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HDL subspecies defined by presence of apolipoprotein C-III and incident coronary heart disease in four cohorts

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Abstract

Background—The causal role of high density lipoprotein (HDL) cholesterol in cardioprotection has been questioned by genetic and randomized studies. Novel measures that relate to HDL function may contribute new information to prediction of cardiovascular risk. Apolipoprotein C-III (apoC-III) is a key regulator of lipoprotein metabolism. We investigated whether subspecies of HDL defined by apoC-III are associated with coronary heart disease (CHD).

Methods—We used immuno-affinity chromatography to measure the apoA-I concentrations of HDL that contains and lacks apoC-III in two prospective studies of adults free of CHD. In the Multi-Ethnic Study of Atherosclerosis (MESA), 5,657 participants (52% women; age 52–72 y) were followed for risk of CHD from 2000–2002 through 2013. In a case-cohort study nested within the Danish Diet, Cancer and Health (DCH) study, 3,642 participants (47% women; age 51–64 y) were followed from 1994–1997 through 2010. Subsequently, we conducted a meta-analysis that combined these results with the previously published findings from two cohort studies that used similar laboratory methodology to measure lipoproteins, totaling 2,997 incident cases.

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Results—ApoC-III was found on 6-8% of apoA-I. The two HDL subspecies showed opposing associations with risk of CHD in each of the individual cohorts and in the meta-analysis (p-heterogeneity between the two subspecies <0.01). HDL that contains apoC-III was associated with higher risk of CHD (pooled relative risk [RR] per SD = 1.09, 95% confidence interval [95% CI] = 1.01 to 1.18), whereas HDL that lacks apoC-III was associated with lower risk (RR= 0.76; 95% CI = 0.70 to 0.83). The relative risk for HDL lacking apoC-III was even more negative than the relative risk for total HDL (RR= 0.80; 95% CI = 0.74 to 0.87).

Conclusions—Our findings from four prospective studies support the hypothesis that apoC-III may mark a subfraction of HDL that is associated with higher risk of CHD. New measures reflecting HDL structure and function may provide novel insights for cardiovascular risk that extend beyond traditional plasma HDL cholesterol concentrations.

Keywords

CHD; cohort study; HDL; biomarker

Introduction

High density lipoprotein (HDL) cholesterol is a strong, inverse predictor of coronary heart disease (CHD).¹ However, the potential causal role of HDL-cholesterol in cardioprotection has been called into question by randomized studies of potent HDL-cholesterol-raising drugs that failed to lower CHD rates.²⁻⁴ Genetic observational studies that do not find fewer cardiovascular events among individuals with genetically determined high HDL-cholesterol levels further highlight the limitations in using HDL-cholesterol to evaluate novel therapeutics aimed at CHD-reduction.^{5, 6}

Emerging measures of key anti-atherogenic properties of HDL, such as its capacity to mediate cholesterol efflux from macrophages, are not captured by average HDL-cholesterol levels.^{7, 8} The strong inverse association between cholesterol efflux capacity and cardiovascular events suggests that markers reflecting functional properties of HDL, rather than its cholesterol concentration alone, may substantially contribute to our understanding of HDL's role in atherosclerosis.

The heterogeneous protein composition of HDL led to the proposal that HDL circulates in subtypes based on the presence and absence of certain apolipoproteins that guide their downstream metabolism and function by mediating their interactions with enzymes and receptors.⁹ Growing evidence suggests apoC-III, a small apolipoprotein present on some but not all VLDL, LDL and HDL particles, may play a key role in lipid metabolism.¹⁰⁻¹² Rare mutations in the *APOC3* gene that lower circulating apoC-III levels also associate with lower triglycerides and a greatly reduced risk of cardiovascular disease.^{13, 14} ApoC-III inhibits hepatic clearance from the circulation of apoB-containing lipoproteins by blocking the interaction of apoE and apoB100 with hepatic receptors, and in large amounts, it can inhibit lipases that metabolize the triglyceride in these lipoproteins.^{12, 15, 16} It is not known if apoC-III affects HDL metabolism. However, apoC-III diminishes the protective functions of HDL that are unrelated to lipoprotein metabolism such as inhibition of monocyte adhesion to endothelial cells, an early event in atherosclerosis.¹⁷ In US male health

professionals and female nurses, we found that the cholesterol concentrations of HDL defined by the presence or absence of apoC-III have opposite associations with CHD.¹⁸ However, this relatively small study was not powered to investigate potential susceptible subgroups and did not include measures of apoA-I, the main structural protein of HDL that is strongly associated with risk of CHD.

To investigate whether subspecies of HDL-apoA-I containing or lacking apoC-III are associated with incident CHD, we prospectively studied this in two large population-based studies with greater racial and ethnic diversity and a large number of well-defined incident events. We further meta-analyzed all current data on HDL subspecies according to apoC-III totaling over 2900 incident events and queried for potential effect modification by important cardiovascular risk factors including obesity, smoking, and clinical HDL-cholesterol measures.

Methods

The data, analytic methods, and study materials will be made available to other researchers for purposes of reproducing the results only with proper IRB approvals and strict adherence to cohort-specific regulations. Requests for data can be directed to senior authors: RMC, KO and EBR.

Study populations and clinical endpoints

Between 2000 and 2002, the Multi-Ethnic Study of Atherosclerosis (MESA) enrolled 6,814 men and women aged 45-84 years from six regions in the U.S. who self-identified as primarily of Caucasian, Chinese, African or Hispanic descent, and were free of clinical CVD.¹⁹ Information on demographics, anthropometrics, and medications were obtained at baseline through questionnaires and physical examinations. Incident CHD events (myocardial infarction [MI], resuscitated cardiac arrest, and CHD death) were ascertained and adjudicated as previously reported.²⁰ By the end of follow-up in December 2013, 300 incident CHD events had occurred.

