Editorial

Does Measurement of Apolipoprotein B Have a Place in Cholesterol Management?

In the accompanying editorial, Sniderman and Silberberg\(^1\) raise the question, "Is It Time To Measure Apolipoprotein B\(^-\)100 (apo B) clinically to estimate the risk for coronary heart disease (CHD) or to guide in lipid-lowering therapy, our answer is an unqualified "No." Whether measurement of apo B will prove valuable in the future is another question; the answer is "Maybe."

Sniderman and associates\(^2\,3\) made an important contribution with the recognition that some patients have high concentrations of apo B in the presence of "normal" serum cholesterol levels. Without question, low density lipoprotein (LDL) apo B levels can be disproportionately high compared to cholesterol levels. The condition of elevated LDL apo B and normal LDL cholesterol they called "hyperapoBetalipoproteinemia." In their initial definition, "elevated" apo B was confined to the LDL fraction. More recently, their definition seemingly has been expanded to include serum total apo B, and a simple term for high total apo B is "hyperapo B." Total apo B includes apo B in LDL, intermediate density lipoprotein (IDL), and very low density lipoprotein (VLDL). For each of these classes, there is one molecule of apo B per lipoprotein particle; hence, measurement of total apo B concentration yields the total number of apo B-containing lipoproteins in a volume of plasma.

The critical question raised by the research of Sniderman et al.\(^2\,3\) is whether total apo B levels better predict CHD than does the total cholesterol level or another cholesterol parameter. What are the data related to this question? The evidence is based on small, retrospective studies and not on large, prospective studies. The latter are required before apo B determination can be considered superior to cholesterol measurement. Retrospective surveys are notorious for giving suggestive evidence that is not confirmed by large prospective studies. At this time, therefore, we cannot assume that total apo B is better than total cholesterol for detection of high-risk individuals. Moreover, the predictive power of total cholesterol measurement is enhanced by adding triglycerides, LDL cholesterol, HDL cholesterol, non-HDL cholesterol, and cholesterol ratios. To justify introducing complex and expensive new methodology for apolipoproteins, it must be demonstrated that the total apo B level is definitely superior to total lipids and lipoprotein cholesterol for predicting CHD risk or for monitoring therapy.

In recommending total apo B measurements, Sniderman and Silberberg seemingly postulate that all apo B-containing lipoproteins are similarly atherogenic. Accordingly, one VLDL particle would have the same atherogeneity as one LDL particle, or within the LDL density class, small LDL particles would have similar atherogeneity as large LDL particles. Although this is an intriguing hypothesis, it is not universally accepted. Apo B-containing lipoproteins vary in size, types of apolipoproteins, and contents of apolipoproteins, triglycerides, and cholesterol. It, therefore, would be surprising if all lipoprotein species are equally atherogenic. This conceptual stumbling block probably stands in the way of universal acceptance of total apo B level as a predictor of CHD risk.

On the other hand, growing evidence supports the concept that all the major species of apo B-containing lipoproteins are atherogenic, although perhaps not equally so. Among these lipoproteins, LDL is the most widely accepted as being an atherogenic particle. Reduction of elevated LDL cholesterol is the foundation of the National Cholesterol Education Program (NCEP).\(^4\) Many reports\(^5\,9\) further suggest strongly that IDL particles can promote atherosclerosis. And more recently, epidemiologic data indicate that high levels of VLDL independently raise coronary risk.\(^10\,13\) Consequently, we agree with Sniderman and Silberberg that combining LDL+IDL+VLDL in some way is a reasonable approach to predicting CHD, or at least to test as a hypothesis. In our view, however, the cholesterol component of all apo B-containing lipoproteins may be just as powerful a predictor of CHD as apo B and easier to obtain as non-HDL cholesterol. Since the cholesterol component of these lipoproteins is what contributes to cellular accumulation of cholesterol in the arterial wall, why would not apo B-linked cholesterol be as predictive of CHD as apo B? In support, as indicated above, prospective data attest to the significant predictive power of cholesterol in LDL, IDL, and VLDL, whereas comparable data for total apo B are lacking.

