

# Clinical Utility of Different Lipid Measures for Prediction of Coronary Heart Disease in Men and Women

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**D**YSLIPIDEMIA IS RECOGNIZED AS one of the major risk factors for coronary heart disease (CHD).<sup>1</sup> The role of low-density lipoprotein cholesterol (LDL-C) in the development and progression of atherosclerosis is well established in experimental studies, and LDL-C and total cholesterol have been associated with CHD risk consistently in multiple clinical investigations.<sup>1</sup> Furthermore, large randomized controlled clinical trials have established the clinical benefits of lowering LDL-C levels in different clinical settings.<sup>2</sup> Hence, current treatment guidelines for dyslipidemia target elevated and borderline-high LDL-C as therapeutic goals. Additionally, present risk-prediction instruments<sup>3,4</sup> and guidelines for CHD prevention<sup>1,5</sup> emphasize the use of LDL-C, total cholesterol, or both as the cornerstone of CHD risk assessment.

**Context** Evidence is conflicting regarding the performance of apolipoproteins vs traditional lipids for predicting coronary heart disease (CHD) risk.

**Objectives** To compare performance of different lipid measures for CHD prediction using discrimination and calibration characteristics and reclassification of risk categories; to assess incremental utility of apolipoproteins over traditional lipids for CHD prediction.

**Design, Setting, and Participants** Population-based, prospective cohort from Framingham, Massachusetts. We evaluated serum total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), non-HDL-C, apolipoprotein (apo) A-I and apo B, and 3 lipid ratios (total cholesterol:HDL-C, LDL-C:HDL-C, and apo B:apo A-I) in 3322 middle-aged white participants who attended the fourth offspring examination cycle (1987-1991) and were without cardiovascular disease. Fifty-three percent of the participants were women.

**Main Outcome Measure** Incidence of first CHD event (recognized or unrecognized myocardial infarction, angina pectoris, coronary insufficiency, or coronary heart disease death).

**Results** After a median follow-up of 15.0 years, 291 participants, 198 of whom were men, developed CHD. In multivariate models adjusting for nonlipid risk factors, the apo B:apo A-I ratio predicted CHD (hazard ratio [HR] per SD increment, 1.39; 95% confidence interval [CI], 1.23-1.58 in men and HR, 1.40; 95% CI, 1.16-1.67 in women), but risk ratios were similar for total cholesterol:HDL-C (HR, 1.39; 95% CI, 1.22-1.58 in men and HR, 1.39; 95% CI, 1.17-1.66 in women) and for LDL-C:HDL-C (HR, 1.35; 95% CI, 1.18-1.54 in men and HR, 1.36; 95% CI 1.14-1.63 in women). In both sexes, models using the apo B:apo A-I ratio demonstrated performance characteristics comparable with but not better than that for other lipid ratios. The apo B:apo A-I ratio did not predict CHD risk in a model containing all components of the Framingham risk score including total cholesterol:HDL-C ( $P=.12$  in men;  $P=.58$  in women).

**Conclusions** In this large, population-based cohort, the overall performance of apo B:apo A-I ratio for prediction of CHD was comparable with that of traditional lipid ratios but did not offer incremental utility over total cholesterol:HDL-C. These data do not support measurement of apo B or apo A-I in clinical practice when total cholesterol and HDL-C measurements are available.

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In the last decade, mounting evidence also implicates higher apolipoprotein (apo) B and lower apo A-I levels in the pathogenesis of CHD.<sup>6-14</sup> Indeed, several recent reports have raised the possibility that these measures might be superior to traditional lipid measures for CHD risk prediction,<sup>6,7,12,13</sup> based on the premise that apo B levels better reflect the number of atherogenic lipoprotein particles in a given volume of plasma. However, the published data are not entirely consistent because in some other studies apo B and apo A-I did not perform better than traditional lipid measures for the purpose of risk prediction,<sup>8-10,14</sup> fuelling an intense debate.<sup>15,16</sup> Previous prospective population-based studies that compared these lipid measures directly have been limited in different ways. Some reports did not evaluate both sexes,<sup>6,9,11</sup> whereas others lacked some of the relevant measures, such as apo A-I,<sup>7,9</sup> LDL-C,<sup>12</sup> or non-HDL-C.<sup>6,10-12</sup> Furthermore, data are sparse regarding important performance measures of prediction models, such as discrimination and calibration characteristics. Few previous studies<sup>9,12-14</sup> assessed model discrimination (evaluated with the C index<sup>17</sup>), whereas none examined calibration (evaluated with, for example, the Hosmer-Lemeshow goodness-of-fit statistic<sup>18</sup> or calibration plots). Additionally, prior studies have not comprehensively evaluated the relative performance of various lipid measures for the purpose of reclassification of CHD risk. The ability to reclassify CHD risk has been described as a critical metric for assessing performance of biomarkers.<sup>19</sup>

Accordingly, we characterized the utility of different lipid measures for predicting CHD risk in a large, population-based prospective cohort of men and women. For this purpose, we examined their correlation and compared the performance of prediction models incorporating these measures. We did this in 2 steps: (1) by comparing the different lipid measures head-

to-head, to evaluate whether the apolipoproteins could be used instead of traditional lipid measures for CHD risk prediction; and (2) by evaluating whether apolipoproteins incrementally predict CHD risk over established risk factors for CHD, including traditional lipid measures.

