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Cholesterol-Overloaded HDL Particles Are Independently Associated With Progression of Carotid Atherosclerosis in a Cardiovascular Disease-Free Population

A Community-Based Cohort Study

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ABSTRACT

BACKGROUND Cholesterol-overloaded high-density lipoprotein (HDL) particles exert a negative impact on the antiatherogenic function of HDL in experimental studies. However, it remains unclear whether cholesterol-overloaded HDL particle is involved in the development of atherosclerosis in humans.

OBJECTIVES The objective of this study was to explore whether cholesterol-overloaded HDL particles are associated with the progression of carotid atherosclerosis in a cardiovascular disease-free population.

METHODS Baseline HDL particle number was measured using nuclear magnetic resonance spectroscopy in 930 participants ages 45 to 74 years in a community-based cohort study. An estimate of cholesterol molecules per HDL particle (HDL-C/P ratio) was calculated as the ratio of HDL cholesterol to HDL particles. HDL-C/P ratio was categorized as $<$41.0 (lowest), 41.0 to 46.9, 47.0 to 52.9, and $\geq$53.0 (highest) using a fixed increment method. Modified Poisson regression was used to assess the association between HDL-C/P ratio and 5-year progression of carotid atherosclerosis as indicated by progression of carotid plaques and change in total plaque area (TPA).

RESULTS Mean baseline HDL-C/P ratio was 46.4 $\pm$ 9.3 (range 23.8 to 86.9). Baseline HDL-C/P ratio was significantly associated with 5-year progression of carotid atherosclerosis. Participants with the highest HDL-C/P ratio had 1.56-fold (95% confidence interval: 1.14 to 2.13; $p = 0.006$) increased progression compared with those with the lowest level. Among participants without baseline plaque, TPA in re-examination was larger by 9.4 mm^2 in the subgroup with the highest level when compared with the lowest level.

CONCLUSIONS Our findings suggest that cholesterol-overloaded HDL particles are independently associated with the progression of carotid atherosclerosis. This may explain why in recent trials raising HDL cholesterol was not beneficial. This study strongly suggests that the combination of cholesterol content and particle number determines the antiatherogenic function of HDLs, rather than either parameter alone. (J Am Coll Cardiol 2015;65:355-63) © 2015 by the American College of Cardiology Foundation.

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ABBREVIATIONS AND ACRONYMS

ASCVD = atherosclerotic cardiovascular disease

BMI = body mass index

BP = blood pressure

CETP = cholesteryl ester transfer protein

CI = confidence interval

CVD = cardiovascular disease

FBG = fasting blood glucose

HDL-C = high-density lipoprotein cholesterol

HDL-C/P ratio = the ratio of HDL-C to HDL-P number

HDL-P = high-density lipoprotein particles

hs-CRP = high-sensitivity C-reactive protein

LDL-P = low-density lipoprotein particle

NMR = nuclear magnetic resonance

RR = relative risk

TPA = total plaque area of maximum plaques

Although observational studies have suggested high-density lipoprotein cholesterol (HDL-C) as an independent inverse predictor for atherosclerotic cardiovascular diseases (ASCVDs) (1), several large randomized controlled trials in which plasma HDL-C was raised failed to show benefit for cardiovascular disease (CVD) events or progression of atherosclerosis (2,3). Recent updated American Heart Association/American College of Cardiology and European Society of Cardiology guidelines do not recommend raising HDL-C as a means to prevent ASCVD (4).

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However, debate regarding the value of HDL-C in CVD prevention continues (5–7). A core question is whether HDL-C fully represents cholesterol reverse transport, an underpinning of the antiatherogenic function of HDL. Experimental studies point out that the concentration of HDL particles (HDL-P), rather than the cholesterol carried by these particles, might be the appropriate parameter for assessing the function of HDL (8,9). Several observational studies have proven HDL-P number to be more strongly associated with CVD risk than HDL-C (10,11). Indeed, cholesteryl ester transfer protein (CETP) inhibitor or niacin was found in recent randomized controlled trials to have little effect on HDL-P number. This was true even if HDL-C was substantially increased, thus resulting in increased cholesterol-overloaded HDL-P (5,12). There is evidence that cholesterol-overloaded HDL-P may be harmful because experimental studies observed that cholesterol-overloaded HDL-P not only exerted a negative impact on the efflux potential of cholesterol from extrahepatic cells (7,13) but also reduced hepatic selective uptake of cholesterol mediated by scavenger receptor class B member 1 (SR-BI) (14–16). However, it remains unclear whether cholesterol-overloaded HDL-P is involved in the development of atherosclerosis in humans.

