

HDL efflux capacity, HDL particle size, and high-risk carotid atherosclerosis in a cohort of asymptomatic older adults: the Chicago Healthy Aging Study

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Abstract HDL efflux capacity and HDL particle size are associated with atherosclerotic CVD (ASCVD) events in middle-aged individuals; however, it is unclear whether these associations are present in older adults. We sampled 402 Chicago Healthy Aging Study participants who underwent a dedicated carotid MRI assessment for lipid-rich necrotic core (LRNC) plaque. We measured HDL particle size, HDL particle number, and LDL particle number with NMR spectroscopy, as well as HDL efflux capacity. We quantified the associations between HDL particle size and HDL efflux using adjusted linear regression models. We quantified associations between the presence of LRNC and HDL and LDL particle number, HDL particle size, and HDL efflux capacity using adjusted logistic regression models. HDL efflux capacity was directly associated with large ($\beta = 0.037$, $P < 0.001$) and medium ($\beta = 0.0065$, $P = 0.002$) HDL particle concentration and inversely associated with small ($\beta = -0.0049$, $P = 0.018$) HDL particle concentration in multivariable adjusted models. HDL efflux capacity and HDL particle number were inversely associated with prevalent LRNC plaque in unadjusted models (odds ratio: 0.5; 95% confidence interval: 0.26, 0.96), but not after multivariable adjustment. HDL particle size was not associated with prevalent LRNC. HDL particle size was significantly associated with HDL efflux capacity, suggesting that differences in HDL efflux capacity may be due to structural differences in HDL particles. Future research is needed to determine whether HDL efflux is a marker of ASCVD risk in older populations.—Mutharasan, R. K., C. S. Thaxton, J. Berry, M. L. Daviglius, C. Yuan, J. Sun, C. Ayers, D. M. Lloyd-Jones, and J. T. Wilkins. **HDL efflux capacity, HDL particle size, and high-risk carotid atherosclerosis**

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Evidence from Mendelian randomization studies (1) and pharmacologic trials (2, 3) suggests that HDL cholesterol (HDL-C) concentration is not causally related to atherosclerosis. However, metrics of HDL structure and function, such as particle size (4), particle concentration (5), and cholesterol efflux capacity (6), may be causal mediators of atheroprotection in young and middle-aged people. Although associations between measures of HDL particle structure/function and atherosclerosis have been described in middle-aged people, the predictive value of these measures in older people, for whom atherosclerotic CVD (ASCVD) risks are highest, are not known. Furthermore, the structural differences in HDL particles that underlie differences in HDL function are not well-understood.

The exact protective effects of HDL efflux may be due to the removal of cholesterol from lipid-laden macrophages, which may lead to a reduction in plaque lipid content and inflammation (7). This flux of cholesterol out of the vascular intima could ultimately protect arteries from forming lipid-rich necrotic core (LRNC) plaque. In vivo detection of LRNC has been possible with clinical imaging modalities, including ultrasound, computed tomography, and MRI (8, 9). To date, the association between the cholesterol

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Abbreviations: ASCVD, atherosclerotic CVD; BP, blood pressure; CHA, Chicago Heart Association Detection Project in Industry; CHAS, Chicago Healthy Aging Study; CI, confidence interval; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; LR, low risk; LRNC, lipid-rich necrotic core; OR, odds ratio.

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efflux function of natural HDL particles and LRNC, as detected by *in vivo* imaging, has not been well-characterized.

Given the emerging data suggesting substantial and potentially clinically significant associations between HDL function and ASCVD risk in younger patients, we chose the richly phenotyped Chicago Healthy Aging Study (CHAS) (10) to investigate the associations between HDL efflux, HDL particle size and number, and the presence of carotid LRNC plaque as assessed by MRI. We hypothesized that HDL efflux would be positively associated with large HDL particle size and that HDL efflux, HDL particle size, and HDL particle number would be inversely associated with LRNC in the carotid arteries.

