Measurement of Apoproteins: Time to Improve the Diagnosis and Treatment of the Atherogenic Dyslipoproteinemias

The articles by Contois et al. [1, 2] describing the reference limits for apolipoprotein (apo) B and apo A-I derived from the Framingham Study population represent another critical advance in the knowledge base necessary if measurement of apoproteins is to become possible in routine clinical practice. We believe it is in the interest of clinical laboratories, physicians, and, most of all, our patients that this should occur. In the first place, measurement of apo B and apo A-I have been standardized [3-6], whereas measurement of the lipoprotein lipids has not. That alone is a major argument in favor of their assay. Moreover, apo B appears to be a more accurate clinical measure of atherogenic risk from low-density lipoproteins (LDL) than is total cholesterol or LDL cholesterol. Given the evidence now in hand that marked lowering of LDL substantially reduces the mortality in a large proportion of those with coronary disease [7], we believe that measurement of apo B should now be available in all clinical laboratories. By contrast, it is not clear that measurement of apo A-I is superior to high-density lipoprotein (HDL) cholesterol as a measure of atherogenic risk. Our comments here, therefore, will center on apo B.

The present diagnostic and therapeutic algorithms are built on LDL cholesterol [8, 9]. As a consequence of the technical weaknesses inherent in its measurement and the physiological limitations of LDL cholesterol as a measure of atherogenic risk, major disadvantages are automatically built into these algorithms—and should no longer be ignored. To begin with, LDL cholesterol is calculated, not measured, and—worse yet—is calculated from several unstandardized assays, each of which necessarily involves its own errors in measurements. Is it any wonder then that unacceptable error rates in classification occur, particularly when triglycerides are even marginally increased [10, 11]? Obviously, errors in diagnosis may well lead to errors in therapy. Of course, direct measurement of LDL cholesterol is now possible. However, only limited experience has yet been gained with this technique, and methodological issues such as the considerable dilution required raise concern. In any case, as we will point out, a direct assay does not overcome the central problem that LDL cholesterol only incompletely estimates the risk due to atherogenic apo B particles.

There are other issues. The first may seem trivial but is not, at least if you are the patient: the necessity in the present scheme for fasting samples. In everyday clinical practice, the actual length of the fast is surely variable, introducing yet another source of error. Moreover, the requirement for a fasting sample sharply limits the time of day when samples can be obtained and more importantly imposes the burden of fasting on the patient. Surely, the cumulative sum of these inconveniences must play an important role in the low compliance rates with hypolipidemic therapy. The next problem pertains to doctors; namely, the present system is complicated in terms of therapeutic decision-making. The present classification system of the dyslipoproteinemias has not been revised since its introduction in 1967 [12]. To be sure, new factors have been recognized in the interim: HDL cholesterol; lipoprotein(a); apo E phenotype; and small, dense LDL, to name only some. But how should these relate to LDL cholesterol in terms of clinical decision-making, and just how many atherogenic phenotypes should a practicing physician be expected to carry in his or her head? Given the complex and convoluted nature of the present diagnostic algorithms, is it any wonder that so few of those who need therapy, and would benefit from it, actually receive it?

