Reduced plasma levels of high-density lipoproteins (HDL) were found to be associated with increased risk of atherosclerosis more than 50 years ago. Diverse methodologies including electrophoresis, ultracentrifugation, and biochemical characterization were used even at that time to examine the association between the cholesterol, phospholipid, and protein composition of HDL, also known as α-lipoproteins, and atherosclerosis.1–4 Within 25 years of these seminal observations, the model was proposed in which atherosclerosis is driven by an excess of atherogenic lipoproteins (primarily low-density lipoprotein), which are deposited in the vessel wall, and a deficiency of protective HDL particles, which control cholesterol efflux from the vessel wall.5

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In our current models of atherosclerosis, the major mechanism for the atheroprotective properties of HDL is thought to be reverse cholesterol transport, a process by which lipid-poor nascent HDL particles accept cholesterol from peripheral cells through the interaction of apolipoprotein A-I (apoA-I), the major protein of HDL, with ATP-binding cassette protein A1. After esterification of the cholesterol by the interaction of lecithin:cholesterol acyltransferase and apoa-I, the HDL particle transfers the cholesteryl esters to the liver via scavenger receptor B1 or to the apoB-containing particles (in exchange for triglycerides) through the action of cholesteryl ester transfer protein.

The cholesterol content in HDL (HDL-C) has been inversely related to risk for coronary heart disease (CHD) in numerous epidemiological studies, with each 1-mg/dL increase in HDL-C associated with an ~2% to 3% decrease in risk for CHD.6 Measurement of HDL-C is recommended for all adults to assess the risk for CHD, and the HDL-C level is to be considered in the development of treatment strategies. Although measurement of HDL-C has proven clinical utility, we know that HDL includes a diverse group of particles with variations in apolipoprotein composition, size, charge, and function. Therefore, one would postulate that more sophisticated methods to subclassify HDL (Figure) would lead to tests that provide better clinical utility to assess risk of cardiovascular disease and response to therapy.7 Density gradient ultracentrifugation has been used to classify HDL on the basis of flotation characteristics: HDL2 is a large, lipid-rich, lower-density particle, and HDL3 is a smaller denser particle. Although many studies comparing HDL subclasses defined by density gradient ultracentrifugation have found the larger HDL2 particles to be more atheroprotective than the smaller HDL3 particles,8,9 in some large prospective epidemiological studies, HDL2 and HDL3 measurements did not provide any additional predictive value beyond that of HDL-C.10

ApoA-I is the major apoprotein in HDL, and measurement of apoA-I, rather than HDL-C, has been proposed as a better method to assess protective levels of HDL. Although some studies have shown that apoA-I is superior to HDL-C, other large prospective studies have shown no additional benefit of apoA-I over HDL-C.10,11 HDL can be classified on the basis of the apolipoprotein composition: some HDL particles contain only apoA-I (LpAI), whereas others contain both apoA-I and apoA-II (LpAI:AII). A number of studies comparing LpAI and LpAI:AII have suggested that LpAI may be more atheroprotective than LpAI:AII, but other studies did not show this distinction.12 Characterization of HDL by electrophoretic mobility or gradient gel electrophoresis has also not consistently shown superiority over measurement of HDL-C. A more sophisticated approach has been developed by Asztalos and colleagues to assess the apoA-I levels in various subclasses of HDL by using native 2-dimensional gel electrophoresis that separates HDL by electrophoresis into pre-β, α, and pre-α in the first dimension followed by nondenaturing 3% to 35% polyacrylamide gel electrophoresis in the second dimension with immunoblotting to quantitate apoA-I in the resulting 12 identified HDL subpopulations.13

In this issue of Atherosclerosis, Thrombosis, and Vascular Biology, Asztalos et al present the results of their study evaluating the measurement of 8 HDL subpopulations (pre-β-1, pre-β-2, α-1, α-2, α-3, pre-α-1, pre-α-2, pre-α-3) determined by 2-dimensional gel electrophoresis and immunoblotting to predict risk of new cardiovascular events in a substudy of the Veterans Affairs HDL Intervention Trial (VA-HIT).14 In VA-HIT, 2531 men with CHD and HDL-C of 40 mg/dL or less, and low-density lipoprotein cholesterol of 140 mg/dL or less were randomized to therapy with gemfibrozil or placebo.15 In the current study, 398 patients with cardiovascular events (CHD death, stroke, or myocardial infarction) and 1097 subjects without cardiovascular events were included. In addition, the authors analyzed 431 patients...
without cardiovascular disease from the Framingham Off-
spring Study.\textsuperscript{16}