## METHODS

### Study Sample

The Framingham Offspring Study was initiated in 1971. The design and selection criteria have been described.<sup>20</sup> Participants who attended the fourth examination cycle (1987 to 1991) were eligible for the present study (n=4019). At the examination (referred to as the baseline for the present investigation), the participants underwent a routine medical history, a physical examination that included blood pressure measurement and anthropometry, and blood sampling (after an overnight fast). We excluded 697 participants for the following reasons: younger than 30 or older than 74 years (n=68) prevalent cardiovascular disease at baseline (n=331); lack of follow-up data (n=16); or serum triglycerides higher than 400 mg/dL (to convert triglycerides from milligrams per deciliter to millimoles per liter, multiply by 0.0113); or missing data on any lipid variable or other covariate (n=282). After these exclusions, 3322 individuals (mean age, 51 years; 53% women) were eligible and constituted the study sample. The study protocol was approved by the Boston University Medical Center Institutional Review Board, and all participants provided written informed consent.

### Lipid and Apolipoprotein Measurements

Twelve-hour fasting venous blood samples were collected in tubes containing 0.1% EDTA. Plasma was separated by ultracentrifugation and plasma lipid concentrations (total cholesterol and high-density lipoprotein cholesterol [HDL-C]) were measured as previously described.<sup>21</sup> High-density lipoprotein cholesterol was measured after

precipitation of apo B-containing lipoproteins.<sup>22</sup> Low-density lipoprotein cholesterol concentrations were estimated using the Friedewald formula.<sup>23</sup> Levels of apo A-I and apo B were measured by immunoturbidimetric assays in plasma stored at -80°C and not previously thawed.<sup>24,25</sup> The average inter-assay coefficients of variation for the lipid measures follow: total cholesterol, 1.8%; HDL-C, 3.2%; apo A-I, 2.9%; and apo B, 6.6%.

### Follow-up and Outcome Events

The follow-up period for the present investigation was defined as from the baseline examination up to December 31, 2005. All Heart Study participants are under longitudinal surveillance for CHD occurrence through periodic examinations at the Framingham Heart Study and through biennial health history updates between examinations. An end point adjudication committee consisting of 3 experienced investigators reviewed hospitalization and physician office visit records for all suspected CHD events. Incident CHD was defined as recognized or unrecognized myocardial infarction, angina pectoris, coronary insufficiency, or CHD death. Diagnosis criteria for these events have been described.<sup>26</sup>

## STATISTICAL METHODS

The lipid measures evaluated in the present investigation included serum total cholesterol, HDL-C, LDL-C, non-HDL-C, apo A-I, and apo B and 3 lipid ratio measures, total cholesterol:HDL-C, LDL-C:HDL-C, and apo B:apo A-I. We evaluated the distribution of the lipid measures and the clinical covariates. Sex-specific pair-wise Spearman correlation coefficients were estimated for the interrelations between the various lipid measurements.

We used sex-specific multivariable Cox proportional hazards regression to investigate the relations of the various lipid measures to CHD incidence, adjusting for age, systolic blood pressure, antihypertensive treatment, diabetes, and smoking (all defined at the

baseline examination). All analyses—including assessment of model discrimination, calibration, and reclassification characteristics—were repeated in a sex-pooled sample (N=3322), with sex-standardized lipid variables and additional adjustment for sex. Cigarette smoking was defined by self-reported cigarette use within the year preceding the baseline examination. Diabetes was defined as a fasting blood glucose 126 mg/dL or higher (to convert fasting blood glucose from milligrams per deciliter to millimoles per liter, multiply by 0.0555), use of insulin, or use of oral hypoglycemic agents. The assumption of proportionality of hazards was confirmed by examining interactions of time-dependent covariates and survival time in Cox models. We estimated hazards ratios (HRs) and their 95% confidence intervals (CIs) for a standard deviation increment of each lipid measure (thereby facilitating comparisons of effect sizes for individual measures). Two-sided *P* values of <.05 were considered statistically significant. All analyses were performed using SAS 9.1 (SAS Institute Inc, Cary, North Carolina).

### Model Discrimination and Goodness-of-Fit Measures

Discrimination is the ability of a prediction model to separate those who experience a CHD event from those who do not. In Cox models, the overall C index is defined as the probability of concordance of time to event, given comparability of individuals.<sup>27</sup> Two individuals can be compared if it can be determined who had a longer time to CHD event (event time vs event time or event time vs censoring time, if censoring time was longer). Participants are considered concordant if their predicted probabilities of CHD-free survival and their actual survival times go in the same direction; if their predicted CHD probabilities are tied, they are considered 0.5 concordant. The C index is the sum of concordance values divided by the number of comparable pairs. The C index from Cox models is conceptually analogous to the area

under a receiver operating characteristic (ROC) curve<sup>17</sup> estimated for logistic models. Sex-specific ROC curves were also generated for lipid variables by plotting sensitivity vs 1-specificity obtained from multivariable logistic regression of 10-year CHD outcome on linear predictors from multivariable Cox models.

