SUPPLEMENTARY MATERIAL

Cholesterol, Lipoproteins and Subclinical Interstitial Lung Disease: The MESA Study

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SUPPLEMENTARY METHODS

Study Participants

The Multi-Ethnic Study of Atherosclerosis (MESA) is a multi-center, prospective cohort study sponsored by the National Heart Lung and Blood Institute to investigate the progression of subclinical cardiovascular disease. The participant selection criteria have previously been described.[1] The study enrolled 6,814 participants between 2000 and 2002 from six centers in the US. Inclusion criteria were age 45-84 and no cardiovascular disease (defined as physician-diagnosed heart attack, angina, stroke, TIA, heart failure, atrial fibrillation) at study entry. Adults who have undergone any procedure related to cardiovascular disease (CABG, angioplasty, valve replacement, pacemaker or defibrillator implantation) were also excluded. Other exclusion criteria included weight greater than 136 kg, pregnancy, any impediment to long term participation, and chest CT within the past year. Notably, there were no selection criteria based on lung disease, respiratory symptoms, or smoking history. Those who had a limited life expectancy and those who had undergone chest CT imaging in the preceding 12 months were not eligible for enrollment in MESA.

In 2004-206, 3,965 MESA participants were enrolled in the MESA Lung Study. Participants were eligible for recruitment if they attended a MESA follow-up exam during the recruitment period, consented to genetic analyses and underwent baseline measures of endothelial function. The 3,965 participants were randomly sampled from this subset of 4,484 eligible participants, with over-sampling of Chinese-Americans. An additional 410 participants were enrolled into the MESA Lung study in 2010-2012. 2,942 of MESA Lung Participants underwent full lung CT scans in 2010-2012, which were visually assessed for interstitial lung abnormalities (Figure S1).

MESA and MESA Lung were approved by Institutional Review Boards at all collaborating centers, and all participants provided written informed consent for participation.

Study Procedures

All participants completed a detailed questionnaire on demographics, family history, medical history, medication use, lifestyle habits and psychosocial factors at the MESA baseline visit in 2000-2002. The physical examination included height, weight and blood pressure measurements. Hypertension (HTN) was defined by the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC) VI criteria as systolic blood pressure ≥140, diastolic blood pressure ≥90 or self-reported history of HTN and the use of hypertensive meds. Diabetes mellitus and the use of statins were self-reported. Blood was drawn for laboratory measurements and participants underwent cardiac CT scans, carotid ultrasonography and examination of ankle-brachial index.

Coronary artery calcium was calculated from cardiac CT scans performed in 2002-2002 on multidetector and electron-beam CT scanners following a standardized protocol.[2 3] A phantom of known physical calcium concentration was included in the field of view for each CT scan. The Agatston score was calculated using the average phantom-adjusted measure from two scans.

Trained technicians performed B-mode ultrasonography of the right and left near and far walls of the internal carotid and common carotid arteries using the Logiq 700 ultrasound device (General Electric Medical Systems, Waukesha, WI). Intima-media thickness (IMT) was determined for

the common and internal carotid arteries as the mean of the maximum IMT of the near and far walls of the right and left sides.[4]

Ankle-brachial index (ABI) was obtained using a hand-held Doppler instrument with a 5-mHz probe (Nicolet Vascular, Golden, CO, USA). Systolic blood pressure was measured at the bilateral brachial, dorsalis pedis and posterior tibial arteries.

Laboratory Measurements

All laboratory measurements were performed on blood samples collected at the MESA baseline visit in 2000-2202. Peripheral blood samples were obtained and processed at each study center using standardized protocols. Samples were frozen and sent to a central laboratory for analysis. Lipoproteins were measured at the Collaborative Studies Clinical Laboratory at Fairview-University Medical Center (Minneapolis, MN). HDL-cholesterol was measured in EDTA plasma using the cholesterol oxidase cholesterol method (Roche Diagnostics) after precipitation of non-HDL-cholesterol with magnesium/dextran. LDL-cholesterol was calculated in plasma specimens having a triglyceride value <400 mg/dL using the formula of Friedewald et al.[5] Lipoprotein particle concentrations and sizes were measured on frozen plasma specimens by proton nuclear magnetic resonance (NMR) spectroscopy (LipoScience Inc., North Carolina), as previously described.[6] The following subclasses were included: small LDL (diameter 18.0-21.2 nm), large LDL (21.2-23 nm), large HDL (8.8-13nm), medium HDL (8.2-8.8 nm), and small HDL (7.3-8.2 nm). Concentrations are reported in nanomoles of particles per liter for LDL and micromoles of particles per liter for HDL.

Apolipoprotein A-1 was measured in 4,884 MESA participants (those with low amounts of stored serum and on lipid lowering therapy were excluded) using reagents from Diazyme at the laboratory of Dr. Alan Remaley at NIH (Bethesda, MD). Levels were quantified using a Siemens Dimension analyzer.

CRP and IL-6 was measured at the Laboratory for Clinical Biochemistry Research (University of Vermont, Burlington, VT) using the BNII nephelometer (N High Sensitivity CRP; Dade Behring Inc., Deerfield, IL) and the ultra-sensitive ELISA (Quantikine HS Human IL-6 Immunoassay; R&D Systems, Minneapolis, MN), respectively. Glomerular filtration rate (GFR) was estimated using the Modification of Diet in Renal Disease (MDRD) equation.[7]

MMP-7 and SP-A were measured in banked baseline serum samples from MESA participants at the MESA Core Laboratory at the University of Vermont's Laboratory for Clinical Biochemistry Research. Samples were stored at -70ºC. MMP-7 was measured using a quantitative sandwich ELISA assay from R&D systems. The lower detection limit for this assay is 0.094 ng/mL and the detection range is 0.2-10 ng/mL. The expected normal range per manufacturer is 1.07-4.40 ng/mL. The mean concentration in our sample was 4.33 ng/mL (4.09 in subcohort only). The laboratory inter-assay CVs range from 2.9 to 6.9%.

