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Lipoprotein Particle Profiles by Nuclear Magnetic Resonance Compared with Standard Lipids and Apolipoproteins in Predicting Incident Cardiovascular Disease in Women

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Abstract

Background—Nuclear magnetic resonance (NMR) spectroscopy measures the number and size of lipoprotein particles, instead of their cholesterol or triglyceride content, but its clinical utility is uncertain.

Methods and Results—Baseline lipoproteins were measured by NMR in 27,673 initially healthy women followed for incident cardiovascular disease (CVD, N=1,015) over 11 years. Adjusting for non-lipid risk factors, hazard ratios (HRs) and 95% confidence intervals (CIs) for top vs bottom quintile of NMR-measured lipoprotein particle concentration (particles/L) were, for low-density lipoprotein (LDL_{NMR}) 2.51 (1.91−3.30), high-density lipoprotein (HDL_{NMR}) 0.91 (0.75−1.12), very-low-density lipoprotein ($VLDL_{NMR}$) 1.71 (1.38−2.12), and LDL_{NMR}/HDL_{NMR} ratio 2.25 (1.80 −2.81). Similarly-adjusted results for NMR-measured lipoprotein particle size (nanometers) were, for LDL_{NMR} size 0.64 (0.52−0.79), HDL_{NMR} size 0.65 (0.51−0.81), and $VLDL_{NMR}$ size 1.37 (1.10 −1.70). Hazard ratios for NMR measures were comparable but not superior to standard lipids: total cholesterol 2.08 (1.63−2.67), LDL cholesterol 1.74 (1.40−2.16), HDL cholesterol 0.52 (0.42−0.64), triglycerides 2.58 (1.95−3.41), non-HDL cholesterol 2.52 (1.95−3.25), total/HDL cholesterol ratio 2.82 (2.23−3.58); and apolipoproteins: B_{100} 2.57 (1.98−3.33), A-1 0.63 (0.52−0.77), B_{100}/A-1 ratio 2.79 (2.21−3.54). There was essentially no reclassification improvement with adding LDL_{NMR} particle concentration or apolipoprotein B_{100} to a model that already included the total/HDL cholesterol ratio and non-lipid risk factors (net reclassification index [NRI], 0% and 1.9%, respectively), nor did the addition of either variable result in a statistically significant improvement in the c-index.

Conclusions—In this prospective study of healthy women, CVD risk prediction associated with lipoprotein profiles evaluated by NMR was comparable but not superior to standard lipids or apolipoproteins.

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Disclosures

Dr Otvos is employed by, is a stockholder of, and serves on the board of directors of LipoScience, Inc., a diagnostic laboratory company that performed the lipoprotein subclass analyses described in the manuscript. Dr Rosenson is a stockholder of LipoScience, Inc., and he serves as a member of its Scientific Advisory Board.

Keywords

Lipoproteins; lipids; apolipoproteins

While current prevention guidelines recommend measurement of standard lipids to assess risk of cardiovascular disease (CVD),[1-3] it has been suggested that alternative lipoprotein measures may improve risk prediction. However, it remains uncertain how well such measures predict CVD when compared with the standard lipids that are routinely obtained in clinical practice.

One method of alternative lipid testing is proton nuclear magnetic resonance (NMR) spectroscopy. This technique simultaneously quantifies the number and size of very low-density lipoprotein ($VLDL_{NMR}$), low-density lipoprotein (LDL_{NMR}), and high-density lipoprotein (HDL_{NMR}) particles, expressed each as a lipoprotein particle concentration (particles/L), or as an average particle size (nanometers).[4] By contrast, standard lipid tests quantify the cholesterol or triglyceride content of lipoproteins, expressed as mg/dL of cholesterol or triglyceride. The cholesterol content of lipoprotein particles varies between individuals because of heterogeneity in particle size and in the relative content of cholesterol ester and triglycerides contained in the particle core.[5,6]

Whether information about lipoprotein particle concentration or size obtained from NMR predicts CVD risk in asymptomatic individuals is uncertain. In addition, direct comparison data with apolipoproteins are scant. Each particle of LDL and VLDL carries 1 molecule of apolipoprotein B_{100} on its surface regardless of its cholesterol or triglyceride content,[7] hence apolipoprotein B_{100} is another measure of atherogenic lipoprotein particle number, obtained by immunoassay, and high levels have been associated with higher CVD risk.[8] Apolipoprotein A-1 is the major molecule that is carried on HDL particles, but because it is not carried in a 1-to-1 fashion, it is not a measure of HDL particle number, although low levels have been associated with higher CVD risk.[9] We conducted this study to evaluate prospectively whether NMR lipoprotein particles predict CVD in initially healthy women, and how they compare with directly-measured standard lipids and immunoassay-measured apolipoproteins.