We also assessed the likelihood ratio  $\chi^2$  statistic as an indicator of the global goodness of fit of predictive models. A higher value indicates a better model fit. Additionally, for models, we estimated the Akaike information criterion,<sup>28</sup> which is a statistical estimate of the trade-off between the likelihood of a model against its complexity, with a lower value indicating a better model. All estimates of model performance were sex-specific and adjusted for age, systolic blood pressure, antihypertensive treatment, diabetes, and smoking.

### Model Calibration

Calibration evaluates the degree of correspondence between the predicted probability of CHD based on a model and the observed CHD incidence and is typically evaluated with the modified Hosmer-Lemeshow  $\chi^2$  statistic.<sup>18,29</sup> Small values indicate a good calibration while values exceeding 20 indicate significant lack of calibration (*P* < .01).

### Reclassification of CHD Risk

Because the lipid ratios performed better than individual lipids in terms of risk prediction (see results below), we chose to compare the ability to reclassify CHD risk for 2 ratios, the total cholesterol:HDL-C and the apo B:apo A-I ratios. We chose the total cholesterol:HDL-C ratio because it performed slightly better than the LDL-C:HDL-C ratio in the risk prediction models and because the former ratio is more frequently used in several settings. Furthermore, the total cholesterol:HDL-C ratio contains information about the very low-density lipoprotein (VLDL), thereby rendering it more comparable with the apo B:apo A-I ratio. It also eliminates the in-

herent variability when LDL-C is calculated, rather than measured.

The reclassification of CHD risk was evaluated by comparing predicted risk estimates based on multivariable models (including age, systolic blood pressure, antihypertensive treatment, diabetes, and smoking) with and without the lipid ratios taking the actual CHD events into account, as recently described.<sup>30</sup> This method differs from previously published reclassification methods, which do not evaluate reclassification rates separately in individuals who develop events and in those who do not, and count all reclassifications as beneficial for risk prediction. The latter is not true, because any upwards movement in categories in individuals who experience a CHD event indicates improved classification, whereas any downward movement in those with CHD events on follow-up implies worse reclassification. The opposite is true for individuals who do not develop an actual CHD event on follow-up, ie, a downward movement in risk category indicates improved classification but an upward movement denotes worse reclassification. The net reclassification improvement is then calculated by summing the reclassification improvements for those experiencing a CHD event on follow-up and for those not experiencing a CHD event on follow-up.

The predicted CHD probabilities were grouped into 10-year risk categories of 0% to less than 5%, 5% to less than 10%, 10% to less than 20%, and 20% or greater based on models with and without lipid variables. The proportions of participants reclassified upward and downward into another 10-year risk category with the incorporation of total cholesterol:HDL-C or apo B:apo A-I ratio to the models were calculated for individuals with and without events during 10 years of follow-up separately. The net reclassification improvements offered by addition of total cholesterol:HDL-C or apo B:apo A-I were calculated separately. Asymptotic tests were used to determine the statistical sig-

nificance of net reclassification improvements for the 2 lipid ratios, separately for each sex, and for the sex-pooled sample. Bootstrap methods were used to derive 95% CIs for the difference in reclassification improvements.

### Evaluation of the Incremental Value of Apo B:Apo A-I Over Established CHD Risk Factors

To address the question whether the apo B:apo A-I ratio has incremental predictive utility over established CHD risk factors including the total cholesterol:HDL-C ratio, we performed multivariable Cox proportional hazards regression to investigate the relations of apo B:apo A-I to CHD incidence adjusting for the individual components of the Framingham risk score, ie, age, systolic blood pressure, antihypertensive treatment, diabetes, smoking, and total cholesterol:HDL-C.

## RESULTS

The baseline clinical characteristics of the study sample are shown in TABLE 1. Many of the lipid measures demonstrated high pairwise correlations. The pairwise correlation coefficients between apo A-I and HDL was 0.82 in men and women; between apo B and total cholesterol, 0.83 in men and 0.82 in women; and between apo B and LDL-C, 0.80 in men and 0.84 in women.

### Incidence of Coronary Heart Disease Associated With Different Lipid Measures

On follow-up (median 15.0 years, maximum 19 years), there were 291 first CHD events (198 in men). TABLE 2 shows the results from the multivariable Cox regression analyses examining different lipid measures as predictors of CHD incidence in men and women.

In men, non-HDL-C, apo B, total cholesterol:HDL-C ratio, LDL-C:HDL-C ratio, and apo B:apo A-I ratio were all positively associated with CHD risk of approximately the same magnitude and statistical significance. Apo A-I and HDL cholesterol were associated with reduced risk of CHD.

**Table 1.** Baseline Characteristics

Clinical Features	Men (n = 1562)	Women (n = 1760)
Age, mean (SD), y	51 (10)	51 (10)
Systolic blood pressure, mean (SD), mm Hg	129 (17)	124 (19)
Risk factors, No. (%)		
Hypertension	597 (38)	502 (29)
Diabetes	76 (5)	50 (3)
Smoking	367 (24)	425 (24)
Lipid measures, median (25th-75th percentile), mg/dL		
Total cholesterol	204 (180-227)	202 (178-229)
LDL-C	135 (112-157)	125 (102-151)
HDL-C	42 (36-50)	54 (46-65)
Apo A-I	133 (120-148)	152 (135-170)
Apo B	102 (87-117)	92 (77-109)
Non-HDL-C	160 (136-184)	144 (119-174)
Ratio		
Total cholesterol:HDL-C	4.8 (3.9-5.8)	3.6 (2.9-4.6)
LDL-C:HDL	3.2 (2.5-4.0)	2.3 (1.7-3.1)
Apo B:apo A-I	0.8 (0.6-0.9)	0.6 (0.5-0.7)

Abbreviations: Apo, apolipoprotein; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

SI conversion factor: To convert total cholesterol, HDL-C, LDL-C from mg/dL to mmol/L, multiply by 0.0259.

