Accumulating evidence indicates that apolipoprotein (apo)B, apoA-I and, particularly, the apoB:apoA-I ratio are strong predictors of risk for coronary heart disease and of benefit in coronary heart disease prevention in lipid-lowering trials. For example, the Apolipoprotein related MOrtality RISk study showed that the apoB:apoA-I ratio was strongly related to increased risk for fatal myocardial infarction, and that plasma apoB and apoA-I levels and apoB:apoA-I ratio were better predictors of risk than levels of total cholesterol or triglycerides. Analysis of lipid variables in the Air Force/Texas Coronary Atherosclerosis Prevention Study population showed that baseline high-density lipoprotein cholesterol (HDL-C), apoA-I, apoB, low-density lipoprotein cholesterol (LDL-C):HDL-C ratio, total cholesterol:HDL-C ratio and apoB:apoA-I ratio significantly predicted first major acute event, whereas LDL-C and total cholesterol did not. For 1-year on-treatment values, only apoA-I, apoB and apoB:apoA-I ratio were significant predictors of a future coronary event. New statins can reduce substantially the apoB:apoA-I ratio. This is particularly evident for rosuvastatin (the latest addition to the class), which both decreases apoB and increases apoA-I. A number of conceptual and practical hurdles need to be overcome before apolipoprotein measurements can be incorporated into routine risk assessment.

Introduction

Apolipoproteins bind with phospholipids to form a surface monolayer in all mature lipoprotein particles. Apolipoprotein (apo)B is found in all atherogenic lipoproteins, including low-density lipoprotein (LDL), small dense LDL, very-low-density lipoprotein (containing cholesterol and triglycerides), remnant particles, intermediate-density lipoprotein and lipoprotein(a). ApoA-I and apoA-II are the major apolipoprotein constituents of the antiatherogenic high-density lipoprotein (HDL) and its subfractions. Whereas early epidemiological studies of risk factors for coronary heart disease (CHD) focused on serum cholesterol and identified high levels of LDL-cholesterol (LDL-C) and low levels of HDL-cholesterol (HDL-C) as important prognostic indicators for future CHD events, more recent studies have suggested that apoB and apoA-I may in fact be better markers of CHD risk. Technical barriers to widespread use have been surmounted, increasing the practical utility of apolipoprotein assays, and they are now widely available and satisfactorily standardized.¹,² The assays have excellent precision, usually better than that for current assays for LDL-C and HDL-C. Clinical applicability of the measures is also likely to be relatively straightforward. As an initial step, target levels could be set by extrapolation from LDL-C and HDL-C targets and on the basis of epidemiological data on apolipoprotein levels.
Apolipoproteins as predictors of risk

A considerable amount of data has accumulated that indicates that plasma apoB is a strong predictor of CHD risk. Numerous case–control studies have reported comparable LDL-C levels but significantly elevated apoB levels in patients with coronary disease as compared with individuals without disease. Indeed, the near normal levels of LDL-C in many CHD patients was a primary motivation in designing the Cholesterol and Recurrent Events (CARE) trial, which showed a significant effect of statin therapy in preventing CHD in individuals with average cholesterol levels. By way of example, in a recently reported case–control study, we found that LDL-C levels (in male patients with angiographic coronary artery disease or post-myocardial infarction patients) did not differ significantly from those in healthy age-matched control individuals. Levels of apoB were significantly higher, however, and HDL-C levels were significantly lower in those with disease (Table 1).

Findings supporting the predictive value of apoB have also been reported in prospective studies. The Quebec Cardiovascular Study evaluated baseline lipid and apolipoprotein levels in 2155 men who were followed for 5 years. It was found that each standard deviation increase in baseline apoB level (i.e. an increment equal to the population standard deviation) was associated with a significant increase in relative risk (RR) for CHD of 1.44 after controlling for age, smoking, diabetes, and systolic blood pressure. Multiple proportional hazards analysis showed that this significant effect was maintained, after adjustment for levels of triglycerides (RR for apoB = 1.48), HDL-C (RR 1.40), and high or low total cholesterol:HDL-C ratio (RR 1.29). Stepwise proportional hazards analysis confirmed the importance of both the total cholesterol:HDL-C ratio and apoB in the prediction of disease. Analysis of the interaction of these two variables by categorizing individuals into those with high or low apoB levels and high or low total cholesterol:HDL-C ratio (median apoB 116 mg/dl and total cholesterol:HDL-C ratio 5.65) showed that the highest ischaemic heart disease rate was among those with high apoB and high total cholesterol:HDL-C ratio values (significant 2.6-fold increase in rate as compared with low apoB/low total cholesterol:HDL-C).

