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

Samia Mora, MD, MHS; James D. Otvos, PhD; Nader Rifai, PhD; Robert S. Rosenson, MD; Julie E. Buring, ScD; Paul M Ridker, MD, MPH

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 up for incident cardiovascular disease (n=11005) over an 11-year period. After adjustment for nonlipid risk factors, hazard ratios and 95% confidence intervals for the top versus the bottom quintile of NMR-measured lipoprotein particle concentration (measured in particles per liter) were 2.51 (1.91 to 3.30) for low-density lipoprotein (LDLNMR), 0.91 (0.75 to 1.12) for high-density lipoprotein (HDLNMR), 1.71 (1.38 to 2.12) for very low–density lipoprotein (VLDLNMR), and 2.25 (1.80 to 2.81) for the LDLNMR/HDLNMR ratio. Similarly adjusted results for NMR-measured lipoprotein particle size (measured in nanometers) were 0.64 (0.52 to 0.79) for LDLNMR size, 0.65 (0.51 to 0.81) for HDLNMR size, and 1.37 (1.10 to 1.70) for VLDLNMR size. Hazard ratios for NMR measures were comparable but not superior to standard lipids (total cholesterol 2.08 [1.63 to 2.67], LDL cholesterol 1.74 [1.40 to 2.16], HDL cholesterol 0.52 [0.42 to 0.64], triglycerides 2.58 [1.95 to 3.41], non-HDL cholesterol 2.52 [1.95 to 3.25], total/HDL cholesterol ratio 2.82 [2.23 to 3.58]) and apolipoproteins (B100 2.57 [1.98 to 3.33], A-1 0.63 [0.52 to 0.77], and B100/A-1 ratio 2.79 [2.21 to 3.54]). Essentially no reclassification improvement was found with the addition of the LDLNMR particle concentration or apolipoprotein B100 to a model that already included the total/HDL cholesterol ratio and nonlipid risk factors (net reclassification index 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, cardiovascular disease risk prediction associated with lipoprotein profiles evaluated by NMR was comparable but not superior to that of standard lipids or apolipoproteins. (Circulation. 2009;119:931-939.)

Key Words: lipoproteins ■ lipids ■ women ■ apolipoproteins

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

Clinical Perspective p 939

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 (VLDLNMR), low-density lipoprotein (LDLNMR), and high-density lipoprotein (HDLNMR) particles, with each expressed as a lipoprotein particle concentration (particles per liter) or as an average particle size (nanometers). By contrast, standard lipid tests quantify the cholesterol or triglyceride content of lipoproteins, expressed as milligrams of cholesterol or triglyceride per deciliter. The cholesterol content of lipoprotein particles varies between individuals because of heterogeneity in particle size and in the relative content of cholesterol ester and triglyceride contained in the particle core.
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\textsubscript{100} on its surface regardless of its cholesterol or triglyceride content; hence, apolipoprotein B\textsubscript{100} is another measure of atherogenic lipoprotein particle number, obtained by immunoassay, and high levels have been associated with higher CVD risk.\textsuperscript{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.\textsuperscript{9} We conducted the present 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-blind, placebo-controlled trial of low-dose aspirin and vitamin E in the primary prevention of CVD and cancer in women.\textsuperscript{10–12} WHS participants were apparently healthy female healthcare professionals 45 years of age 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 the present study, we excluded women with missing data on baseline lipids or apolipoproteins (n = 236), which left 27 673 women for analysis. The study was approved by the institutional review board of the Brigham and Women’s Hospital (Boston, Mass).

**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, separated into 200-μL aliquots, 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.\textsuperscript{5} Particle diameters and coefficients of variation are shown in supplemental Table I. The NMR lipoprotein variables that we examined are those that are provided when an NMR lipoprotein profile is ordered for clinical use.

In a laboratory (N. Rifai, Children’s Hospital, Boston, Mass) 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 measured directly with reagents from Roche Diagnostics (Indianapolis, Ind), with coefficients of variation <3%. Apolipoproteins B\textsubscript{100} and A-1 were measured with immunoturbidimetric assays (DiaSorin, Stillwater, Minn), with coefficients of variation of 5% and 3%, respectively.