In women, apo B, total cholesterol:HDL-C ratio, LDL-C:HDL-C ratio, and apo B:apo A-I ratio non-HDL-C were all associated with an increased CHD risk. High-density lipoprotein cholesterol was associated with reduced risk of CHD, whereas apo A-I was not significantly associated with incident CHD. Total cholesterol and LDL-C were not significantly associated with CHD incidence in either men or women, in part because of limited statistical power. With our sample size and at an  $\alpha$  of .05, we had statistical power to detect an HR of 1.25, 1.5, and 1.75 per SD increment of 31%, 72%, and 92%, respectively, in men; and 16%, 39%, and 61%, respectively, in women.

The results from sex-pooled analyses were similar to the sex-specific models, with better discrimination (higher C index) and goodness of fit but increased model complexity (data not shown.). Of note, total cholesterol and LDL-C were significant predictors of CHD in the pooled sample.

In secondary analyses, we examined the non-HDL-C:HDL-C ratio as a predictor of CHD incidence. The results were identical to those for total cholesterol:HDL-C, which should be expected because non-HDL-C:

HDL-C translates into (total cholesterol:HDL-C)  $-1$  mathematically. In additional secondary analyses, we explored whether our results would be influenced by the inclusion of treatment with  $\beta$ -blockers ( $n=245$ ; 7.4%) and lipid-lowering agents ( $n=91$ ; 2.7%) at baseline as covariates in the multivariable analyses because these agents have been shown to affect different lipid measures.<sup>31-34</sup> These analyses demonstrated essentially the same results as the main analyses (data not shown).

### Discrimination and Calibration Characteristics of Different Lipid Measures

The C index for multivariable models incorporating age, systolic blood pressure, antihypertensive treatment, diabetes, smoking, and one of the lipid variables at a time (Table 2) was highest for the apo B:apo A-I ratio in both men and women (0.74 and 0.76, respectively). However, the differences in the C index in models with different lipid measures were small and statistically nonsignificant ( $P > .70$  for comparisons with total cholesterol:HDL-C ratio, LDL-C:HDL-C ratio, and non-HDL-C in both men and women). The likelihood ratio  $\chi^2$  statistics

were highest for apo B:apo A-I ratio in men and for total cholesterol:HDL-C for women, but these differences were also small (Table 2). The Akaike information criterion estimates were similar for all lipid measures, with the lowest values for the apo B:apo A-I ratio in men and for the total cholesterol:HDL-C ratio for women, (Table 2). The FIGURE displays the ROC curves for the total cholesterol:HDL-C and apo B:apo A-I ratios and indicates near equivalence of performance for these 2 lipid ratios.

The modified Hosmer-Lemeshow  $\chi^2$  statistics are shown in Table 2, and all lipid variables demonstrated adequate model calibration.

### Reclassification of CHD Risk

In men, both the total cholesterol:HDL-C ratio and the apo B:apo A-I ra-

tio offered an improvement in reclassification of CHD risk at 10 years that was statistically significant. Improvement in CHD risk reclassification was numerically better for the total cholesterol:HDL-C ratio compared with the apo B:apo A-I ratio (TABLE 3), but the difference was not statistically significant (4.7%; 95% CI, -2.7 to 12.1). In women, there were only 50 CHD events at 10 years of follow-up; neither of the lipid ratios demonstrated a statistically significant net improvement in CHD risk reclassification (TABLE 4). The net reclassification improvement was numerically better for the apo B:apo A-I ratio, but the difference did not reach statistical significance (10.0%; 95% CI, -19.6 to 0.1).

In sex-pooled analyses, the difference in net reclassification indices between the total cholesterol:HDL-C

and apo B:apo A-I ratios was very small and statistically nonsignificant (0.1%; 95% CI, -5.9 to 6.1; data not shown).

### Incremental Value of Apo B:Apo A-I Over Established CHD Risk Factors

In multivariable models that incorporated the components of the Framingham risk score including total cholesterol:HDL-C, apo B:apo A-I was not significantly associated with CHD during follow-up (HR, 1.23; 95% CI, 0.95-1.60 in men; HR, 1.14; 95% CI, 0.72-1.82 in women). Similarly, the change in likelihood ratio  $\chi^2$  with the addition of the apo B:apo A-I ratio to the multivariable models was not statistically significant in either sex (change in  $\chi^2=2.41$  in men,  $P=.12$ ; change in  $\chi^2=0.31$  in women,  $P=.58$ ).