METHODS

Study Population

Study participants were drawn from the Women's Health Study (WHS), a recently completed randomized, double-blinded, placebo-controlled trial of low-dose aspirin and vitamin E in the primary prevention of CVD and cancer in women.[10-12] WHS participants were apparently healthy female health care professionals, ages 45 years or older, who were free of self-reported CVD and cancer at study entry (1992−1995). Women gave written informed consent and completed questionnaires at the time of enrollment on demographics, anthropometrics, medical history, and lifestyle factors. They were also asked to provide a baseline blood sample; 28,345 women did so, and of these, 98.5% (N=27,909) had NMR measurements. For this study, we excluded women with missing data on baseline lipids or apolipoproteins (N=236), leaving 27,673 women for analysis. The study was approved by the institutional review board of the Brigham and Women's Hospital (Boston, Mass). Drs. Mora and Ridker had full access to the data and take full responsibility for its integrity. All authors have read and agree to the manuscript as written.

Laboratory Measurements

EDTA blood samples were obtained at the time of enrollment into the WHS and stored in vapor phase liquid nitrogen (−170° C). Samples for lipoprotein particle analysis by proton NMR spectroscopy were thawed, aliquoted (200 ul), refrozen, and shipped on dry ice to LipoScience,

Inc. (Raleigh, NC). Particle concentrations of lipoproteins of different sizes were calculated from the measured amplitudes of their spectroscopically distinct lipid methyl group NMR signals. Weighted-average lipoprotein particle sizes are derived from the sum of the diameter of each subclass multiplied by its relative mass percentage based on the amplitude of its methyl NMR signal.[5] Particle diameters and coefficients of variation (CVs) are shown in Supplementary Table 1. The NMR lipoprotein variables that we examined are those that are provided when ordering an NMR lipoprotein profile for clinical use.

In a laboratory (N. Rifai, Children's Hospital, Boston, MA) certified by the National Heart, Lung, and Blood Institute/Centers for Disease Control and Prevention Lipid Standardization program, baseline samples were thawed and analyzed for standard lipids and apolipoproteins. Standard lipids were directly measured using reagents from Roche Diagnostics (Indianapolis, IN), with CVs <3%. Apolipoproteins B_{100} and A-1 were measured using immunoturbidimetric assays (DiaSorin, Stillwater, Minn), with CVs of 5% and 3%, respectively.

Ascertainment of CVD Events

The primary endpoint of interest was a composite endpoint of incident CVD (nonfatal myocardial infarction, percutaneous coronary intervention, coronary artery bypass grafting, nonfatal ischemic stroke, or cardiovascular death). During the 11 year follow-up period, women reported the endpoints of interest on follow-up questionnaires every 6 or 12 months, and medical records were obtained to confirm events by a blinded end-points committee of physicians as previously described.[12]

Statistical Analysis

Statistical analyses were performed using STATA version 8.2 (STATA Corporation, College Station, Texas). We calculated Spearman rank correlation coefficients to evaluate the interrelations between the measured lipid biomarkers. Following guidelines from the Department of Health and Human Services,[13] lipid biomarkers were divided into quintiles based on the distribution among women not taking hormone replacement. Cox proportional hazard regression models were used to calculate the hazard ratios (HRs) and 95% confidence intervals (CIs) according to these quintiles. The proportional hazard assumption was satisfied using Schoenfeld residuals and the natural logarithm of follow-up time.

To examine the extent to which each lipid biomarker was associated with incident events, we initially considered each lipid variable in a separate model that adjusted for non-lipid risk factors (age, randomized treatment assignment, smoking status, menopausal status, postmenopausal hormone use, blood pressure, diabetes, and body mass index). Removing body mass index and diabetes from the multivariable analyses did not substantially affect the findings, nor did the addition of physical activity or alcohol use. Excluding the 883 women who were on baseline lipid lowering therapy did not change the results, and hence these women were included in the analyses. Analyses were also stratified according to fasting/nonfasting status based on our prior work in this cohort.[14] P value for linear trend was obtained using the median value for each quintile. All P-values were two-tailed. Since lipoprotein particles are metabolically interrelated and their concentrations are not independent,[4,15] NMR lipoproteins were also analyzed in a model that included the 9 NMR lipoprotein subclasses (large and small LDL_{NMR}, IDL_{NMR}, 3 HDL_{NMR} and 3 $VLDL_{NMR}$ lipoprotein subclasses). We also analyzed LDL_{NMR} lipoprotein concentration in multivariate Cox models that adjusted for other lipids.

The likelihood ratio χ^2 statistic was used to evaluate the goodness-of-fit of predictive models. Model discrimination was examined using the c-index,[16] a generalization of the area under the receiver operator characteristic curve. Model calibration was assessed using the Hosmer-Lemeshow goodness-of-fit test.[17] Risk reclassification was assessed by categorizing the

predicted 10-year risk for each model into categories of less than 5%, 5% to less than 10%, 10% to less than 20%, and 20% or higher. We calculated the proportion of participants who were reclassified by the comparison model as compared to the reference model. We computed the Net Reclassification Improvement (NRI),[18] which compares the shifts in reclassified categories by observed outcome, and the Integrated Discrimination Improvement (IDI),[18] which compares the integrals of sensitivity and specificity under two models.

