 0.06)
Ratio of HDL-C/HDL-P		
Minimally adjusted Model	0.03 (0.01, 0.05)	0.04 (0.03, 0.05)
Final Model	0.03 (0.02, 0.05)	0.03 (0.02, 0.04)

*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

Supplementary Table 3. Associations between 2-week averaged air pollutants and HDL - MESA Air

Two week Averages	PM2.5 (5µg/m3)	Outdoor PM2.5 (5µg/m3)
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 - excluding those with TG >400mg/dL and CRP >3mg/L	-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 - excluding those with TG >400mg/dL and CRP >3mg/L	-0.23 (-0.53, 0.06)	-0.04 (-0.24, 0.16)
Ratio of HDL-C/HDL-P		
Minimally adjusted Model	-0.01 (-0.02, 0.003)	0.01 (-0.002, 0.014)
Final Model	0.005 (-0.01, 0.02)	0.01 (0.0001, 0.02)

* 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

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