SP-A was measured using a quantitative sandwich ELISA from Biovendor. The lower detection limit for this assay is 0.16 ng/mL and the upper limit of detection is 500 ng/mL. The expected normal range per manufacturer is 13-65 ng/mL. The mean concentration in our sample was 76.25 ng/mL (79.87 in subcohort only). The laboratory inter-assay CVs range from 10.9 to 14.2%.

Cardiac MRI

Cardiac magnetic resonance imaging (MRI) was performed at the MESA baseline visit in 5,098 participants, of whom 5,004 had interpretable images of the left ventricle (LV). The MRI protocol has been described previously.[8] Images were acquired using 1.5-T magnets, using a four-element, phased-array surface coil placed anteriorly and posteriorly, with ECG gating. Imaging was consisted of fast gradient echo cine images with temporal resolution of 50 msec or less. Data were analyzed using MASS software, version 4.2 (Medis) at a single reading center.[9] LV end-diastolic volume was calculated as the summation of areas on each separate slice multiplied by the sum of slice thickness and image gap. LV mass was determined by the sum of the myocardial areas times slice thickness plus image gap in the end-diastolic phase, multiplied by the specific gravity of the myocardium.[8] LV ejection fraction was calculated as the stroke volume divided by the end-diastolic volume.[9]

Lung Attenuation on CT Scan

Lung attenuation was measured on cardiac CT scans performed in years 2000-2002. Scans imaged approximately 66% of the lung volume from the carina to lung bases. We have previously shown good correlation between attenuation measurements on cardiac and full lung CT scans.[10 11] High attenuation areas (HAA) were defined as the volume of imaged lungs having CT attenuation values between -600 and -250 Hounsfield Units (HU), as previously described.[10] Percent emphysema was defined as the percentage of the voxels in the lung below -950 HU, as previously described.[11] Quantitative image attenuation was measured using a

modified version of the Pulmonary Analysis Software Suite at a single reading center by trained readers.

Interstitial Lung Abnormalities

ILA was visually assessed on full lung CT scans in 2,942 MESA participants during the 2010-2012 MESA follow-up exam enrolled in the MESA Lung Study. MESA Lung participants were randomly sampled from the parent cohort in 2004-2006. Chinese-Americans were oversampled. Scans were performed without intravenous contrast at suspended full inspiration on 64-slice scanners (GE and Siemens) using the MESA-Lung/SPIROMICS protocol.[12] Images were reconstructed using 0.625 mm slice thickness. ILA was visually assessed by one of five expert radiologists and was defined as the presence of ground-glass, reticular abnormality, diffuse centrilobular nodularity, honeycombing, traction bronchiectasis, non-emphysematous cysts, or architectural distortion in at least 5% of nondependent portions of the lung, as previously described.[13-16]

Statistical Analysis

We used generalized linear and additive models (with loess smoothing functions for continuous variables) to examine associations between CVD risk factors and each outcome of interest. Models examining HAA included age, gender, race/ethnicity, educational attainment, height, BMI, waist circumference, smoking status, cigarette pack-years, presence of hypertension, presence of diabetes, statin use, diuretic use, coronary artery calcium, c-reactive protein (CRP), interleukin-6 (IL-6), glomerular filtration rate (GFR), study site, milliAmpere (mA) dose, total volume imaged lung and percent emphysema on CT. We adjusted for percent emphysema in

light of prior work showing associations between emphysema on CT, subclinical atherosclerosis and HDL-C.[17 18] We adjusted for CRP and IL-6 to control for systemic inflammation. We used multivariable logistic regression to examine the association of each exposure with ILA. Given the smaller sample size of models examining ILA, MMP-7 and SP-A, we limited covariates to age, gender, race/ethnicity, BMI, smoking status, cigarette pack-years, statin use, coronary artery calcium, CRP and IL-6. HAA, MMP-7 and SP-A were natural log-transformed to improve model fit and we used inverse natural log transformed beta coefficients to express the adjusted percent increase in HAA, MMP-7 and SP-A per standard deviation change in each independent variable. MMP-7 and SP-A models included inverse probability weighting to account for the probability of selection for biomarker measurement. Effect modification by smoking status and other factors was tested in full multivariable models using likelihood ratio tests. We performed sensitivity analyses adjusting for left ventricular function, since elevated left atrial pressure could increase extravascular lung water and thereby increase CT lung attenuation. Statistical significance was defined by two-tailed tests with a p-value of <0.05. In each analysis, participants with missing exposure or outcome data were excluded. Multiple imputation using chained equations of 10 datasets (SAS PROC MI and PROC MIANALYZE) was performed to account for missing covariate data. Analyses were performed using SAS 9.3 (SAS Institute, Cary, NC, USA) and the gam function in R 2.6 (R Foundation, Vienna, Austria). Drs. Podolanczuk and Lederer had full access to all the data in the study and take responsibility for the integrity of the data and the data analysis.[19 20] Drs. Podolanczuk and Lederer had full access to all the data in the study and take responsibility for the integrity of the data and the data analysis.

SUPPLEMENTARY RESULTS

The flow diagram of study participants is shown in Supplementary Figure S1. Of the 6,814 MESA participants, there were 6,700 with available lipid measurements included in HAA analyses, 2,391 in ILA analyses, and 1,216 in MMP-7 and SP-A analyses, with sampling frame previously described. [21] The median time between baseline measurements and ILA assessment was 9.5 years (range 8.0 to 11.4 years). The baseline characteristics of MESA participants are shown in Supplementary Table S1. In the overall cohort, the mean (± standard deviation; SD) age was 62 ± 10 years, 47% were male, 41% were former smokers and 14% were current smokers, with a median of 15 pack-years. The median (IQR) HAA was 4.2% (3.5-5.4%) of total imaged lung.