RESULTS

During a mean follow-up of 11 years (302,399 person-years), a total of 1,015 first CVD events occurred, with "hard" events comprising 74% of these events (155 CVD deaths, 265 myocardial infarctions, and 334 strokes). In comparison to the standard lipid measurements which reflect the cholesterol or triglyceride content of lipoprotein particles, the NMR-measured lipoprotein particle concentrations of total LDL_{NMR} and $VLDL_{NMR}$ were higher in the women who developed CVD (Table 1), but no difference in total HDL_{NMR} was found. Women with CVD had significantly smaller LDL_{NMR} and HDL_{NMR} particle sizes and larger $VLDL_{NMR}$ particle size.

Table 2 shows the Spearman correlation coefficients for NMR lipoproteins with each other and with standard lipids and apolipoproteins. Total LDL_{NMR} particle concentration correlated positively with LDL cholesterol ($r = 0.62$) but correlated more closely with apolipoprotein B_{100} ($r = 0.83$), non-HDL cholesterol ($r = 0.74$), total/HDL cholesterol ratio ($r = 0.80$), and apolipoprotein B_{100}/A-1 ratio ($r = 0.80$), all $P<0.001$.

Association of NMR Lipoproteins, Lipids, and Apolipoproteins with CVD

Table 3 shows the association of each of the NMR lipoproteins, standard lipids, and apolipoproteins with CVD examined in separate Cox regression models that adjusted for non-lipid risk factors. Of the NMR measures, total LDL_{NMR} particle concentration had the largest hazard ratio and best goodness-of-fit likelihood ratio χ^2. The concentration of small LDL_{NMR} particles was associated with higher CVD, but large LDL_{NMR} was not. However, when small and large LDL_{NMR} were examined in a model that included all 9 NMR-measured lipoprotein particle concentrations (data not shown), both large and small LDL_{NMR} were significantly associated with CVD to a similar degree.

Of the HDL_{NMR} measures, the total concentration of HDL_{NMR} particles was not significantly associated with CVD. Large HDL_{NMR} particles were significantly and inversely associated with CVD, while medium and small HDL_{NMR} particles had no significant associations. All $VLDL_{NMR}$ particles were associated with higher CVD. Associations of NMR lipoproteins with CVD, analyzed according to self-reported fasting/nonfasting status ($<$ or ≥ 8 hours to last meal) resulted in stronger associations for large and medium $VLDL_{NMR}$ particles with CVD in the nonfasting state.

LDL_{NMR} and HDL_{NMR} particle size were inversely associated, and $VLDL_{NMR}$ particle size directly associated, with CVD. After adjusting for LDL_{NMR} particle concentration, there was no additional contribution of LDL_{NMR} size to CVD risk (P for trend=0.25), while HDL_{NMR} and $VLDL_{NMR}$ particle size remained significantly associated with CVD after adjustment for the respective concentrations.

When we removed body mass index and diabetes from the adjusted models, the adjusted HRs for top vs bottom quintiles were: total LDL_{NMR} particle concentration 2.92 (2.24−3.81), apolipoprotein B_{100} 2.89 (2.24−3.72), non-HDL cholesterol 2.61 (2.04−3.35), and the total/HDL cholesterol ratio 3.19 (2.54−3.99).

As shown in Table 3 and summarized in the Figure, hazard ratios for NMR measures were of approximately similar magnitude as those for standard lipids and apolipoproteins, although the total/HDL cholesterol ratio had the largest hazard ratio for any lipid or lipoprotein measure with CVD and the best goodness-of-fit likelihood ratio χ^2.

Multivariate Lipid Models

In models that included non-lipid risk factors plus other lipids, the association of LDL_{NMR} particle concentration with CVD was attenuated (Supplementary Table 2). In particular, after adjustment for the total/HDL cholesterol ratio, the association of LDL_{NMR} examined as quintiles was attenuated but remained significant (top quintile HR 1.63; 95% CI 1.18−2.25). However, when LDL_{NMR} was examined as a continuous variable there was no significant association after including the total/HDL cholesterol ratio.

Model Discrimination, Calibration, and Reclassification

Finally, we compared measures of model discrimination, calibration, and reclassification (Table 4). The referent model was comprised of the total/HDL cholesterol ratio and non-lipid risk factors, and compared to two other models: one that additionally incorporated LDL_{NMR} particle concentration, and the other additionally incorporated apolipoprotein B_{100}. All three models were well-calibrated.[19] There was no statistically significant difference in the c-index for the models that added LDL_{NMR} or apolipoprotein B_{100} to the referent model. There was essentially no reclassification improvement with adding LDL_{NMR} particle concentration or apolipoprotein B_{100} to the referent model (NRI 0% and 1.9%, respectively).

DISCUSSION

In this prospective cohort of 27,673 initially healthy women, we found that NMR-measured lipoproteins were significantly associated with incident CVD after adjusting for non-lipid risk factors, with a magnitude of risk comparable but not superior to standard lipids or immunoassay-measured apolipoproteins. Even though NMR-measured LDL_{NMR} particle concentration performed well for CVD risk prediction in this study, and was similar in risk to apolipoprotein B_{100}, neither measurement was better than the total/HDL cholesterol ratio which is readily obtained from a standard lipid panel. These data support current guidelines that recommend the use of a standard lipid panel, in particular the total/HDL cholesterol ratio, for CVD risk assessment in clinical practice.