**Ascertainment of CVD Events**

The primary end point of interest was a composite end point 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 end points 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 described previously.\textsuperscript{12}

**Statistical Analysis**

Statistical analyses were performed with STATA version 8.2 (STATA Corporation, College Station, Tex). 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,\textsuperscript{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 and 95% confidence intervals (CIs) according to these quintiles. The proportional hazard assumption was satisfied with 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 nonlipid risk factors (age, randomized treatment assignment, smoking status, menopausal status, postmenopausal hormone use, body mass index, diabetes mellitus, and body mass index). The removal of body mass index and diabetes mellitus from the multivariable analyses did not substantially affect the findings, nor did the addition of physical activity or alcohol use. Exclusion of the 883 women who were taking 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.\textsuperscript{14} The P value for linear trend was obtained with the median value for each quintile. All P values were 2-tailed. Because lipoprotein particles are interrelated metabolically and their concentrations are not independent,\textsuperscript{4,15} NMR lipoproteins were also analyzed in a model that included the 9 NMR lipoprotein subclasses (large and small LDL\textsubscript{NMR}, IDL\textsubscript{NMR}, and 3 HDL\textsubscript{NMR} and 3 VLDL\textsubscript{NMR} lipoprotein subclasses). We also analyzed LDL\textsubscript{NMR} lipoprotein concentration in multivariate Cox models that adjusted for other lipids.

The likelihood ratio χ\textsuperscript{2} statistic was used to evaluate the goodness-of-fit of predictive models. Model discrimination was examined with the c-index,\textsuperscript{16} a generalization of the area under the receiver operator characteristic curve. Model calibration was assessed with the Hosmer-Lemeshow goodness-of-fit test.\textsuperscript{17} Risk reclassification was assessed by categorizing the predicted 10-year risk for each model into categories of <5%, 5% to <10%, 10% to <20%, and ≥20%. We calculated the proportion of participants who were reclassified by the comparison model compared with the reference model. We computed the net reclassification improvement,\textsuperscript{18} which compares the shifts in reclassified categories by observed outcome, and the integrated discrimination improvement,\textsuperscript{18} which compares the integrals of sensitivity and specificity under 2 models.

Dr’s Mora and Ridker had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

**Results**

During a mean follow-up of 11 years (302 399 person-years), a total of 1015 first CVD events occurred, with “hard” events constituting 74% of these events (155 CVD deaths, 265 myocardial infarctions, and 334 strokes). Compared with the standard lipid measurements, which reflect the cholesterol or triglyceride content of lipoprotein particles, the NMR-measured lipoprotein particle concentrations of total LDL\textsubscript{NMR} and VLDL\textsubscript{NMR} were higher in the women who developed CVD (Table 1), but no difference in total HDL\textsubscript{NMR} was found. Women with CVD had significantly smaller LDL\textsubscript{NMR} and HDL\textsubscript{NMR} particle sizes and larger VLDL\textsubscript{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 B100 ($r=0.84$), 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 nonlipid risk factors. Of the NMR measures, total LDL$_{\text{NMR}}$ particle concentration had the largest hazard ratio and best goodness-of-fit likelihood ratio $\chi^2$. The concentration of small LDL$_{\text{NMR}}$ 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, 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, whereas 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 (<8 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 was directly associated with CVD. After adjustment for LDL_{NMR} particle concentration, no additional contribution of LDL_{NMR} size to CVD risk was found (P for trend=0.25), whereas 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 mellitus from the adjusted models, the adjusted hazard ratios for top versus bottom quintiles were 2.92 (2.24 to 3.81) for total LDL_{NMR} particle concentration, 2.89 (2.24 to 3.72) for apolipoprotein B_{100}, 2.61 (2.04 to 3.35) for non-HDL cholesterol, and 3.19 (2.54 to 3.99) for total cholesterol/HDL cholesterol ratio.

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 of any lipid or lipoprotein measure with CVD and the best goodness-of-fit likelihood ratio $\chi^2$.

### Multivariate Lipid Models

In models that included nonlipid risk factors plus other lipids, the association of LDL_{NMR} particle concentration with CVD was attenuated (online-only Data Supplement Table II). 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 hazard ratio 1.63, 95% CI 1.18 to 2.25). However, when LDL_{NMR} was examined as a continuous variable, no significant association was found after inclusion of 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 comprised the total/HDL cholesterol ratio and nonlipid risk factors and was compared with 2 other models, 1 that additionally incorporated LDL_{NMR} particle concentration and another that additionally incorporated apolipoprotein B_{100}. All 3 models were well calibrated.$^{19}$ No statistically significant difference was found in the c-index for the models that added LDL_{NMR} or apolipoprotein B_{100} to the referent model. Essentially no reclassification improvement was found with the addition of LDL_{NMR} particle concentration or apolipoprotein B_{100} to the referent model (net reclassification index 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 adjustment for nonlipid risk factors, with a magnitude of risk comparable but not superior to standard lipids or immunoassay-measured apolipoproteins. Even though LDL_{NMR} particle concentration performed well for CVD risk pre-
Table 3. Associations of Lipoprotein and Lipid Measures With Incident CVD, Adjusted for Nonlipid Risk Factors