Measures of Subclinical Atherosclerosis

In fully adjusted models, there was a significant association between the presence of CAC, HAA and ILA (Supplementary Tables S2 and S3). The presence of CAC was associated with a 1.47% increase in HAA (95% CI 0.19 to 2.77). Among those with CAC, each 1 unit increase in Agatston calcium score was associated with a 0.79% increase in HAA (95% CI -0.04 to 1.62). The presence of CAC was also associated with a 57% increase in the odds of ILA (95% CI 1.18 to 2.08). There were no meaningful associations of common carotid IMT, internal carotid IMT, or ABI with HAA or ILA.

High Attenuation Areas

In a multivariable-adjusted model, greater total cholesterol level was associated with lower HAA (1.32% decrease in HAA per SD increase in total cholesterol, 95% CI 0.75 to 1.89; Table 1).

Both greater HDL-C and greater LDL-C concentrations were associated with lower HAA (Supplementary Figure S2a and S2b; Table 2). HAA decreased by 2.12% per SD increase in HDL-C (95% CI 1.44 to 2.79%) and by 0.84% per SD increase in LDL-C (95% CI 0.28 to 1.41%). Greater triglyceride levels were also associated with lower HAA (0.82% decrease in HAA per SD increase in triglycerides, 95% CI 0.18 to 1.45). Adjustment for measures of left ventricular function slightly attenuated the association between HDL-C and HAA (1.94% decrease in HAA per SD, 95% CI 1.18 to 2.69) but it remained significant, while the association between LDL-C and HAA was no longer significant (HAA decrease of 0.46 per SD, 95% CI -0.17 to 1.09; Supplementary Table S4). The association between triglycerides and HAA was borderline significant in models adjusted for left ventricular function (0.74% decrease in HAA per SD, 95% CI 0.03 to 1.44).

The association between higher HDL and lower HAA was strongest for small HDL particles and medium HDL particles and weaker for large HDL particles (Table 1). The association between HAA and large LDL was stronger than for small LDL particles. There was an association between higher ApoA-1 levels and lower HAA: HAA decreased by 1.66% per SD increase in ApoA-1 (95% CI 0.97 to 2.36%; Table 1 and Supplementary Figure S2c). There was also a borderline significant association between higher ApoB levels and lower HAA: HAA decreased by 0.69% per SD increase in ApoB (95% CI 0.01 to 1.37). In multivariable-adjusted models, there were no significant associations between HAA and the presence of DM, HTN, or statin use (Supplementary Table S5).

Interstitial Lung Abnormalities

In a multivariable-adjusted model, there were no associations of baseline HDL-C, LDL-C, triglycerides, ApoA-1, ApoB, presence of DM, HTN, or statin use with the presence of ILA assessed at 10 year follow-up (Supplementary Tables S6 and S7).

Biomarkers of lung injury and remodeling

In a multivariable-adjusted model, MMP-7 decreased by 3.53% (95% CI 0.93 to 6.07%) per SD increase in HDL-C (Table 2). SP-A decreased by 6.37% per SD increase in HDL-C (95% CI 1.35 to 11.13%). The association between HDL and MMP-7 was similar across HDL particle sizes, while the association between HDL and SP-A was strongest for large and small HDL particles and weakest for medium HDL particles (Table 2). There was a significant inverse association between ApoA-1 and MMP-7 (2.83% decrease in MMP-7 per SD increase in ApoA-1, 95% CI 0.27 to 5.32%) and a non-significant inverse association between ApoA-1 and SP-A (4.79 decrease in SP-A per SD increase in ApoA-1, 95% CI -0.12 to 9.56%). There was also a significant positive association between triglycerides and MMP-7 (2.81% increase in MMP-7 per SD increase in triglycerides, 95% CI 0.30 to 5.37%), but not SP-A. There were no significant associations of total cholesterol, LDL-C or ApoB with MMP-7 or SP-A, but there was a significant inverse association between large LDL particles and MMP-7 (4.86% decrease in MMP-7 per SD increase in large LDL, 95% CI 2.15 to 7.50%).

Stratified Analyses

Analyses stratified on demographic factors and smoking (Supplementary Tables S8 and S9) suggested that gender modified the association between HDL-C and HAA (p for interaction 0.006). There was a stronger association between HDL-C and HAA among women (2.49%

decrease in HAA per SD increase in HDL-C, 95% CI -3.34 to -1.64%) than among men (0.59% decrease in HAA per SD increase in HDL-C, 95% CI -1.74 to 0.57%). There was no evidence of effect modification by age, race, BMI, smoking status or statin use on the relationship between HDL-C or LDL-C and HAA.

Supplementary Table S1: Baseline characteristics of 6,700 MESA participants by HDL-C quartiles.