MATERIALS AND METHODS

CHAS cohort description

The design of the CHAS has been described elsewhere (10). Briefly, CHAS investigators examined 1,395 participants originally enrolled in the Chicago Heart Association Detection Project in Industry (CHA) cohort, whose cardiovascular risk profiles were determined from 1967 to 1973 when participants were 25–44 years old. Investigators recontacted surviving CHA participants for reexamination, selectively oversampling 421 participants who were “low risk” (LR) at baseline and 974 participants who were not LR. LR was defined as no history of diabetes mellitus, no history of myocardial infarction, no smoking, blood pressure (BP) <120/80 mmHg, no BP-lowering medications, total cholesterol <200 mg/dl, and no lipid-lowering medications. The reexamination included collection of participants’ health histories, CVD risk factor measurements, and blood samples. Of the 1,395 CHAS participants, 440 randomly selected individuals underwent MRI of their carotid and coronary arteries. Of these, 38 people were excluded for inadequate serum or MRI measurements, leaving 402 for this analysis. CHAS was reviewed and approved by the Northwestern IRB.

Exposures of interest

CVD risk factors were measured using standard methods. Demographics, smoking status, and current medications were obtained via participant self-report. Height, weight, and waist circumference were measured using protocols and equipment adapted from the MESA (11) and CARDIA (12) studies. Height and weight measurements were made with participants wearing light clothing and no shoes. Height was measured by standard stadiometer. After resting for 5 min BP was measured three times in the right arm with the participants seated. The reported BP is an average of the last two seated measurements (12). After a 10 h fast, approximately 40 ml of blood were drawn by peripheral venipuncture. Blood samples were analyzed for glucose, hs-CRP, and standard lipid fractions. The remaining serum was stored at –80°C.

Cholesterol efflux assay

Frozen serum aliquots from banked serum were gently thawed and apoB-depleted serum was prepared using the polyethylene glycol precipitation method. A total of 402 participants had banked serum in sufficient quantity (60 microl) to run the cholesterol efflux assay in technical triplicate. The cholesterol efflux assay used has been described previously (13, 14). Briefly, J774 mouse macrophages radiolabeled with ³H-cholesterol at 2 microCi/

ml and treated with 2 microg/ml ACAT inhibitor (Sandoz 58-035; Sigma-Aldrich, St. Louis, MO) and 30 microM of cAMP analog [8-(4-chlorophenylthio)adenosine 3',5'-cyclic monophosphate sodium salt; Sigma-Aldrich] were incubated with a 2.0% final concentration of apoB-depleted serum for 4 h. After 4 h, radioactivity in both the cell culture media and the cells was assessed and percent efflux was calculated as follows: (radioactivity in the medium after efflux – radioactivity in blank medium lacking serum)/total radioactivity in labeled cells. The percent efflux for technical triplicates was averaged together. To normalize across assay runs, a serum sample pool drawn from healthy volunteers was run with each sample. Normalized efflux was calculated as percent efflux for the sample divided by percent efflux for the sample pool. This normalized efflux value for each participant was then used in statistical analyses.

NMR spectroscopy

NMR spectroscopy was performed by LipoScience (Raleigh, NC) (15). This technique uses the NMR signature of serum to deconvolute concentrations of lipoprotein species of different sizes. NMR data analysis was performed by LipoScience to determine the concentration of HDL particles in participant serum, as well as mean HDL particle size. Further analysis was performed to determine concentration of small-sized, medium-sized, and large-sized HDL particles.

MRI protocol

A 1.5 Tesla cardiovascular MRI system (MAGNETOM Avanto; Siemens Medical Solutions, Erlangen, Germany) equipped with bilateral carotid coils (MACHNET) was used for all MRI studies. Fifteen slices (2 mm thickness) were obtained of the index artery, centered at the bifurcation. All black blood and bright blood sequences were acquired with a standard matrix size of 256 × 256, and with Fourier transform to create a 512 × 512 matrix. The field of view was 16 × 16 cm. MRI sequences were obtained with the parameters that follow: bright-blood gradient echo: time-of-flight: TR 32 ms, TE 10 ms, saturation band on veins; black-blood fast spin echo: T1-weighted: TR 780 ms, TE 11 ms, with blood and fat suppression; T2-weighted: TR 4000, TE 53 ms, with blood and fat suppression.

