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High-Density Lipoprotein Subclass Measurements Improve Mortality Risk Prediction, Discrimination and Reclassification in a Cardiac Catheterization Cohort

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Abstract

Background and Aims—Recent failures of HDL cholesterol (HDL-C)-raising therapies to prevent cardiovascular disease (CVD) events have tempered the interest in the role of HDL-C in clinical risk assessment. Emerging data suggest that the atheroprotective properties of HDL depend on specific HDL particle characteristics not reflected by HDL-C. The purpose of this study was to determine the association of HDL particle concentration (HDL-P) and HDL subclasses with mortality in a high-risk cardiovascular population and to examine the clinical utility of these parameters in mortality risk discrimination and reclassification models.

Methods—Using nuclear magnetic resonance spectroscopy, we measured HDL-P and HDL subclasses in 3972 individuals enrolled in the CATHGEN coronary catheterization biorepository; tested for association with all-cause mortality in robust clinical models; and examined the utility of HDL subclasses in incremental mortality risk discrimination and reclassification.

Results—Over an average follow-up of eight years, 29.6% of the individuals died. In a multivariable model adjusted for ten CVD risk factors, HDL-P [HR, 0.71 (0.67-0.76), p= 1.3e-24] had a stronger inverse association with mortality than did HDL-C [HR 0.93 (0.87-0.99), p=0.02]. Larger HDL size conferred greater risk and the sum of medium- and small-size HDL particles (MS-HDL-P) conferred less risk. Furthermore, the strong inverse relation of HDL-P levels with mortality was accounted for entirely by MS-HDL-P; HDL-C was not associated with mortality after adjustment for MS-HDL-P. Addition of MS-HDL-P to the GRACE Risk Score significantly improved risk discrimination and risk reclassification.

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Conflict of Interest Statement:

The authors declare that no conflict of interest exists

Conclusion—HDL-P and smaller HDL subclasses were independent markers of residual mortality risk and incremental to HDL-C in a high-risk CVD population. These measures should be considered in risk stratification and future development of HDL-targeted therapies in high-risk populations.

Keywords

biomarkers; HDL; HDL subclasses; risk prediction; mortality

Introduction

The biological role of HDL in cardiovascular disease (CVD) remains unclear. Whereas epidemiological studies consistently demonstrate an inverse relation of HDL cholesterol (HDL-C) with CVD, pharmacological interventions that raise HDL-C fail to result in improved cardiovascular outcomes.[1–4] Moreover, a recent large Mendelian randomization study failed to identify any relation between genetic variants of high HDL-C and improved CVD risk.[5] These findings have raised serious doubts about the biological relation between HDL-C and CVD; they clearly demonstrate that the health benefits of HDL metabolism extend beyond HDL-C alone.

New data suggest that the atheroprotective properties of HDL – such as its antioxidant effects, removal of cellular cholesterol and production of nitric oxide – depend on specific HDL particle characteristics that are not well represented by HDL-C (a measure of HDL dominated by the contribution of larger, more cholesterol-rich HDL subclasses).[6] HDL particle concentration (HDL-P), an alternate measure of HDL that attributes equal weight to all HDL subclasses, may better represent the biological relation between HDL and clinical risk. For instance, in individuals not on lipid-lowering medications in the Multi-Ethnic Study of Atherosclerosis (MESA), even after adjustment for HDL-C and LDL particles (LDL-P), HDL-P is inversely associated with carotid intimal thickness and incident CVD.[7] Similarly, in the Justification for the Use of statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) study, HDL-P is inversely associated with incident CVD in both placebo- and rosuvastatin-treated individuals. This association persists after adjustment for HDL-C, suggesting that HDL-P may be a marker of residual CVD risk in individuals on statin therapy.[8] Notably, in both of these studies, HDL-C is no longer associated with CVD after adjusting for HDL-P. There are similar findings in the Heart Protection Study (HPS) and VA-HIT study in patients with established coronary disease.[9,10] This apparently discordant relation between HDL-C and HDL-P is further illustrated in MESA and in a recent Chinese study that demonstrate a positive association between the HDL-C/HDL-P ratio and risk of progression of carotid atherosclerosis. This suggests that cholesterol-overloaded HDL particles may have impaired atheroprotective properties and therefore that HDL subclasses differing in size or density may have differential associations with clinical outcomes.[7,11]

The data are conflicting regarding which HDL subclasses are associated with decreased risk of clinical outcomes. Earlier studies indicate that larger HDL subclasses confer more cardioprotection; however, more recent studies suggest that smaller HDL subclasses are

associated with improved cardiovascular risk.[9,12–15] While HDL-P and HDL subclasses have been considered in relation to intermediate CVD phenotypes and CVD outcomes, the few studies that have examined all-cause mortality as an endpoint suggest that HDL subclasses have different relations with CVD than with mortality.[16]

The CATHGEN biorepository at Duke University collected blood samples from 9344 individuals presenting to the catheterization laboratory from 2001-2010 for concern of ischemic heart disease. These individuals were followed after enrollment for clinical events. The overall 5-year mortality rate was 21%.[17] As such, this high-risk population was ideal for further investigating the relation of HDL-P and HDL subclasses with all-cause mortality.

We tested the hypothesis that HDL-P and HDL subclasses would be associated with all-cause mortality, independent of HDL-C levels; we also hypothesized that in clinical risk prediction models HDL-P and HDL subclasses would improve mortality risk discrimination and reclassification.