Our findings have direct clinical relevance on several fronts. First, major European and North American guidelines have endorsed the use of standard lipids for CVD risk prediction in asymptomatic individuals.[1-3] By contrast, a recent statement involving an international panel of lipid experts proposed that CVD risk may be more closely related to atherogenic lipoprotein particle number than to LDL cholesterol.[8] Atherogenic particle concentration may be measured by NMR, which provides the number per unit volume of lipoprotein particles of varying size, or by immunoassay measurement of apolipoprotein B_{100}, since each VLDL, IDL, and LDL particle carries on its surface only one molecule of apolipoprotein B_{100}.[8]

Previous studies, predominantly cross-sectional or case-control studies found that NMR-measured LDL_{NMR} particle concentration may predict atherosclerotic diseases better than LDL cholesterol levels.[15,20-24] Data from INTERHEART[25] and other studies[26-29] have found that apolipoprotein B_{100} or the apolipoprotein B_{100}/A-1 ratio predict CVD. However, direct prospective comparison data for NMR measurements with apolipoproteins and standard lipid ratios are scarce.

To our knowledge, this study is the first large prospective comparison of associations of NMR-measured lipoproteins with both standard lipids and immunoassay-measured apolipoproteins for predicting incident CVD. We found that NMR-measured total LDL_{NMR} particle concentration was similar in CVD risk prediction to apolipoprotein B_{100} and both measurements performed better than LDL cholesterol. However, the differences compared with triglycerides, non-HDL cholesterol and the total/HDL cholesterol ratio were small and do not support the routine measurement of NMR lipoproteins or immunoassay apolipoproteins when a standard lipid panel is available. The data from this study, along with our prior findings[14,29] and recent data from the Framingham Study,[30] provide evidence-based confirmation for guidelines that are based on the use of standard lipid measurements, particularly the total/HDL cholesterol ratio.

Second, our study provides new data regarding the potential atherogenicity of the various HDL particles, which are heterogeneous in size and composition, carrying variable amounts of cholesterol and apolipoprotein A-1 molecules.[31] In this population of women, only large HDL_{NMR} particles were associated with lower CVD risk. The magnitude of the inverse association of large HDL_{NMR} particles with CVD was similar to that of apolipoprotein A-1 or HDL cholesterol, suggesting that the potentially protective effects of HDL cholesterol may be due to the large HDL_{NMR} particles. Prior studies have demonstrated strong inverse relationships between insulin resistance and the large HDL_{NMR} subclass as measured by NMR,[32] or the corresponding HDL_2 (sometimes referred to as "buoyant" HDL) as measured by ultracentrifugation.[33] This observation of the potential cardioprotective role of large HDL_{NMR} but not smaller HDL_{NMR} particles may have clinical implications for developing therapeutic agents that target HDL metabolism, such as CETP inhibitor drugs.[31] CETP inhibitors, such as torcetrapib, increase HDL cholesterol, predominantly altering the large HDL subclass, but there is controversy as to whether this results in reduced or enhanced cholesterol efflux from macrophages.[34,35]

While our study addresses primary prediction of CVD with NMR-based lipoprotein testing, our data should not be construed to exclude possible utility in this setting for alternative lipid or lipoprotein testing assessed by other measurement methods. Since our study is largely limited to Caucasian women, these data may not be generalizable to men or other patient groups. In particular, since we studied an apparently healthy cohort at low overall risk for CVD, our data do not address the question of whether or not lipoprotein testing with NMR has clinical utility for risk assessment and treatment strategies for higher risk patients, such as those with known cardiovascular disease, diabetes/insulin resistance, dyslipidemia, or for the monitoring of patients taking lipid altering therapy. Such studies need to be performed in the appropriate patient settings, preferably within the context of randomized trials of primary or secondary prevention.