	All MESA participants	HDL-C Quartiles			
		Q1	Q2	Q3	Q4
Mean HDL-C, mg/dL (range)	51 (15-142)	35 (15-40)	44 (41-48)	54 (49-59)	72 (60-142)
Age, years	62±10	61±10	62±10	62±10	64±10
Male	3213 (47)	1243 (74)	897 (54)	671 (39)	345 (21)
Race/ethnicity					
White	2622 (38)	599 (36)	609 (36)	662 (38)	708 (44)
African-American	1892 (28)	401 (24)	456 (27)	509 (29)	503 (31)
Hispanic	1495 (22)	477 (28)	394 (24)	344 (20)	248 (15)
Asian (Chinese)	804 (12)	201 (12)	212 (13)	227 (13)	150 (9)
Body mass index, kg/m^2	28±5	29±5	29±5	28±6	27±5
Height, cm	166±10	170±10	167±10	165±10	163±9
Weight, kg	78±17	85±17	81±17	77±17	71±15
Waist circumference, cm	98±14	102±13	100±14	98±15	92±14
Smoking					
Never smokers	3082 (45)	677 (40)	727 (43)	834 (48)	798 (50)
Former smokers	2764 (41)	704 (42)	699 (42)	677 (39)	645 (40)
Current smokers	967 (14)	297 (18)	245 (15)	231 (13)	166 (10)
Cigarette pack-years*	14.5 (3.3-33.0)	16 (4-35)	15 (4-34)	13 (3-31)	14 (3-30)
Hypertension	3057 (45)	753 (45)	756 (45)	768 (44)	724 (45)
Systolic blood pressure, mmHg	127±21	126±21	126±21	127±21	127±24
Diastolic blood pressure, mmHg	72±10	74±10	72±10	71±10	70±10
Diabetes mellitus	775 (11)	279 (17)	195 (12)	167 (10)	111 (7)
LDL-C, mg/dL	117±31	115±31	121±32	120±32	113±31
Fasting plasma glucose, mg/dL	97±30	104±35	98±30	96±28	91±21
Glomerular filtration rate†	81±18	82±19	82±18	81±17	80±20
Statin use	1012 (15)	220 (13)	259 (16)	290 (17)	223 (14)
Diuretic use	918 (13)	213 (13)	221 (13)	236 (14)	229 (14)
Spirometry‡					
FEV1, mL	2380±731	2613±721	2434±746	2293±727	2145±637
FEV1, percent predicted	93±18	92±17	93±18	94±18	95±19
FVC, mL	3187±955	3507±923	3270±977	3066±957	2862±829
FVC, percent predicted	95±16	94±15	95±17	96±16	97±17
FEV1/FVC ratio, %	75±9	74±8	75±9	75±9	75±8
Percent emphysema	2.9 (1.2-5.7)	3.2 (1.4-6.0)	3.0 (1.2-5.9)	2.7 (1.1-5.8)	2.7 (1.2-5.2)
Percent HAA	4.2 (3.5-5.4)	4.4 (3.6-5.6)	4.3 (3.6-5.5)	4.2 (3.5-5.3)	4.1 (3.4-5.1)
HAA volume on CT, mL	119 (100-143)	133 (113-158)	125 (106-148)	115 (98-137)	105 (91-124)
Imaged lung volume on CT, mL	2691 (2208-3253)	2969 (2425-3519)	2754 (2256-3316)	2595 (2167-3139)	2512 (2118-2955)

Data presented as mean±SD, n (%) or median (interquartile range), unless otherwise stated. LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol; FEV1: forced expiratory volume in 1 second; FVC: forced vital capacity; CT: computed tomography

*Among ever-smokers

† Calculated in mL/min/1.73m^2 using the simplified MDRD (Modification of Diet in Renal Disease study) equation

‡Among subset of participants with acceptable spirometry measurements

Supplementary Table S2: Associations of baseline measures of subclinical atherosclerosis with HAA.

	% change in HAA (95% CI)*	P value
Presence of CAC	1.47 (0.19 to 2.77)	0.02
Agatston score†	0.79 (-0.04 to 1.62)	0.06
Common carotid IMT, mm	0.22 (-0.42 to 0.87)	0.50
Internal carotid IMT, mm	0.17 (-0.46 to 0.79)	0.60
Ankle-brachial index	0.20 (-0.42 to 0.82)	0.53

CAC: coronary artery calcium; IMT: intima-media thickness;

Each row in the table presents the results of a single regression model that includes adjustment for age, gender, race/ethnicity, educational attainment, height, body mass index, waist circumference, smoking status, cigarette pack-years, presence of hypertension, presence of diabetes, low density lipoprotein, high density lipoprotein, triglycerides, glomerular filtration rate, statin use, diuretic use, study site, milliampere dose, total volume imaged lung, percent emphysema on CT, IL-6 and CRP.

*Reported per standard deviation in continuous variables and for the presence vs. absence of CAC.

†Among those with detectable CAC.

Supplementary Table S3: Associations of baseline measures of subclinical atherosclerosis with ILA at 10 year follow-up.

	OR for ILA (95% CI)*	P value
Presence of CAC	1.57 (1.18 to 2.08)	0.002
Agatston score†	1.07 (0.91 to 1.25)	0.40
Common carotid IMT, mm	1.09 (0.96 to 1.25)	0.19
Internal carotid IMT, mm	1.05 (0.93 to 1.18)	0.42
Ankle-brachial index	1.00 (0.88 to 1.14)	0.97

CAC: coronary artery calcium; IMT: intima-media thickness

Each row in the table presents the results of a single regression model that includes adjustment for age, gender, race/ethnicity, body mass index, smoking status, cigarette pack-years, presence of hypertension, presence of diabetes, low density lipoprotein, high density lipoprotein, triglycerides, statin use, IL-6 and CRP.

* Reported per standard deviation in continuous variables and for the presence vs. absence of CAC.

†Among those with detectable CAC.

Supplementary Table S4: Associations between plasma lipids and HAA with serial adjustment for confounding and precision variables.

	HDL-C		LDL-C		TG	
	% change in HAA (95% CI)*	**P value**	**% change in HAA (95% CI)***	**P value**	**% change in HAA (95% CI)***	**P value**
Unadjusted	-8.62 (-9.42 to -7.83)	<0.001	-0.94 (-1.55 to -0.33)	0.004	-0.65 (-1.92 to 0.63)	0.01
+ imaged lung volume	-7.81 (-8.61 to -7.01)	<0.001	-0.94 (-1.55 to -0.33)	0.01	-0.65 (-1.92 to 0.63)	0.17
+ demographics†	-4.43 (-5.13 to -3.72)	<0.001	-1.00 (-1.60 to -0.39)	0.001	-0.24 (-0.94 to 0.46)	0.50
+ smoking	-4.35 (-5.05 to -3.64)	<0.001	-0.92 (-1.52 to -0.31)	0.003	-0.38 (-1.08 0.31)	0.28
+ anthropometrics‡	-2.50 (-3.20 to -1.80)	<0.001	-0.77 (-1.34 to -0.20)	0.008	-0.93 (-1.58 to -0.27)	0.006
+ emphysema, mA dose	-2.24 (-2.91 to -1.57)	<0.001	-0.81 (-1.36 to -0.26)	0.004	-1.02 (-1.65 to -0.39)	0.002
+ GFR	-2.26 (-2.93 to -1.59)	<0.001	-0.72 (-1.27 to -0.17)	0.01	-0.95 (-1.58 to -0.32)	0.003
+ HTN, DM, statin, diuretic use	-2.24 (-2.91 to -1.56)	<0.001	-0.85 (-1.40 to -0.28)	0.003	-0.88 (-1.51 to -0.25)	0.007
+ coronary artery calcium	-2.22 (-2.89 to -1.54)	<0.001	-0.91 (-1.47 to -0.34)	0.002	-0.89 (-1.52 to -0.26)	0.006
+ IL-6, CRP	-2.12 (-2.79 to -1.44)	<0.001	-0.84 (-1.41 to -0.28)	0.004	-0.82 (-1.45 to -0.18)	0.01
+ LVEF, LVEDM§	-1.94 (-2.69 to -1.18)	<0.001	-0.46 (-1.09 to 0.17)	0.15	-0.74 (-1.44 to -0.03)	0.04

HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol; TG: triglycerides; HAA: high attenuation areas; mA: milliampere (CT radiation) dose); GFR: glomerular filtration rate; HTN: hypertension; DM: diabetes mellitus; IL-6: interleukin-6; CRP: c-reactive protein; LVEF: left ventricular ejection fraction; LVEDM: left ventricular end-diastolic mass

*Reported per standard deviation in each exposure variable
†Includes age, gender, race, study site and educational attainment
‡Includes height, BMI and waist circumference
§Measured by cardiac MRI in n=4,928 MESA participants with complete lipid data and HAA analysis

Supplementary Table S5: Associations between HTN, DM, statin use and HAA.

	% change in HAA (95% CI)	P value
Presence of hypertension	-1.15 (-2.41 to 0.14)	0.08
Presence of diabetes mellitus	0.14 (-1.68 to 2.00)	0.88
Statin use	-1.47 (-3.06 to 0.14)	0.07

Each model includes all other variables the table and is additionally adjusted for age, gender, race/ethnicity, educational attainment, height, body mass index, waist circumference, smoking status, cigarette pack-years, glomerular filtration rate, diuretic use, presence of coronary artery calcium, HDL-C, LDL-C, TG, study site, milliampere dose, total volume imaged lung, percent emphysema on CT, interleukin-6 and c-reactive protein.

Supplementary Table S6: Associations of cholesterol and lipoproteins with ILA.

	OR for ILA (95% CI)*	P value
Total cholesterol	0.95 (0.83 to 1.09)	0.45
HDL-C, mg/dL	0.97 (0.83 to 1.13)	0.66
LDL-C, mg/dL	0.95 (0.83 to 1.09)	0.46
Triglycerides	1.01 (0.87 to 1.17)	0.89
Large HDL, 9.4-14 nm	1.08 (0.89 to 1.30)	0.45
Medium HDL, 8.2-9.4 nm	0.93 (0.78 to 1.11)	0.44
Small HDL, 7.3-8.2 nm	0.87 (0.73 to 1.04)	0.13
Large LDL, 20.5-23 nm	0.87 (0.74 to 1.02)	0.09
Small LDL, 18-20.5 nm	1.07 (0.88 to 1.30)	0.49
Apolipoprotein A-1	0.88 (0.69 to 1.11)	0.28
Apolipoprotein B	0.93 (0.78 to 1.10)	0.40

Each model includes all exposure variables listed and is additionally adjusted for age, gender, race/ethnicity, body mass index, smoking status, cigarette pack-years, presence of coronary artery calcium, statin use, interleukin-6 and c-reactive protein.

*Reported per standard deviation in each exposure variable.

Supplementary Table S7: Associations between HTN, DM, statin use, and ILA.

	OR for ILA (95% CI)	P value
Presence of hypertension	0.92 (0.70 to 1.22)	0.57
Presence of diabetes mellitus	1.36 (0.91 to 2.04)	0.14
Statin use	0.95 (0.68 to 1.35)	0.79

Each model includes all other variables the table and is additionally adjusted for age, gender, race/ethnicity, body mass index, smoking status, cigarette pack-years, presence of coronary artery calcium, HDL-C, LDL-C, TG, interleukin-6 and c-reactive protein.

Supplementary Table S8: Associations between HDL-C and HAA stratified on selected demographic factors.

	% change in HAA (95% CI)*	P value	P for interaction
Age			0.15
<55	-1.62 (-2.76 to -0.47)	0.01	
55-64	-2.05 (-3.19 to -0.91)	0.001	
65-74	-1.91 (-3.04 to -0.76)	0.002	
≥75	-2.49 (-4.45 to -0.49)	0.02	
Gender			0.006
Female	-2.49 (-3.34 to -1.64)	<0.001	
Male	-0.59 (-1.74 to 0.57)	0.25	
Race			0.38
White	-1.47 (-2.33 to -0.61)	<0.001	
African-American	-2.05 (-3.19 to -0.91)	0.001	
Hispanic	-2.78 (-4.46 to -1.07)	0.001	
Asian (Chinese)	-1.03 (-3.59 to 1.59)	0.44	
BMI category			0.99
<20	-3.21 (-6.26 to -0.07)	0.06	
20-24	-1.47 (-2.61 to -0.32)	0.009	
25-29	-2.20 (-3.33 to -1.06)	<0.001	
≥30	-2.34 (-3.75 to -0.92)	<0.001	
Smoking status			0.67
Never smokers	-1.76 (-2.90 to -0.61)	<0.001	
Ever smokers	-2.20 (-3.05 to -1.34)	<0.001	
Statin Use			0.36
No	-2.05 (-2.62 to -1.48)	<0.001	
Yes	-2.05 (-3.75 to -0.33)	0.03	

Model is adjusted for age, gender, race/ethnicity, educational attainment, height, body mass index, waist circumference, smoking status, cigarette pack-years, presence of hypertension, presence of diabetes, low density lipoprotein, triglycerides, glomerular filtration rate, statin use, diuretic use, study site, milliampere dose, total volume imaged lung, percent emphysema on CT, coronary artery calcium, c-reactive protein and IL-6.

*Reported per standard deviation in HDL-C.