Methods

Study Population

The CATHeterization GENetics (CATHGEN) biorepository at Duke University has collected blood samples from sequential consenting individuals undergoing coronary catheterization for suspicion of ischemic heart disease from 2001-2010. Details of the biorepository have been previously described.[17,18] Clinical information was obtained from the Duke Databank for Cardiovascular Disease. Available data include symptom histories; clinical characteristics and medical history; angiographic data; and in most subjects, fasting chemistry data within 1 year preceding cardiac catheterization. Individuals enrolled in the biorepository had routine yearly follow-up after enrollment catheterization. Follow-up included mortality (verified via National Death Index search and supplemented by Social Security Death Index search), myocardial infarction (MI), stroke, rehospitalization, coronary revascularization procedures, smoking, and medication use. Coronary artery disease (CAD) was defined as ≥1 epicardial vessel with ≥75% stenosis on enrollment catheterization in individuals with no history of CAD or coronary artery bypass grafting (CABG). Incident events were defined as all-cause death at any time during the follow-up period.

Laboratory Methods

Lipoprotein particle concentrations and sizes were measured in 3972 CATHGEN individuals by NMR spectroscopy at LipoScience, Inc (Raleigh, NC) using the LipoProfile-3 algorithm.[19,20] The 3 measured HDL subclasses had the following estimated particle diameter ranges: large HDL-P, 9.4-14 nm; medium HDL-P, 8.2-9.4 nm; small HDL-P, 7.3-8.2 nm. In some analyses, the medium and small HDL subclasses (HDL particles with diameters <9.4 nm) were combined and named MS-HDL-P. Mean HDL sizes are mass-weighted averages.[14] Standard lipids including triglycerides were measured with an Olympus AU680 chemistry analyzer using Beckman Coulter reagents. LDL-C was measured using a direct homogeneous assay.

Statistics

Continuous variables are presented as mean ± SD and dichotomous variables as percentages. Follow-up time is presented as median time with interquartile range. Lipoprotein particle levels were Z-transformed to obtain hazard ratios in terms of each population standard deviation change in particle value.

Differences in baseline characteristics between those who did and did not experience all-cause death were determined using Student's t-test. The associations of baseline HDL parameters with time to all-cause death were quantified using Cox proportional hazard models, adjusted for age, race, sex, diabetes, hypertension, LDL-C, smoking status, BMI, CAD and EF. Proportional hazard assumptions were tested by introducing time-dependent covariates into the model and testing for the interaction of time and particle measures. Pearson correlation coefficients were used to determine the correlations between HDL parameters.

Likelihood ratio tests, yielding χ^2 statistics, were used to assess the improvement in risk discrimination resulting from the addition of HDL parameters to multivariable clinical models and from the addition of MS-HDL-P to the GRACE Risk Score in individuals with complete data available on all of the variables used in the GRACE Risk Score (N=3209). The GRACE Risk Score is a registry-based clinical risk prediction tool originally developed to estimate the cumulative six-month risk of death or MI in individuals presenting with acute coronary syndrome.[21] An updated GRACE Risk Score was developed to estimate all-cause mortality or the combined outcome of all-cause mortality or MI at 1 and 3 years.[22] This revised score improves risk discrimination to a greater degree for all-cause mortality than it does for the combined outcome: it was thus suitable for our current study. Variables include: age, heart rate, systolic blood pressure, creatinine, cardiac arrest at admission, ST segment deviation on electrocardiogram, abnormal cardiac enzymes and signs/symptoms of CHF. To assess the usefulness of MS-HDL-P in risk discrimination, we determined the χ^2 statistic from the likelihood ratio test of models containing the GRACE Risk Score variables with and without the addition of MS-HDL-P. Using the risk categories of <5%, 5% to <10%, 10% to <20% and ≥20%, the net reclassification index (NRI) and integrated discrimination improvement (IDI) were calculated for individuals who experienced all-cause death and for those who did not during the follow-up period.

Statistical analyses were performed using SAS Version 9.4 (Cary, NC). All reported P values are two-sided.

Study Approval

The CATHGEN biorepository is monitored and approved by the Duke University Institutional Review Board. Written informed consent was received from participants prior to inclusion in the study.

Results

Table 1 shows the characteristics of the CATHGEN cohort (N=3972) according to all-cause mortality during the follow-up period. The overall cohort was predominantly men (61.8%)

with a mean age of 60.2 years and was enriched for CVD risk factors including hypertension (67.4%), smoking (49.8%), diabetes (28.8%), obesity (mean BMI = 30.4 kg/m^2), family history of CAD (36.6%) and hyperlipidemia (59.4%). The mean levels of LDL-C (98.2 ± 33.2 mg/dL) and HDL-C (38.1 ± 11.2 mg/dL) were relatively low. At index catheterization, 2571 (64.4%) individuals had CAD. During an average follow-up time of 8.1 years, 1181 individuals (29.6%) died. As expected, individuals who died were overall older and had a higher prevalence of CVD risk factors compared to individuals who had not died. Mean LDL-C, HDL-C, and non-HDL-C levels were lower in those who died. There was no difference in triglycerides between the two groups.

Association of HDL-P and HDL Subclasses with All-Cause Mortality

HDL subclasses have different associations with mortality than they do with cardiovascular disease prevalence.[16] Using Cox proportional hazards models adjusted for clinical risk factors, HDL-P had a stronger inverse association with all-cause mortality than did HDL-C [HR 0.71 (0.67-0.76), p<0.0001 and HR 0.93 (0.87-0.99), p=0.02, respectively; **Table 2**].