<table>
<thead>
<tr>
<th>NMR lipoprotein particle concentrations</th>
<th>Quintile 1</th>
<th>Quintile 2</th>
<th>Quintile 3</th>
<th>Quintile 4</th>
<th>Quintile 5</th>
<th>LR $\chi^2$</th>
<th>P for Linear Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LDL$_{NMR}$ particles</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>&lt;963</td>
<td>963–1165</td>
<td>1166–1387</td>
<td>1388–1703</td>
<td>≥1704</td>
<td>1107.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HR (95% CI)</td>
<td>1.00</td>
<td>1.37 (1.01–1.85)</td>
<td>1.35 (1.01–1.81)</td>
<td>1.80 (1.36–2.38)</td>
<td>2.51 (1.91–3.30)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large</td>
<td>&lt;354</td>
<td>354–471</td>
<td>472–574</td>
<td>575–695</td>
<td>≥696</td>
<td>1050.4</td>
<td>0.21</td>
</tr>
<tr>
<td>HR (95% CI)</td>
<td>1.00</td>
<td>0.69 (0.56–0.84)</td>
<td>0.67 (0.54–0.82)</td>
<td>0.75 (0.61–0.91)</td>
<td>0.86 (0.72–1.03)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small</td>
<td>&lt;352</td>
<td>352–564</td>
<td>565–794</td>
<td>795–1172</td>
<td>≥1173</td>
<td>1089.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HR (95% CI)</td>
<td>1.00</td>
<td>0.89 (0.69–1.15)</td>
<td>0.96 (0.75–1.22)</td>
<td>1.24 (0.99–1.56)</td>
<td>1.76 (1.41–2.18)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>VLDL$_{NMR}$ particles</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>&lt;46.0</td>
<td>46.0–62.1</td>
<td>62.2–77.6</td>
<td>77.7–97.5</td>
<td>≥97.6</td>
<td>1062.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HR (95% CI)</td>
<td>1.00</td>
<td>1.18 (0.93–1.50)</td>
<td>1.22 (0.97–1.55)</td>
<td>1.53 (1.23–1.92)</td>
<td>1.71 (1.38–2.12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large</td>
<td>&lt;0.2</td>
<td>0.2–0.7</td>
<td>0.8–1.9</td>
<td>2.0–4.0</td>
<td>≥4.1</td>
<td>1056.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HR (95% CI)</td>
<td>1.00</td>
<td>1.15 (0.86–1.54)</td>
<td>1.42 (1.06–1.90)</td>
<td>1.48 (1.11–1.97)</td>
<td>1.77 (1.34–2.33)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>&lt;8.4</td>
<td>8.4–16.1</td>
<td>16.2–24.1</td>
<td>24.2–34.4</td>
<td>≥34.5</td>
<td>1042.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HR (95% CI)</td>
<td>1.00</td>
<td>1.13 (0.89–1.44)</td>
<td>1.30 (1.03–1.64)</td>
<td>1.29 (1.02–1.62)</td>
<td>1.46 (1.17–1.82)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small</td>
<td>&lt;31.8</td>
<td>31.8–42.2</td>
<td>42.3–51.6</td>
<td>51.7–63.3</td>
<td>≥63.4</td>
<td>1054.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HR (95% CI)</td>
<td>1.00</td>
<td>1.01 (0.81–1.27)</td>
<td>1.31 (1.06–1.62)</td>
<td>1.34 (1.08–1.65)</td>
<td>1.56 (1.27–1.91)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(Continued)
diction in the present study and was similar in risk to apolipoprotein B100, neither measurement was better than the total/HDL cholesterol ratio that 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.

The present 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. 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 B100, because each VLDL, IDL, and LDL particle carries on its surface only 1 molecule of apolipoprotein B100.

Previous studies, predominantly cross-sectional or case-control studies, found that NMR-measured LDL particle concentration may predict atherosclerotic diseases better than LDL cholesterol.