**Table 2.** Incidence, Discrimination, and Calibration Estimates of Different Lipid Measures for Coronary Heart Disease During Follow-up<sup>a</sup>

	Incidence <sup>b</sup>		Discrimination, Overall C Index (95% CI) <sup>c</sup>	Goodness-of-Fit, Likelihood Ratio $\chi^2$ <sup>d</sup>	Model Complexity, AIC <sup>e</sup>	Calibration, Cox $\chi^2$ <sup>f</sup>
	HR (95% CI)	P Value				
Men (n = 1562)						
Total cholesterol	1.12 (0.97-1.28)	.11	0.71 (0.67-0.75)	93.4	2743	9.4
LDL-C	1.11 (0.97-1.27)	.14	0.71 (0.67-0.75)	93.1	2743	9.6
HDL-C	0.71 (0.60-0.83)	<.001	0.73 (0.69-0.77)	109.8	2726	14.7
Apo A-I	0.83 (0.72-0.96)	.01	0.72 (0.68-0.76)	97.2	2739	11.8
Apo B	1.37 (1.20-1.57)	<.001	0.73 (0.69-0.77)	110.9	2725	13.4
Non-HDL-C	1.22 (1.06-1.40)	.005	0.72 (0.68-0.76)	98.7	2737	11.3
Ratio						
Total cholesterol:HDL-C	1.39 (1.22-1.58)	<.001	0.73 (0.70-0.77)	114.2	2722	15.4
LDL-C:HDL-C	1.35 (1.18-1.54)	<.001	0.73 (0.69-0.77)	109.7	2726	10.4
Apo B:apo A-I	1.39 (1.23-1.58)	<.001	0.74 (0.70-0.78)	115.5	2720	11.3
Women (n = 1760)						
Total cholesterol	1.18 (0.96-1.44)	.11	0.74 (0.69-0.80)	59.2	1306	8.8
LDL-C	1.20 (0.99-1.46)	.06	0.74 (0.69-0.80)	60.1	1305	9.2
HDL-C	0.72 (0.57-0.92)	.007	0.74 (0.68-0.80)	64.8	1300	6.3
Apo A-I	0.85 (0.68-1.07)	.16	0.74 (0.68-0.80)	58.9	1306	18.5
Apo B	1.38 (1.15-1.67)	<.001	0.76 (0.70-0.81)	67.1	1298	8.5
Non-HDL-C	1.28 (1.06-1.56)	.01	0.75 (0.69-0.80)	62.7	1302	10.4
Ratio						
Total cholesterol:HDL-C	1.39 (1.17-1.66)	<.001	0.75 (0.70-0.81)	69.0	1296	11.4
LDL-C:HDL-C	1.36 (1.14-1.63)	<.001	0.75 (0.69-0.80)	67.3	1298	8.3
Apo B:apo A-I	1.40 (1.16-1.67)	<.001	0.76 (0.70-0.81)	68.4	1297	9.8

Abbreviations: AIC, Akaike information criterion; apo, apolipoprotein; CI, confidence interval; HDL-C, high-density lipoprotein cholesterol; HR, hazard ratio; LDL-C, low-density lipoprotein cholesterol.

<sup>a</sup>All estimates reported are from multivariate analyses, adjusted for age, systolic blood pressure, antihypertensive treatment, diabetes, and smoking.

<sup>b</sup>Values are HRs (95% CIs), and P values from Cox proportional hazards analyses for a 1-SD increase of the lipid measures.

<sup>c</sup>Values are C index (95% CI; a higher value indicates a better discrimination) calculated at 10 years of follow-up.

<sup>d</sup>Values are likelihood ratio  $\chi^2$  statistics from the Cox model (a higher value indicates a better fit).

<sup>e</sup>Values are AIC<sup>28</sup> (a lower value indicates a better tradeoff between the likelihood of a model against its complexity).

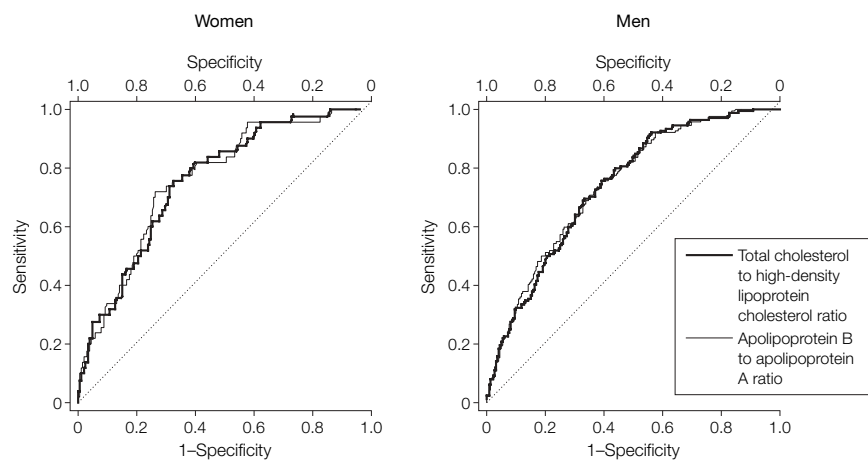
<sup>f</sup>Values are modified Hosmer-Lemeshow  $\chi^2$  statistics<sup>18,29</sup> (comparing the differences between predicted and actual event rates, where small values indicate a good calibration and values exceeding 20 indicate significant lack of calibration) calculated at 10 years of follow-up.