Considering all three HDL subclasses together in the same model, small and medium HDL-P had comparably strong inverse associations with all-cause mortality [HR 0.64 (0.6-0.69), p<0.0001 and HR 0.73 (0.68-0.78), p<0.0001, respectively; **Table 2**], while large HDL-P had no association with mortality [HR 1.03 (0.97-1.1), p=0.29]. Combining levels of medium and small HDL particles (MS-HDL-P) yielded a model with discrimination similar to the model containing all three subclasses. HDL size was also associated with all-cause mortality [HR 1.16 (1.09-1.23), p<0.0001] and modestly improved model discrimination when included with MS-HDL-P.

Consistent with the fact that HDL-C primarily reflects levels of larger HDL particles, inclusion of HDL-C in a model containing MS-HDL-P did not improve discrimination. Taken together, these results suggest that smaller HDL subclasses, represented by MS-HDL-P, were associated with decreased risk of mortality while larger HDL subclasses had no significant association. The positive association of HDL size with mortality may be related to its negative correlation with protective smaller HDL particles rather than with its positive correlation with large HDL particles.

Further illustrating the different contributions of HDL subclasses to HDL-P and HDL-C measurements, MS-HDL-P was highly correlated with HDL-P (r = 0.90, p<0.0001; **Supplementary Table 1**) while large HDL-P - which has no association with mortality - was highly correlated with HDL-C (r = 0.79, p<0.0001).

HDL Subclasses and Mortality Risk Discrimination and Reclassification

Given the strong inverse, independent association of MS-HDL-P with all-cause mortality even after adjustment for clinical risk factors and other lipoprotein parameters, we hypothesized that the addition of MS-HDL-P to an established clinical risk model would improve prediction of mortality in our study population.

To perform these analyses, we used a common clinical risk prediction tool, the GRACE Risk Score, which estimates the risk of all-cause mortality in individuals presenting with

acute coronary syndrome.[22] As seen in **Table 3**, the addition of MS-HDL-P to the GRACE model significantly improved mortality risk prediction as reflected by an improvement in model fit.

We then evaluated the usefulness of MS-HDL-P in risk reclassification. **Table 4** illustrates the risk classification of individuals based on the GRACE model alone and with the addition of MS-HDL-P. Of the 2706 individuals who remained free of death, 644 were reclassified into a lower-risk category and 341 were reclassified into a higher-risk category. Of the 503 individuals who had died, 51 were reclassified into a lower-risk category and 70 were reclassified into a higher-risk category. The net reclassification index (NRI) and integrated discrimination improvement (IDI) after addition of MS-HDL-P to the GRACE model were 0.13 ($p<0.0001$) and 0.03 ($p<0.0001$), respectively (**Table 3**).

Discussion

Capitalizing on a large, high-risk cardiovascular cohort with long-term follow-up, detailed angiographic data and a high mortality rate, we observed that NMR-derived HDL-P and HDL subclasses were significantly associated with all-cause mortality. To date, this is the largest study using NMR-derived lipoprotein measurements to examine HDL particle associations specifically with mortality. We observed a strong inverse relation of HDL-P levels with mortality; this was accounted for entirely by the subset of smaller particles with diameters <9.4 nm (MS-HDL-P). In contrast, there was only a weak association of low HDL-C with death; this relation was abolished upon adjustment for MS-HDL-P. We also demonstrated, for the first time, that inclusion of HDL subclasses (MS-HDL-P) in an established clinical risk model significantly improved risk discrimination and reclassification indexes. Our findings suggest that low levels of small- and medium-size HDL particles are important markers of residual mortality risk above and beyond HDL-C: these measures should be considered in risk stratification and future development of HDL-targeted therapies in high-risk individuals.

These observations add to a growing body of evidence indicating that HDL-C levels, which are a measure of the amount of cholesterol contained within HDL particles, provide only a crude, and sometimes misleading, indication of the extent to which HDL levels confer cardioprotection. It is becoming clear that HDL-C does not correlate well with HDL function. For example, in the Atherothrombosis Intervention in Metabolic syndrome with low HDL/high triglycerides: impact on Global Health outcomes (AIM-HIGH) trial, despite a 25% increase in HDL-C levels in niacin-treated individuals, there was no reduction in the combined cardiovascular endpoint.[3] Importantly, in smaller studies, treatment with niacin has no effect on total HDL particle number but changes HDL composition by increasing the amount of large HDL-P and decreasing the amount of small HDL-P.[23,24] Similar changes to HDL particle composition, increasing HDL-C concentration by increasing HDL particle size, are observed in treatment with the cholesteryl ester transfer protein (CETP) inhibitors evacetrapib and torcetrapib.[25,26] Based on the results of our study, these changes to HDL particle composition do not translate into an improved risk profile and, given the increase in HDL size, may even negatively impact clinical outcomes.