Image analysis

Images were transferred for review by the core laboratory using custom-designed image analysis software (Cascade; University of Washington, Seattle, WA) (16). All images were analyzed by trained readers who were blinded to clinical data. Plaque measurement reproducibility of MRI of the carotid artery analyzed by using Cascade has been reported previously (17–19). Each case was analyzed by two reviewers who arrived at a consensus opinion before recording the data. During image analysis, multiple weightings were registered using the carotid artery bifurcation as a landmark. LRNC was detected as hypointense areas within the carotid wall on T2-weighted images. Calcification is also hypointense on T2-weighted images but could be differentiated by using matched time-of-flight and T1-weighted images. This technique has been found to be accurate and reproducible when compared with histology sections obtained from the carotid artery of humans (20). All MRI readers were blinded to risk factor and HDL efflux status of participants. In a subset of 177 participants, both the right and left carotid artery were analyzed. Study procedures were approved by the Institutional Review Boards at Northwestern University and the University of Texas Southwestern Medical Center.

Statistical analysis

Descriptive statistics for demographics, CVD risk factors, NMR spectroscopy, efflux, and MRI measures of atherosclerosis were

generated using standard methods. Comparisons between groups were made using a Student's *t*-test or chi-squared test as appropriate. Spearman correlation coefficients were used to describe the correlation between NMR-derived HDL particle number and the concentrations of small, medium, and large HDL particles. Separate linear regression models were used to assess the associations between HDL particle number and size and HDL efflux capacity. Separate logistic regression models were used to assess the association between HDL efflux and the presence or absence of LRNC on carotid MRI. All models were adjusted as follows: model 1, unadjusted; model 2, age, sex, and race adjusted; model 3, model 2 plus additional adjustment for systolic BP, diabetes mellitus, smoking status, HDL-C concentration, LDL cholesterol (LDL-C) concentration, lipid-lowering medications, and BP-lowering medications. For models in which HDL particle number-related parameters or LDL particle number-related parameters were the exposure of interest, HDL-C or LDL-C, respectively, were removed from model 3 to prevent over-adjustment. The study had 87% power to detect a difference of 0.1 in normalized HDL efflux between participants with and without LRNC at an α level of 0.05. $P < 0.05$ was used to define significance. All analyses were run in SAS version 9.4 (SAS Institute, Cary, NC).

RESULTS

Baseline characteristics

Baseline characteristics of the 402 study participants, stratified by LRNC status, are presented in **Table 1**. Ninety-three (23%) of the 402 participants in our sample had LRNC in their carotid arteries. The mean age of the participants was 73 years and 65% were men. Individuals with LRNC tended to be men; have lower HDL-C; and have higher triglycerides, fasting glucose, LDL particle number, lower HDL particle number, and a lower concentration of large HDL particles. In individuals with LRNC, there was a trend toward lower mean HDL particle size and lower normalized HDL efflux capacity.

Correlations between NMR spectroscopy-derived parameters and efflux

Normalized efflux was significantly and positively correlated with both total HDL particle concentration (**Fig. 1A**) and mean HDL particle size (**Fig. 1B**). Concentrations of large- (8.8–13 nm) and medium-sized (8.2–8.8 nm) HDL particles were significantly and positively correlated with normalized efflux (**Fig. 1C, D**). Concentration of small-sized (7.3–8.2 nm) HDL particles was inversely correlated with normalized efflux capacity (**Fig. 1E**). After multivariable adjustment for demographics and traditional risk factors, HDL particle number ($\beta = 0.013$, $P < 0.001$) and a higher concentration of large- ($\beta = 0.037$, $P < 0.001$) and medium-sized particles ($\beta = 0.0065$, $P = 0.002$) were all positively associated with HDL efflux capacity. Higher concentrations of small HDL particles were inversely associated with HDL efflux capacity ($\beta = -0.0049$, $P = 0.018$) (**Table 2**).