The Diet, Cancer, and Health (DCH) is a population-based study started in 1993-1997 when 57,053 Danish born residents, aged 50 to 65 years and free of cancer, participated in a clinical examination and detailed lifestyle survey.²¹ All incident CHD events occurring between baseline and May 2008 (MI, resuscitated cardiac arrest, and fatal CHD) were included by linking participants to Danish registers via the unique identification number assigned to all Danish citizens (see online supplement for details on registers).²²⁻²⁴ The total number of confirmed incident cases of CHD between study entry and May 2010 was 2,063.

Study designs and laboratory measurements

Blood was sampled at the baseline study visit in both studies and stored at -70°C in MESA and -150°C in the Danish study. Our lipid laboratory at the Harvard T.H. Chan School of Public Health received plasma from all MESA participants except 1000 participants excluded by the study Steering Committee due to extensive laboratory testing on baseline samples, leading to depletion of stored plasma in these randomly chosen individuals (included $n=5,796$). In the DCH study, a case-cohort was designed for the assessment of

blood markers.²⁵ Plasma samples were received from all incident CHD cases and a sub-cohort of randomly sampled participants drawn from the study population who were free of CHD at baseline (n=1,824).

HDL subspecies according to apoC-III were assessed by concentrations of apoA-I with and without apoC-III in both studies. In MESA, a modified sandwich ELISA first fractionates plasma by binding lipoproteins containing apoC-III using affinity-purified anti-human apoC-III and subsequently quantifies the concentration of apoA-I in the apoC-III-bound and unbound fractions by standard ELISA assays. Additionally, the concentration of total plasma apoC-III was measured by ELISA separately. Upon exclusion of 45 participants with implausible apolipoprotein ranges, 27 with missing information on CHD events, 67 with missing covariate data, 5,657 MESA participants remained for our analyses.

In the assessment of DCH samples, we used immuno-affinity chromatography with anti-apoC-III antibody to separate the plasma into fractions with and without apoC-III. Subsequently, concentrations of apoA-I in the apoC-III-bound and unbound fractions were assessed by standard ELISA. To compare with our earlier report where HDL was quantified using measures of HDL-cholesterol concentrations rather than apoA-I concentrations,¹⁸ we also assessed the concentrations of cholesterol (in HDL) containing and lacking apoC-III upon precipitation of the apoB-containing lipoproteins from the apoC-III-defined immunofractions. Total plasma levels of triglycerides, apoB, apoC-III, and apoC-III in the HDL fraction were also assessed in our lipid laboratory. From the original 3,830 participants in the DCH case-cohort, 161 did not have enough samples left for qualitative analyses in the lipid laboratory. We also excluded 27 samples that did not pass quality control and therefore the final case-cohort set included 1,949 incident CHD cases and 1,693 non-cases. Due to the case-cohort design, 57 incident CHD cases also belonged to the reference sub-cohort (total n=1,750). More details on laboratory methodology are available in online supplement.

Additional Cohorts for Meta-analysis

To provide the most robust estimate of risk, we considered all available studies of incident CHD that incorporated the measures of HDL subspecies according to apoC-III content and conducted a cumulative meta-analysis. The inclusion of our previous investigations of HDL-cholesterol containing and lacking apoC-III in case control studies nested within the Nurses' Health Study [NHS] and the Health Professionals Follow-Up Study [HPFS] (details in supplement),¹⁸ summed to a total of 2,997 incident CHD events.

In a subset of the HPFS controls (n=301), cholesterol efflux from macrophages was also measured using methods previously described.⁸

Statistical Analyses

We assessed characteristics of the MESA participants and the random sub-cohort of the DCH using means (medians of skewed variables) and proportions of covariates of interest. In both MESA and DCH, risk of incident CHD was analyzed using Cox proportional hazards regression where participants contributed person-time from baseline until the date of an event, death, or end of follow-up, whichever occurred first. In the DCH, Kalbfleisch and Lawless weights and robust variance suitable for the case-cohort data were applied.²⁶ All

analyses adjusted for age and sex; multivariable-adjusted models also included all traditional ASCVD (atherosclerotic cardiovascular disease) risk factors except for HDL cholesterol. Thus, prevalent diabetes, treatment with anti-hypertensive medication, race/ethnicity (MESA only), systolic blood pressure, smoking, and total cholesterol were included in multivariable models.²⁷ The two subfractions of HDL-apoA-I were simultaneously included in all analyses of these subspecies as they sum to total HDL-apoA-I. In each study, we assessed the heterogeneity between the two associations of HDL containing and lacking apoC-III with risk of CHD in Cox regression models with both subfractions included as linear terms per study-specific SDs as well as per 5 mg/dL. We then tested the linear hypothesis that the regression coefficients for the two subfractions were equal (using a 1 d.f. Wald test).

Initial evaluations found no statistically significant difference in associations of HDL subspecies with risk of CHD across sex and race/ethnicity (MESA only), and therefore results from the total study population are presented. No evidence for non-proportionality of hazards was found by examining martingale residuals. A subsequent model additionally included log-transformed plasma triglycerides and LDL-cholesterol (available in MESA) or total apolipoprotein B (available in DCH) concentrations to address whether the associations were independent of adjustment for these clinical risk factors.