In sum, CVD risk prediction associated with NMR lipoprotein profiles in this large prospective cohort of women was comparable but not superior to standard lipids or immunoassay-measured apolipoproteins. Thus, our data support the use of standard lipids, in particular the total/HDL cholesterol ratio, which are highly effective and readily available, for routine CVD risk assessment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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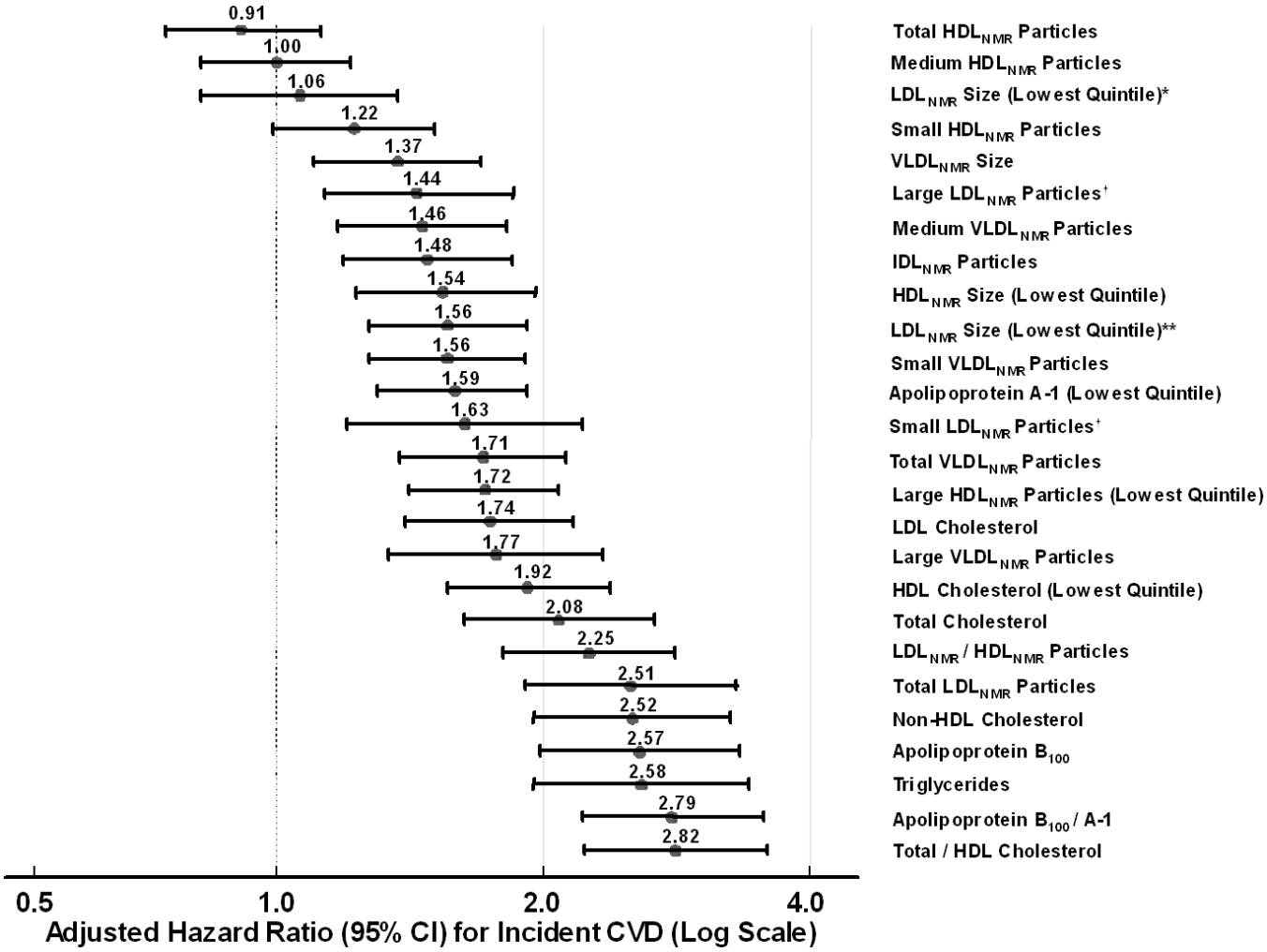

1. .

Hazard ratios and 95% CIs for the top vs bottom quintile, unless otherwise noted, adjusted for non-lipid risk factors (age, randomized treatment assignment, smoking status, menopausal status, postmenopausal hormone use, blood pressure, diabetes, and body mass index).

*LDL_{NMR} size adjusted for non-lipid risk factors and additionally for total LDL_{NMR} particle concentration.

†Large and small LDL_{NMR} particles were adjusted for non-lipid risk factors and additionally for the other NMR lipoproteins.

**LDL_{NMR} size adjusted for non-lipid risk factors but not for total LDL_{NMR} particle concentration.

Table 1

Baseline characteristics of participants according to incident cardiovascular disease

	No CVD N=26,658	CVD N=1,015	P Value
Age, mean (SD), y	54.5 (7.0)	59.8 (8.1)	<0.001
Current smoking, %	11.2	22.4	<0.001
Hypertension, %	24.2	48.5	<0.001
Diabetes, %	2.3	15.2	<0.001
Postmenopausal status, %	53.6	72.0	<0.001
Postmenopausal hormone use, %	43.7	41.1	0.11
Body mass index, mean (SD), kg/m^2	25.9 (4.9)	27.3 (5.4)	<0.001
NMR lipoprotein particle concentrations, nmol/L			
Low-density lipoprotein particles (LDL_{NMR})			
Total	1277 (1033−1600)	1557 (1251−1943)	<0.001
Large	541 (401−684)	518 (315−690)	<0.001
Small	655 (396−1020)	955 (577−1461)	<0.001
Intermediate-density lipoprotein particles (IDL_{NMR})	33 (11−68)	50 (20−91)	<0.001
High-density lipoprotein particles (HDL_{NMR})			
Total	35000 (31100−39400)	34800 (31000−39200)	0.59
Large	7500 (5000−10400)	5900 (3400−9000)	<0.001
Medium	2800 (800−6000)	2600 (700−6100)	0.77
Small	23700 (20000−27300)	24700 (21300−28400)	<0.001
Very low-density lipoprotein particles ($VLDL_{NMR}$)			
Total	68.3 (49.1−90.2)	80.3 (58.8−102.6)	<0.001
Large	1.4 (0.4−3.7)	2.8 (0.9−5.4)	<0.001
Medium	20.7 (11.2−31.9)	24.2 (14.3−36.7)	<0.001
Small	44.6 (32.4−57.8)	50.7 (37.9−64.3)	<0.001
NMR average particle size, nm			
LDL_{NMR} size	21.4 (20.8−21.8)	20.9 (20.3−21.6)	<0.001
HDL_{NMR} size	9.0 (8.6−9.4)	8.8 (8.5−9.1)	<0.001
$VLDL_{NMR}$ size	46.7 (42.2−52.1)	48.6 (43.4−55.1)	<0.001
Standard chemical lipids, mg/dL			
Total cholesterol	208 (183−235)	223 (199−252)	<0.001
Low-density lipoprotein cholesterol (LDL-C)	121 (100−144)	133 (113−156)	<0.001
High-density lipoprotein cholesterol (HDL-C)	52 (43−63)	46 (39−56)	<0.001
Triglycerides (TG)	118 (83−173)	158 (109−239)	<0.001
Apolipoproteins, mg/dL			
Apolipoprotein B_{100}	100 (84−120)	118 (97−137)	<0.001
Apolipoprotein A-1	149 (133−168)	143 (127−164)	<0.001
Combined measures			
LDL_{NMR}/HDL_{NMR} ratio	36.2 (28.9−46.2)	44.0 (34.6−57.7)	<0.001
Non-high-density lipoprotein cholesterol (non-HDL-C)	154 (129−181)	174 (150−202)	<0.001