HDL efflux capacity, particle number, and carotid LRNC

In unadjusted models, the highest quartile of LDL particle number was significantly associated with LRNC [odds ratio (OR): 2.3; 95% confidence interval (CI): 1.15, 4.65] and there were progressive increases in ORs with higher quartiles of LDL particle number (**Table 3**). This association was not statistically significant in the fully adjusted model controlling for traditional risk factors (model 3 OR = 2.62; 95% CI: 0.95, 7.23). The monotonic trend in OR increases with higher quartiles of LDL particle number is present in model 1 and model 2. Compared with the lowest quartile, the highest quartile of HDL efflux capacity was significantly and inversely associated with LRNC in unadjusted models (OR = 0.50; 95% CI: 0.26, 0.96), but not after adjustment for age, sex, race, and traditional risk factors (model 3 OR = 0.89; 95% CI: 0.39, 2.04). No clear dose-effect was seen for the association between HDL efflux capacity

TABLE 1. Cohort demographics and traditional risk factors by carotid LRNC status

	LRNC Absent (n = 309)	LRNC Present (n = 93)	P
Age (years)	73 (5.1)	73.2 (4.15)	0.49
Men (%)	62	78	0.001
Total cholesterol (mg/dl)	182.1 (33.6)	184.2 (39.5)	0.57
HDL-C (mg/dl)	59.0 (18.0)	52.5 (15.7)	<0.001
LDL-C (mg/dl)	109.1 (33.1)	110.2 (27.7)	0.35
Triglycerides (mg/dl)	101.5 (49.0)	118.6 (64.9)	0.046
Systolic BP (mmHg)	124.5 (14.2)	126.3 (16.0)	0.27
Diastolic BP (mmHg)	74.3 (9.6)	74.5 (9.2)	0.96
BMI (kg/m ²)	24.0 (3.4)	24.7 (3.4)	0.16
Glucose (mg/dl)	112.3 (28.0)	123.1 (35.8)	0.01
Smoking (%)	21	30	0.02
Hypertension medication use (%)	54	49	0.04
Cholesterol medication use (%)	56	59	0.7
Diabetes mellitus medication use (%)	10	11	0.8
LDL particle number (nmol/l)	1,044.1 (314.8)	1,107.3 (280.2)	0.02
HDL particle number (micromole/l)	36.3 (5.9)	34.9 (6.2)	0.02
HDL particle size (nm)	9.3 (0.54)	9.2 (0.50)	0.10
Large HDL particle concentration (micromole/l)	6.52 (3.8)	5.31 (3.25)	0.006
Medium HDL particle concentration (micromole/l)	9.81 (5.68)	9.12 (4.73)	0.49
Small HDL particle concentration (micromole/l)	19.97 (5.81)	20.51 (5.29)	0.45
Efflux capacity	1.19 (0.22)	1.15 (0.26)	0.07

Data restricted to all participants with scaled efflux measurements. SD in parentheses.