Similar findings have been made with regard to the predictive ability of apo A-I. In one case–control study of 184 patients with angiographic coronary disease and 191 age- and sex-matched control individuals, HDL-C (39 vs 43 mg/dl, respectively), apoA-I (117 vs 137 mg/dl), HDL particles containing only apoA-I (LpA-I; 37 vs 45 mg/dl) and HDL particles containing both apoA-I and apoA-II (LpA-I:A-II; 80 vs 92 mg/dl) were all significantly lower (P<0.001 for all except HDL-C, which was P=0.03) in patients with disease. Total cholesterol (201 vs 205 mg/dl) and triglyceride (142 vs 133 mg/dl) levels did not differ significantly between the two groups. Among the HDL measures, total plasma apoA-I was the single best predictor of disease, with neither LpA-I nor LpA-I:A-II adding to predictive strength.

The recently reported Prospective Epidemiological Study of Myocardial Infarction (PRIME) study assessed the predictive ability of HDL constituents in more than 9000 men without CHD who were followed for CHD events over 5 years. CHD events occurred in 289 men during follow-up, and HDL-C, apoA-I, LpA-I and LpA-I:A-II were all significantly lower in those with events than in those without. Division of the entire cohort into quintiles according to the level of each HDL variable showed that there was a significant linear increase (P<0.0001) in RR across quintiles for all variables. However, logistic regression analysis...
showed that apoA-I was the strongest predictor (Table 2). Neither measurements of LpA-I or those of LPA-I:A-II added to predictive ability. Such findings suggest that the best predictor of risk among the HDL parameters is the total amount of apoA-I rather than the amount found in any particular form of HDL.

Perhaps the strongest evidence of the predictive power of the apolipoproteins comes from the recently reported Apolipoprotein related MOrtality RISk (AMORIS) study,11 in which apolipoproteins and other lipid measures were related to fatal myocardial infarction in 175,553 individuals. This cohort of 98,722 men and 76,831 women was followed up for approximately 5.5 years; 864 men and 395 women had fatal myocardial infarction, with risk being 2.5-fold greater in men. Univariate analysis showed that risk increased from lowest to highest quartile of plasma apoB by approximately 2.7-fold in both men and women, and by about 3.6-fold in individuals aged younger than 70 years. The risk ratio for myocardial infarction decreased to 0.5 or less in the highest compared with the lowest quartile of apoA-I for both men and women. Figure 1 shows apoB and apoA-I quartiles in individuals younger than 70 years adjusted for age and total cholesterol and triglycerides. There was a stepwise increase in risk ratio, with both males and females in the highest apoB/lowest apoA-I quartiles having the greatest risk. There were similar findings in individuals aged 70 years or older, in whom cholesterol loses power as a risk marker. Overall, the greatest increase in RR on univariate analysis was observed for the apoB:apoA-I ratio (risk ratios of 4.8 for men and 4.0 for women; Fig. 2). In stepwise regression models, apoB, apoA-I or both, or the apoB:apoA-I ratio were confirmed as stronger predictors of risk than total cholesterol or triglyceride levels. Receiver operating characteristics analysis showed that apoB had a higher sensitivity and specificity than LDL-C, especially in men and women whose LDL-C was below the population median (Fig. 3). Testing for interaction between LDL-C and apoB indicated that both variables contribute information on risk, with the nature of the interaction indicating that apoB is a better predictor than LDL-C at low LDL-C or apoB concentrations. The reason for the superior predictive ability of apoB may be that the apolipoprotein serves as a marker for the number of atherogenic particles because one apoB peptide is present on each particle. Thus, whereas LDL-C may be a good marker of the

### Table 2 Multivariate comparison of incident coronary heart disease patients and individuals without coronary artery disease by logistic regression