Studies are conflicting about whether HDL size and subclasses are associated with clinical outcomes (reviewed in [27]). In both JUPITER and HPS, there are no associations of HDL size with CVD in multivariable-adjusted models.[8,9] On the other hand, in the Lipoprotein Investigators Collaborative low concentrations of HDL_3-C (the smaller of the two major HDL cholesterol fractions) are associated with increased risk of CVD and mortality in both primary and secondary prevention populations.[16,28] Likewise, in two recent population-based studies, HDL_3-C levels are negatively associated with prevalent carotid atherosclerosis.[29,30] These results contradict earlier studies wherein decreased risk is associated with greater concentrations of the larger HDL_2-C subfractions and increased risk is associated with greater concentrations of HDL_3-C.[12–14] Such discordances may be explained, in part, by the various methods used to characterize HDL subclasses (ultracentrifugation, gel electrophoresis, ion mobility, NMR). For instance, in JUPITER, NMR-measured HDL-P is strongly related to residual cardiovascular risk while ion mobility-measured HDL-P has no association.[31] Additionally, due to the complex biology and function of HDL particles - which are influenced by other lipoproteins and by conditions such as diabetes and systemic inflammatory states - the predictive capacity of HDL subclasses in CVD and mortality may vary depending on the populations examined and the potential confounders considered in statistical modeling.

Studies of individuals who carry rare mutations in genes involved in HDL metabolism that alter HDL subclass composition indicate that smaller HDL particles likely represent the more functional and atheroprotective subclass of HDL.[32] These findings are supported by mechanistic *in vitro* studies.[27,33,34] Du et al. showed that small, dense HDL subfractions (i.e., HDL_3-C) are the most efficient mediators of macrophage cholesterol efflux; they conclude that HDL-directed therapies should focus on increasing this HDL subclass.[33]

HDL-P is an alternate measure of HDL that attributes equal weight to all HDL subclasses. While we showed that HDL-P had a stronger inverse association with mortality than HDL-C, further analysis demonstrated unique associations of HDL subclasses with mortality. In considering all HDL-P subclasses (small, medium and large) in the multivariable clinical model, small and medium HDL-P had a stronger inverse association with mortality than did HDL-P; large HDL-P had no association with mortality (**Table 2**). These associations explain why adding large HDL-P to small and medium HDL-P (MS-HDL-P) weakened the association of HDL-P with mortality compared to MS-HDL-P. Moreover, these associations explain why HDL-P had a stronger association with mortality than HDL-C, which is a measure of HDL dominated by the contribution of large cholesterol-rich HDL particles. In sum, HDL-C alone may not fully quantify an individual's HDL-related risk.

HDL subclasses may have different relations with CVD than with mortality or non-atherosclerotic outcomes: therefore, HDL-P and HDL subclasses may play unique roles in mortality risk prediction. The positive association of HDL size with mortality (observed here) is consistent with other studies that have investigated HDL parameters in relation to mortality or non-atherosclerotic outcomes, a finding that is not consistently demonstrated when utilizing CVD events as an outcome.[8,9,16] For instance, the HPS study separated outcomes into coronary events and non-coronary outcomes.[9] Non-coronary outcomes have a strong positive association with HDL size that is not observed with coronary events; non-

coronary outcomes also demonstrate a more marked negative association with HDL-P than does coronary events.

HDL particle composition, and therefore function, can be significantly influenced and modified by coexisting metabolic and lipoprotein variables; therefore, these factors must be considered when analyzing the relation of HDL subclasses to outcomes. Metabolic risk factors such as diabetes, BMI, apolipoprotein B and triglyceride levels can affect HDL size.[35] Previous studies, after accounting for these covariables, have noted attenuation of the association of HDL subclasses, but not HDL-P, with CVD.[36,37] In contrast, inclusion of other metabolic and lipoprotein variables, including LDL-C, in our fully adjusted model did not significantly modify the association of HDL-P, HDL size or smaller HDL subclasses with outcomes. In addition to the interaction of HDL with metabolic conditions, gathering evidence suggests that systemic processes such as inflammation have the potential to modify HDL particles into dysfunctional or even proatherogenic forms, without impacting the particle size.[38,39] Before these measurements are to be adopted in routine clinical practice, there is a need for ongoing epidemiological and mechanistic studies to further understand the complex functional heterogeneity of HDL subclasses.

To our knowledge, this report is the first to perform detailed risk discrimination and reclassification analyses with HDL subclasses. Not only did we observe that MS-HDL-P significantly improved mortality risk prediction when added to the GRACE Risk Score, but it also correctly reclassified risk in our study population, indicating the potential utility of MS-HDL-P in mortality risk assessment in high-risk populations. Importantly, addition of MS-HDL-P had a large effect in reclassifying individuals in an intermediate (10% to <20%) risk category (**Table 4**). These individuals are the most likely to benefit from tests that can effectively increase or decrease risk assessment to help tailor risk-modifying therapies.

Our study population had an overall risk profile similar to the GRACE cohort: albeit during a longer follow-up time period, our measured outcome was identical to that in the revised GRACE model. Although not an ACS cohort, our study population includes individuals presenting for coronary catheterization due to suspicion for ischemic heart disease and is enriched for those with ACS (**Table 1**). It is important to note that lipoprotein measurements were not included as variables in the development of the GRACE Risk Score, so it is unclear *a priori* what predictive effect the addition of HDL subclasses to this model would have had in the GRACE study population.