	No CVD N=26,658	CVD N=1,015	P Value
Total/high-density lipoprotein cholesterol ratio (TC/HDL-C)	3.9 (3.2−4.9)	4.7 (3.8−5.9)	<0.001
Apolipoprotein B_{100}/A-1 ratio	0.67 (0.54−0.84)	0.82 (0.65−1.02)	<0.001

Values are median (25^{th}–75^{th} percentile) unless otherwise indicated. P values for age and body mass index were obtained from t tests. P values for categorical variables were obtained from chi-square tests. P values for the lipid biomarkers were obtained from the Wilcoxon rank-sum test.

Table 2

Spearman correlation coefficients for NMR lipoproteins with lipids and apolipoproteins

	Total Chol	LDL-C	HDL-C	Trigs	Apo B_{100}	Apo A-1	Non HDL-C	Total Chol/HDL-C	Apo B_{100}/A-1
Low-density lipoprotein particles (LDL_{NMR})									
Total	0.57	0.62	−0.48	0.62	0.84	−0.23	0.74	0.80	0.80
Large	0.35	0.34	0.46	−0.27	0.17	0.37	0.19	−0.19	−0.05
Small	0.28	0.33	−0.62	0.61	0.58	−0.39	0.50	0.74	0.68
IDL_{NMR}	0.33	0.34	−0.20	0.37	0.44	−0.02	0.40	0.39	0.37
High-density lipoprotein particles (HDL_{NMR})									
Total	0.23	0.00	0.41	0.25	0.10	0.69	0.09	−0.22	−0.26
Large	−0.03	−0.22	0.82	−0.41	−0.37	0.73	−0.32	−0.76	−0.67
Medium	−0.06	−0.16	−0.07	0.26	−0.03	0.08	−0.04	0.02	−0.07
Small	0.29	0.26	−0.07	0.34	0.36	0.18	0.32	0.25	0.20
Very-low-density lipoprotein particles ($VLDL_{NMR}$)									
Total	0.46	0.50	−0.40	0.64	0.61	−0.20	0.61	0.66	0.60
Large	0.24	0.14	−0.40	0.85	0.41	−0.07	0.38	0.51	0.37
Medium	0.28	0.22	−0.30	0.68	0.39	−0.09	0.39	0.45	0.36
Small	0.48	0.60	−0.34	0.38	0.60	−0.24	0.62	0.62	0.61
LDL_{NMR} size	−0.06	−0.12	0.64	−0.55	−0.36	0.43	−0.29	−0.62	−0.52
HDL_{NMR} size	−0.14	−0.31	0.75	−0.54	−0.47	0.57	−0.42	−0.77	−0.68
$VLDL_{NMR}$ size	0.01	−0.14	−0.20	0.59	0.11	0.05	0.07	0.18	0.06
LDL_{NMR}/HDL_{NMR}	0.41	0.57	−0.64	0.45	0.72	−0.55	0.64	0.85	0.88

Abbreviations: Trigs: triglycerides; IDL_{NMR} intermediate-density lipoprotein particles.

All P values for the correlation coefficients were <0.01, except for $VLDL_{NMR}$ size with total cholesterol (P=0.22).