Table 4. Comparison of Models Based on Discrimination, Calibration, and Reclassification Measures

<table>
<thead>
<tr>
<th>Models</th>
<th>Discrimination C-Index*</th>
<th>Goodness-of-Fit, Likelihood Ratio $\chi^2$ (P)†</th>
<th>Calibration Cox $\chi^2$‡</th>
<th>NRI§, % (P)</th>
<th>IDI∥, % (P)</th>
<th>Percentage Reclassified¶</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonlipid covariates and total/HDL cholesterol ratio (referent model)</td>
<td>0.784</td>
<td>Referent</td>
<td>16.7</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td>Referent model covariates plus LDLNMR</td>
<td>0.785</td>
<td>2.01 (0.16)</td>
<td>17.2</td>
<td>0 (0.52)</td>
<td>0.0 (0.65)</td>
<td>1.1</td>
</tr>
<tr>
<td>Referent model covariates plus apolipoprotein B$_{100}$</td>
<td>0.786</td>
<td>10.5 (0.001)</td>
<td>14.3</td>
<td>1.9 (0.02)</td>
<td>0.1 (0.12)</td>
<td>2.6</td>
</tr>
</tbody>
</table>

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

*The c-index for the referent model (nonlipid covariates and total/HDL cholesterol ratio) was not statistically significantly different from the models that additionally included LDLNMR or apolipoprotein B$_{100}$.

†Values are likelihood ratio $\chi^2$ and P values obtained from the Cox proportional hazards regression comparing models that added either LDLNMR or apolipoprotein B$_{100}$ to the referent model (nonlipid covariates and total/HDL cholesterol ratio). A higher $\chi^2$ value indicates a better model fit.

‡Values are modified Hosmer-Lemeshow $\chi^2$ comparing differences between predicted and actual event rates ($\chi^2$ values >.20 indicate poor calibration).

§NRI is the net reclassification index, which compares the proportions moving up or down in clinical categories in cases vs controls, comparing models that added either LDLNMR or apolipoprotein B$_{100}$ to the referent model.

∥IDI is the integrated discrimination improvement, which compares the integrals of sensitivity and specificity under 2 models (referent model compared with the model that added either LDLNMR or apolipoprotein B$_{100}$).

¶The proportion of individuals who move up or down in a risk category according to the model that incorporates either LDLNMR or apolipoprotein B$_{100}$ compared with the referent model.
cholesterol levels.\textsuperscript{15,20–24} Data from INTERHEART\textsuperscript{25} and other studies\textsuperscript{26–29} have found that apolipoprotein B\textsubscript{100} or the apolipoprotein B\textsubscript{100}/A-1 ratio predicts CVD; however, direct prospective comparison data for NMR measurements with apolipoproteins and standard lipid ratios are scarce.

To the best of 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\textsubscript{NMR} particle concentration was similar in CVD risk prediction to apolipoprotein B\textsubscript{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 the present study, along with our prior findings\textsuperscript{14,29} and recent data from the Framingham Study,\textsuperscript{30} provide evidence-based confirmation for guidelines that are based on the use of standard lipid measurements, particularly the total/HDL cholesterol ratio.

Second, the present study provides new data on 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.\textsuperscript{31} In the present study population of women, only large HDL\textsubscript{NMR} particles were associated with lower CVD risk. The magnitude of the inverse association of large HDL\textsubscript{NMR} particles with CVD was similar to that of apolipoprotein A-1 or HDL cholesterol, which suggests that the potentially protective effects of HDL cholesterol may be due to the large HDL particles. Prior studies have demonstrated strong inverse relationships between insulin resistance and the large HDL subclass as measured by NMR\textsuperscript{32} or the corresponding HDL\textsubscript{2} (sometimes referred to as “buoyant” HDL) as measured by ultracentrifugation.\textsuperscript{33} This observation of the potential cardioprotective role of large HDL\textsubscript{NMR} but not smaller HDL\textsubscript{NMR} particles may have clinical implications for the development of therapeutic agents that target HDL metabolism, such as cholesteryl ester transfer protein inhibitor drugs.\textsuperscript{31} Cholesteryl ester transfer protein inhibitors, such as torcetrapib, increase HDL cholesterol, predominantly altering the large HDL subclass, but controversy exists as to whether this results in reduced or enhanced cholesterol efflux from macrophages.\textsuperscript{34,35}

Although the present 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. Because the present study is largely limited to white women, these data may not be generalizable to men or other patient groups. In particular, because we studied an apparently healthy cohort at low overall risk for CVD, the present 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 CVD, diabetes mellitus/insulin resistance, or 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 summary, CVD risk prediction associated with NMR lipoprotein profiles in the present large prospective cohort of women was comparable but not superior to standard lipids or immunoassay-measured apolipoproteins. Thus, the present 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.