Strengths of our study include the size of the cohort and the detailed clinical characteristics and outcomes gathered during long-term follow-up. Our results are generalizable to high-risk individuals with established CVD or multiple CVD risk factors. Despite adjustment for established CVD risk factors in our clinical models, there is the possibility that important confounding variables that have an effect on HDL parameters were not measured or considered in our analyses. Detailed information regarding the specific cause of death is not readily available in our cohort so it is not possible to determine if these HDL parameters are more strongly associated with specific etiologies of mortality. The relation of HDL to heart failure-related deaths in our population would be an interesting area of future study given the evidence that HDL appears to have beneficial effects on ventricular remodeling and

myocardial tissue repair.[40] Due to insufficient adjudicated incident CVD events - such as myocardial infarction - we did not have the power to perform a comparative analysis of HDL subclasses in relation to CVD events versus all-cause mortality. Overall, the differential associations of HDL parameters with specific clinical outcomes warrant further investigation. HDL particles are constantly remodeled, with changing compositions of lipids, phospholipids and apolipoproteins. Therefore, the static measures of HDL particles, such as HDL-P and HDL subclasses used here, do not fully capture the complexity of HDL metabolism and composition. Though not feasible in this study, the integration of HDL-P and HDL subclass data along with HDL functional measurements are likely to provide the biggest utility in CVD risk assessment in the future.[41]

Conclusion

In conclusion, despite optimal control of modifiable risk factors in individuals with high cardiovascular risk, the chance of subsequent clinical events, such as MI or death, remains significant.[42] Low HDL-C has traditionally indicated increased risk in secondary prevention populations; however, efforts to raise HDL-C have not shown improvement in clinical outcomes. We observed that smaller HDL subclasses, which more closely correlate with HDL function, are better markers of residual mortality risk and better predictors of mortality than is HDL-C or HDL-P in a high-risk CVD population. Taken together with previously published data in primary and secondary prevention populations[7,8,36] , there is now evidence to suggest that HDL-P and HDL subclasses have utility in characterizing clinical risk across a broad spectrum of individuals. These findings have important implications for the future development of HDL-targeted drugs and the use of this lipoprotein in risk stratification.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Schwartz GG, Olsson AG, Abt M, et al. Effects of dalcetrapib in patients with a recent acute coronary syndrome. N. Engl. J. Med. 2012; 367(22):2089–99. doi:10.1056/NEJMoa1206797. [PubMed: 23126252]
2. Barter PJ, Caulfield M, Eriksson M, et al. Effects of torcetrapib in patients at high risk for coronary events. N. Engl. J. Med. 2007; 357(21):2109–22. doi:10.1056/NEJMoa0706628. [PubMed: 17984165]
3. Boden WE, Probstfield JL, Anderson T, et al. Niacin in patients with low HDL cholesterol levels receiving intensive statin therapy. N. Engl. J. Med. 2011; 365(24):2255–67. doi:10.1056/NEJMoa1107579. [PubMed: 22085343]

4. Landray MJ, Haynes R, Hopewell JC, et al. Effects of extended-release niacin with laropiprant in high-risk patients. N. Engl. J. Med. 2014; 371(3):203–12. doi:10.1056/NEJMoa1300955. [PubMed: 25014686]
5. Voight BF, Peloso GM, Orho-Melander M, et al. Plasma HDL cholesterol and risk of myocardial infarction: A mendelian randomisation study. Lancet. 2012; 380(9841):572–580. doi:10.1016/S0140-6736(12)60312-2. [PubMed: 22607825]
6. Rader DJ, Hovingh GK. HDL and cardiovascular disease. Lancet. 2014; 384(9943):618–625. doi:10.1016/S0140-6736(14)61217-4. [PubMed: 25131981]
7. Mackey RH, Greenland P, Goff DC, Lloyd-Jones D, Sibley CT, Mora S. High-density lipoprotein cholesterol and particle concentrations, carotid atherosclerosis, and coronary events: MESA (multi-ethnic study of atherosclerosis). J. Am. Coll. Cardiol. 2012; 60(6):508–16. doi:10.1016/j.jacc.2012.03.060. [PubMed: 22796256]
8. Mora S, Glynn RJ, Ridker PM. High-density lipoprotein cholesterol, size, particle number, and residual vascular risk after potent statin therapy. Circulation. 2013; 128(11):1189–1197. doi:10.1161/CIRCULATIONAHA.113.002671. [PubMed: 24002795]
9. Parish S, Offer A, Clarke R, et al. Lipids and lipoproteins and risk of different vascular events in the MRC/BHF Heart Protection Study. Circulation. 2012; 125(20):2469–78. doi:10.1161/CIRCULATIONAHA.111.073684. [PubMed: 22539783]
10. Otvos JD, Collins D, Freedman DS, et al. Low-density lipoprotein and high-density lipoprotein particle subclasses predict coronary events and are favorably changed by gemfibrozil therapy in the Veterans Affairs High-Density Lipoprotein Intervention Trial. Circulation. 2006; 113(12):1556–63. doi:10.1161/CIRCULATIONAHA.105.565135. [PubMed: 16534013]
11. Qi Y, Fan J, Liu J, et al. Cholesterol-overloaded HDL particles are independently associated with progression of carotid atherosclerosis in a cardiovascular disease-free population: a community-based cohort study. J. Am. Coll. Cardiol. 2015; 65(4):355–63. doi:10.1016/j.jacc.2014.11.019. [PubMed: 25634834]
12. Lamarche B, Moorjani S, Cantin B, Dagenais GR, Lupien PJ, Després JP. Associations of HDL2 and HDL3 subfractions with ischemic heart disease in men. Prospective results from the Québec Cardiovascular Study. Arterioscler. Thromb. Vasc. Biol. 1997; 17(6):1098–105. [PubMed: 9194760]
13. Stampfer MJ, Sacks FM, Salvini S, Willett WC, Hennekens CH. A prospective study of cholesterol, apolipoproteins, and the risk of myocardial infarction. N. Engl. J. Med. 1991; 325(6):373–81. doi:10.1056/NEJM199108083250601. [PubMed: 2062328]
14. Sweetnam PM, Bolton CH, Yarnell JW, et al. Associations of the HDL2 and HDL3 cholesterol subfractions with the development of ischemic heart disease in British men. The Caerphilly and Speedwell Collaborative Heart Disease Studies. Circulation. 1994; 90(2):769–74. [PubMed: 8044946]
15. Yu S, Yarnell JWG, Sweetnam P, Bolton CH. High density lipoprotein subfractions and the risk of coronary heart disease: 9-years follow-up in the Caerphilly Study. Atherosclerosis. 2003; 166(2):331–8. [PubMed: 12535746]
16. Martin SS, Khokhar A a, May HT, et al. HDL cholesterol subclasses, myocardial infarction, and mortality in secondary prevention: the lipoprotein investigators collaborative. Eur. Heart J. 2014:1–9. doi:10.1093/eurheartj/ehu264. [PubMed: 24382633]
17. Shah SH, Granger CB, Hauser ER, et al. Reclassification of cardiovascular risk using integrated clinical and molecular biosignatures: Design of and rationale for the Measurement to Understand the Reclassification of Disease of Cabarrus and Kannapolis (MURDOCK) Horizon 1 Cardiovascular Disease . Am. Heart J. 2010; 160(3):371–379.e2. doi:10.1016/j.ahj.2010.06.051. [PubMed: 20826242]
18. Kraus W, Granger C, Sketch M, et al. A guide for cardiovascular genomics biorepository: the CATHGEN experience. J Cardiov Transl Res. 2015 In press.
19. Jeyarajah EJ, Cromwell WC, Otvos JD. Lipoprotein particle analysis by nuclear magnetic resonance spectroscopy. Clin. Lab. Med. 2006; 26(4):847–70. doi:10.1016/j.cll.2006.07.006. [PubMed: 17110242]