Subjects from VA-HIT who developed new cardiovascular
events were found to have more apoA-I in the poorly
lipidated, small $\alpha$-3, and pre-$\beta$-1 HDL particles (3% and 9%,
respectively) and less apoA-I in the better lipidated and larger
$\alpha$-1 and $\alpha$-2 particles (12% and 7%, respectively) compared
with subjects without new cardiovascular events. For every
SD increase in $\alpha$-1 and HDL-C, the hazard for new events
decreased by 18\% ($P=0.002$) and 15\% ($P=0.015$), respec-
tively. The results were similar in the placebo arm (ie, no
gemfibrozil therapy) of VA-HIT, which had a 12\% decrease
in new cardiovascular events for each 1-SD increase in
$\alpha$-1 levels. Among HDL-related variables, the $\alpha$-1 apoA-I level
was the most significant risk factor measured for develop-
ment of a new cardiovascular event.

These results add to a growing body of evidence in
different populations and circumstances for the importance
of HDL subfractions, in particular $\alpha$-1, for predicting CHD risk.
The same group has shown that simvastatin–niacin combina-
tion therapy significantly increased apoA-I–containing $\alpha$-1
HDL concentration in 123 subjects with CHD in the HDL-
Atherosclerosis Treatment Study (HATS) and in turn was
associated with less progression of coronary stenosis.\textsuperscript{17} Similar-
ly, a severe depletion in $\alpha$-1 and pre-$\alpha$ species of apoA-
I–containing HDL subpopulations was noted in CHD cases
compared with controls even after stratifying by HDL-C <35
mg/dL and $>35$ mg/dL.\textsuperscript{18}

The question that needs to be given further consideration is
how best to use this observation: should HDL subclasses be
used primarily for research, only in high-risk patients, or in
routine clinical practice? Researchers like Ridker\textsuperscript{19} and
Manolio\textsuperscript{20} have suggested a useful framework when consi-
dering the clinical utility of a new risk marker, including
answering certain questions: (1) is there consensus as to the
best way to measure the risk marker, (2) are the results of
series of prospective epidemiological studies consistent with
each other, (3) is the marker able to add to our ability to
predict risk over and above existing tools, (4) is the marker
able to account for a large proportion of risk associated with
the disease, (5) is the assay reproducible (ie, low coefficient
of variation), (6) what are the sensitivity and specificity of the
marker in predicting disease, and (7) what are the cost,
availability, and ease of performance of the test?

This study adds more support to a growing body of
epidemiological data that have demonstrated that large cho-
lesterol-rich HDL subspecies such as $\alpha$-1 are cardioprotec-
tive. Although the inter- and intraassay coefficients of var-
iation in this study were reported to be <10\%, it is not clear
whether this method could be implemented in routine clinical
chemistry labs with coefficients of variation remaining
<10\%. In contrast, because of extensive standardization and
automation, measurement of HDL-C routinely has coeffi-
cients of variation $\leq$5\% and is relatively inexpensive.

In addition to helping with risk assessment, measurement of
HDL subspecies may help to determine the efficacy of
therapies (ie, $\alpha$-1 apoA-I may be an improved surrogate
marker for efficacy of lipid-modifying drugs). Statins (albeit
in varying degrees) have been shown to modify and signifi-
cantly increase $\alpha$-containing and pre–$\alpha$–containing HDL
subpopulations.\textsuperscript{21,22} Although statins have been shown to
increase $\alpha$-1 apoA-I, we do not know whether the increased
levels of $\alpha$-1 apoA-I on statin therapy are associated with a
reduction in events. In contrast, we do have data that the
change in apoA-I on statin therapy was associated with event
reduction.\textsuperscript{23} Prior attempts that have used HDL subspecies
to predict risk have yielded mixed results. In an earlier report
from VA-HIT, HDL$_3$ and not HDL$_2$ was found to be predic-
tive of CHD events.\textsuperscript{24} The current study reports only baseline
values and does not provide information as to whether
changes in HDL subpopulations were associated with the
event reduction with gemfibrozil. Clearly, future studies that
examine the association between change in $\alpha$-1 apoA-I level
with statins, fibrates, or other agents and reduction in cardio-
vascular events are needed.

Treatment of patients with low HDL-C levels is now the
focus of major efforts in the development of new therapies,
and new therapies such as cholesteryl ester transfer protein inhibitors and apoA-I infusions will provide further key information on HDL and atherosclerosis. Identifying and understanding the various steps in HDL metabolism and quantitative assessment of HDL subspecies will continue to be an important research area and may provide improved risk assessment and better surrogates for monitoring therapy.

References


Fat, Fit, and Leading the Charge: The Evolution of Measuring High-Density Lipoprotein Subpopulations
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