**COMMENT****Principal Findings**

In this large, population-based prospective cohort of men and women, we have comprehensively characterized the CHD risk associated with different lipid measures and assessed the clinical performance of these measures, using the appropriate metrics of model discrimination, calibration, and reclassification characteristics. Our principal findings are 3-fold. First, even though the apo B:apo A-I ratio performed well overall in terms of CHD risk prediction and model performance measures in both sexes, the differences compared with other lipid variables were small and statistically nonsignificant. Non-HDL-C performed relatively less well compared with the lipid ratios. Second, when CHD risk reclassification was evaluated, the differences in net reclassification improvement offered by the total cholesterol:HDL-C ratio vs the apo B:apo A-I ratio were small and statistically nonsignificant in both sexes. Third, the apo B:apo A-I ratio was not significantly associated with CHD incidence in either sex when added to a model that incorporated components of the Framingham risk score, including total cholesterol:HDL-C. This observation suggests that apo B:apo A-I ratio does not provide incremental predictive utility over and established CHD risk factors including traditional lipid measures.

**Previous Studies**

Previous studies have rendered conflicting results about which lipid measure is the best predictor of future CHD risk. Some recent reports have indicated that the apolipoproteins are better risk predictors than the traditional lipid measures,<sup>6,7,12,13</sup> whereas other studies do not support this notion.<sup>8-10,14</sup> These discrepancies could be the result of inherent differences in the study samples or to varying statistical methods used in different reports. Another proposed reason for the inconsistent results is that the study samples evaluated have varied with regard to baseline CHD risk. Thus, apo B might

**Figure.** ROC Curves for Predicting 10-Year Coronary Heart Disease Risk

The diagonal line indicates a test with an area under the receiver operating characteristic (ROC) curve of 0.5.

not perform better than lipid fractions for identifying CHD risk in low-risk populations, whereas it may outperform these fractions in high-risk samples.<sup>16</sup>

As noted previously, some prior prospective studies comparing lipid measures may have been limited by evaluation of only 1 sex<sup>6-9,11</sup> or by the lack of assessment of 1 or more of the relevant lipids or assessment of measures of model performance.<sup>6,7,9-12,14</sup> For example, in the large Apolipoprotein-related Mortality Risk Study (AMORIS),<sup>12</sup> HDL-C was measured only in a small subset (<10%) of the study population and formulae for calculating HDL-C and LDL-C were developed for the rest of the population. Also, several prior studies have compared the lipid measures by including several of them in the same multivariable models or by use of stepwise selection from such models.<sup>6,9,11,12</sup> This statistical approach is challenged by the high degree of correlation among the lipid measures and may be prone to overfitting of models. Also, given the focus on prediction of future CHD events, an analysis of the comparative performance of lipid measures in terms of discrimination, calibration, and risk reclassification may be more important than a comparison of relative risk estimates for different lipids.

Only 4 of the previous population-based studies that compared apolipoproteins with traditional lipid measures assessed model discrimination.<sup>9,12-14</sup> In the AMORIS study,<sup>12</sup> apo B was demonstrated to have a significantly higher C index than LDL-C in univariate models (0.65 in men and 0.69 in women for apo B vs 0.60 for LDL-C in both men and women). Similar estimates were reported from the Copenhagen City Heart Study.<sup>13</sup> No multivariable C index was reported in either of these studies. In a recent report from the Nurses' Health Study,<sup>9</sup> HDL-C was the primary contributor to a stepwise prediction model evaluated with the C index, whereas apo B was less important. In another recent study, the apo B:apo A-I ratio and total cholesterol:HDL-C ratio were comparable.<sup>14</sup> Of note, no prior study has examined model calibration, a measurement of how closely predicted outcomes agree with observed outcomes, and none has comprehensively assessed the relative ability of lipids to reclassify absolute CHD risk, which recently has been described as a critical metric for assessing biomarker performance.<sup>19</sup>

Another recent study, using data from the Framingham Heart Study, showed that non-HDL-C was a better predictor of CHD than LDL-C.<sup>35</sup> These findings are consistent with the find-

ings of the present study, in which non-HDL-C was shown to outperform LDL-C in a similar fashion. However, the 2 studies used different study samples, different time periods of observation, and varying analytical methods and thus are not strictly comparable.

### Advantages and Disadvantages for Apolipoproteins vs Traditional Lipid Measures

Current guidelines for CHD prevention<sup>1,5</sup> emphasize the use of total cho-

lesterol and LDL-C in CHD risk assessment and identify non-HDL-C as a secondary target for therapy.<sup>1</sup> The importance of LDL-C in CHD development is unequivocal, but there has been an ongoing debate on whether apo B, or rather the apo B:apo A-I ratio would be a more appropriate measure for estimating CHD risk associated with dyslipidemia.<sup>15,16,36,37</sup> The observational studies have rendered conflicting results in this matter, as discussed above. The advocates for apo B have emphasized that apo B is a better indicator of

the total number of atherogenic particles because each of VLDL—intermediate-density lipoprotein (IDL), LDL-C, and lipoprotein A [Lp(a)] particles contains 1 molecule of apo B-100.<sup>36</sup> Even though non-HDL-C contains information about VLDL, IDL, and Lp(a), it has been argued that a direct measurement of the number of atherogenic particles would be superior for risk prediction. The measurements of apo B and apo A-I have been standardized and are easily accomplished with an automated assay.<sup>38</sup> Furthermore,