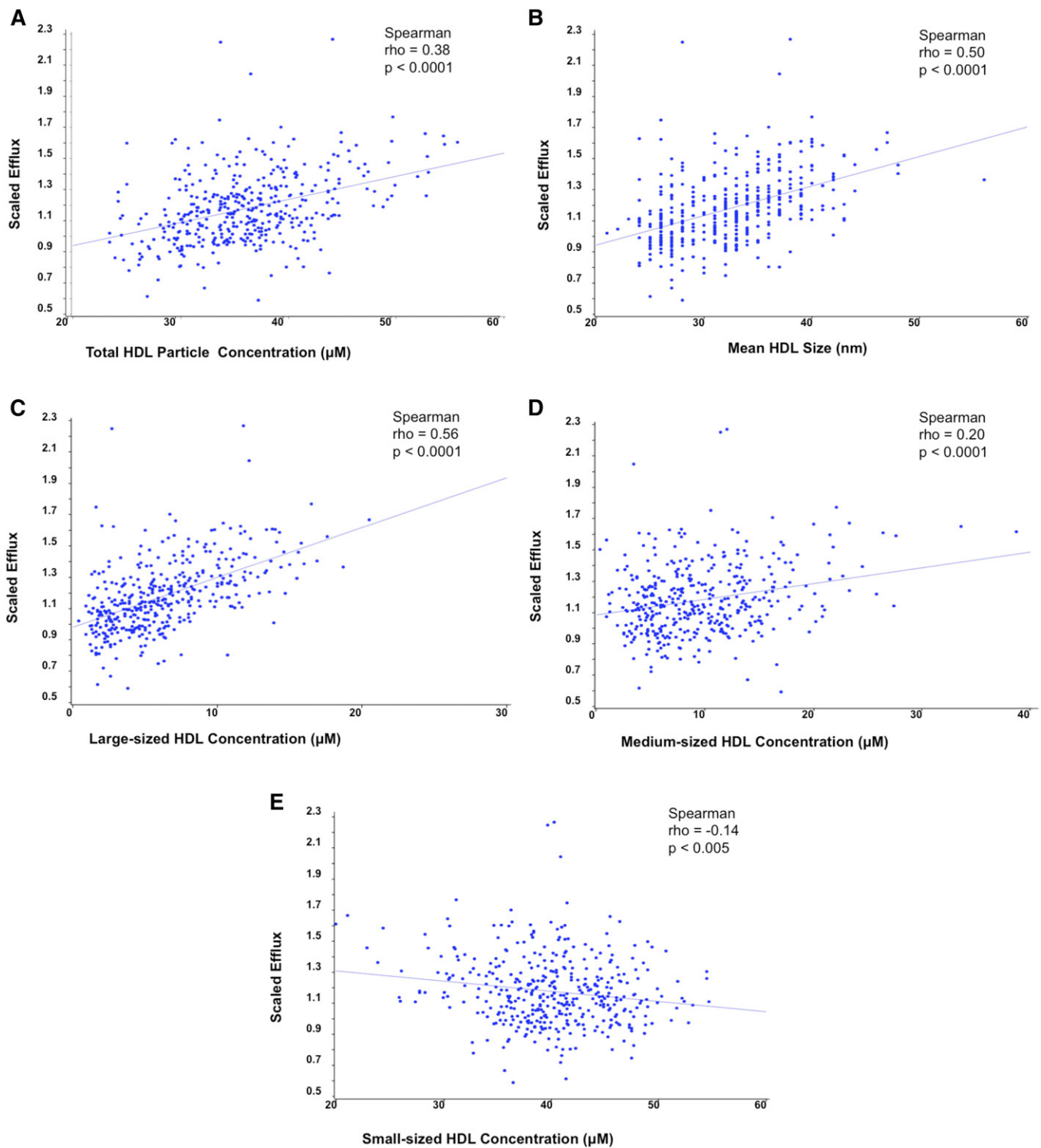


Fig. 1. Correlations between HDL efflux and total HDL particle concentration (A), mean HDL particle size (B), large HDL particle concentration (C), medium HDL particle concentration (D), and small HDL particle concentration (E).

and LRNC. HDL particle number was not significantly associated with LRNC.

DISCUSSION

In this sample of healthy older adults, HDL efflux capacity was positively associated with a higher concentration of

large HDL particles and inversely associated with small HDL particles. Compared with the lowest quartiles, the highest quartiles of efflux capacity and LDL particle number were strongly associated with LRNC in unadjusted models. However, these associations were attenuated and not statistically significant in multivariable-adjusted models.