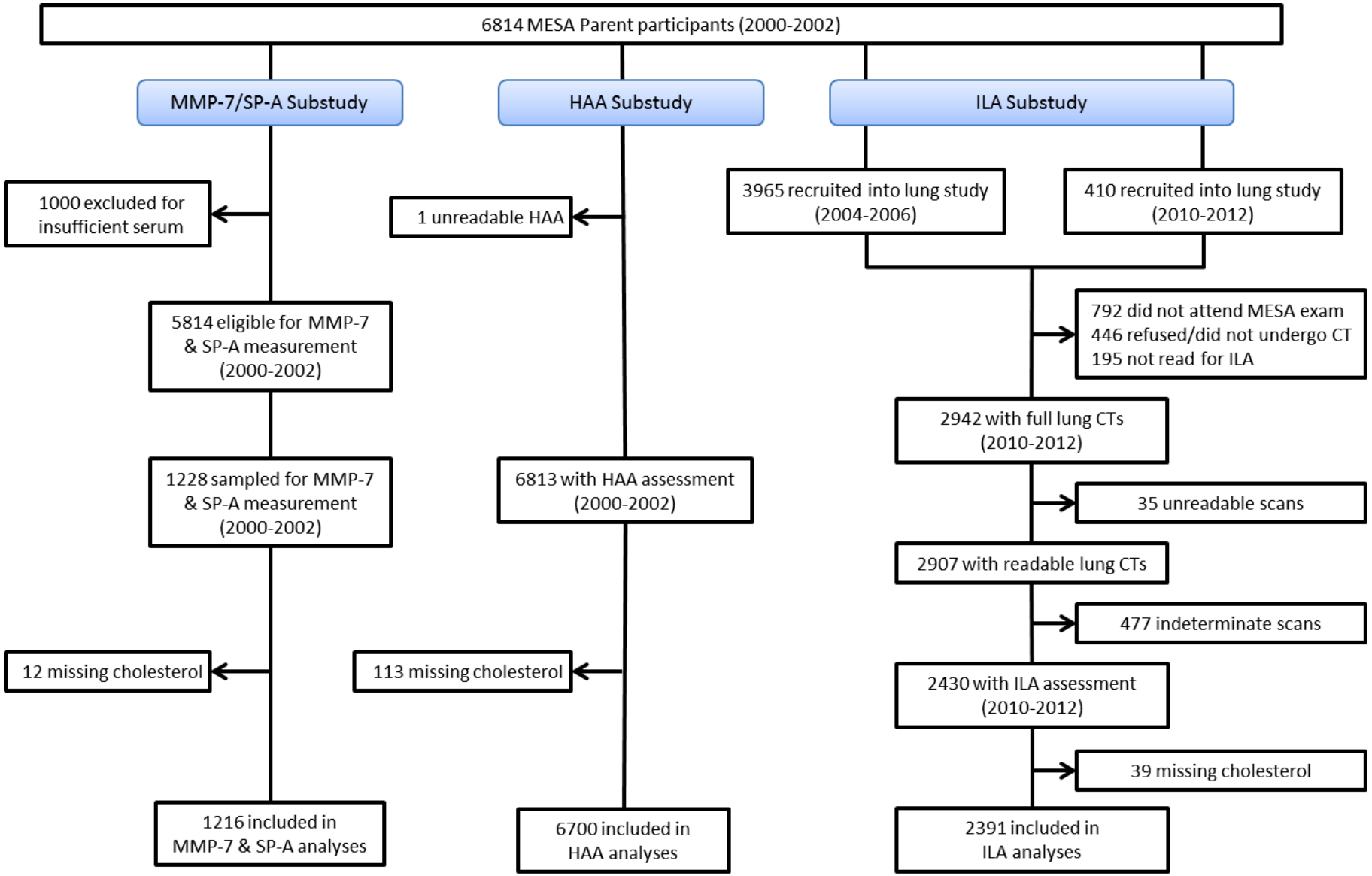
Supplemental Table S9: Associations between LDL-C and HAA stratified on selected demographic factors.

	% change in HAA (95% CI)*	P value	P for interaction
Age			0.69
<55	-0.31 (-1.54 to 0.92)	0.40	
55-64	0.00 (-1.23 to 1.24)	0.79	
65-74	-0.94 (-2.15 to 0.29)	0.08	
≥75	-1.56 (-3.36 to 0.28)	0.10	
Gender			0.27
Female	-0.63 (-1.24 to -0.01)	0.20	
Male	-0.94 (-1.55 to -0.33)	0.01	
Race			0.88
White	-0.63 (-1.24 to -0.01)	0.11	
African-American	-0.94 (-2.15 to 0.29)	0.06	
Hispanic	-0.94 (-2.15 to 0.29)	0.21	
Asian (Chinese)	-0.63 (-2.45 to 1.23)	0.45	
BMI category			0.88
<20	0.32 (-2.73 to 3.46)	0.76	
20-24	-0.31 (-1.54 to 0.92)	0.54	
25-29	-0.94 (-1.55 to -0.33)	0.04	
≥30	-0.63 (-1.85 to 0.61)	0.20	
Smoking status			0.68
Never smokers	-0.63 (-1.24 to -0.01)	0.14	
Ever smokers	-0.63 (-1.24 to -0.01)	0.06	
Statin Use			0.07
No	-0.94 (-1.55 to -0.33)	0.003	
Yes	0.32 (-1.52 to 2.19)	0.70	

Model is adjusted for age, gender, race/ethnicity, educational attainment, height, body mass index, waist circumference, smoking status, cigarette pack-years, presence of hypertension, presence of diabetes, high density lipoprotein, triglycerides, glomerular filtration rate, statin use, diuretic use, study site, milliampere dose, total volume imaged lung, percent emphysema on CT, coronary artery calcium, c-reactive protein, IL-6.