The results based on the NHS/HPFS data were used as previously reported (also available in supplement),¹⁸ and with the additional measures of cholesterol efflux in a subset of HPFS controls, we estimated the age-adjusted spearman correlations with the apoC-III based HDL subspecies.

Meta-analysis

To test relationships with CHD across the diverse measures and study designs, we performed meta-analyses per study-specific SDs, quintiles, and per 5 mg/dL fixed increment of the HDL measures containing or lacking apoC-III. In the absence of between-study heterogeneity, we estimated the fixed-effects.²⁸ We also pooled all estimates from cohort-specific analyses of HDL subspecies with risk of CHD in strata of CVD risk factors; sex, obesity, smoking, diabetes, use of cholesterol-lowering medication, and clinically-defined low HDL-cholesterol (HDL-cholesterol < 40 mg/dL).²⁹

Statistical analyses were performed using SAS 9.4 (SAS Institute; Cary, NC) and STATA 11 (STATA Corp., College Station, TX).

The first author had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Written informed consent was obtained from all participants and the protocol was approved by the institution review board at the Harvard T. H. Chan School of Public Health, all MESA study sites, the Danish National Committee on Health Research Ethics (journal nr. (KF) 01-345/93), and the Danish Data Protection Agency.

Results

The mean age at study entry was 63 years in MESA and 57 years in DCH. In MESA, 52% were female, and 37% self-identified as Caucasians, 12% as Chinese, 29% as African-Americans, and 22% as Hispanics, whereas DCH participants were Danish-born residents (Table 1). At baseline, 17% of MESA participants reported use of lipid-lowering medication, whereas this was uncommon in Denmark (<1% reported use). Similarly, despite higher average systolic blood pressure in Danes, fewer reported a physician diagnosis of hypertension, and taking medicine for this was uncommon. The median apoA-I levels were 125 mg/dL in MESA and 136 mg/dL in DCH and, on average, 6-8% of the HDL-apoA-I contained apoC-III.

The age-adjusted spearman correlation (in a subset of the HPFS participants) between cholesterol efflux and the proportion of HDL cholesterol that has apoC-III was 0.12 ($p=0.2$). Age- and sex-adjusted spearman correlations of the HDL subspecies amongst each other (0.7, $p < 0.01$) and with triglycerides and total apoC-III in Supplemental Table S1 were based on the MESA cohort. While total apoC-III was moderately correlated with triglycerides (0.6, $p < 0.01$), the HDL subspecies containing or lacking apoC-III were weakly or not correlated with triglycerides.

Associations with risk of coronary heart disease in MESA and DCH

The concentration of total HDL-apoA-I was inversely associated with the risk of CHD in both cohorts (Table 2). However, the two HDL subspecies defined by the presence versus absence of apoC-III were differentially associated with the risk of CHD. Only HDL-apoA-I lacking apoC-III was inversely associated with risk of CHD (hazard ratio per SD = 0.83; 95% confidence interval [CI], 0.71 to 0.96 in MESA and 0.75, 95% CI, 0.66 to 0.84 in DCH), whereas the risk associated with each SD increment in HDL-apoA-I containing apoC-III was 1.09 (0.95 to 1.25) in MESA and 1.03 (0.92 to 1.15) in DCH. The relative risks associated with HDL containing and lacking apoC-III were significantly different from each other (p for heterogeneity in multivariable-adjusted models was 0.03 in MESA and 0.01 in DCH).

Measures of apoC-III itself, including the absolute concentration of apoC-III within the plasma HDL fraction (only available in DCH) and total plasma apoC-III both showed tendencies for higher risk of CHD with higher levels, but neither of them were statistically significant in the fully-adjusted models (Table 2).

In the DCH, we obtained measures of apoC-III defined HDL subspecies by assessing both HDL-cholesterol concentrations and apoA-I concentrations. Results for HDL-cholesterol containing and lacking apoC-III were similar to those reported for HDL subspecies based on apoA-I measures (Supplemental Table S2). Cholesterol concentrations of the HDL subspecies were not measured in MESA.

Meta-analysis of four studies

The divergent associations observed for HDL-apoA-I containing and lacking apoC-III in MESA and DCH were similar to the results observed using HDL-cholesterol measures in

both DCH (Supplemental Table S3) and the previous report from the Nurses' Health and Health Professionals Follow-Up Studies (results from all four cohorts shown using study-specific quintiles and SDs in fig 1).¹⁸ We also repeated the analysis in each cohort using a fixed increment of 5 mg/dL for both HDL subfractions. The results, which were very similar to those shown in fig 1, are included in online supplement Table S4. Thus, we pooled all cohorts with analyses of HDL subspecies according to apoC-III and found the multivariable-adjusted relative risk for CHD per each SD higher HDL lacking apoC-III was 0.76 (0.70 to 0.83) and the relative risk for HDL containing apoC-III was 1.09 (1.01 to 1.18). (fig 2, and study-specific quintile medians presented in supplemental Table S5 and forest plots in figures S1 and S2) Using 5 mg/dL increments, the relative risks were 0.96 (0.93 to 0.98) and 1.13 (0.96 to 1.30), respectively. The association with CHD for HDL containing apoC-III was statistically significantly different than for HDL lacking apoC-III (p for heterogeneity <0.01). Adjustment for triglycerides and LDL cholesterol attenuated the results but HDL lacking apoC-III remained inversely associated with risk of CHD (relative risk per SD =0.84; 0.75 to 0.95) and the relative risk per each SD of HDL containing apoC-III was 1.05 (0.96 to 1.14) (p for heterogeneity=0.04).