**Table 3.** Predicted Risks and Reclassification of Coronary Heart Disease Risk in Men Using a Multivariate Risk Prediction Model With and Without Inclusion of Total Cholesterol:HDL-C Ratio or Apo B:Apo A-I Ratio (N = 1562)<sup>a</sup>

	No. of Men				Reclassified		Net Correctly Reclassified, % <sup>c</sup>
	0-<5%	5-<10%	10-<20%	≥20%	Increased Risk <sup>b</sup>	Decreased Risk <sup>b</sup>	
<b>Predicted 10-Year CHD Risk With Total Cholesterol:HDL-C Ratio</b>							
Individuals with events during follow-up (n = 133)							
Predicted 10-y CHD risk without total cholesterol:HDL-C							
0-<5%	6	4	0	0	27	14	9.8
5-<10%	3	35	15	1			
10-<20%	0	7	32	7			
≥20%	0	0	4	19			
Individuals without events during follow-up (n = 1429)							
Predicted 10-y CHD risk without total cholesterol:HDL-C							
0-<5%	371	63	3	0	177	236	4.1
5-<10%	139	406	79	4			
10-<20%	0	83	190	28			
≥20%	0	0	14	49			
Net reclassification improvement <sup>d</sup>							13.9
P value							.006
<b>Predicted 10-Year CHD Risk With Apo B:Apo A-I Ratio</b>							
Individuals with events during follow-up (n = 133)							
Predicted 10-y CHD risk without apo B:apo A-I							
0-<5%	6	4	0	0	28	20	6.0
5-<10%	4	34	15	1			
10-<20%	0	9	29	8			
≥20%	0	0	7	16			
Individuals without events during follow-up (n = 1429)							
Predicted 10-y CHD risk without apo B:apo A-I							
0-<5%	365	72	0	0	188	234	3.2
5-<10%	135	408	80	5			
10-<20%	1	83	186	31			
≥20%	0	0	15	48			
Net reclassification improvement <sup>d</sup>							9.2
P Value							.09

Abbreviations: Apo, apolipoprotein; CHD, coronary heart disease; HDL-C, high-density lipoprotein cholesterol.

<sup>a</sup>The reclassification of CHD risk was evaluated by comparing predicted risk estimates based on multivariate models (including age, systolic blood pressure, antihypertensive treatment, diabetes, and smoking) with and without the lipid ratios, separately for individuals with and without CHD events during follow-up. Data shown are the number of individuals cross-classified by their predicted 10-year risk using the model with and without the lipid ratio.

<sup>b</sup>The number of individuals that were reclassified upwards and downwards, respectively, when the lipid ratio is added to the model.

<sup>c</sup>The proportion correctly reclassified is in those who experience a CHD event during 10 years of follow-up, the proportion of individuals reclassified to a *higher* risk minus the proportion reclassified to a *lower* risk; in those who do not experience a CHD event during 10 years of follow-up, the proportion of individuals reclassified to a *lower* risk minus the proportion reclassified to a *higher* risk. Thus, for the upper half of the table (data for total cholesterol:HDL-C ratio), the percentage correctly reclassified in people with events is (27-14)/133 or 9.8%, and in people without events is (236-177)/1429, or 4.1%.

<sup>d</sup>The net reclassification improvement is the sum of correctly reclassified individuals with and without CHD events.

fasting samples are not needed for assays of apolipoproteins, which clearly is an advantage over calculated LDL-C. Non-HDL-C and total cholesterol also can be evaluated without fasting. Another advantage of the apolipoproteins is that they are better predictors of CHD risk of patients who are taking lipid-lowering treatment (vs traditional lipid measures).<sup>32</sup> Also, the INTERHEART study has clearly established the strong association of the apo B:apo A-I ratio with the incidence of myocardial infarction.<sup>39</sup> In this large

study of about 30 000 individuals from 52 countries, apo B:apo A-I was the risk factor accounting for most of the risk of myocardial infarction (population-attributable risk, 49.2% when adjusting for all other risk factors). However, this study did not compare the predictive value of apo B:apo A-I with that of total cholesterol:HDL-C, which was the purpose of the current investigation.

The main arguments against the routine clinical use of apolipoproteins (in preference to established lipid mark-

ers) are that apolipoprotein assays are not as widely available relative to standard lipid fractions; there is more information about the distribution of cholesterol fractions in populations and therapeutic cutpoints for apolipoproteins that may not be defined as readily; extensive educational campaigns have taken place for health professionals and for the public regarding the importance of measuring serum cholesterol, so replacing cholesterol measurement in clinical practice may create confusion.<sup>15</sup>

**Table 4.** Predicted Risks and Reclassification of Coronary Heart Disease Risk in Women Using a Multivariate Risk Prediction Model With and Without Inclusion of Total Cholesterol:HDL-C Ratio or Apo B:Apo A-I Ratio (N = 1760)<sup>a</sup>