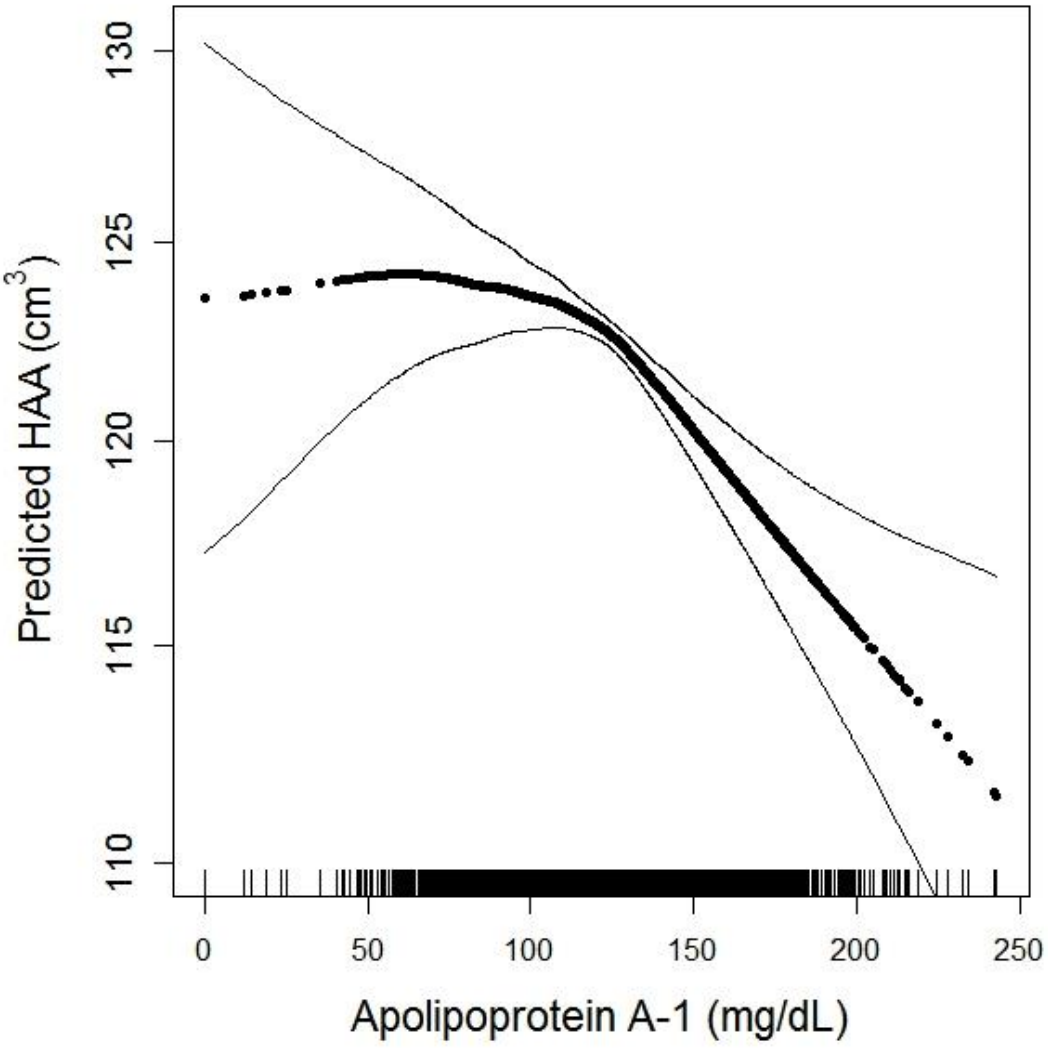
*Reported per standard deviation in LDL-C.

Supplementary Figure S1. Flow diagram of study participants. The year of each substudy is listed in parentheses.