In this study, we found significant correlations between cholesterol efflux capacity and HDL particle number and

TABLE 2. Associations between HDL particle characteristics and normalized HDL efflux capacity

	Model 1	Model 2	Model 3 ^a
HDL particle number (micromole/l)	0.01485 (0.00173, <i>P</i> < 0.01)	0.01214 (0.00187, <i>P</i> < 0.001)	0.01339 (0.0019, <i>P</i> < 0.001)
Large HDL particle concentration (micromole/l)	0.03187 (0.00257, <i>P</i> < 0.001)	0.02900 (0.00281, <i>P</i> < 0.001)	0.037 (0.003, <i>P</i> < 0.001)
Medium HDL particle concentration (micromole/l)	0.01000 (0.00199, <i>P</i> < 0.001)	0.00671 (0.00204, <i>P</i> = 0.001)	0.00646 (0.0021, <i>P</i> = 0.002)
Small HDL particle concentration (micromole/l)	-0.00659 (0.00194, <i>P</i> = 0.001)	-0.00484 (0.00188, <i>P</i> = 0.011)	-0.00492 (0.0021, <i>P</i> = 0.018)

Data expressed as beta coefficients (SE, *P* value)

^aModel 3 does not include HDL-C.

size. We further observed that while medium and large HDL particles are positively correlated with efflux capacity, small HDL particles are inversely correlated with cholesterol efflux capacity. These associations may imply that the mechanism of cholesterol efflux capacity is driven by mass effect, wherein the critical parameter is not HDL-C, but, in fact, the number of HDL particles of a particular size phenotype. Associations between HDL size and efflux capacity have been reported previously, albeit in different contexts. El Khoudary et al. (21) evaluated changes in cholesterol efflux capacity and HDL particle size as assessed by electrophoretic mobility in pre- and postmenopausal women. Similar to our findings, a strong positive correlation between cholesterol efflux capacity and concentration of large and medium HDL particles was seen, whereas no correlation was seen between the concentration of small HDL particles and cholesterol efflux capacity. Rohatgi et al. (6) noted a linear positive correlation of both particle concentration and particle size with cholesterol efflux capacity in the Dallas Heart Study, a cohort of younger adults. From a mechanistic perspective, Du et al. (22) evaluated HDL efflux in vitro to various HDL acceptors, including HDL2 and HDL3 subfractions isolated from human plasma as well as reconstituted HDL of varying diameters. The investigators found that with both human HDL subfractions and reconstituted HDL, smaller acceptors mediated more efflux (22). Taken together, our findings and the findings of others support the notion that small HDL particles are excellent mediators of cholesterol efflux and, furthermore, that the presence of high concentrations of small HDL particles in the serum may mark a block in maturation of small HDL particles.

The measurement of subfractions of HDLs is complex and their associations with HDL function and risk are incompletely understood. Furthermore, how subfractions

obtained by one analytical technique (such as NMR) compare with those of another (such as gradient gel electrophoresis or density gradient ultracentrifugation) is not precisely defined (23). Nevertheless, others have observed through at least two different analytical techniques that pre β HDL, an HDL fraction presumably contained in the small HDL fraction by NMR, is positively correlated with the presence of coronary heart disease (22, 24–27). Our data may suggest a link between HDL particle concentration and efflux capacity and support a possible block in maturation of HDL particles in individuals with lower HDL efflux capacity.

Previous studies have observed associations between HDL efflux and carotid atherosclerosis (28, 29). One study in particular, by Doonan et al. (28), demonstrated a strong inverse association between efflux and ultrasound-measured atheromatous plaque. We did not observe a significant association between HDL efflux and a MRI-based measure of high risk atherosclerosis, LRNC, in adjusted models. This seeming discordance in our findings may be explained by the use of a clinical and partially symptomatic sample by Doonan et al. (28), and different techniques (MRI vs. ultrasound) to identify high risk plaque. We contend that our findings add to our understanding of the role of HDL efflux in the maturation and development of high-risk atherosclerosis across the life course. Of note, this is the first study to quantify the associations between novel markers of HDL structure and function in exclusively older (>70 years) adults; previous studies assessing these associations have included middle-aged participants from either hospital- or community-based samples. We believe that age-specific findings may provide key insights into the importance of HDL efflux to cardiovascular risk. For example, HDL efflux may be a stronger determinant of atherosclerosis initiation than it is of maturation into high-risk plaque. Thus,


TABLE 3. Associations between HDL efflux capacity, HDL particle number, LDL particle number, and prevalent LRNC