In this study, we calculated the ratio of HDL-C to HDL-P number (HDL-C/P ratio) to estimate the cholesterol content per HDL-P in a CVD-free population. Specifically, a high HDL-C/P ratio indicates cholesterol-overloaded HDL-P. We therefore tested the hypothesis that cholesterol-overloaded HDL-P may be associated with the progression of carotid atherosclerosis in asymptomatic individuals from a community-based cohort study of the Chinese Multiprovincial Cohort Study (CMCS)-Beijing Project.

MATERIALS AND METHODS

STUDY PARTICIPANTS. Study participants were recruited from the CMCS-Beijing Project, which is a generally healthy population-based study (17). Of 1,982 participants originally enrolled from a community in Beijing in 1992, a total of 1,324 participants ages 45 to 74 years completed examinations on demographic characteristics, measurements of traditional risk factors, and carotid ultrasound from September to November 2002. After excluding participants with established CVD (n = 68), unavailable blood samples (n = 7), and incomplete data (n = 14) at baseline, 1,235 participants were followed up for the occurrence of CVD and then invited to repeat examinations for risk factors and carotid ultrasound in 2007. Sixty-two participants were lost to follow-up, 15 died, and 228 did not participate at re-examinations. Thus, 930 (418 men and 512 women) participants with complete data from 2 examinations were eligible for final analysis.

All participants gave written informed consent, and this study was approved by the ethics committee of Beijing An Zhen Hospital, Capital Medical University, and was performed in accordance with the Declaration of Helsinki.

RISK FACTOR SURVEY. This study complied with the protocol of the World Health Organization-MONICA (Monitoring of Trends and Determinants in Cardiovascular Disease). Demographic characteristics and personal medical history were collected by a standard questionnaire. Anthropometric measurements and blood pressure (BP) levels were recorded during physical examination. Body mass index (BMI) was calculated as weight in kilograms divided by height squared in meters. The survey method and definition of risk factors, including hypertension, diabetes, and current smoking, were previously described (17).

LABORATORY ASSAYS. Venous blood samples were drawn from the antecubital vein and collected in lavender-top tube(s) containing EDTA anticoagulant in the morning after fasting at least 8 h. Lipid profiles, fasting blood glucose (FBG), and high-sensitivity C-reactive protein (hs-CRP) were measured on fresh samples at the same day of collection in 2002 according to previous reports (17). HDL-C levels were measured by homogeneous assays (Daiichi, Tokyo, Japan). The remaining samples were aliquoted and stored at −80°C. Plasma lipoprotein particle numbers were measured in 2013 using a commercially available nuclear magnetic resonance (NMR) spectroscopy assay at LipoScience (Raleigh, North Carolina). Briefly, samples for lipoprotein particle analysis using

proton NMR spectroscopy were thawed, separated into 500-μl aliquots, refrozen, and shipped on dry ice to LipoScience. This method uses the characteristic signals broadcast by lipoprotein subfractions of different size as the basis for quantification.

CAROTID ATHEROSCLEROSIS MEASUREMENTS. Carotid atherosclerosis was measured using high-resolution B-mode ultrasound. The method and the measurement validation are detailed in the Online Appendix. Briefly, the presence of plaque was defined as an intima-media thickness ≥1.5 mm, or a focal structure that encroached into the arterial lumen ≥0.5 mm, or ≥50% of the surrounding intima-media thickness (18). Progression of carotid plaque was defined as the appearance of at least 1 plaque at re-examination in a previously plaque-free arterial segment. Additionally, Vascular Research Tools 6 carotid analyzer (Medical Imaging Applications, Coralville, Iowa) was used to measure the total plaque area of maximum plaques (TPA), which can evaluate the progression of carotid atherosclerosis among participants with new-onset plaque who had no baseline plaque.

STUDY POWER ESTIMATION. No studies have yet investigated the association between baseline HDL-P number and carotid atherosclerosis progression. Based on a recent meta-analysis (19), risk estimates between baseline HDL-P number and CVD risk ranged from 0.62 to 0.82. In this study, the 5-year progression rate of carotid plaque was 52.9%. The estimated sample size was 208 if risk estimate was 0.62, and 904 if risk estimate was 0.82, assuming an alpha (probability of type I error) of 0.05 and delta (admissible error) of 0.10. The actual sample size of 930 in this study enabled us to have sufficient statistical power.

STATISTICAL ANALYSIS. Continuous variables, expressed either as mean ± SD in case of normally distributed variables or as median (interquartile ranges), were compared using unpaired Student *t* test or Mann-Whitney *U* test or 1-way analysis of variance, where appropriate. Categorical variables, expressed as number (percent), were compared using the chi-square test. Correlations were estimated using Spearman rank method while adjusting for age and sex.