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Table 3

Associations of lipoprotein and lipid measures with incident cardiovascular disease, adjusted for non-lipid risk factors

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	LR χ^2	P for Linear Trend
NMR Lipoprotein Particle Concentrations							
LDL particles (LDL_{NMR})							
Total							
Range, nmol/L	<963	963−1165	1166−1387	1388−1703	≥1704		
HR (95% CI)	1.00	1.37 (1.01−1.85)	1.35 (1.01−1.81)	1.80 (1.36−2.38)	2.51 (1.91−3.30)	1107.6	<0.001
Large							
Range, nmol/L	<354	354−471	472−574	575−695	≥696		
HR (95% CI)	1.00	0.69 (0.56−0.84)	0.67 (0.54−0.82)	0.75 (0.61−0.91)	0.86 (0.72−1.03)	1050.4	0.21
Small							
Range, nmol/L	<352	352−564	565−794	795−1172	≥1173		
HR (95% CI)	1.00	0.89 (0.69−1.15)	0.96 (0.75−1.22)	1.24 (0.99−1.56)	1.76 (1.41−2.18)	1089.8	<0.001
IDL particles (IDL_{NMR})							
Range, nmol/L	<6	6−20	21−40	41−74	≥75		
HR (95% CI)	1.00	1.16 (0.91−1.49)	1.06 (0.83−1.35)	1.26 (1.00−1.58)	1.48 (1.19−1.84)	1047.0	<0.001
HDL particles (HDL_{NMR})							
Total							
Range, nmol/L	<29200	29200−31900	32000−34400	34500−37400	≥37500		
HR (95% CI)	1.00	1.01 (0.80−1.26)	0.83 (0.66−1.05)	0.91 (0.73−1.13)	0.91 (0.75−1.12)	1031.8	0.34
Large							
Range, nmol/L	<3900	3900−5800	5900−7600	7700−9900	≥10000		
HR (95% CI)	1.00	0.74 (0.61−0.89)	0.62 (0.51−0.75)	0.56 (0.45−0.69)	0.58 (0.48−0.71)	1070.6	<0.001
Medium							
Range, nmol/L	<400	400−1400	1500−3000	3100−5600	≥5700		
HR (95% CI)	1.00	0.90 (0.73−1.11)	0.89 (0.72−1.10)	0.82 (0.66−1.02)	1.00 (0.82−1.21)	1033.6	0.60
Small							
Range, nmol/L	<18900	18900−21900	22000−24500	24600−27400	≥27500		
HR (95% CI)	1.00	1.24 (0.98−1.56)	1.26 (1.01−1.59)	1.16 (0.93−1.46)	1.22 (0.99−1.51)	1033.3	0.19
VLDL particles ($VLDL_{NMR}$)							

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	LR χ^2	P for Linear Trend
Total							
Range, nmol/L	<46.0	46.0−62.1	62.2−77.6	77.7−97.5	≥97.6		
HR (95% CI)	1.00	1.18 (0.93−1.50)	1.22 (0.97−1.55)	1.53 (1.23−1.92)	1.71 (1.38−2.12)	1062.2	<0.001
Large							
Range, nmol/L	<0.2	0.2−0.7	0.8−1.9	2.0−4.0	≥4.1		
HR (95% CI)	1.00	1.15 (0.86−1.54)	1.42 (1.06−1.90)	1.48 (1.11−1.97)	1.77 (1.34−2.33)	1056.0	<0.001
Medium							
Range, nmol/L	<8.4	8.4−16.1	16.2−24.1	24.2−34.4	≥34.5		
HR (95% CI)	1.00	1.13 (0.89−1.44)	1.30 (1.03−1.64)	1.29 (1.02−1.62)	1.46 (1.17−1.82)	1042.7	<0.001
Small							
Range, nmol/L	<31.8	31.8−42.2	42.3−51.6	51.7−63.3	≥63.4		
HR (95% CI)	1.00	1.01 (0.81−1.27)	1.31 (1.06−1.62)	1.34 (1.08−1.65)	1.56 (1.27−1.91)	1054.9	<0.001
NMR Lipoprotein Particle Size							
LDL_{NMR} size							
Range, nm	<20.6	20.6−21.0	21.2−21.5	21.6−21.9	22.0−23.0		
HR (95% CI)	1.00	0.68 (0.56−0.82)	0.57 (0.47−0.69)	0.56 (0.45−0.69)	0.64 (0.52−0.79)	1022.7	<0.001
HDL_{NMR} size							
Range, nm	<8.6	8.6−8.7	8.8−9.0	9.1−9.4	9.5−10.8		
HR (95% CI)	1.00	1.02 (0.85−1.23)	0.81 (0.67−0.97)	0.65 (0.53−0.79)	0.65 (0.51−0.81)	1059.1	<0.001
$VLDL_{NMR}$ size							
Range, nm	<40.7	40.7−44.1	44.2−47.5	47.6−52.3	52.4−131.2		
HR (95% CI)	1.00	1.14 (0.90−1.44)	0.97 (0.76−1.23)	0.98 (0.77−1.23)	1.37 (1.10−1.70)	1048.1	0.002
Standard Chemical Lipid Concentrations							
Total cholesterol							
Range, mg/dL	<176	176−197	198−216	217−242	≥243		
HR (95% CI)	1.00	1.35 (1.03−1.78)	1.57 (1.21−2.04)	1.84 (1.43−2.36)	2.08 (1.63−2.67)	1075.8	<0.001
LDL cholesterol							
Range, mg/dL	<97.7	97.7−115.4	115.5−132.1	132.2−153.9	≥154		
HR (95% CI)	1.00	1.12 (0.88−1.42)	1.31 (1.04−1.64)	1.56 (1.25−1.94)	1.74 (1.40−2.16)	1064.7	<0.001
HDL cholesterol	<39.5	39.5−45.9	46.0−52.7	52.8−61.5	≥61.6		