Sources of Funding
The research for this article was supported by a grant to Dr Mora from the American Heart Association (0670007N). Dr Mora is also supported by the National Heart, Lung, and Blood Institute (K08 HL094375), the Sandra A. Daugherty Foundation, and the Lerner Research Young Investigator Award. The WHS is supported by grants HL 43851, HL 080467, and CA 47988 from the National Heart, Lung, and Blood Institute and the National Cancer Institute; the Donald W. Reynolds Foundation (Las Vegas, Nev); and the Leducq Foundation (Paris, France). The funding agencies played no role in the design, conduct, data management, or analysis related to this manuscript or in the manuscript preparation.

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 herein. Dr Rosenson is a stockholder of LipoScience Inc and serves as a member of its Scientific Advisory Board. The remaining authors report no conflicts.

References
11. Lee IM, Cook NR, Gaziano JM, Gordon D, Ridker PM, Mazzio JM, Gordon D, Ridker PM, Manson JE, Hennekens CH, Buring JE. Vitamin E in the


CLINICAL PERSPECTIVE

Although current prevention guidelines recommend measurement of standard lipids to assess risk of cardiovascular disease, it has been suggested that alternative lipoprotein measures may improve risk prediction; however, it remains uncertain how well such measures predict cardiovascular disease compared with the standard lipids that are obtained routinely in clinical practice. One method of alternative lipid testing is proton nuclear magnetic resonance spectroscopy. This technique simultaneously quantifies the number (concentration) and size of very low–density lipoprotein, low-density lipoprotein, and high-density lipoprotein particles. Apolipoprotein B\textsubscript{100} is another measure of atherogenic lipoprotein particle number, obtained by immunoassay. By contrast, standard lipid tests quantify the cholesterol or triglyceride content of lipoproteins. In this prospective cohort of 27 673 initially healthy women, nuclear magnetic resonance–measured nuclear magnetic resonance–measured low-density lipoprotein particle concentration performed well for cardiovascular disease risk prediction in the present study and was similar in risk to apolipoprotein B\textsubscript{100}, neither measurement was better than the total/high-density lipoprotein cholesterol ratio that is readily obtained from a standard lipid panel. Essentially no reclassification improvement was found with the addition of nuclear magnetic resonance–measured low-density lipoprotein particle concentration or apolipoprotein B\textsubscript{100} to a model that already included the total/high-density lipoprotein cholesterol particle and nonlipid risk factors. These data support current guidelines that recommend the use of standard lipids, in particular the total/high-density lipoprotein cholesterol ratio, which are highly effective and readily available, for routine cardiovascular disease risk assessment.


Lipoprotein Particle Profiles by Nuclear Magnetic Resonance Compared With Standard Lipids and Apolipoproteins in Predicting Incident Cardiovascular Disease in Women
Samia Mora, James D. Otvos, Nader Rifai, Robert S. Rosenson, Julie E. Buring and Paul M. Ridker

Circulation. 2009;119:931-939; originally published online February 9, 2009; doi: 10.1161/CIRCULATIONAHA.108.816181

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2009 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/119/7/931

Data Supplement (unedited) at:
http://circ.ahajournals.org/content/suppl/2009/02/13/CIRCULATIONAHA.108.816181.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org/subscriptions/
**Supplemental Material**