20. Rosenson RS, Brewer HB, Chapman MJ, et al. HDL measures, particle heterogeneity, proposed nomenclature, and relation to atherosclerotic cardiovascular events. Clin. Chem. 2011; 57(3):392–410. doi:10.1373/clinchem.2010.155333. [PubMed: 21266551]
21. Fox, K a a; Dabbous, OH.; Goldberg, RJ., et al. Prediction of risk of death and myocardial infarction in the six months after presentation with acute coronary syndrome: prospective multinational observational study (GRACE). BMJ. 2006; 333(7578):1091. doi:10.1136/bmj.38985.646481.55. [PubMed: 17032691]
22. Fox KAA, Fitzgerald G, Puymirat E, et al. Should patients with acute coronary disease be stratified for management according to their risk? Derivation, external validation and outcomes using the updated GRACE risk score. BMJ Open. 2014; 4(2):e004425. doi:10.1136/bmjopen-2013-004425.
23. Airan-Javia SL, Wolf RL, Wolfe ML, Tadesse M, Mohler E, Reilly MP. Atheroprotective lipoprotein effects of a niacin-simvastatin combination compared to low- and high-dose simvastatin monotherapy. Am. Heart J. 2009; 157(4):687.e1–8. doi:10.1016/j.ahj.2009.01.001. [PubMed: 19332196]
24. Jafri H, Alsheikh-Ali A a, Mooney P, Kimmelstiel CD, Karas RH, Kuvin JT. Extended-release niacin reduces LDL particle number without changing total LDL cholesterol in patients with stable CAD. J. Clin. Lipidol. 2009; 3(1):45–50. doi:10.1016/j.jacl.2008.12.003. [PubMed: 21291788]
25. Nicholls SJ, Brewer HB, Kastelein JJP, et al. Effects of the CETP inhibitor evacetrapib administered as monotherapy or in combination with statins on HDL and LDL cholesterol: a randomized controlled trial. JAMA. 2011; 306(19):2099–109. doi:10.1001/jama.2011.1649. [PubMed: 22089718]
26. Kastelein JJP, van Leuven SI, Burgess L, et al. Effect of torcetrapib on carotid atherosclerosis in familial hypercholesterolemia. N. Engl. J. Med. 2007; 356(16):1620–30. doi:10.1056/NEJMoa071359. [PubMed: 17387131]
27. Rizzo M, Otvos JD, Nikolic D, Montalto G, Toth PP, Banach M. Subfractions And Subpopulations Of HDL: An Update. Curr. Med. Chem. 2014; 21(1):1–11. [PubMed: 24466585]
28. Martin SS, Jones SR, Toth PP. High-density lipoprotein subfractions: current views and clinical practice applications. Trends Endocrinol. Metab. 2014; 25(7):329–36. doi:10.1016/j.tem.2014.05.005. [PubMed: 24931711]
29. Tiozzo E, Gardener H, Hudson BI, et al. High-density lipoprotein subfractions and carotid plaque: the Northern Manhattan Study. Atherosclerosis. 2014; 237(1):163–8. doi:10.1016/j.atherosclerosis.2014.09.002. [PubMed: 25240111]
30. Kim DS, Burt AA, Rosenthal EA, et al. HDL-3 is a superior predictor of carotid artery disease in a case-control cohort of 1725 participants. J. Am. Heart Assoc. 2014; 3(3):e000902. doi:10.1161/JAHA.114.000902. [PubMed: 24965026]
31. Mora S, Caulfield MP, Wohlgemuth J, et al. Atherogenic Lipoprotein Subfractions Determined by Ion Mobility and First Cardiovascular Events After Random Allocation to High-Intensity Statin or Placebo: The JUPITER Trial. Circulation. 2015 doi:10.1161/CIRCULATIONAHA.115.016857.
32. Calabresi L, Gomaraschi M, Simonelli S, Bernini F, Franceschini G. HDL and atherosclerosis: Insights from inherited HDL disorders. Biochim. Biophys. Acta. 2014 doi:10.1016/j.bbalip.2014.07.015.
33. Du X, Kim M-J, Hou L, et al. HDL Particle Size is a Critical Determinant of ABCA1-Mediated Macrophage Cellular Cholesterol Export. Circ. Res. 2015; 116(7):1133–1142. doi:10.1161/CIRCRESAHA.116.305485. [PubMed: 25589556]
34. Camont L, Chapman MJ, Kontush A. Biological activities of HDL subpopulations and their relevance to cardiovascular disease. Trends Mol. Med. 2011; 17(10):594–603. doi:10.1016/j.molmed.2011.05.013. [PubMed: 21839683]
35. Mackey RH, Mora S, Bertoni AG, et al. Lipoprotein particles and incident type 2 diabetes in the multi-ethnic study of atherosclerosis. Diabetes Care. 2015; 38(4):628–36. doi:10.2337/dc14-0645. [PubMed: 25592196]
36. El Harchaoui K, Arsenault BJ, Franssen R, et al. High-density lipoprotein particle size and concentration and coronary risk. Ann. Intern. Med. 2009; 150(2):84–93. [PubMed: 19153411]