In meta-analyses, the proportion of HDL containing apoC-III was associated with higher risk of CHD (relative risk per SD=1.09; 1.03 to 1.16), while each SD higher total HDL was associated with a relative risk of 0.80 (0.74 to 0.87). (Supplemental fig S3)

Analyses in strata of CVD risk factors

We analyzed the risk of CHD for each SD higher HDL containing and lacking apoC-III according to CVD risk factors in each of the four cohorts and pooled the data using fixed-effects meta-analysis. Shown in fig 3, HDL lacking apoC-III was generally inversely associated with risk of CHD across all participant characteristics, whereas HDL containing apoC-III was associated with higher risk of CHD. As indicated by the confidence intervals, some subgroups had few cases (women, prevalent diabetes, use of cholesterol lowering medication, and low HDL-cholesterol level). Nevertheless, the stratified results suggested that HDL subspecies are differentially associated with CHD risk broadly across several participant characteristics associated with CHD risk. The results appeared to be slightly stronger in groups of participants characterized by lower cardiovascular risk, such as among those who were normal-weight, non-smokers, free of diabetes, who did not use cholesterol-lowering medication, or had HDL-cholesterol greater than 40 mg/dL, but the differences were not significant. Analyses in strata of self-reported race/ethnicity of the MESA participants only were not statistically powered to highlight potential differences across these subgroups (Supplemental Table S6).

Discussion

In this investigation of two large prospective studies, separation of HDL-apoA-I according to the presence and absence of apoC-III identified two subtypes of HDL that had qualitatively different associations with risk of CHD. In a cumulative meta-analysis of all current data on these HDL subspecies, the statistically significant direct association of HDL containing apoC-III with risk of CHD was strikingly different from the strong, inverse association for

HDL lacking apoC-III, which appeared even stronger than for the association with total HDL.

While the fraction of HDL that contains apoC-III is small (approximately 5-6% of apoA-I or 10-15% of HDL cholesterol has apoC-III), these investigations may shed light on ways in which the HDL proteome could result in HDL particles with more or less cardioprotective functions. Thus far, little is known about the potential effects of apoC-III on important functional aspects of HDL. We have previously demonstrated that apoC-III inhibits the clearance from the circulation of apoB-containing lipoproteins by interfering with the interaction of apoE with hepatic receptors,¹² and preliminary data from our laboratory support a similar delayed clearance of HDL containing apoC-III. We observed a weak correlation between the proportion of HDL that contains apoC-III and cholesterol efflux (0.12), consistent with their separate biological roles. As such, the measure of HDL subspecies according to apoC-III may be viewed as complementary to that of cholesterol efflux capacity via ABCA1, which is focused on the initial lipidation of the apoA-I particle, but does not reflect the delivery of cholesterol to the liver. Further, apoC-III may have influence beyond cholesterol transport. Riwanto *et al.* found that compared to healthy patients, HDL from CHD patients had elevated apoC-III levels and had lost its capacity to inhibit endothelial cell apoptosis.³⁰ Earlier, we found that HDL that lacks apoC-III inhibits the monocyte-endothelial cell interaction, leading to a lowered inflammatory response, whereas HDL with apoC-III did not diminish this interaction.³¹ Thus, even if total HDL levels are high, this would not translate into a lower risk of CHD in individuals who have a large proportion of HDL containing apoC-III.

We also measured the total plasma concentration of apoC-III and the concentration of apoC-III in HDL in the Danish study. Consistent with the literature, we observed that while apoC-III is directly associated with CHD risk, it is not robust to adjustment for clinical risk factors and other lipids,^{18, 32} suggesting that the concentrations of lipoproteins (HDL and LDL) that contain *any* apoC-III may be more relevant cardiovascular risk measures than are total plasma apoC-III or the concentration of apoC-III in LDL and HDL.^{18, 33}

Strengths of this study include its large number of cases and the opportunity to look into potential effect modification by race/ethnicity and sex. However, we had limited statistical power for stratified analyses by prevalent diabetes status and use of lipid-lowering medications. In the meta-analysis, we included two prior publications where the surrogate measure of HDL was HDL-cholesterol concentration, rather than apoA-I. Hence, we used study-specific SDs and quintiles as our primary units of measurement as no universal cutpoints have been identified. Similar results ensued from using fixed increments of 5 mg/dL and overall the two measures of HDL, cholesterol and apoA-I, provided associations of same magnitude and direction. The direct measure of the concentration of apoA-I containing and lacking apoC-III using two antibodies has shortened the process of measuring the HDL subspecies considerably and reduced measurement error, making this biomarker measure well-suited for assessment in future large-scale epidemiologic settings.

We had information on LDL-cholesterol or apoB in both MESA and DCH, but did not measure LDL with and without apoC-III in those studies. However, we previously found in

NHS and HPFS that adjustment for LDL subspecies had virtually no effect on estimates for HDL subspecies after adjustment for total LDL-cholesterol.¹⁸

While our data suggest that apoC-III may confer pro-atherogenic properties to HDL, further research is necessary to establish how measures of HDL containing and lacking apoC-III relate to functional properties of HDL, such as cholesterol efflux from cells, inflammation, or cholesterol excretion.

Lastly, our HDL measurements from a single time point may not reflect long-term exposure, and does not allow the evaluation of associations between changes in HDL subspecies distribution with the risk of CHD.

In conclusion, our findings from four prospective studies support the hypothesis that apoC-III may mark a subfraction of HDL that is associated with higher risk of CHD. New measures reflecting HDL structure and function may provide novel insights for cardiovascular risk that extend beyond traditional plasma HDL concentrations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Clinical Perspective

What is new?

