Can Measurement of Apolipoprotein B Replace the Lipid Profile in the Follow-up of Patients with Lipoprotein Disorders?

Apolipoprotein B (apo B) was so called because it was the principal protein found in β lipoprotein (LDL). It was later discovered that it is the major protein component of chylomicrons, chylomicron remnants, VLDL, intermediate density lipoprotein, LDL, and lipoprotein(a); it is absent from HDL. Thus apo B is present in lipoproteins that are potentially atherogenic and absent from those that are antiatherogenic. This prompted Fredrickson to write in 1974, “Its resistance to characterization, its seeming essentiality for glyceride transport, and perhaps the added suspicion that it has something to do with atherogenesis have all transformed apo B into one of the central mysteries of lipoprotein physiology” (1). This statement was remarkably prescient; apo B proved to be essential for hepatic and intestinal triglyceride secretion and for LDL catabolism and to be intimately involved in atherogenesis.

In the human, circulating hepatic apo B has 4536 amino acids and a molecular mass of ~540 000 Da, whereas intestinal apo B present in chylomicrons has a molecular mass that is 48% that of hepatic apo B (2). Hence hepatic and intestinal apo B are termed apo B_{100} and apo B_{48}, respectively. Both are the products of a single gene, but its message is edited in the enterocyte after translation of the N-terminal 2152 codons representing the amino acid sequences necessary for triglyceride-rich lipoprotein assembly and secretion. Hepatic and intestinal steatosis occur both in a-β-lipoproteinemia (reflecting mutation of the microsomal triglyceride transfer protein, which allows apo B to associate physically with triglycerides in the liver and gut) and in homozygous hypo-β-lipoproteinemia when mutations truncate apo B early in its sequence (2). The additional part of the molecule present in apo B_{100} is necessary for LDL-receptor binding and thus for the receptor-mediated catabolism of LDL. Mutations of apo B in this region can decrease the fractional catabolic rate of LDL, producing hypercholesterolemia. Probably the most common of these mutations, seen in the condition called familial defective apo B, is a mutation at position 3500. This affects ~1 in 500–600 people in the US. Its clinical expression is varied, but at its most severe it can produce a phenotype similar to that in heterozygous familial hypercholesterolemia resulting from a mutation of the LDL receptor itself. Of even greater potential significance is the increase of serum apo B that arises from overproduction of apo B_{100}-containing lipoproteins. This is the etiology of the dyslipoproteinemias most commonly associated with atherosclerosis (2–5). Furthermore, in recent years it has been discovered that fragmentation of apo B by lipid peroxidation products can block the uptake of lipoproteins by the LDL receptor and lead to their recognition by scavenger and other receptors of cells involved in atherogenesis, such as arterial wall monocytes and macrophages and endothelial and smooth muscle cells (6, 7).

Because each VLDL, intermediate-density lipoprotein, and LDL particle contains only a single apo B molecule, the serum apo B concentration is a close reflection of the number of such particles present. Except in the most severe hypertriglyceridemia, >90% of the apo B-containing lipoproteins are LDL particles (3). On first principles, it may be better to measure a substance, such as apo B, that is directly involved in a pathologic process, like atheroma, rather than a surrogate, such as cholesterol. Is there, however, any more pragmatic reason why we should depart from the time-honored measurement of LDL cholesterol? Sniderman and colleagues (4, 8) have been at the heart of this controversy for many years. In support of these authors, evidence has progressively accumulated that LDL cholesterol, particularly when hypertriglyceridemia is present, is a poor reflection of the LDL concentration (8), because a small dense LDL subfraction depleted in cholesterol is present particularly when coronary risk is increased (9). This LDL subfraction is poorly cleared by the physiologic LDL receptor and is susceptible to oxidative modification. Until recently, calibration of apo B assays was not good, but this is no longer a reason for clinging to LDL cholesterol measurement (10). It is true that apo B measurement is less precise than total serum cholesterol. However, it is better than LDL cholesterol, which, whether estimated indirectly by the Friedewald formula, or assayed directly by ultracentrifugation, has greater inter- and intraassay CVs than modern apo B immunoassays. Whatever battles have been won or lost previously (8), in this issue of Clinical Chemistry (11), Sniderman and his colleagues argue that, at least in the monitoring of lipid-lowering drug therapy, patients would benefit from the measurement of apo B.