37. Akinkuolie AO, Paynter NP, Padmanabhan L, Mora S. High-density lipoprotein particle subclass heterogeneity and incident coronary heart disease. Circ. Cardiovasc. Qual. Outcomes. 2014; 7(1): 55–63. doi:10.1161/CIRCOUTCOMES.113.000675. [PubMed: 24248942]

38. Ansell BJ, Fonarow GC, Fogelman AM. The paradox of dysfunctional high-density lipoprotein. Curr. Opin. Lipidol. 2007; 18(4):427–34. doi:10.1097/MOL.0b013e3282364a17. [PubMed: 17620860]

39. G HB, Rao VS, Kakkar VV. Friend Turns Foe: Transformation of Anti-Inflammatory HDL to Proinflammatory HDL during Acute-Phase Response. Cholesterol. 2011; 2011:274629. doi: 10.1155/2011/274629. [PubMed: 21490770]

40. Van Linthout S, Frias M, Singh N, De Geest B. Therapeutic potential of HDL in cardioprotection and tissue repair. Handb. Exp. Pharmacol. 2015; 224:527–65. doi:10.1007/978-3-319-09665-0_17. [PubMed: 25523001]

41. Rohatgi A, Khera A, Berry JD, et al. HDL Cholesterol Efflux Capacity and Incident Cardiovascular Events. N. Engl. J. Med. 2014 141118051511004. doi:10.1056/NEJMoa1409065.

42. Mora S, Wenger NK, Demicco D a, et al. Determinants of residual risk in secondary prevention patients treated with high-versus low-dose statin therapy: the Treating to New Targets (TNT) study. Circulation. 2012; 125(16):1979–87. doi:10.1161/CIRCULATIONAHA.111.088591. [PubMed: 22461416]

- HDL-P is strongly and inversely associated with all-cause mortality
- The HDL-P association is independent of HDL cholesterol (HDL-C)
- Moreover, the HDL-P association is accounted for by smaller HDL-P
- Smaller HDL-P improves mortality risk discrimination and reclassification
- Smaller HDL-P may help to further risk stratify high-risk individuals

Table 1

Baseline Characteristics of the CATHGEN Cohort (n = 3992)