- We identified a subspecies of HDL that contains apoC-III. HDL containing apoC-III comprises 5-6% of apoA-I or 10-15% of HDL cholesterol.
- In four prospective studies, HDL containing apoC-III was associated with a greater risk of coronary heart disease, whereas HDL that lacked apoC-III was inversely associated with risk, more strongly than the total HDL.

What are the clinical implications?

- ApoC-III may adversely affect anti-atherogenic properties of HDL.
- HDL containing apoC-III may be useful to evaluate response to future HDL-raising therapies.
- Measures that reflect function or structure of HDL may provide information on cardiovascular risk beyond traditional HDL concentrations.

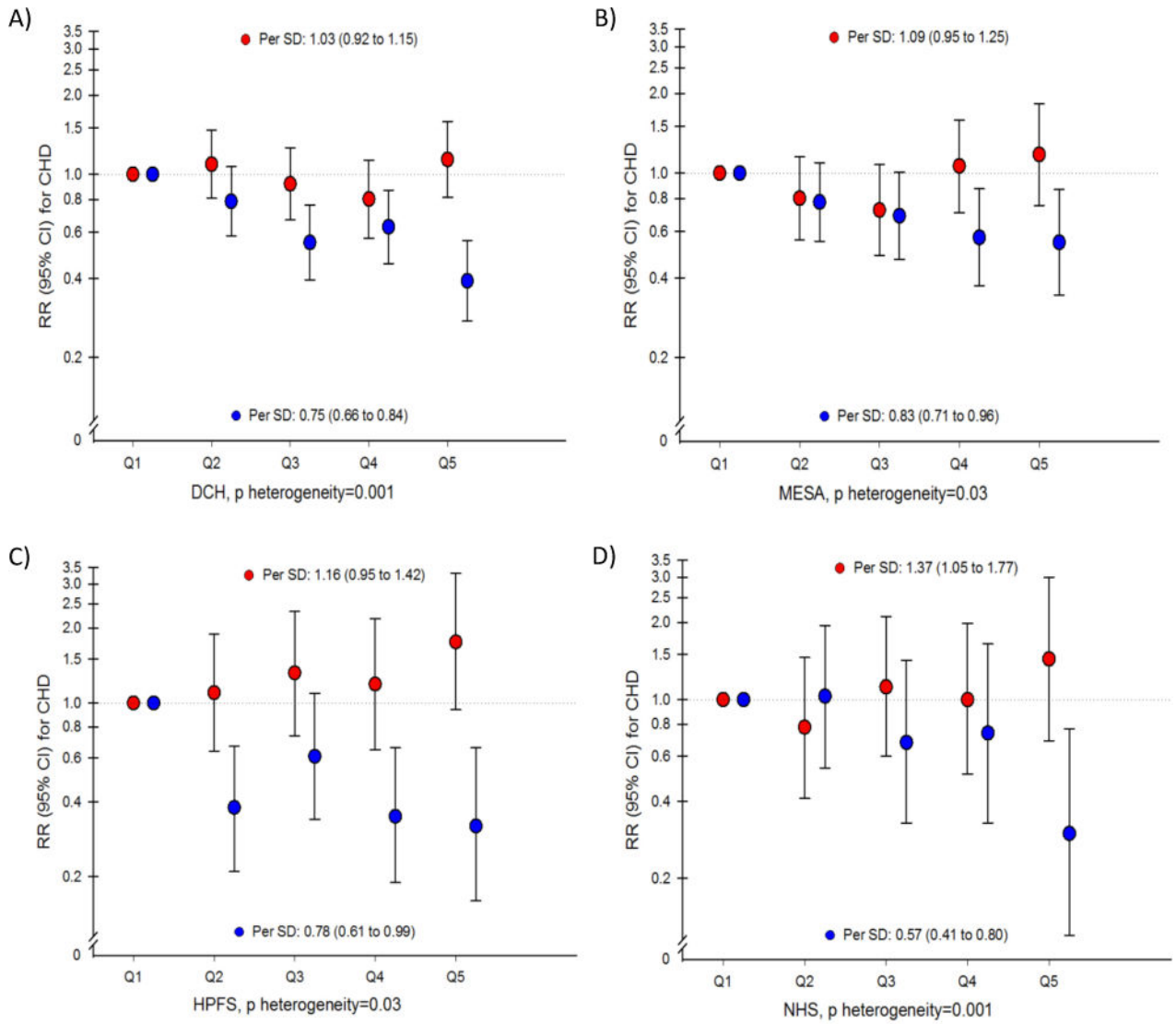


Fig 1. Coronary heart disease risk associated with HDL containing (red) and HDL lacking apoC-III (blue) in four prospective studies

Relative risks with 95% CI for risk of CHD estimated in four prospective studies (A) the Diet, Cancer and Health [DCH] Study, B) the Multi-Ethnic Study of Atherosclerosis [MESA], C) the Health Professionals Follow-Up Study [HPFS], and D) the Nurses' Health Study [NHS]) per SD and using study-specific quintiles. Adjusted for ASCVD (atherosclerotic cardiovascular disease) risk factors: age, sex, prevalent diabetes, treatment with anti-hypertensive medication, (race/ethnicity in MESA), systolic blood pressure, smoking, and total cholesterol. Both subtypes of HDL (containing and lacking apoC-III) are simultaneously included in all models. P heterogeneity = test of the linear hypothesis that the regression coefficients for the two subfractions were equal (1 d.f. Wald test).