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	LR χ^2	P for Linear Trend
Range, mg/dL							
HR (95% CI)	1.00	0.85 (0.71−1.03)	0.70 (0.58−0.86)	0.59 (0.48−0.73)	0.52 (0.42−0.64)	1075.8	<0.001
Triglycerides							
Range, mg/dL	<72	72−96	97−128	129−182	≥183		
HR (95% CI)	1.00	1.34 (0.98−1.83)	1.87 (1.39−2.50)	1.89 (1.42−2.52)	2.58 (1.95−3.41)	1097.4	<0.001
Apolipoprotein Concentrations							
Apolipoprotein B_{100}							
Range, mg/dL	<79.1	79.1−93.3	93.4−108.9	109.0−126.2	≥126.3		
HR (95% CI)	1.00	1.29 (0.96−1.73)	1.48 (1.12−1.96)	1.65 (1.26−2.17)	2.57 (1.98−3.33)	1117.2	<0.001
Apolipoprotein A-1							
Range, mg/dL	<124.1	124.1−135.4	135.5−146.2	146.3−159.8	≥159.9		
HR (95% CI)	1.00	0.93 (0.76−1.13)	0.69 (0.55−0.85)	0.64 (0.52−0.80)	0.63 (0.52−0.77)	1059.2	<0.001
Combined Measures							
LDL_{NMR}/HDL_{NMR} ratio							
Range, mg/dL	<29.0	29.0−34.9	35.0−41.5	41.6−52.0	≥52.1		
HR (95% CI)	1.00	1.23 (0.96−1.57)	1.29 (1.01−1.64)	1.61 (1.28−2.03)	2.25 (1.80−2.81)	1098.2	<0.001
Non-HDL cholesterol							
Range, mg/dL	<123.3	123.3−144.9	145.0−165.6	165.7−191.1	≥191.2		
HR (95% CI)	1.00	1.19 (0.89−1.59)	1.87 (1.43−2.43)	1.94 (1.49−2.52)	2.52 (1.95−3.25)	1115.9	<0.001
Total/HDL cholesterol ratio							
Range	<3.20	3.20−3.81	3.82−4.49	4.50−5.44	≥5.45		
HR (95% CI)	1.00	1.42 (1.10−1.84)	1.54 (1.20−1.98)	1.87 (1.46−2.39)	2.82 (2.23−3.58)	1125.5	<0.001
Apolipoprotein B_{100}/A-1 ratio							
Range	<0.54	0.54−0.64	0.65−0.77	0.78−0.94	≥0.95		
HR (95% CI)	1.00	1.38 (1.07−1.79)	1.63 (1.28−2.09)	1.78 (1.40−2.27)	2.79 (2.21−3.54)	1122.0	<0.001

*Adjusted for age, randomized treatment assignment, smoking status, menopausal status, postmenopausal hormone use, blood pressure, diabetes, and body mass index. P value for linear trend was obtained using the median value for each quintile. LR χ^2 is the likelihood ratio chi-square statistic, which evaluates goodness of fit of predictive models associated with each lipid markers separately, with higher values consistent with better goodness of fit.

Table 4

Comparison of models based on discrimination, calibration, and reclassification measures

Models	Discrimination C-Index[a]	Goodness-of-Fit, Likelihood Ratio X^2 (p value)[b]	Calibration Cox X^{2c}	NRI[d] (p value)	IDI[e] (p value)	Percent reclassified[f]
Non-lipid covariates and total/HDL cholesterol ratio (referent model)	0.784	Referent	16.7	Referent	Referent	Referent
Referent model covariates *plus* LDL_{NMR}	0.785	2.01 (0.16)	17.2	0% (0.52)	0.0% (0.65)	1.1
Referent model covariates *plus* apolipoprotein B_{100}	0.786	10.5 (0.001)	14.3	1.9% (0.02)	0.1% (0.12)	2.6

All statistical measures were calculated at 10-years of follow-up.

[a] The c-index for the referent model (non-lipid covariates and total/HDL cholesterol ratio) was not statistically significantly different from the models that additionally included LDL_{NMR} or apolipoprotein B_{100}.

[b] Values are likelihood ratio X^2 and p values obtained from the Cox proportional hazards regression comparing models that added either LDL_{NMR} or apolipoprotein B_{100} to the referent model (non-lipid covariates and total/HDL cholesterol ratio). A higher X^2 value indicates a better model fit.

[c] Values are modified Hosmer-Lemeshow X^2, comparing differences between the predicted and actual event rates (X^2 values greater than 20 indicate poor calibration).[19]

[d] NRI is the net reclassification index, which compares the proportions moving up or down in clinical categories in cases versus controls, comparing models that added either LDL_{NMR} or apolipoprotein B_{100} to the referent model.

[e] IDI is the integrated discrimination improvement, comparing the integrals of sensitivity and specificity under two models (referent model compared with the model that added either LDL_{NMR} or apolipoprotein B_{100}).

[f] The proportion of individuals that move up or down a risk category using the model that incorporates either LDL_{NMR} or apolipoprotein B_{100} compared with the referent model.