There has been much discussion of what are appropriate target LDL cholesterol cutoffs for statin therapy. However, whether LDL cholesterol cutoffs of 130 or 100 mg/dL (3.4 or 2.6 mmol/L, respectively) are chosen (12), there is considerable individual variation in the LDL particle concentration at these low concentrations, particularly when they have been achieved with statins. We have, for example, recently found that serum apo B concentrations were 650 ± 180 mg/L (mean ± SD) in 91 type 2 diabetic patients receiving statin treatment despite having achieved LDL cholesterol <40 mg/dL (mean, 28 ± 12 mg/dL, 0.7 ± 0.3 mmol/L) and serum triglycerides of 98 mg/dL (1.1 nmol/L; median range: 35–310 mg/dL, 0.4–3.4 mmol/L); Menys V, Mackness MI, Durrington PN, unpublished observation). This issue should undoubtedly be more thoroughly assessed given the finding of several studies that the concentrations of lipids, including LDL cholesterol, achieved on statins may not predict outcome as well as serum apo B (13–15). It has not been widely appreciated that the Friedewald formula, by which LDL cholesterol is generally estimated in clinical laboratories, may not hold true at the low concentrations of LDL cholesterol that are presently achieved regularly.
with statin therapy (16). The answer is not necessarily to use a direct chemical or immunologic method to measure LDL cholesterol because such methods have not been sufficiently validated in, for example, hypertriglyceridemia and diabetes, and, of course, the criticism that cholesterol may not reflect particle number still applies to them. Even measurement of LDL cholesterol by ultracentrifugation may not be the best approach, given that the highly atherogenic cholesterol-depleted LDL that persists despite therapy may still be missed, unless the highly specialized methods for LDL subtraction analysis are used (9). Thus in comparison with all these, the immunoassay of apo B is looking decidedly robust (10). Assays of apo B can be performed in any clinical chemistry laboratory using standard automation. Fasting is unnecessary both because of the relatively small contribution of apo B_{48}-containing lipoproteins to serum apo B and because epitopes for antibodies used in immunoassays that have been raised against apo B_{100} may be missing from apo B_{48}. Of course, fasting is necessary for the Friedewald formula because it depends on measurement of fasting triglycerides.

For monitoring statin therapy, therefore, there is much support for apo B assay. It must be conceded that the response to fibrate drugs cannot be monitored by measuring total serum apo B because their effect is not so much to alter the LDL particle concentration (5, 17) as to increase the size of the smaller LDL particles (9). More precise monitoring of their effects than is possible with standard triglyceride and HDL cholesterol measurement must await more widely applicable methods for detecting small, dense LDL. Furthermore, apo B assay cannot yet replace lipid measurement in the assessment of coronary heart disease risk in primary prevention. There is no risk prediction equation similar to that from the Framingham study, which allows apo B to be included in risk estimation for men and women over a wide age range, taking into account smoking and blood pressure. Thus the third National Cholesterol Education Program (18) recommends a scoring system based on the Framingham risk equation using lipid measurements. Therefore, for the foreseeable future these will be necessary in assessing coronary heart disease risk. Once the decision to introduce statin therapy has been taken, however, apo B measurement requires careful consideration as a means of monitoring such therapy. In the future, it may be possible to develop apo B immunoassays that are even more informative about coronary heart disease risk by, for example, designing them to be particularly sensitive to epitopes displayed in small, dense LDL or oxidatively modified LDL.

References