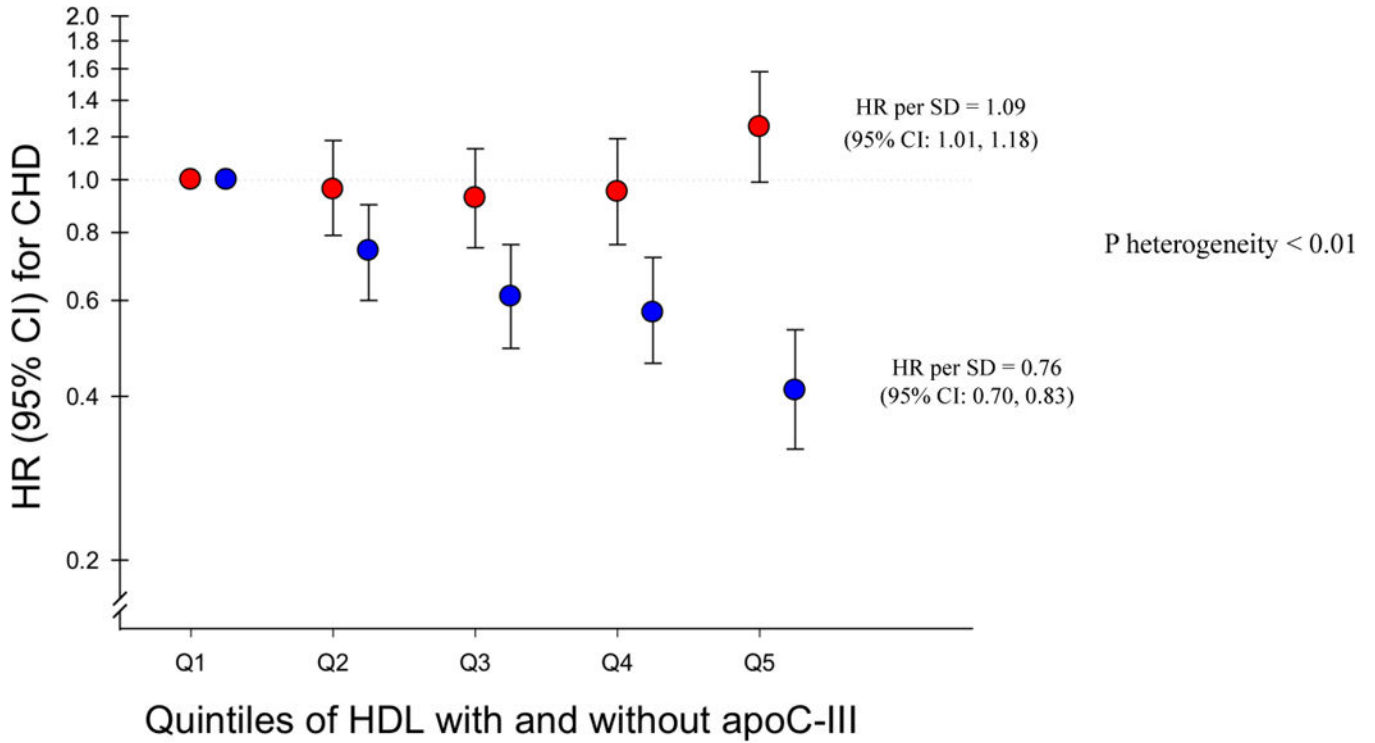


Fig 2. Coronary heart disease risk associated with HDL containing (red dot) and HDL lacking apoC-III (blue dot) in meta-analysis of four prospective studies
 Pooled relative risks with 95% CI for risk of CHD estimated in fixed-effects meta-analysis using study-specific SDs and study-specific quintiles in four prospective studies (the Diet, Cancer and Health [DCH] Study, the Multi-Ethnic Study of Atherosclerosis [MESA], the Health Professionals Follow-Up Study [HPFS], and the Nurses' Health Study [NHS]). Adjusted for ASCVD (atherosclerotic cardiovascular disease) risk factors: age, sex, prevalent diabetes, treatment with anti-hypertensive medication, (race/ethnicity in MESA), systolic blood pressure, smoking, and total cholesterol. HDL assessed per apoA-I concentrations in MESA and DCH and per HDL-cholesterol concentrations in NHS and HPFS. Both subtypes of HDL (containing and lacking apoC-III) are simultaneously included in all models. P heterogeneity = test of the linear hypothesis that the regression coefficients for the two subfractions were equal (1 d.f. Wald test).