We calculated proportions within subgroups of HDL-C level among subgroups of HDL-P numbers. HDL-C and HDL-P number were categorized using the fixed-increment method. For HDL-C, the cutoff point of the lowest level was defined by the criterion of low HDL-C (<1.04 mmol/l). A fixed increment of 0.25 mmol/l was then used and study participants classified into 3 groups (<1.04, 1.04 to 1.29, ≥1.30 mmol/l). For HDL-P number, the cutoff point of the lowest level was set at 28.0, which is the cutoff point of the lowest tertile. A fixed increment of 4 μmol/l was used and study participants classified into 3 groups (<28.0, 28.0 to 31.9, ≥32.0 μmol/l). An estimate of cholesterol molecules per HDL-P (HDL-C/P ratio) can be obtained by calculating the ratio of HDL-C to HDL-P numbers. The units of HDL-C were transformed to μmol/l from the original mmol/l unit when calculating the HDL-C/P ratio. HDL-C/P ratio was categorized into 4 groups (<41.0, 41.0 to 46.9, 47.0 to 52.9, ≥53.0). The cutoff point of the lowest level was set at 41.0, which is the cutoff point of the lowest quartile. The increment value was 6.

Relative risk (RR) for 5-year progression of carotid plaque within combined subgroups of HDL-C, and HDL-P number was calculated using a modified Poisson regression model while adjusting for known CVD risk factors (age, sex, BMI, systolic BP, diabetes, current smoking, and triglycerides), low-density lipoprotein particle (LDL-P) numbers, HDL-P size, and 5-year changes in systolic BP, total cholesterol, HDL-C, and FBG. Risk estimates were calculated in each combined subgroup using the subgroup with both highest level of HDL-C and HDL-P number as a reference.

Furthermore, associations between HDL-C/P ratio and the risk for 5-year progression of carotid plaque were analyzed to evaluate the impact of the cholesterol amount per HDL particle after adjusting for HDL-C and HDL-P number. Risk estimates were then calculated for participants in each subgroup of HDL-C/P ratio, HDL-C and HDL-P number using the lowest category as references, respectively. Additionally, association between HDL-C/P ratio and changes in TPA was analyzed using multiple linear regression models among those with new-onset carotid plaque who had no baseline plaque in all segments of carotid artery. Additional adjustment was performed for baseline LDL-C, hs-CRP levels, and lipid-lowering medication.

To test whether missing data would yield potential bias, comparisons were performed between 930 participants who were eligible in final analyses, lost to follow-up, and unavailable for re-examinations, and no significant differences were observed (Online Table 1).

Statistical analyses were performed using SAS software (version 9.3, SAS Institute, Cary, North Carolina). Values of $p < 0.05$ were considered statistically significant. Sample size estimation was calculated using PASS software (version 8.0, NCSS, Kaysville, Utah).

RESULTS

BASELINE CHARACTERISTICS OF THE STUDY PARTICIPANTS. A total of 930 participants (mean

age, 59.6 ± 7.8 years; 55.1% women) were enrolled, and their baseline characteristics are shown in Table 1. Mean baseline HDL-C level and HDL-P number were 1.40 ± 0.31 (range 0.62 to 2.93) mmol/l and 30.3 ± 4.6 (range 18.3 to 53.6) μmol/l, respectively. The prevalence of carotid plaque was 19.5% at baseline.

HDL-C, HDL-P, AND HDL-C/P RATIO. HDL-C and HDL-P number were positively correlated (partial r = 0.378) (Online Table 2). HDL-C and HDL-P number were categorized and combined into 9 groups (Figure 1). Participants in the highest category of HDL-P number included 95.0% of participants with HDL-C level ≥1.04 mmol/l, and 47.0% of participants with HDL-C level ≥1.55 mmol/l. However, participants with the lowest category of HDL-P number included 80.9% of participants with HDL-C level ≥1.04 mmol/l and 16.3% of participants with HDL-C level ≥1.55 mmol/l. Moreover, the levels of large HDL-P number (median [interquartile range]: 8.6 [6.9 to 9.4] μmol/l) and HDL-P size (9.9 ± 0.3 nm) were the highest in the subgroup of the lowest HDL-P number and the highest HDL-C level.

An estimation of cholesterol molecules per HDL-P (HDL-C/P ratio) was calculated. Mean HDL-C/P ratio was 46.4 ± 9.3 (range 23.8 to 86.9) (Online Figure 1). HDL-C/P ratio was positively correlated with HDL-C (partial r = 0.755), but negatively correlated with HDL-P number (partial r = −0.300) (Online Table 2).