**Supplementary Table 1.** Diameter ranges of lipoprotein particles measured by NMR, with inter-assay reproducibility

<table>
<thead>
<tr>
<th>NMR Lipoprotein Parameter</th>
<th>Diameter range (nm)</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL particles (LDL(_{NMR}), nmol/L)</td>
<td>18-23</td>
<td>2.1</td>
</tr>
<tr>
<td>Total</td>
<td>21.2-23</td>
<td>6.3</td>
</tr>
<tr>
<td>Large</td>
<td>18-21.2</td>
<td>4.7</td>
</tr>
<tr>
<td>Small</td>
<td>23-27</td>
<td>13.1</td>
</tr>
<tr>
<td>IDL particles (IDL(_{NMR}))</td>
<td>7.3-13</td>
<td>1.5</td>
</tr>
<tr>
<td>Total</td>
<td>8.8-13</td>
<td>5.9</td>
</tr>
<tr>
<td>Large</td>
<td>8.2-8.8</td>
<td>&lt;30</td>
</tr>
<tr>
<td>Medium</td>
<td>7.3-8.2</td>
<td>3.7</td>
</tr>
<tr>
<td>Small</td>
<td>&gt;60</td>
<td>5.1</td>
</tr>
<tr>
<td>Medium</td>
<td>35-60</td>
<td>4.1</td>
</tr>
<tr>
<td>Small</td>
<td>27-35</td>
<td>7.1</td>
</tr>
<tr>
<td>VLDL particles (VLDL(_{NMR}), nmol/L)</td>
<td>≥27</td>
<td>3.1</td>
</tr>
<tr>
<td>Total</td>
<td>&gt;60</td>
<td>5.1</td>
</tr>
<tr>
<td>Large</td>
<td>35-60</td>
<td>4.1</td>
</tr>
<tr>
<td>Medium</td>
<td>27-35</td>
<td>7.1</td>
</tr>
<tr>
<td>Small</td>
<td>**</td>
<td>0.4</td>
</tr>
<tr>
<td>LDL(_{NMR}) size, nm</td>
<td>**</td>
<td>0.6</td>
</tr>
<tr>
<td>HDL(_{NMR}) size, nm</td>
<td>**</td>
<td>1.8</td>
</tr>
<tr>
<td>VLDL(_{NMR}) size, nm</td>
<td>**</td>
<td>1.8</td>
</tr>
</tbody>
</table>

CV: coefficient of variation. Interassay precision was derived from the analysis of frozen aliquots of each of two plasma pools for 20 days across six instruments.¹
**Supplementary Table 2.** Multivariate models of NMR LDL particle concentration (LDL<sub>NMR</sub>) with incident cardiovascular disease, additionally adjusted for lipid risk factors

<table>
<thead>
<tr>
<th>Quintile 1</th>
<th>Quintile 2</th>
<th>Quintile 3</th>
<th>Quintile 4</th>
<th>Quintile 5</th>
<th>P for Linear Trend</th>
<th>Per 1-SD Increase in LDL&lt;sub&gt;NMR&lt;/sub&gt;</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-lipid covariates&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.00</td>
<td>1.37 (1.01-1.85)</td>
<td>1.35 (1.01-1.81)</td>
<td>1.80 (1.36-2.38)</td>
<td>2.51 (1.91-3.30)</td>
<td>&lt;0.001</td>
<td>1.27 (1.21-1.35)</td>
</tr>
<tr>
<td>plus LDL-C&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.00</td>
<td>1.36 (1.00-1.84)</td>
<td>1.33 (0.98-1.79)</td>
<td>1.77 (1.31-2.37)</td>
<td>2.44 (1.80-3.31)</td>
<td>&lt;0.001</td>
<td>1.26 (1.18-1.35)</td>
</tr>
<tr>
<td>plus LDL-C, HDL-C, triglycerides&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.00</td>
<td>1.25 (0.92-1.69)</td>
<td>1.13 (0.83-1.54)</td>
<td>1.37 (1.00-1.87)</td>
<td>1.57 (1.10-2.24)</td>
<td>0.006</td>
<td>1.05 (0.95-1.16)</td>
</tr>
<tr>
<td>plus Total cholesterol/HDL-C ratio&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.00</td>
<td>1.27 (0.94-1.72)</td>
<td>1.17 (0.87-1.58)</td>
<td>1.43 (1.06-1.92)</td>
<td>1.63 (1.18-2.25)</td>
<td>0.001</td>
<td>1.07 (0.98-1.16)</td>
</tr>
<tr>
<td>plus apolipoprotein B&lt;sub&gt;100&lt;/sub&gt;&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.00</td>
<td>1.23 (0.91-1.67)</td>
<td>1.12 (0.83-1.52)</td>
<td>1.38 (1.02-1.88)</td>
<td>1.63 (1.16-2.30)</td>
<td>0.001</td>
<td>1.08 (0.98-1.19)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Adjusted for non-lipid risk factors (age, randomized treatment assignment, smoking status, menopausal status, postmenopausal hormone use, blood pressure, diabetes, and body mass index)

<sup>b</sup> Adjusted for non-lipid risk factors plus LDL cholesterol (LDL-C) as a continuous variable

<sup>c</sup> Adjusted for non-lipid risk factors plus LDL cholesterol, HDL cholesterol (HDL-C), and triglycerides, all three expressed as continuous variables

<sup>d</sup> Adjusted for non-lipid risk factors plus the total/HDL cholesterol ratio as a continuous variable

<sup>e</sup> Adjusted for non-lipid risk factors plus apolipoprotein B<sub>100</sub> as a continuous variable
Supplemental Reference