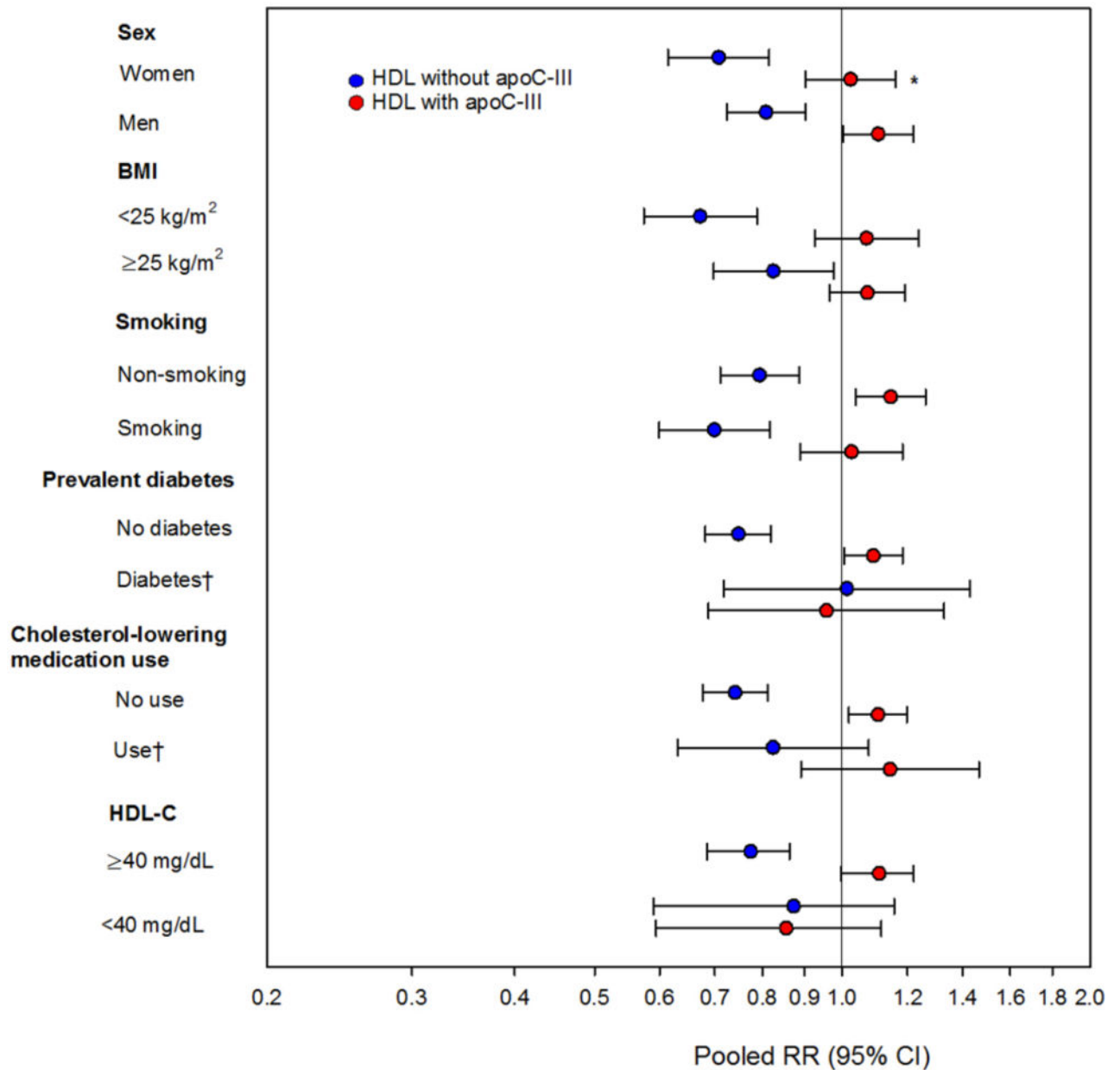


Fig 3. Coronary heart disease risk per standard deviation of HDL containing (red) and lacking (blue) apoC-III in strata of CVD-risk factors in four prospective studies
 Pooled relative risks with 95% CI for risk of CHD estimated in fixed-effects meta-analysis using study-specific SDs in four prospective studies (the Diet, Cancer and Health [DCH] Study, the Multi-Ethnic Study of Atherosclerosis [MESA], the Health Professionals Follow-Up Study [HPFS], and the Nurses' Health Study [NHS]). Adjusted for ASCVD (atherosclerotic cardiovascular disease) risk factors: age, sex, prevalent diabetes, treatment with anti-hypertensive medication, (race/ethnicity in MESA), systolic blood pressure, smoking, and total cholesterol.
 Both subtypes of HDL (containing and lacking apoC-III) are simultaneously included in all models.

*Between-study heterogeneity, $p < 0.05$.

†Estimates not available in NHS and HPFS.

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Table 1

Baseline characteristics of participants from the Multi-Ethnic Study of Atherosclerosis (MESA) and the randomly selected sub-cohort from Diet, Cancer and Health (DCH) study

Variables	MESA N=5657	DCH sub-cohort N=1750
Age, mean years (SD)	62.7 (10.3)	56.5 (4.4)
Female, <i>No.</i> (%)	2941 (52.0)	817 (46.7)
Postmenopausal (females only), <i>No.</i> (%)	2444 (83.2)	479 (58.6)
<i>Race/ethnicity, No. (%)</i>		
Caucasian	2102 (37.2)	1750 (100)
Chinese-American	694 (12.3)	0
African-American	1617 (28.6)	0
Hispanic	1244 (22.0)	0
Carotid intima-media thickness, mean μm^* (SD)	978 (361)	N/A
Diabetes, <i>No.</i> (%)	721 (12.8)	31 (1.8)
Hypertension, <i>No.</i> (%)	2578 (45.6)	324 (18.5)
Use of blood pressure lowering drugs, <i>No.</i> (%)	2136 (38)	192 (11)
Hypercholesterolemia, <i>No.</i> (%)	542 (9.6)	140 (8.0)
Use of lipid lowering drugs, <i>No.</i> (%)	953 (16.9)	14 (0.8)
Systolic blood pressure, mean mmHg (SD)	127 (22)	139 (20)
Waist circumference, mean cm (SD)	98.2 (14.3)	89.4 (12.8)
Body mass index, mean kg/m^2 (SD)	28.3 (5.4)	26.1 (4.0)
Physical activity, median MET-hr/wk (IQR) [†]	58 (73)	60 (49)
Current smoker, <i>No.</i> (%)	712 (12.6)	662 (37.8)
No alcohol intake, <i>No.</i> (%)	2547 (45.0)	39 (2.2)
Alcohol intake, mean drinks/wk (SD)	2.3 (5.3)	10.7 (11.1)
HDL-C, median mg/dL (IQR)	48 (19)	52 (25)
HDL-C containing apoC-III, median mg/dL (IQR)	N/A	7.7 (6.7)
HDL-C lacking apoC-III, median mg/dL (IQR)	N/A	43.4 (22.3)
Proportion of HDL-C that contains apoC-III, mean % (IQR)	N/A	14.9 (10.4)
ApoA-I, median mg/dL (IQR)	125 (41)	135.5 (57.3)
ApoA-I containing apoC-III, median mg/dL (IQR)	7.9 (3.6)	11.3 (8.2)
ApoA-I lacking apoC-III, median mg/dL (IQR)	118 (38)	123.4 (52.1)
Proportion of apoA-I that contains apoC-III, mean % (IQR)	6.3 (1.9)	8.2 (4.8)
Total apoC-III, median mg/dL (IQR)	8.7 (4.1)	11.4 (7.5)
ApoC-III in HDL, median mg/dL (IQR)	N/A	5.6 (4.9)
Triglycerides, median mg/dL (IQR)	111 (84)	80.7 (67.4)
Total cholesterol, median mg/dL (IQR)	192 (45)	235.5 (57.9)