TABLE 1 Baseline Characteristics of the Study Participants

	Total (N = 930)	Men (n = 418)	Women (n = 512)
Age, yrs	59.6 ± 7.8	61.1 ± 7.4	58.3 ± 8.0
Systolic blood pressure, mm Hg	129.6 ± 18.2	132.3 ± 17.6	127.5 ± 18.5
Diastolic blood pressure, mm Hg	80.8 ± 10.1	83.2 ± 10.2	78.8 ± 9.5
Body mass index, kg/m^2	25.0 ± 3.3	25.1 ± 2.9	24.9 ± 3.5
Fasting blood glucose, mmol/l	4.90 ± 0.99	4.85 ± 1.04	4.95 ± 0.93
Hs-CRP, mg/l	0.84 (0.38-1.77)	0.80 (0.40-1.63)	0.88 (0.37-1.92)
Obesity	161 (17.3)	59 (14.1)	102 (19.9)
Current smoking	89 (9.6)	88 (21.1)	1 (0.2)
Hypertension	445 (47.8)	227 (54.3)	218 (42.6)
Diabetes	99 (10.6)	50 (12.0)	49 (9.6)
Lipid-lowering treatment	110 (11.8)	44 (10.5)	66 (12.9)
Prevalence of carotid plaque	181 (19.5)	100 (23.9)	81 (15.8)
Baseline maximal IMT, mm	0.90 (0.70-1.20)	1.00 (0.80-1.40)	0.90 (0.70-1.10)
Standard chemical lipids			
Total cholesterol, mmol/l	5.57 ± 1.02	5.39 ± 0.96	5.72 ± 1.04
LDL cholesterol, mmol/l	3.35 ± 0.83	3.30 ± 0.81	3.40 ± 0.85
HDL cholesterol, mmol/l	1.40 ± 0.31	1.29 ± 0.26	1.78 ± 0.32
Triglycerides, mmol/l	1.29 (0.94-1.86)	1.27 (0.96-1.83)	1.29 (0.92-1.88)
NMR lipoprotein particle number			
HDL particle number, μmol/l			
Total	30.3 ± 4.6	28.7 ± 4.1	31.6 ± 4.7
Large	4.5 (3.1-6.4)	3.5 (2.4-5.1)	5.3 (3.9-7.0)
Medium	5.3 (3.5-7.6)	4.9 (3.3-6.6)	6.0 (3.8-8.2)
Small	19.6 (17.3-21.7)	19.6 (17.5-21.7)	19.7 (17.0-22.0)
LDL particle number, nmol/l			
Total	1,088.0 ± 272.2	1,075.4 ± 262.8	1,098.1 ± 278.7
Large	253.5 (129.7-369.0)	258.0 (126.0-371.0)	249.0 (132.0-367.0)
Small	585.5 (411.7-807.0)	591.0 (438.0-795.0)	576.0 (394.0-818.0)
NMR lipoprotein particle size, nm			
HDL	9.1 ± 0.4	8.9 ± 0.4	9.2 ± 0.4
LDL	20.6 ± 0.6	20.5 ± 0.6	20.6 ± 0.6

Values are mean ± SD, median (interquartile range), or n (%). Obesity was defined by BMI ≥28 kg/m^2. Smoking was defined as smoking 1 or more cigarettes per day for 3 months. Hypertension was defined as blood pressure <140 mm Hg systolic, <90 mm Hg diastolic, and/or current antihypertensive treatment. Diabetes mellitus was defined by fasting plasma glucose ≥7.0 mmol/l or currently on glucose-lowering medical treatment.

HDL = high-density lipoprotein; Hs-CRP = high-sensitivity C-reactive protein; IMT = intima-media thickness; LDL = low-density lipoprotein; NMR = nuclear magnetic resonance.

ASSOCIATION BETWEEN HDL-C/P RATIO AND THE PROGRESSION OF CAROTID ATHEROSCLEROSIS. Of 930 participants, 52.9% developed new-onset plaque during 5-year follow-up. The RRs for progression within combined subgroups of HDL-C and HDL-P number were calculated (Central Illustration). In any category of the HDL-C subgroup, the progression risk increased with a decrease in HDL-P numbers, despite being significant only at the highest level of HDL-C. By contrast, within HDL-P subgroups, HDL-C was generally not inversely associated with the progression risk. At the highest level of HDL-P number, the risk increased with a decrease in HDL-C level. However, at the lowest level of HDL-P number, the risk increased with an increase in HDL-C level. Specifically, participants who simultaneously had both the highest HDL-C and the lowest HDL-P number had higher progression risk than those who simultaneously had both the highest HDL-C and highest HDL-P number (RR: 1.55, 95% confidence interval [CI]: 1.05 to 1.92; p = 0.006). Although the combined subgroup of HDL-C and HDL-P number *qualitatively* reflected the unmatched phenomena of HDL-C and HDL-P number and possible association with atherosclerosis, it was not a *quantitative* indicator for the mean level of cholesterol per HDL particle for each study participants.