Diagnostic and therapeutic algorithms work better if they are simple. This is a major advantage for measuring apo B: High concentrations of apo B confer increased risk for vascular disease, and lowering apo B concentrations brings benefit. This simple but not simplistic statement describes the precise relation between apo B concentration and the number of apo B particles in plasma. Each particle of very-low-density lipoproteins (VLDL) secreted by the liver contains one molecule of apo B, which stays with the particle during its lifetime in plasma [13]. The total number of VLDL, intermediate-density lipoproteins (IDL), and LDL particles is therefore given by the total plasma apo B concentration, a value that changes relatively little postprandially that fasting samples are not necessary [14-16]. Because the time to halve the concentration of LDL in plasma is so much longer than that for VLDL, the two clearly atherogenic B100-containing lipoprotein particles—IDL and LDL—make up >90% of the total number of B100 particles present in plasma at any time. This holds even for hypertriglyceridemic patients [17, 18] and means that, for clinical purposes, plasma apo B concentration is equivalent to the number of atherogenic apo B particles. There are, in fact, two forms of apo B: apo B100 and apo B48. All the apo B particles secreted by the liver contain one molecule of apo B100; those secreted by the intestine contain one molecule of apo B48. Thus, whenever chylomicron particles are present, apo B48 is also, and the commercially available assays almost certainly recognize it as well as apo B100. Nevertheless, this does not pose a problem because, even at peak postprandial concentrations, the mass of apo B48 present is trivial (1% or less) compared with the mass of apo B100; consequently, no error of significance is introduced [19, 20]. Thus, LDL cholesterol is inadequate as a measure of LDL because of LDL heterogeneity. That LDL particles differ in composition is now well established [21, 22], as is the fact that coronary patients frequently have increased numbers of smaller, denser LDL particles [23, 24]. Because smaller, denser LDL particles contain less cholesterol than do normal LDL, the LDL cholesterol measured in such patients inadequately reflects the atherogenic risk of the LDL present. Measurement of apo B is key because, particle for particle, the smaller, denser LDL appear to be more atherogenic than normal LDL particles [25].

We believe that measurement of apo B in hypertriglyceridemic patients has become essential. Whether hypertriglyceridemia per se increases the risk of coronary disease is a vexing and much disputed issue. Much of the debate, we believe, is generated by the reality that type IV hyperlipoproteinemia, as defined in 1967 [12], includes a very heterogeneous collection of disorders, particularly with respect to apo B. About one-third of patients with hypertriglyceridemia have an apo B concentration above the 90th percentile of the population [17, 26]. Given the marked increase of apo B particle numbers in such patients, their marked increase in coronary risk compared with that of hypertriglyceridemic patients with normal apo B numbers should not be surprising. This difference in risk has been demonstrated in
several cross-sectional studies [26–30], and now even clearer
evidence is available from a recently completed prospective
study [31]. This study—the Quebec Heart Study—
demonstrates a threefold increase of risk in type II hypercholester-
olemia, in type IV hyperlipoproteinemia with increased apo B, and
in normolipidemia with increased apo B. All three phenotypes
share a common denominator—an increased number of LDL
particles. By contrast, hypertriglyceridemia with a normal apo B
number was not associated with any increase in risk.

Not only does measurement of apo B identify those at
increased risk, it also points the way to therapy. With the results
of the WOSCOPS trial now in hand [32], plus those of the 4S
[7], plus the host of angiographic trials [33], surely the prece-
dence in therapy must be to lower an increase in LDL particle
numbers. The preceding wide and deep array of evidence is the
basis for the view that, in hypertriglyceridemic patients with
high apo B concentrations, statins—not fibrates—should be the
preferred therapeutic route [34]. Similarly, statins are the ther-
apy we prefer for the normolipidemic patient with high apo B
who requires therapy. Even with moderate hypercholesterol-
emia, the degree of abnormality of LDL may be masked if the
apo B content is not known. Thus, under virtually any circum-
stance—the sole exception being marked hypercholesterolemia
such as in familial hypercholesterolemia—apo B adds important
information. Simply put: Until the measurement of apo B is
available in routine clinical laboratories, doctors cannot make
the best choice of therapy for most of their patients.

Cutpoints are essential for clinical decision-making and we
present these as suggestions to be considered. But several
important issues must be thought through before any specific
cutoff values are adopted. For example, it is excellent to have
data separated by age and sex because these make it obvious that
apo B increases importantly with age and differs with sex [1]. If,
however, one chooses age-adjusted cutpoints, one may easily
start to think that apo B becomes less dangerous with age—an
assumption that seems most unlikely. Therefore, we suggest that
the percentile distribution of values for (e.g.) the 50-year-olds be
used, after having averaged the results for men and women. In
the absence of other risk factors, an apo B value greater than the
75th percentile should be regarded as high risk and a value
greater than the 50 percentile as moderate. We have picked the
50th percentile on the basis of our study [35], which showed that
<10% of patients with coronary disease had an apo B value less
than this. In the presence of other risk factors, a value greater
than the 50th percentile should qualify as high risk. In patients
at high risk, pharmacological therapy should be considered in
addition to diet and weight-loss therapy.