<table>
<thead>
<tr>
<th>Model 1</th>
<th>HDL-C +0.187</th>
<th>2.7</th>
<th>NS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td>ApoA-I −0.510</td>
<td>21.7</td>
<td>0.0001</td>
</tr>
<tr>
<td>Model 2</td>
<td>HDL-C +0.185</td>
<td>2.5</td>
<td>NS</td>
</tr>
<tr>
<td>Model 2</td>
<td>ApoA-I −0.511</td>
<td>19.4</td>
<td>0.0001</td>
</tr>
<tr>
<td>Model 2</td>
<td>LpA-I +0.015</td>
<td>0.0</td>
<td>NS</td>
</tr>
<tr>
<td>Model 3</td>
<td>HDL-C +0.185</td>
<td>2.5</td>
<td>NS</td>
</tr>
<tr>
<td>Model 3</td>
<td>ApoA-I −0.510</td>
<td>20.9</td>
<td>0.0001</td>
</tr>
<tr>
<td>Model 3</td>
<td>LpA-I:apoA-I −0.002</td>
<td>0.0</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data from the Prospective Epidemiological Study of Myocardial Infarction (PRIME) study. Model 1: high-density lipoprotein cholesterol (HDL-C) and apolipoprotein (apo)A-I included after adjustment for lipid (LDL-C and triglycerides) and non-lipid parameters (study center, diabetes, smoking, high blood pressure). Model 2: HDL-C, apoA-I and HDL particles containing only apoA-I (LpA-I) were included. Model 3: HDL-C, apoA-I and LpA-I:apoA-I ratio were included. NS=non-significant. (Reproduced with permission.10)

**Fig. 1** Relative risk ratios for fatal myocardial infarction by quartiles of apolipoprotein (apo)B and apoA-I in subjects in the Apolipoprotein related MOrtality RISk (AMORIS) study aged under 70 years. (Reproduced with permission.11)
number of atherogenic particles in a normo-
triglyceridaemic individual, in a hypertriglyceri-
daemic individual LDL particles have reduced
cholesterol content (i.e. LDL is small and dense)
and so more atherogenic particles will be present
in the circulation than are revealed by measure-
ment of LDL-C. The number of atherogenic
particles in these circumstances will be more
accurately reflected by the plasma apoB level.

Despite the strength of the accumulating
evidence supporting predictive power of the
apolipoproteins, it needs to be noted that not all
studies have found apoB and apoA-I to be stronger
predictors of risk than conventional lipid
measures. For example, in the Quebec Cardio-
vascular Study, adjustment for other lipid
measures eliminated the association between
apoA-I and disease risk. Likewise, the large
Atherosclerosis Risk in Communities (ARIC) study
followed 12,339 individuals without CHD at
baseline for 10 years, with 725 coronary events
occurring during follow-up. LDL-C, HDL-C and
triglyceride levels (in women) were independent
predictors of risk. When variables were assessed
by risk decile, the addition of apoB and apoA-I to
a model consisting of age, race, LDL-C, HDL-C and
triglycerides produced no improvement in
prediction.

Fig. 2  Age-adjusted relative risk ratios by apolipoprotein (apo)B:apoA-I ratio quartiles in men and women in the Apolipoprotein related MOrtality RISk (AMORIS) study (triglycerides <4.5 mmol/l). (Reproduced with permission.)

Fig. 3  Receiver-operating characteristics curves for low-density lipoprotein cholesterol (LDL-C) and apolipoprotein (apo)B in men and women with LDL-C levels below the population median in the Apolipoprotein related MOrtality RISk (AMORIS) study. Also shown in diagrammatic form is the concept that in hypertriglyceridaemic subjects apoB, rather than LDL-C, is a truer index of the number of atherogenic particles in the circulation. (Reproduced with permission.)
Apolipoproteins as predictors of treatment benefit