We further explored the association of cholesterol molecules per particle (indicated by HDL-C/P ratio) with 5-year progression of carotid plaque. Modified Poisson regression analysis showed that the RR for progression at the highest level of HDL-C/P ratio was 1.56 (95% CI: 1.14 to 2.13; p = 0.006) (Table 2) compared with the lowest level while adjusting for HDL-C and HDL-P number. The trend test indicated that with increasing HDL-C/P ratio, the progression risk was significantly increased (p for trend = 0.006). Additionally, baseline HDL-C was significantly and

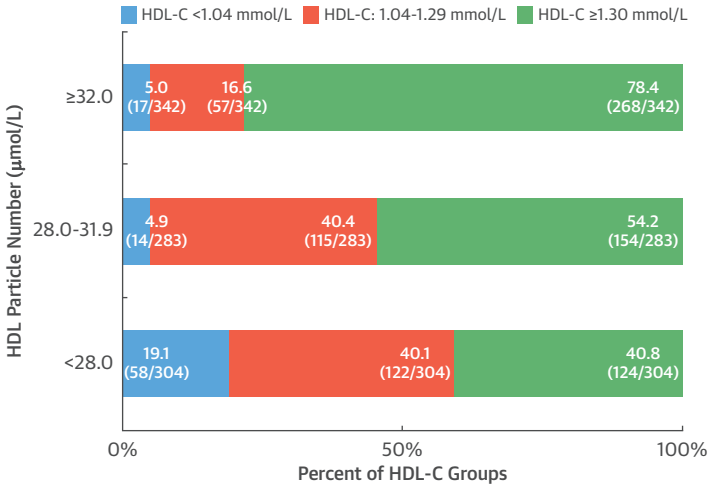
negatively associated with 5-year progression of carotid plaque after adjusting for HDL-C/P ratio. RRs for carotid plaque progression at the highest level of HDL-C compared with the lowest level was 0.74 (95% CI: 0.61 to 0.94; p = 0.03).

Of 749 participants who had no baseline plaque in all segments of carotid artery, 48.7% had incidence of new-onset plaque. We investigated the association between baseline HDL-C/P ratio and incidence of new-onset plaque, and found the same relationship (**Table 2**).

Among participants without baseline plaque, mean TPA was 27.0 ± 20.4 and a median 21.6 mm^2 in re-examination. Online Table 3 showed multiple adjusted TPA according to the subgroups of baseline HDL measures. Baseline HDL-C/P ratio was significantly associated with the TPA. In participants without baseline plaque, the TPA in re-examination was larger by 9.4 mm^2 in the subgroup with the highest level of HDL-C/P ratio than the lowest level. However, baseline HDL-C was significantly and negatively associated with the TPA, even after adjusting for HDL-C/P ratio. In participants without baseline plaque, the TPA in re-examination was larger by 7.8 mm^2 in the subgroup with the lowest level of HDL-C than the highest level.

Further adjustment for baseline LDL-C, hs-CRP levels, and lipid-lowering medication had no effect on risk estimates for HDL-C/P ratio (Online Table 4).

FIGURE 1 Percentage of HDL-C Subgroups Among Subgroups of HDL-P Numbers



HDL-C level was categorized as <1.04, 1.04 to 1.29, ≥1.30 mmol/l, and HDL-P number as <28.0, 28.0 to 31.9, ≥32.0 μmol/l. Results are presented as percentages (subgroup number/total number). HDL = high-density lipoprotein; HDL-C = high-density lipoprotein cholesterol; HDL-P = high-density lipoprotein particle.