We might further note that apo B-associated cholesterol (non-HDL cholesterol) is strongly correlated with total apo B levels. This correlation is shown in Figure 1, which plots values for these two parameters obtained on 113 patients from our laboratory. The top graph shows the correlation for 72 patients with plasma triglycerides below 300 mg/dl and the bottom graph, for 41 patients with triglyceride levels over 300 mg/dl. The method used for estimating apo B was a modification of the Lowry procedure; apo B measurements were made on separate lipoprotein fractions and combined, as previously published.\(^14\) For people with triglyceride levels below 300 mg/dl, who make up about 97% of the American population, the correlation coefficient is very high (0.951). For those with hypertriglyceridemia, the correlation is relatively high, although less strong (0.801). The explanation for the latter good correlation is that as the
cholesterol content of LDL particles declines in hypertriglyceridemic patients, the cholesterol concentration in VLDL increases to compensate. As indicated by Figure 1, if total apo B has any advantage over apo B-associated cholesterol, it is for hypertriglyceridemic patients and not for the general population. Still, even for patients with elevated triglycerides, total apo B levels may not provide any advantage over apo B-associated cholesterol concentrations, and it is doubtful that prospective epidemiologic studies in hypertriglyceridemic patients could unequivocally prove such an advantage. From Figure 1, the apo B level can be approximated from the apo B-associated cholesterol by dividing 1.54 (or multiplying 0.65).

Thus, we contend that until better epidemiologic data become available, the apo B-associated cholesterol level can be used if it is desired to combine the atherogenic potential of LDL+IDL+VLDL. This approach may not give any better predictability than LDL cholesterol levels in hypercholesterolemic patients with normal triglyceride levels, but it could be useful for patients with hypertriglyceridemia, mixed hyperlipidemia, and diabetic dyslipidemia. Apo B-associated cholesterol (non-HDL cholesterol) has the further advantage of being obtained directly in analysis and not calculated indirectly, as is the case for LDL cholesterol. Table 1 shows how non-LDL cholesterol would compare to the NCEP guidelines for LDL cholesterol, and the table also gives corresponding approximate values for total apo B. The upshot of our argument is that little is to be gained at present by measurement of total apo B beyond that which is already available, that is, various cholesterol fractions including apo B-associated cholesterol.

Another critical problem for the clinical use of apo B measurements is that of methodology. Reliable measurements of total apo B currently are not available, nor will they be so in the near future. Several methodologic problems stand in the way of accurate measurement and, hence, clinical application. First, automated techniques for rapidly processing large numbers of samples of apo B levels are not available. Second, a variety of immunological techniques currently are employed, but each has imperfections. And third, suitable standards have not been developed to accurately calibrate immunological methods. The nature of these problems can be reviewed briefly.

The immunological methods currently available for apo B are radial immunodiffusion,\textsuperscript{2,3} radioimmunoassay,\textsuperscript{16} electroimmunoassay,\textsuperscript{17,18} enzyme-linked immunosorbent assay,\textsuperscript{19} and nephelometric immunoassay.\textsuperscript{20} Opinion differs as to which of these approaches is preferable. Most provide greater accuracy for LDL apo B than for VLDL apo B, and yet the clinical value of total apo B measurement may be greatest for patients with hypertriglyceridemia, that is, those with elevated VLDL apo B (Figure 1). Certainly, among many of the commercially available kits for apo B, the accuracy for absolute concentrations varies considerably, and most absolute values are suspect. In a few "reference" laboratories, accurate estimations for total apo B levels may be available, but this is not the case for most values obtained in standard commercial laboratories.