	Full Cohort	All-cause Mortality at Mean of Eight Years		
	(n = 3992)	No (n = 2811)	Yes (n = 1181)	p Value
Age, y	60.2 ± 11.4	58.2 ± 11	64.9 ± 11.1	<0.0001
Male	2467 (61.8)	1716 (61.1)	751 (63.6)	0.13
Race/ethnicity				0.03
Black	772 (19.3)	541 (19.3)	231 (19.6)	
White	2952 (74.0)	2067 (73.5)	885 (74.9)	
Native American	152 (3.8)	107 (3.8)	45 (3.8)	
Other race	116 (2.9)	96 (3.4)	20 (1.7)	
BMI, kg/m^2	30.4 ± 7.4	30.7 ± 7.3	29.5 ± 7.4	<0.0001
Smoking	1988 (49.8)	1325 (47.1)	663 (56.1)	<0.0001
HTN	2692 (67.4)	1861 (66.2)	831 (70.4)	0.01
Diabetes	1149 (28.8)	722 (25.7)	427 (36.2)	<0.0001
Family history of CAD	1460 (36.6)	1016 (36.1)	444 (37.6)	0.38
History of prior MI	1144 (28.7)	720 (25.6)	424 (35.9)	<0.0001
CAD present on angiography	2571 (64.4)	1701 (60.5)	870 (73.7)	<0.0001
Number of diseased vessels (≥75% stenosis)				<0.0001
0	1638 (42.8)	1259 (46.7)	379 (33.5)	
1	921 (24.1)	676 (25.1)	245 (21.7)	
2	619 (16.2)	386 (14.3)	233 (20.6)	
3	651 (17)	377 (14)	274 (24.2)	
Heart failure	1027 (26.4)	570 (20.8)	457 (39.6)	<0.0001
Left ventricular EF	55.8 ± 13.5	57.5 ± 12.5	51.7 ± 14.9	<0.0001
Chronic kidney disease	72 (1.8)	27 (1.0)	45 (3.8)	<0.0001
Hyperlipidemia	2372 (59.4)	1664 (59.2)	708 (60)	0.66
Follow-up, days	2942 (2244-3664)	3296 (2619-3781)	1714 (769-2578)	<0.0001
All-cause death	1181 (29.6)	0	1181	
MI	273 (6.8)	168 (6)	105 (8.9)	<0.001
Total cholesterol, mg/dL	158.3 ± 40	160.3 ± 38.6	153.4 ± 42.9	<0.0001
LDL-C, mg/dL	98.2 ± 33.2	99.9 ± 32.3	94 ± 34.8	<0.0001
LDL-P, nmol/L	1165.3 ± 387.2	1176.3 ± 378	1139 ± 407.1	<0.001
HDL-C, mg/dl	38.1 ± 11.2	38.4 ± 10.9	37.4 ± 11.9	<0.001
HDL-P, umol/L	28.9 ± 6.2	29.6 ± 5.9	27.2 ± 6.5	<0.0001
Large HDL-P, umol/L	4.4 ± 2.7	4.2 ± 2.6	4.7 ± 2.8	<0.0001
Medium HDL-P, umol/L	10.3 ± 5.9	10.5 ± 6.0	9.8 ± 5.4	<0.01
Small HDL-P, umol/L	14.3 ± 6.1	14.9 ± 6.0	12.7 ± 6.2	<0.0001
HDL size, nm	9.2 ± 0.5	9.1 ± 0.4	9.3 ± 0.6	<0.0001
Non-HDL-C, mg/dL	120.1 ± 37.8	121.8 ± 36.7	115.9 ± 40	<0.0001
Triglycerides, mg/dL	134.1 ± 93.7	133.1 ± 93.5	136.5 ± 94.2	0.22

Values are mean ± SD, n (%), or median (interquartile range)

Table 2

Cox Proportional Hazards Models Showing the Association of HDL-C, HDL-P and HDL Subclasses with All-Cause Mortality

	All-Cause Mortality (n=1181/3972)		
Parameter	**Model‡ LR χ2**	**HR (95%CI)***	**p Value**
HDL-C	552	0.93 (0.87-0.99)	0.02
HDL-P	654	0.71 (0.67-0.76)	<0.0001
Large HDL-P	709	1.03 (0.97-1.1)	0.29
Medium HDL-P		0.73 (0.68-0.78)	<0.0001
Small HDL-P		0.64 (0.6-0.69)	<0.0001
MS-HDL-P	698	0.68 (0.63-0.72)	<0.0001
HDL size	631	1.33 (1.25-1.41)	<0.0001
MS-HDL-P	719	0.73 (0.68-0.78)	<0.0001
HDL size		1.16 (1.09-1.23)	<0.0001
HDL-C	699	1.02 (0.96-1.09)	0.51
MS-HDL-P		0.67 (0.63-0.72)	<0.0001

*Hazard ratio and 95% confidence interval per 1.0 population standard deviation

‡Adjusted for age, race, sex, diabetes, HTN, LDL-C, smoking status, BMI, CAD, ejection fraction

Table 3

Improvement of Discrimination and Reclassification of Mortality Risk with the Use of MS-HDL-P

Model	χ^2	$\Delta\chi 2$	p Value	NRI	p Value	IDI	p Value
GRACE model	343						
GRACE model + MS-HDL-P	480	13 7	<0.0001	0.1 3	<0.0001	0.0 3	<0.0001

NRI denotes net reclassification index; IDI, integrated discrimination improvement

Table 4

Reclassification of Patients Into Risk Categories of Death by Addition of MS-HDL-P to the GRACE Model

Participants With Death Outcome (n = 503)

	GRACE Model Plus MS-HDL-P				
GRACE Model	**Total**	**<5%**	**5% to <10%**	**10% to <20%**	**≥20%**
<5%	6	4	2	0	0
5% to <10%	57	9	27	20	1
10% to <20%	210	2	21	129	58
≥20%	230	0	1	37	192
Total	503	15	51	186	251

51 Moved to lower risk

70 Moved to higher risk

Participants Free From Death Outcome (n = 2706)

	GRACE Model Plus MS-HDL-P				
GRACE Model	**Total**	**<5%**	**5% to <10%**	**10% to <20%**	**≥20%**
<5%	126	85	40	1	0
5% to <10%	835	147	530	152	6
10% to <20%	1151	20	299	690	142
≥20%	594	0	5	173	416
Total	2706	252	874	1016	564

644 Moved to lower risk

341 Moved to higher risk

All Participants With Actual Event Rate in Parentheses (n = 320 9)

	GRACE Model Plus SM-HDL-P				
GRACE Model	**Total**	**<5%**	**5% to <10%**	**10% to <20%**	**≥20%**
<5%	132	89(4.5)	42(4.8)	1(0)	0(0)
5% to <10%	892	156(5.8)	557(4.9)	172(11.6)	7(14.3)
10% to <20%	1361	22(9.1)	320(6.6)	819(15.8)	200(29.0)
≥20%	824	0(0)	6(16.7)	210(17.6)	608(31.6)
Total	3209	267	925	1202	815