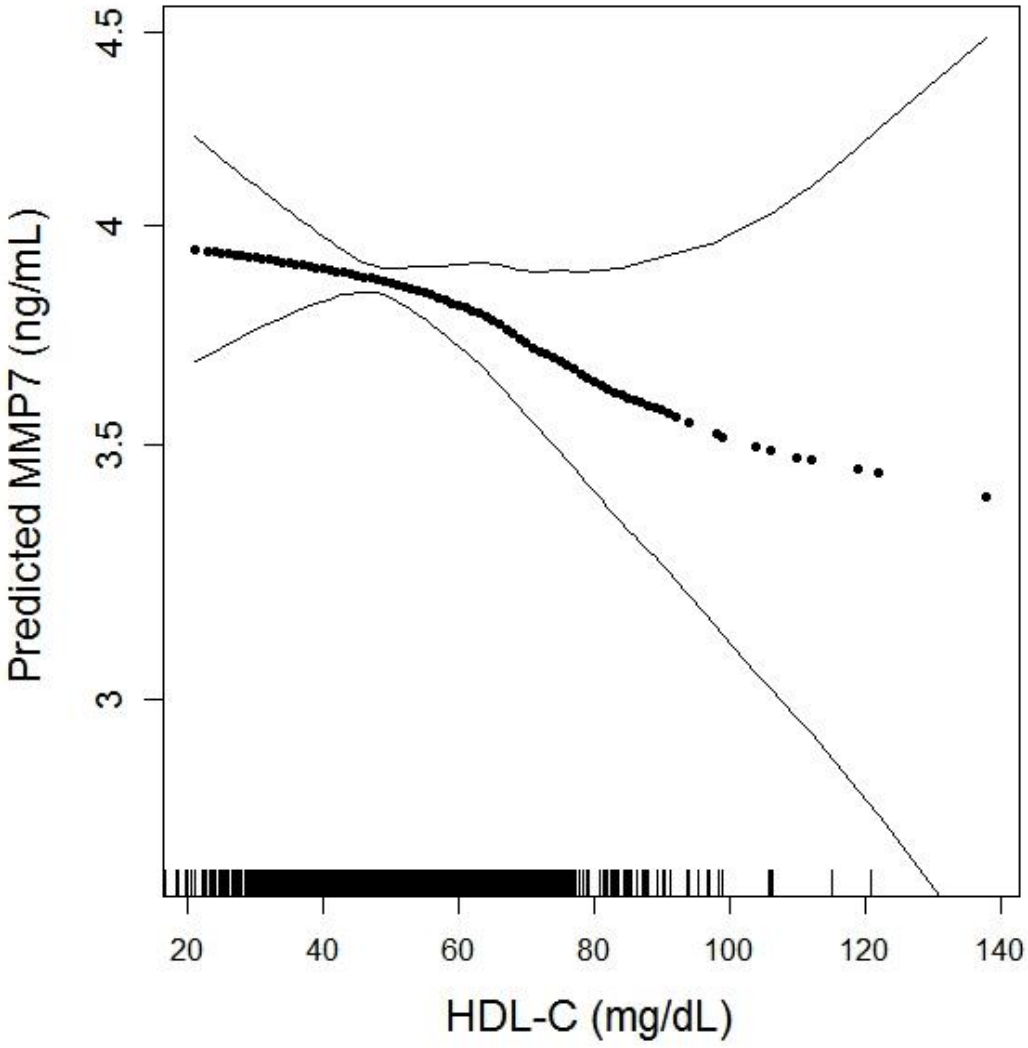


Supplementary Figure S2: Continuous association between (a) ApoA-1 and HAA (p for nonlinearity 0.003, p for association <0.001), (b) HDL-C and MMP-7 (p for nonlinearity 0.28, p for association 0.008), and (c) HDL-C and SP-A (p for nonlinearity 0.15, p for association 0.01). HAA model adjusted for age, gender, race/ethnicity, educational attainment, height, body mass index, waist circumference, smoking status, cigarette pack-years, presence of hypertension, presence of diabetes, low density lipoprotein, triglycerides, c-reactive protein, interleukin-6, glomerular filtration rate, statin use, diuretic use, coronary artery calcium, study site, milliAmpere dose, total volume imaged lung and percent emphysema on CT. MMP-7/SP-A models adjusted for age, gender, race/ethnicity, BMI, smoking status, cigarette pack-years, statin use, coronary artery calcium, low-density lipoprotein, triglycerides, CRP and IL-6. Dark dotted line is the continuous association. Thin solid lines are the 95% confidence bands. Each point in the graphs and each vertical hashmark in the rug plot along the x-axis represent one study participant.

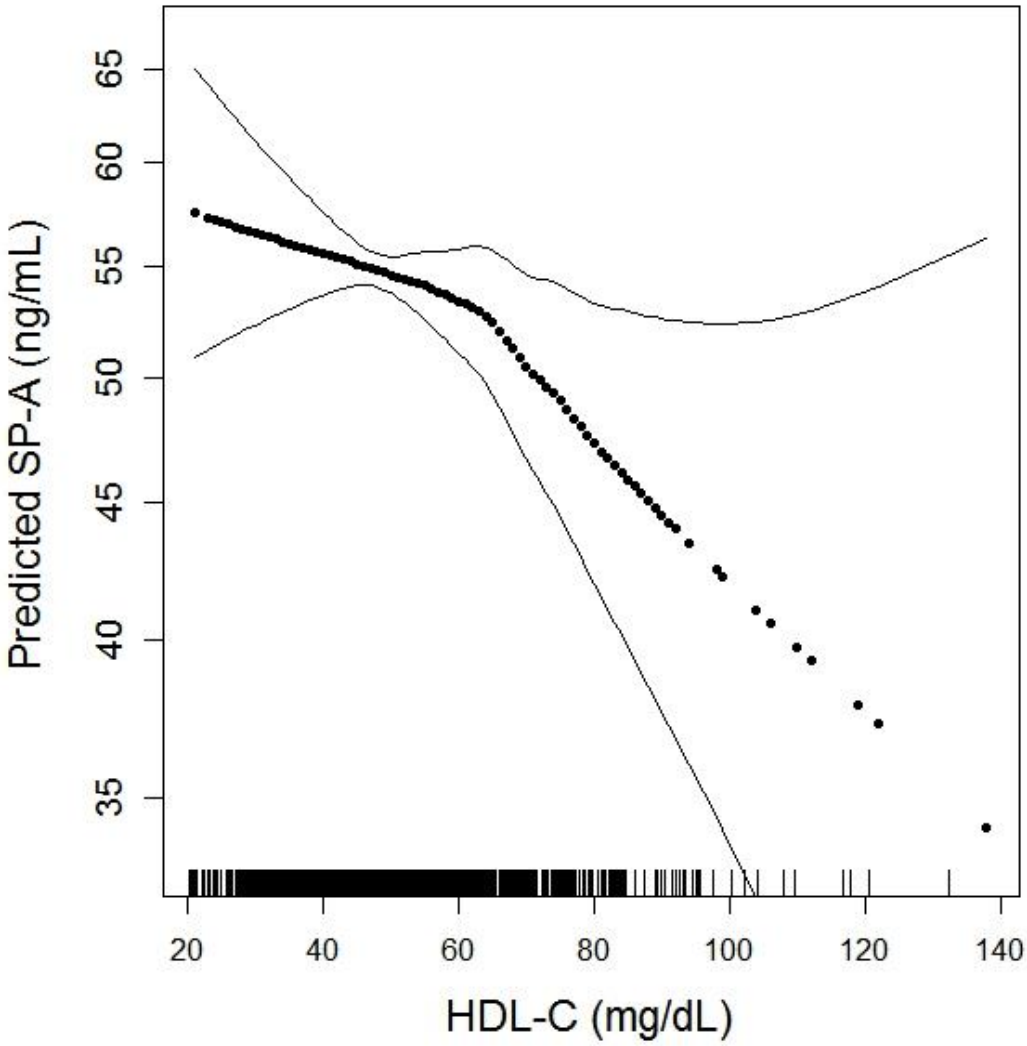
(a)


Predicted HAA (cm3)
110
115
120
125
130
0
50
100
150
200
250
Apolipoprotein A-1 (mg/dL)

(b)



(c)


Predicted SP-A (ng/mL)
35
40
45
50
55
60
65
20
40
60
80
100
120
140
HDL-C (mg/dL)

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