With respect to secondary prevention, apo B concentrations
should be lowered to at least the 50th percentile. If data from
recent studies such as the 4S [7] point to an incremental benefit
with even greater lowering of apo B, then we should amend this
recommendation. We anticipate that target values for apo B will
approximate the 30th percentile of the population.

Three arguments remain against measuring apo B: Do data
from prospective studies support this approach? Would using
this approach create unacceptable cost? Would any change in
what we measure diminish the credibility of the public campaign
against cholesterol? As to the first, here we disagree with the
views of Contois et al. [1]. To be sure, negative studies exist—or
rather, studies that have been interpreted as negative, the most
influential being that by Stampfer et al. [36]. However, in that
study, nonfasting samples were analyzed, the effect of which was
to exaggerate the triglyceride and HDL cholesterol deviance
from normal. In addition, the question tested was not whether
apo B was a better index of risk than total cholesterol or LDL
cholesterol but whether apo B was better than the total prog-
nostic information available from the ratio of total to HDL
cholesterol plus the clinical risk factors—a tough test indeed!
Moreover, at that time, apo B assays were not as reliable as now
and certainly were not standardized. More recent studies have
reached different conclusions. For example, Wald et al. [37]
found apo B to be the variable most closely associated with risk.
More recent data from the Quebec Heart Study [38] confirm
and extend these findings. The Quebec Heart Study differs
importantly from those previously conducted, which, although
prospective, used a case control design. This means that the
results for only a small proportion of those in the study were
used in the analysis. Usually, such studies include all the cases
and a small but variable number of controls. The weakness
of this approach is that those chosen to be controls may not
adequately reflect the much larger group they are selected from.
In the study of Wald et al. [37], five times as many controls as
cases were used—a traditional approach—whereas Stamper et al.
[36] used an equal number of cases and controls—a riskier
approach. In any case, in the Quebec Heart Study, the analysis
included every participant in the analysis and demonstrated that
not only was apo B the single most important determinant of
risk but, in fact, was an even better marker of risk than the LDL
cholesterol/HDL cholesterol ratio [38]. Also of interest, the
EARS study, the largest and most systematic examination of risk
factors in the offspring of fathers with a documented history of
early coronary disease [39], likewise showed apo B to be the best
separator of affected subjects from controls of similar age. Thus,
apo B better demonstrated risk of coronary disease, whether in
the parents or in the progeny.

The second concern is cost. Measurement of apo B is easy to
automate, which reduces labor costs. Measurement of HDL
cholesterol, on the other hand, has not yet been automated, so
labor costs must be added. Furthermore, test costs are also
obviously a function of volume, and savings are certainly possi-
ble as volume increases. But testing could also change in other
ways. For example, if lipids, lipoproteins, and apo B were
measured at the beginning of a patient's treatment for a complete
assessment, why would plasma and lipoprotein lipids have to be
measured on follow-up? If these latter analytes are not needed at
follow-up, the necessity for fasting is avoided, and the
number of measurements is reduced. The process of therapy
becomes simpler and cheaper.

The third concern is almost always unstated but is perhaps the
greatest impediment to change: After so much emphasis on
cholesterol as the hallmark for risk assessment, is it possible to
change to apo B without confusion and loss of credibility?
Fortunately, to state the problem is to overcome it. If change is
in the patient's interest, if change leads to more precise diagnosis
treatment, if change makes our practice simpler and in the
end less expensive, should we not welcome change rather than
resist it? The views we express may challenge conventional
practice, but they do not represent a challenge to conventional
wisdom. On the contrary, they reemphasize that LDL are key to
the pathogenesis of arteriosclerosis and that lowering LDL is
key to prevention of its complications. Measuring apo B is
simply a better way of measuring LDL than is assaying LDL
cholesterol or total cholesterol.
References


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