* cIMT only available in 5,494 of the MESA participants.

[†] Physical activity includes moderate and vigorous activity. Participants reporting more than 18 hrs/day of total physical activity excluded (MESA, N=4,894 after exclusions).

In DCH hypertension, diabetes, and hypercholesterolemia were self-reported physician-diagnoses.

To convert apoA-I and apoC-III to g/L multiply by 0.01; HDL-C and total cholesterol to mmol/L multiply by 0.0259; triglycerides to mmol/L multiply by 0.0113.

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Table 2
 Apolipoprotein A-I concentrations in total plasma and in subspecies of HDL containing and lacking apoC-III and risk of coronary heart disease. The Multi-Ethnic Study of Atherosclerosis and the Diet, Cancer and Health Study*

	MESA			DCH		
	Per SD	Q5 vs Q1	P	Per SD	Q5 vs Q1	P
Total apoA-I	SD=36.4 mg/dL			SD=45.8 mg/dL		
Age- and sex-adjusted	0.85 (0.74, 0.96)	0.60 (0.41, 0.88)	0.01	0.74 (0.66, 0.83)	0.33 (0.23, 0.45)	<0.0001
+ ASCVD factors	0.87 (0.76, 1.00)	0.65 (0.44, 0.95)	0.04	0.76 (0.67, 0.85)	0.35 (0.25, 0.50)	<0.0001
ApoA-I lacking apoC-III	SD=34.3 mg/dL			SD=41.7 mg/dL		
Age- and sex-adjusted	0.80 (0.69, 0.92)	0.50 (0.32, 0.78)	0.003	0.72 (0.64, 0.81)	0.36 (0.26, 0.51)	<0.0001
+ ASCVD factors	0.83 (0.71, 0.96)	0.55 (0.35, 0.87)	0.01	0.75 (0.66, 0.84)	0.39 (0.28, 0.56)	<0.0001
ApoA-I containing apoC-III	SD=3.3 mg/dL			SD=7.5 mg/dL		
Age- and sex-adjusted	1.10 (0.96, 1.26)	1.26 (0.82, 1.95)	0.16	1.05 (0.95, 1.17)	1.19 (0.85, 1.65)	0.3
+ ASCVD factors	1.09 (0.95, 1.25)	1.18 (0.76, 1.83)	0.24	1.03 (0.92, 1.15)	1.14 (0.82, 1.59)	0.6
Proportion of apoA-I that contains apoC-III	SD=3 %			SD=4 %		
Age- and sex-adjusted	1.05 (0.95, 1.15)	1.27 (0.89, 1.83)	0.34	1.11 (1.01, 1.22)	1.19 (0.87, 1.63)	0.03
+ ASCVD factors	1.03 (0.94, 1.14)	1.20 (0.83, 1.74)	0.49	1.08 (0.98, 1.19)	1.17 (0.85, 1.42)	0.1
Total apoC-III	SD=3.9 mg/dL			SD=6.4 mg/dL		
Age- and sex-adjusted	1.06 (0.94, 1.19)	1.16 (0.82, 1.65)	0.33	1.12 (1.01, 1.25)	1.35 (0.97, 1.90)	0.04
+ ASCVD factors	1.00 (0.88, 1.14)	0.98 (0.67, 1.44)	0.97	1.00 (0.89, 1.13)	0.97 (0.67, 1.42)	0.9
ApoC-III in HDL mg/dL		NA		SD=4.3 mg/dL		
Age- and sex-adjusted		NA		1.09 (0.97, 1.22)	1.00 (0.71, 1.42)	0.16
+ ASCVD factors		NA		1.02 (0.91, 1.15)	0.80 (0.56, 1.14)	0.72

* Hazard Ratios and 95% confidence intervals for coronary heart disease per 1 SD (in mg/dL) increments and for comparison of extreme quintiles. Multivariable model adjusted for ASCVD (atherosclerotic cardiovascular disease) risk factors: for age, sex, prevalent diabetes, treatment with anti-hypertensive medication, race/ethnicity (MESA), systolic blood pressure, smoking, and total cholesterol. P-heterogeneity between continuous measures of apoA-I with and without apoC-III: age- and sex-adjusted model, MESA=0.01, DCH=0.0001; ASCVD factors model, MESA = 0.03; DCH=0.01.