	Quartile 1 (Referent)	Quartile 2	Quartile 3	Quartile 4	<i>P</i> for Trend
HDL efflux capacity					
Model 1	1.0	0.46 (0.24, 0.89)	0.66 (0.36, 1.23)	0.50 (0.26, 0.96)	0.087
Model 2	1.0	0.49 (0.25, 0.95)	0.76 (0.40, 1.43)	0.67 (0.34, 1.34)	0.415
Model 3	1.0	0.52 (0.26, 1.04)	0.99 (0.43, 1.99)	0.89 (0.39, 2.04)	0.87
HDL particle number					
Model 1	1.0	0.75 (0.41, 1.39)	0.41 (0.21, 0.81)	0.53 (0.27, 1.02)	0.012
Model 2	1.0	0.82 (0.44, 1.54)	0.48 (0.24, 0.97)	0.73 (0.36, 1.49)	0.134
Model 3	1.0	0.86 (0.43, 1.70)	0.54 (0.25, 1.20)	0.89 (0.36, 2.20)	0.439
LDL particle number					
Model 1	1.0	1.69 (0.82, 3.48)	1.86 (0.92, 3.80)	2.32 (1.15, 4.65)	0.028
Model 2	1.0	1.80 (0.87, 3.73)	1.92 (0.94, 3.95)	2.49 (1.22, 5.06)	0.02
Model 3	1.0	2.16 (0.98, 4.77)	2.27 (0.93, 5.52)	2.62 (0.95, 7.23)	0.14

when efflux is studied in younger populations, it has more easily detectable associations with subclinical atherosclerosis and events than are seen in this older sample (29). However, we were unable to test this hypothesis directly with the current dataset, as CHAS is composed exclusively of older participants (mean age 71, SD 5 years); thus, no younger age group was available for comparison. Similar attenuation of ASCVD risk associated with total cholesterol and HDL-C levels in people >70 years old has been reported as well. Thus, age-specific risks for atherosclerosis may not be unique to HDL efflux capacity. Furthermore, if efflux is a stronger risk determinant in younger adults, studies such as ours may be particularly susceptible to attenuation of effect by survivor bias. Thus, findings in the literature that greater age is associated with higher efflux may be due to age-dependent changes in efflux or survivor bias, as individuals with low efflux may have died at younger ages or have been too sick to participate in community-based studies.

The association between hypercholesterolemia and LRNC was also seen in previous epidemiological studies (30, 31). Our finding of a dose-dependent pattern of LDL particle number and LRNC extends previous observations by demonstrating the importance of LDL particle number. Notably, the association was seen in the context of normal cholesterol-emia, as the average LDL-C level was about 110 mg/dl. Therefore, LDL particle number may be of prognostic value in subjects with normal LDL-C level, which has been observed in other cohort studies as well (32). The relatively normal cholesterol level may be another reason to account for the weak association between HDL-C efflux and LRNC.

This study should be viewed in the context of its limitations. First, this analysis is cross-sectional, thus the direction of causality cannot be determined. Second, LRNC plaque is a surrogate and understudied marker of overall atherosclerotic burden and vulnerability to ASCVD events. If efflux triggers plaque rupture, it could be associated with ASCVD events (including stroke) and not LRNC in older populations. Finally, the limited sample size prevented a more rigorous exploration of potential confounding factors, as evidenced by the wide CIs present in some of our fully adjusted analyses.

In summary, this study demonstrates, for the first time, correlations between NMR-derived HDL particle size concentrations and cholesterol efflux. Though LRNC was found in 23% of this sample of otherwise healthy older adults, HDL-C efflux was not independently associated with this marker of high-risk carotid atherosclerosis. It is possible that LRNC, once formed, is less amenable to the protective effect of HDL-C efflux, as histopathologic evaluation of LRNC suggests little cellular activity in the core of these plaques that are full of cell debris and cholesterol clefts. Future research is needed to determine whether HDL particle structural and functional characteristics are determinants of ASCVD risk in older populations. 

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