Apolipoproteins have also been found to be strong predictors of CHD prevention benefit in lipid-lowering trials. The Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS)\textsuperscript{13} was a primary prevention study of lovastatin conducted in 6605 patients with average total and LDL-C and below average HDL-C levels. Lovastatin treatment was associated with a 37\% reduction in risk for first major acute coronary event in the setting of a 25\% reduction in LDL-C and a 6\% increase in HDL-C. In analyses of baseline and on-treatment (1-year) lipid levels, a Cox backward stepwise regression model was used to identify predictors of outcome, and logistic regression models were used to examine the relationship between lipid variables and CHD event risk. A model without apolipoprotein data showed that, among baseline lipid factors, LDL-C ($P=0.029$) and HDL-C ($P=0.010$) levels were significant predictors of risk. When baseline apolipoproteins were included, the apoB:apoA-I ratio ($P<0.001$) replaced LDL-C and HDL-C in the model, with the latter two factors no longer being significant; each 0.25 increment in baseline ratio was associated with a 36\% incremental risk for a CHD event. For 1-year on-treatment values, only apoA-I ($P=0.013$), apoB ($P<0.001$) and apoB:apoA-I ratio ($P<0.001$) values were significant predictors of an event. The ability of apoB and, particularly, the apoB:apoA-I ratio to predict risk is illustrated by the logistic regression lines shown for on-treatment values in Fig. 4. The separation and difference in slope between lovastatin treatment and placebo curves for LDL-C values indicate an absence of a continuous relationship between values and risk in the two study groups (e.g. there are differences in risk levels associated with identical LDL-C values in the two groups during treatment). The regression lines for apoB for the two groups are nearly overlapping and continuous, indicating a better absolute relationship between apoB levels and risk (i.e. particular values are associated with nearly identical risk, irrespective of active or placebo treatment, and the gradient of risk is nearly continuous from lower values [in the lovastatin group] to higher values [in the placebo group]). The curves for apoB:apoA-I ratio are virtually superimposed, indicating a continuous gradient of risk according to ratio value from low to high.

Analysis of the predictive ability of baseline and on-treatment lipid levels was also performed in the secondary prevention Long-term Intervention with Pravastatin in Ischemic Disease (LIPID) trial population.\textsuperscript{14} In that trial, pravastatin treatment

![Fig. 4](image-url) Logistic regression models of the relationship between first acute major coronary event and 1-year on-treatment values for low-density lipoprotein cholesterol (LDL-C), apolipoprotein (apo)B and apoB:apoA-I ratio, adjusted for age, sex, marital status, hypertension, smoking and family history, in the Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS) population. Solid line indicates lovastatin treatment; dashed line indicates placebo. (Reproduced with permission.\textsuperscript{13})
was associated with a 24% reduction in risk for fatal CHD or non-fatal myocardial infarction among 9014 hypercholesterolaemic patients with CHD. Adjusted analysis of baseline lipids showed significant associations of levels of total cholesterol, LDL-C, HDL-C, apoA-I, apoB and total cholesterol:HDL-C with risk in the placebo group (n=4502). Adjusted analysis of lipid values at 1 year on-treatment showed significant associations for total cholesterol, LDL-C, triglycerides, apoA-I, apoB and total cholesterol:HDL-C ratio in the pravastatin group (n=4386). Analysis of the proportion of treatment effect (PTE; the total treatment effect is the 24% reduction in risk) explained by on-treatment lipid levels showed that apoB was the most important single measure accounting for the PTE. On-treatment apoB levels accounted for 67% of the treatment effect. This matched the PTE explained by the combination of total cholesterol and HDL-C, and was greater than the PTE for levels of LDL-C (52%), total cholesterol (48%), HDL-C (11%), apoA-I (11%) and triglycerides (9%).

**Effects of statins on apolipoproteins**

Newer representatives of the statin class, including atorvastatin and rosuvastatin, produce remarkably large decreases in LDL-C levels. In light of the clinical trial findings noted above, it is of interest to determine how these agents affect apoA-I and apoB levels and the apoB:apoA-I ratio. Rosuvastatin has been reported to reduce LDL-C significantly more than other statins and to increase HDL-C to a greater degree in individual comparative trials and in pooled analyses of clinical trial data.15–21

In a 6-week trial conducted in hypercholesterolaemic patients,22 rosuvastatin 10 mg and 40 mg reduced apoB levels by 39% and 47%, respectively, and increased apoA-I levels by 5% and 6%, respectively (Fig. 5). When the apoB:apoA-I ratios for rosuvastatin were compared across the dose range with those for atorvastatin, the former achieved a significantly lower reduction from baseline (−7.8%, P<0.001).