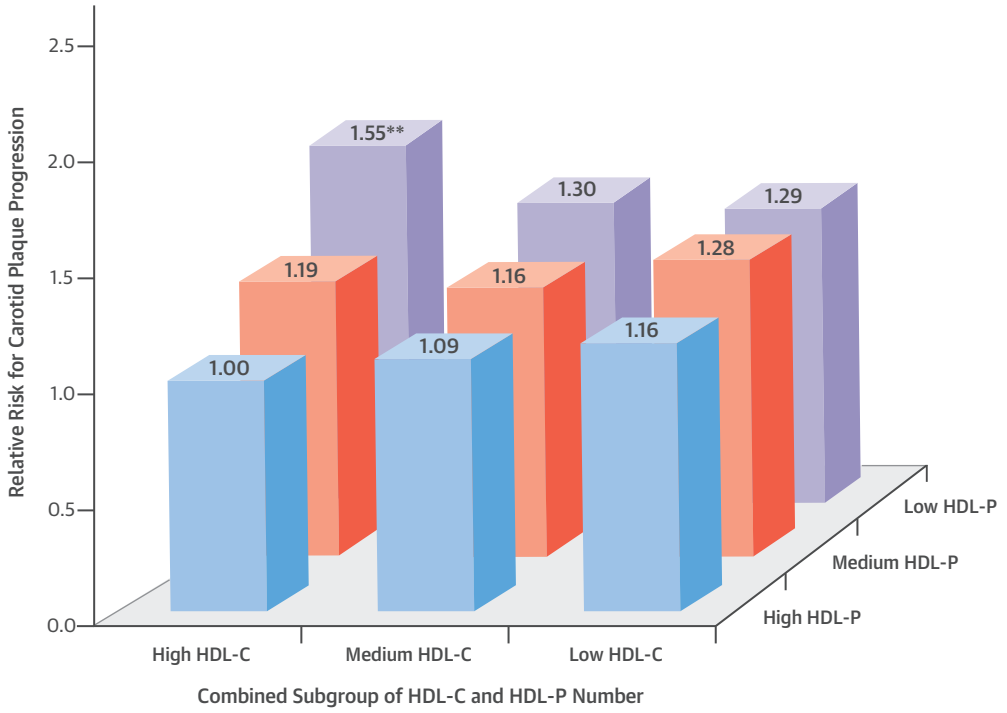
DISCUSSION

In this community-based cohort study, the cholesterol content of HDL-P was highly variable, suggesting the substantial existence of cholesterol-overloaded particles among asymptomatic individuals. Moreover and importantly, we reported for the first time that cholesterol-overloaded HDL-P was associated with increased progression of carotid atherosclerosis (**Central Illustration**).

SUBSTANTIAL EXISTENCE OF CHOLESTEROL-OVERLOADED HDL PARTICLES. In this study, we calculated the HDL-C/P ratio to assess the content of cholesterol per particles, and found 3.78-fold variation among asymptomatic individuals. One participant may have cholesterol-overloaded HDL-P, whereas another may have cholesterol-poor HDL-P. From a biochemical perspective, HDL-C is merely an index reflecting how much cholesterol is carried by the pool of HDL-P in the blood. However, the terms HDL-C and HDL-P are not synonymous, as illustrated in our study, and there are substantial inter-individual variations. The reasons are understood mechanistically, either naturally or as a result of HDL-raising therapy. The HDLs in human plasma vary in quantitative and qualitative content of lipids. Subjects with genetic CETP deficiency exhibited high levels of large cholesterol-enriched HDL-P (20). Moreover, interventions that raise HDL-C levels, including CETP inhibitors and niacin, had little effect on HDL function (21), and tend to increase cholesterol-overloaded particles (12). However, the evidence linking functions to this specific form of HDLs in humans is sparse.

CHOLESTEROL-OVERLOADED HDL PARTICLES AND ATHEROSCLEROSIS. Our findings suggested that very high levels of cholesterol-overloaded HDL-P are independently associated with the increased risk of carotid atherosclerosis in asymptomatic individuals. It is possible that HDL-P may be dysfunctional despite high levels of HDL-C. Previous data in humans have shown that low levels of the small, denser HDL (HDL3) subfraction carried higher risk than the large, less dense HDL (HDL2) subfraction in primary prevention (22) and secondary prevention (23). This could be in accordance with our findings, because these individuals would be overall shifted toward a more cholesterol-loaded state, with potentially less capacity for cholesterol efflux (24). Moreover, other observational studies, consistent with our findings, have suggested that high HDL-C levels or larger size of HDL-P, when not accompanied by a correspondingly high level of HDL-P number or

CENTRAL ILLUSTRATION **Cholesterol-Overloaded HDL-P and Atherosclerosis: Relative Risk for Carotid Plaque Progression Among Subgroups Defined by Levels of HDL-P Number and HDL-C**



Qi, Y. et al. J Am Coll Cardiol. 2015; 65(4):355-63.