	No. of Women				Reclassified		Net Correctly Reclassified, % <sup>c</sup>
	0-<5%	5-<10%	10-<20%	≥20%	Increased Risk <sup>b</sup>	Decreased Risk <sup>b</sup>	
<b>Predicted 10-y CHD Risk With Total Cholesterol:HDL-C Ratio</b>							
No. of Individuals with events during follow-up (n = 50)							
Predicted 10-y CHD risk without total cholesterol:HDL-C							
0-<5%	32	2	2	0	7	5	4.0
5-<10%	3	3	3	0			
10-<20%	0	1	1	0			
≥20%	0	0	1	2			
Individuals without events during follow-up (n = 1710)							
Predicted 10-y CHD risk without total cholesterol:HDL-C							
0-<5%	1418	68	5	0	104	69	-2.0
5-<10%	57	97	29	0			
10-<20%	0	11	20	2			
≥20%	0	0	1	2			
Net reclassification improvement <sup>d</sup>							2.0
P Value							.78
<b>Predicted 10-Year CHD Risk With Apo B:Apo A-I Ratio</b>							
Individuals with events during follow-up (n = 50)							
Predicted 10-y CHD risk without apo B:apo A-I							
0-<5%	29	6	1	0	9	2	14.0
5-<10%	2	5	2	0			
10-<20%	0	0	2	0			
≥20%	0	0	0	3			
Individuals without events during follow-up (n = 1710)							
Predicted 10-y CHD risk without apo B:apo A-I							
0-<5%	1419	69	3	0	101	65	-2.1
5-<10%	53	104	26	0			
10-<20%	0	11	19	3			
≥20%	0	0	1	2			
Net reclassification improvement <sup>d</sup>							11.9
P Value							.08

Abbreviations: Apo, apolipoprotein; CHD, coronary heart disease; HDL-C, high-density lipoprotein cholesterol.

<sup>a</sup>The reclassification of CHD risk was evaluated by comparing predicted risk estimates based on multivariate models (including age, systolic blood pressure, antihypertensive treatment, diabetes, and smoking) with and without the lipid ratios, separately for individuals with and without CHD events during follow-up. Data shown are the number of individuals cross-classified by their predicted 10-year risk using the model with and without the lipid ratio.

<sup>b</sup>The number of individuals that were reclassified upwards and downwards, respectively, when the lipid ratio is added to the model.

<sup>c</sup>The proportion correctly reclassified is in those who experience a CHD event during 10 years of follow-up, the proportion of individuals reclassified to a *higher* risk minus the proportion reclassified to a *lower* risk; in those who do not experience a CHD event during 10 years of follow-up, the proportion of individuals reclassified to a *lower* risk minus the proportion reclassified to a *higher* risk. Thus, for the upper half of the table (data for total cholesterol:HDL-C ratio), the percentage correctly reclassified in people with events is (7-5)/50 or 4%, and in people without events is (69-104)/1710, or -2.0%.

<sup>d</sup>The net reclassification improvement is the sum of correctly reclassified individuals with and without CHD events

### Strengths and Limitations

The strengths of our study include the large, population-based sample consisting of men and women, the continuous surveillance for CVD events blinded to lipid status, comprehensive assessment of several lipid measures, and inclusion of several important measures of test performance to compare the various lipids. Nevertheless, several limitations of our investigations must be recognized. Our sample consists predominantly of middle-aged whites of European descent, limiting the generalizability of our findings to other age groups and ethnicities. Specifically, the mean age of our study participants was 51 years, and it is accepted that the incidence of CHD in women escalates after menopause. Indeed, the overall incidence rate was higher in men than in women. Moreover, total cholesterol and LDL-C did not predict CHD in sex-specific models, but we observed statistically significant associations in the sex-pooled analyses. The lack of associations in the sex-specific analyses could possibly be due to insufficient statistical power to detect modest associations. Another possibility of the weak association of LDL-C with CHD risk may be the increasing proportion of participants receiving lipid-lowering treatment during follow-up (5.8% after 4 years, 10.9% after 8 years, and 18.9% after 12 years); such increasing trends for lipid-lowering treatment may have influenced our results relating LDL-C and total cholesterol to CHD incidence. Previous reports from the Framingham Heart Study, based on larger samples and more events, have established the associations of total cholesterol and LDL-C levels with CHD risk.<sup>4</sup>

### CONCLUSIONS

In our large, population-based prospective cohort of men and women, the performance of the apo B:apo A-I ratio for CHD risk prediction was comparable with that of other lipid ratios with respect to model discrimination, calibration, and reclassification

characteristics in both sexes. Furthermore, apo B:apo A-I did not provide incremental value for CHD risk prediction over established risk factors, including total cholesterol: HDL-C. Given overall equal performance of various lipids ratios, other factors will be critical in guiding the choice of lipid measures that should be used for CHD risk prediction. These factors include the costs and availability of assays, educational needs for health care professionals and the public for interpreting apolipoprotein measures, the possibility of obtaining valid measurements for risk prediction in nonfasting samples or in patients receiving lipid-lowering treatment, and the availability of appropriate therapeutic cutpoints and clinical evidence of benefits accruing from lowering levels (based on randomized, controlled clinical trials). However, with regard to test performance characteristics, our data do not support the need for measuring apo B or apo A-I in clinical practice when traditional lipid measurements are obtained routinely.

**Author Contributions:** Dr Vasani 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.

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**Acquisition of data:** Schaefer, Contois, McNamara, Wilson, D'Agostino, Vasani.

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**Study supervision:** Schaefer, D'Agostino, Vasani.

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