The calibration of the secondary standard with a primary standard also presents problems. The primary standard for most apo B methods is bovine serum albumin (BSA). This is related to the secondary standard of lipoprotein apo B by determining protein with a modified Lowry method.\textsuperscript{21} LDL is most often used as the secondary standard because almost all LDL protein is apo B. Unfortunately, even expert lipoprotein chemists report different relative chromogenecities for apo B and BSA in the Lowry reaction. Estimates for the apo B-to-BSA chromogenecity ratio vary from 0.78\textsuperscript{22} to 1.0.\textsuperscript{23} This discrepancy alone can produce a difference in absolute values for total apo B of 22%.

If apo B is to have utility in clinical practice, its absolute concentration must be reported accurately. Absolute values are required for risk assessment, recommendations for therapy, and monitoring of response. The same, of course, is true for total cholesterol and lipoprotein cholesterol; and even for cholesterol measurements, there is considerable room for improvement. Without accurate estimates for absolute concentrations of cholesterol fractions, the guidelines of the NCEP are difficult to follow in the clinical setting. Still, quality control and
assurance for cholesterol measurements are improving rapidly and certainly are far ahead of those for apo B. Without availability of accurate measurements for apo B, it is impossible to make categorical recommendations about cutpoints for diagnosis and therapy.

When considering the issue of apo B measurement, the apo B level must be distinguished from that of LDL particle size. Many patients with hyperapo B have small, dense LDL particles, and recent reports suggest that small, dense LDL raise the risk for CHD. Such LDL particles are low in cholesterol, and hence appear to be relatively "enriched" in apo B, although in fact there is still only one apo B molecule per particle. Without question, determination of LDL cholesterol level gives an underestimated total number of LDL particles in such patients. Many patients having small, dense LDL also have hypertriglyceridemia, and since hypertriglycerideemic patients may have hyperapo B, some people have mistakenly concluded that small, dense LDL in the presence of elevated triglycerides is synonymous with hyperapo B. The two are not synonymous, and patients can have small, dense LDL particles without having hyperapo B. Seemingly, some immunosassays for apo B "over-read" small, dense LDL and thus give erroneously high values for apo B in their presence. Part of the increased risk for CHD previously attributed to hyperapo B, therefore, may be due instead to the presence of small, dense LDL.

Finally, we can speculate on the potential value of apo B measurement for the future. The first step necessary for its use is to standardize methodology. Without accurate methods, systematic evaluation of the utility of apo B measurement is impossible. Once standardized methods become available, the predictive power of apo B in prospective studies can be compared with standard cholesterol measurements including lipoprotein cholesterol and cholesterol ratios. In addition, a search can be made for subgroups of patients who have levels of apo B that are increased outside of proportion to cholesterol levels. If such groups can be identified, they may deserve drug therapy for lowering of lipoproteins even in the presence of normal lipid concentrations. Identification of such patients, however, without appropriate apo B methodology is suspect, and for now, measurement of apo B on a routine clinical basis cannot be recommended.

### Table 1. Comparable Values for Low Density Lipoprotein Cholesterol, Apolipoprotein B-associated Cholesterol, and Total Apolipoprotein B according to Risk Category

<table>
<thead>
<tr>
<th>Category</th>
<th>LDL cholesterol</th>
<th>Apo B-associated cholesterol (LDL+IDL+VLDL)</th>
<th>Estimated total apo B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desirable</td>
<td>&lt;130</td>
<td>&lt;160</td>
<td>&lt;105</td>
</tr>
<tr>
<td>Borderline high</td>
<td>130–159</td>
<td>160–189</td>
<td>105–124</td>
</tr>
<tr>
<td>High risk</td>
<td>160–189</td>
<td>190–209</td>
<td>125–139</td>
</tr>
<tr>
<td>Very high risk</td>
<td>≥190</td>
<td>≥210</td>
<td>≥140</td>
</tr>
</tbody>
</table>

The values are given as mg/dl.

Apolipoprotein B-associated cholesterol is non-HDL (LDL+IDL+VLDL) cholesterol. The risk categories are from the National Cholesterol Education Program guidelines.

apo = apolipoprotein, LDL = low density lipoprotein, IDL = intermediate density lipoprotein, VLDL = very low density lipoprotein, HDL = high density lipoprotein.

### References


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