Data were compared between participants with high HDL-P and high HDL-C and the others, after adjusting for age, sex, smoking, diabetes, body mass index, systolic blood pressure (SBP), low-density lipoprotein particle, triglyceride, HDL-P size, and 5-year changes in SBP, total cholesterol, HDL-C, and fasting blood glucose. HDL-C level was categorized as <1.04 (low), 1.04 to 1.29, ≥1.30 (high) mmol/l, and HDL-P number as <28.0 (low), 28.0 to 31.9, ≥32.0 (high) μmol/l. **Risk estimates were calculated in each subgroup using the subgroup with both highest level of HDL-C and HDL-P number as a reference. $p < 0.01$. HDL-C = high-density lipoprotein cholesterol; HDL-P = high-density lipoprotein particle.

apolipoprotein A-I (a rough measure of HDL-P), may be significantly associated with increased rather than decreased CVD risk (25). This epidemiological observation paralleled the findings of some experimental studies, and several potential biological mechanisms explain how this type of HDL can become proatherogenic. First, enrichment of HDL-P in cholesterol leads to impairments in HDL reverse cholesterol transport capacity (7,13). Moreover, exchange of cholesterol esters between HDL and peripheral cells is known to be bidirectional, in part mediated by SR-BI (26,27). Thus, cholesterol-overloaded HDL-P may at some point become cholesterol donors instead of acceptors. Additionally, cholesterol-overloaded HDL-P have also been shown to be ineffective in SR-BI-mediated cholesterol selective uptake in the liver, and filtration and clearance in the kidney (14-16). Second, although it is acknowledged that the anti-inflammatory capacity of HDL contributes to its antiatherogenic potency, HDL may become proinflammatory when in the large and cholesterol-enriched form (28). Third, large, less dense HDL impairs protection from oxidation (29). The accumulation of oxidized HDL results in impairments of cholesterol efflux (30), and interferes with SR-BI-mediated cholesterol selective transfer to hepatocytes (31). Conceivably, a very high level of cholesterol content per HDL-P may induce a proatherogenic lipoprotein profile via these 3 mechanisms. However, whether these mechanisms have physiological relevance in humans requires further investigation.

This study has shown that baseline HDL-C was significantly and negatively associated with carotid atherosclerosis progression, independent of HDL-C/P

ratio. This suggests that HDL-C indicates the cargo of HDL particles and may, therefore, reflect the potential for reverse cholesterol transport if the undesirable effects of cholesterol-overloaded HDL-P can be removed. This association can account for why recent clinical trials using CETP inhibitors that increase the level of HDL-C have failed to demonstrate any benefit for atherosclerosis. Additionally, although baseline HDL-P number was nonsignificantly associated with carotid plaque progression after adjusting for HDL-C/P ratio in this study, stratified analyses showed that at any given category of HDL-C level, this progression risk increased with decreasing HDL-P numbers. These findings suggest that HDL-P numbers may still be a proper metric of HDL, and provide more information beyond HDL-C. Indeed, ex vivo studies showed that a greater HDL-P number may indicate high reverse cholesterol transport capacity independent of HDL-C, and increasing binding to SR-BI and high efficiency of subsequent lipid transfer (8,9,27,32).

STUDY LIMITATIONS. The present study is the first large-scale study conducted in a general Chinese population to assess the cholesterol content per HDL-P, calculated by the ratio of HDL-C to HDL-P number, and we found substantial existence of cholesterol-overloaded HDL-P in asymptomatic individuals from a community-based cohort study. Moreover, we investigated the relation between cholesterol-overloaded HDL-P and carotid atherosclerosis, and found an independent positive association. Despite these clear strengths, our study has several possible limitations. First, HDL-C quantified in the clinical laboratory came from the various HDL subclasses. It is unknown which HDL functions are clinically important, and whether some functions are more antiatherogenic than others. However, we used the ratio of HDL-C to HDL-P number to assess the cholesterol content per HDL-P, and found an independent association for atherosclerosis after adjusting for HDL-P size. Although our findings suggest that the amount of cholesterol molecules per HDL-P exerts an important effects on atherosclerosis, the composition of cholesterol molecules carried by HDL-P, such as the differences between HDL subpopulations in the abundance of cholesteryl ester and free cholesterol (7), is also a crucial research frontier. This needs to be studied further, and may provide important insights into the multifarious antiatherogenic effects of HDL. Second, the present results were observational, which precluded any causal inference. Although we adjusted for many confounding factors, residual confounding by imperfectly measured or unmeasured confounders cannot be excluded, such as dietary factors and the types of lipid-lowering medications. However, this is a common limitation of a nonrandomized study, mandating the need for further studies. Third, the sample size, although reasonably large, reflects only a proportion of the original cohort. To ascertain whether the missing data would yield a potential bias, we compared the baseline characteristics among study participants who were eligible, lost to follow-up, and unavailable for re-examination, and we found no significant differences. Nevertheless, this finding still highlights the need for extensive replication and clinical validation of our results in larger studies. Finally, the fact that our study participants were Chinese may limit the generalizability of our findings, necessitating further confirmation in other ethnic populations.