Likewise, in a 12-week trial comparing rosuvastatin 10 mg (n=129) and atorvastatin 10 mg (n=127) in hypercholesterolaemic patients,15 rosuvastatin treatment significantly reduced apoB (33% vs 26%; P<0.001), significantly increased apoA-I (7% vs 3%; P<0.05) and significantly reduced apoB:apoA-I ratio (37% vs 28%; P<0.001) as compared with atorvastatin. Data have also been reported comparing rosuvastatin 10 mg (n=111) with pravastatin 20 mg (n=136) and simvastatin 20 mg (n=129).16 Rosuvastatin significantly reduced apoB (40% vs 21% and 30%, respectively; P<0.001 for both comparisons) and apoB:apoA-I ratio (42% vs 23% and 32%; P<0.001 for both) as compared with the other two statins, and increased apoA-I to a comparable degree (5% vs 4% and 4%, respectively). Similar results were reported in a pooled analysis of 12-week treatment periods in comparative trials with rosuvastatin and other statins (Fig. 6).23

**Conclusion**

There is increasing evidence that apoB, apoA-I and, particularly, the apoB:apoA-I ratio may be more sensitive than LDL-C and HDL-C in predicting CHD risk. It is arguable that use of these measures is a next natural step in assessing patient risk, and
would represent an alternative to use of non-HDL-C, for example, as a risk measure. It is also clear that statin treatment produces sizable reductions in apoA-I, thereby improving the apoB:apoA-I ratio, which is consistent with the observed effects of statins in reducing CHD risk. It remains to be determined whether apolipoproteins should replace or complement LDL-C and HDL-C as risk markers and whether apolipoproteins should be measured in all patients or, if not, whether treatment decisions in specific subgroups of patients would be facilitated by these measurements. It also needs to be determined whether apolipoprotein goals should be incorporated into future lipid-lowering guidelines and, if so, precisely what these goals should be.

References


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Discussion

Dr Pedersen: As a cardiologist, I have to say that it has taken us a long time to get comfortable with measuring total cholesterol and HDL-C, and now you want to introduce us to something new. In the way of possible analogy, I think the reason that use of troponins in diagnosis of myocardial infarction was adopted so quickly is that labs simply stopped giving out the old enzyme values and started giving out just the troponin values. Do you think there is a case for stopping measurement of cholesterol and just measuring apoB and apoA-I?

Dr Packard: I think that apoB and apoA-I are actually easier and better to measure than lipid fractions. Whether you could get the audience here, for example, to stop measuring cholesterol ... well, you can see the horror on some faces. But, there are extremely good reasons to adopt such an approach. I don’t think it will happen today or tomorrow, but it will be interesting to see what happens over the next 3 to 5 years in this regard. I think the problem with cardiologists and lipoproteins is that cardiologists didn’t invent them. I’m wondering, Mr Chairman, whether cardiologists invented troponins?

Dr Pedersen: What would the target levels be for apoB and apoA-I?

Dr Packard: Well, an extremely simple starting point would be 1 and 1: if apo B is above 1 g/l, do something about it; if apoA-I is below 1 g/l, do something about it.

Dr Pedersen: [Reads a question from the audience.] Given the limited resources for health care, is it realistic to consider spending more money to measure apolipoproteins rather than just continuing to measure total cholesterol?

Dr Packard: Most people measure total cholesterol, and now we do direct measurement of HDL-C in the lab, which is actually more expensive than measuring apolipoproteins. So, if I were to suggest a replacement, it might be to measure total cholesterol, apoB and apoA-I. That might be sufficient and would probably be less expensive than getting a full lipid profile, and would give provide as much information — If not more. So, I don’t think cost is a central issue.

Dr Stein: [Reads a question from the audience.] The proportion of treatment effect analysis in the LIPID trial that you discussed showed that on-treatment apoB accounted for two-thirds of the treatment effect and apoA-I accounted for 11%. Did that analysis also look at the apoB:apoA-I ratio?

Dr Packard: As far as I know the authors have not looked at the ratio; it’s not in the Simes article[14]. But, it would be nice to have the analysis done to see if it agrees with the AFCAPS/ TexCAPS analysis.