TABLE 2 Modified Poisson Regression Analysis of the Associations Between HDL-C/P Ratio, HDL-P Number, HDL-C, and the Progression of Carotid Atherosclerosis

	Progression of Carotid Plaque		Incidence of New-Onset Carotid Plaque	
	RR (95% CI)	p Value*	RR (95% CI)	p Value*
HDL-C/P ratio				
<41.0	Ref		Ref	
41.0–46.9	1.20 (1.00-1.44)	0.05	1.17 (0.93-1.48)	0.17
47.0–52.9	1.32 (1.04-1.67)	0.02	1.23 (0.91-1.67)	0.06
≥53.0	1.56 (1.14-2.13)	0.006	1.47 (1.00-2.16)	0.04
HDL-P number, μmol/l				
<28.0	Ref		Ref	
28.0–32.9	1.03 (0.88-1.22)	0.70	0.94 (0.77-1.15)	0.55
≥33.0	0.95 (0.77-1.18)	0.64	0.88 (0.68-1.14)	0.33
HDL-C, mmol/l				
<1.04	Ref		Ref	
1.04–1.29	0.87 (0.74-1.03)	0.11	0.93 (0.75-1.16)	0.55
≥1.30	0.74 (0.61-0.94)	0.03	0.83 (0.66-0.99)	0.05
Age, 5-yr	1.17 (1.11-1.22)	<0.001	1.15 (1.09-1.21)	<0.001
SBP, 5 mm Hg	1.04 (1.02-1.06)	<0.001	1.05 (1.02-1.08)	<0.001
LDL-P, 200 nmol/l	1.10 (1.04-1.15)	0.001	1.11 (1.04-1.19)	0.002
Smoking	1.24 (1.03-1.50)	0.03	1.34 (1.06-1.70)	0.02
Diabetes	1.32 (1.09-1.60)	0.004	1.38 (1.11-1.72)	0.004
5-yr SBP change, 5 mm Hg	1.03 (1.01-1.05)	0.006	1.04 (1.01-1.07)	0.003

*All substantial models are additionally adjusted for sex, body mass index (1 kg/m^2), triglyceride, HDL-P size, and changes in total cholesterol, HDL-C, and fasting blood glucose. Smoking was defined as smoking 1 or more cigarettes per day for more than 3 months. Diabetes was defined by fasting plasma glucose ≥7.0 mmol/l or currently on glucose-lowering medical treatment.

CI = confidence interval; HDL-C = high-density lipoprotein cholesterol; HDL-C/P ratio = the ratio of HDL-C to HDL-P number (giving the content of cholesterol per HDL-P); HDL-P = high-density lipoprotein particle; LDL-P = low-density lipoprotein particle; RR = relative risk; SBP = systolic blood pressure.

CONCLUSIONS

Our findings suggest that there are substantial variations in cholesterol content per HDL-P, and individuals may have cholesterol-overloaded HDL-P. Moreover, cholesterol-overloaded HDL-P may be a strong predictor of carotid atherosclerosis risk, implying that in cases of high levels of cholesterol-overloaded HDL-P,

an individual may be more prone to atherosclerosis risk despite high HDL-C levels. The cholesterol content and particle number of HDL codetermined the antiatherogenic potency. These findings may help direct future efforts to reduce ASCVD beyond the isolated increase in cholesterol content of HDLs, and the focus of future intervention studies should be, not only on raising HDL-C levels, but also on lowering cholesterol-overloaded HDL-P.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Cholesterol-overload HDL particles may predict atherosclerotic risk despite high HDL-C levels and might explain why high plasma HDL-C levels have failed to avoid ischemic events in some clinical trials.

TRANSLATIONAL OUTLOOK: Consideration of the combination of cholesterol content and HDL particle number, rather than either parameter alone, may help direct future efforts to reduce atherogenesis by lowering the cholesterol content of HDL particles.

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KEY WORDS carotid atherosclerosis, cholesterol-overloaded high-density lipoprotein particle, high-density lipoprotein cholesterol

APPENDIX For an expanded methods section and supplemental tables and a figure, please see the online version of this article.