In Search of the Ideal Measure of High-Density Lipoprotein

Measurement of any heterogeneous substance poses a challenge. HDL-cholesterol (HDL-C) is no exception. Numerous methods, based on different principles, have been widely adopted and these different methods often give significantly different values for HDL-C. The paper of Okazaki et al. [1], in this issue, demonstrates the inadequacy of a phosphotungstate precipitation method for HDL-C and proposes the use of HPLC as a tool to compare analytical methods for HDL-C.

Interest in developing and evaluating methods for the measurement of HDL-C has origins in two separate arenas—one clinical, reflecting increased attention to HDL-C as a risk factor for coronary heart disease (CHD), and the other economic, reflecting increased emphasis on finding more-cost-effective ways to measure HDL-C.

Although HDL-C has long been recognized as a factor in the development of CHD, it has not received nearly the attention given to LDL-cholesterol. Much of the primary and secondary prevention effort is based on risk as defined by LDL-cholesterol. Treatment is targeted at reducing LDL-cholesterol, and success of therapies is judged by their impact on LDL-cholesterol. Nevertheless, in many studies, HDL-C has been shown to be a stronger predictor of risk than CHD and LDL-cholesterol. For example, in the Framingham Heart Study, a 1% increase in LDL-cholesterol translated to a 2% increase in risk, whereas a 1% decrease in HDL-C translated into a 3–4% increase in risk [2]. In the Helsinki Heart Study, in those subjects with high LDL-cholesterol (Frederickson Type IIa hyperlipidemia), the greatest predictive value for CHD was low HDL-C, not the severity of the LDL-cholesterol increase [3]. In a review of nine different prospective studies in women, HDL cholesterol emerged as the single most important lipid risk factor in women [4].

Recently, the National Cholesterol Education Panel released the Adult Treatment Panel II Guidelines (NCEP ATP II) [5], a revision of earlier guidelines, that place new emphasis on HDL-C. The ATP II guidelines recommend the addition of HDL-C to initial cholesterol testing, despite the lack of direct treatment data. Low HDL-C (<350 mg/L), as an independent indicator of risk, is equivalent to nonlipid risk factors such as smoking or hypertension. High HDL-C (>600 mg/L) is a negative risk factor, thereby compensating for the presence of a positive risk factor. The increased clinical importance attributed to HDL-C concentrations now places additional burden on the clinical laboratory to provide accurate and precise measures of HDL-C, especially at the decision points of 350 and 600 mg/L.

Traditional measurement of HDL-C has been a cumbersome, labor-intensive process involving pretreatment of the serum with a precipitating agent to remove apolipoprotein (apo) B-containing lipoproteins, followed by analysis of the isolated supernatant for cholesterol. In the presence of high triglycerides, precipitation is often incomplete, requiring manual dilution, repeat precipitation, and reanalysis. The requirements for manual handling and special treatment translate into an expensive test, compared with other lipid measures such as total cholesterol or triglycerides. In an era of managed care and decreasing reimbursement, laboratories must identify more cost-effective ways to perform high-volume tests. The inclusion of HDL-C in screening protocols and the use of HDL-C to calculate LDL-cholesterol make HDL-C a high-volume test and a natural target for cost-cutting measures.

To overcome inefficiencies associated with traditional testing for HDL-C, several new, direct methods have been developed. These methods eliminate the need for pretreatment steps by performing enzymatic analysis for cholesterol in a system where the reaction of cholesterol in the non-HDL lipoproteins is selectively inhibited. One direct HDL method, for example, uses a-cyclodextrin sulfate as a sequestering agent and polyethylene glycol (PEG)-modified cholesterol esterase to restrict the reaction to the cholesterol contained in HDL particles [6]. Another method combines the use of PEG 4000 to sequester VLDL and LDL and antibodies to apoprotein B and C-III to produce aggregates of the chylomicrons, VLDL, and LDL that will not react with cholesterol esterase [7]. Additional methods include direct inhibition of the reaction of non-HDL cholesterol by use of antibodies to beta lipoproteins [8] or the use of polyanions and polymers to complex apo B-containing lipoproteins, which are stabilized in an unreactive form with a detergent that solubilizes HDL [9].

The major advantage of direct methods is the elimination of manual pretreatment. In direct (homogeneous) reaction systems, an inhibitor is mixed with the serum and, after appropriate incubation time for complexation of beta lipoproteins, enzymatic reagents for the determination of cholesterol are mixed directly with the treated serum sample. Most automated analyzers can perform these steps in a single cuvette; hence HDL-C can be determined directly from the same serum sample as that used for determination of cholesterol and triglyceride, resulting in increased efficiency. Another advantage of some direct HDL-C methods is the absence of interferences from high concentrations of triglycerides. Triglycerides as high as 18 000 mg/L have been demonstrated to have minimal effect in two different direct methods [6, 10], although a third such method demonstrated considerable bias with triglycerides >4000 mg/L [9].

The introduction of any new method for HDL-C raises several questions. How accurate is the new method? How well does the new method compare with older, more established methods—those on which CHD risk is defined? Only a limited number of studies have compared a direct method with the currently recommended ultracentrifugation Reference Method [9–11]. Most correlations have been limited to comparisons of direct methods with various precipitation methods. The tediousness of ultra-
centrifugation and its requirement for relatively large sample volumes discourage routine cross-over studies between new HDL methods and the recommended Reference Method.

The article by Okazaki et al. [1] describes a new approach to the measurement of HDL-C with an HPLC system that physically separates the lipoprotein fractions and detects and quantifies cholesterol in the effluent. This system offers the advantages that it is less tedious than ultracentrifugation and requires only 20 µL of serum. The authors use this system to demonstrate the limitations of a commonly used precipitation step as a function of the concentration of MgCl₂ in the precipitating reagent. They show that too little MgCl₂ (<22 mmol/L) under the conditions of their assay results in underprecipitation of non-HDL, whereas too much MgCl₂ (>22 mmol/L) results in overprecipitation of HDL. Because the commercially marketed form of this reagent includes 44 mmol/L MgCl₂, these findings suggest that routine use of the commercially formulated reagent will result in a systematic error and consequently reporting of low HDL-C values. The authors also demonstrate the robustness of a direct method for HDL-C, with results close to target HDL-C values over a wide range of HDL concentrations and across a broad range of triglyceride concentrations. Regrettably, the authors did not include comparison of this HPLC method and the ultracentrifugation method that is currently endorsed as the Reference Method for HDL-C to provide evidence of accuracy of the assigned target values [11].

The biases among different HDL-C methods are generally attributed to preferential measurement of different subpopulations of HDL, especially those that differ in apo E content [12, 13]. Although identification of subpopulations of HDL has not yet found use in clinical settings, subpopulations of HDL have been a focus of research aimed at understanding metabolism of HDL. The hope was that a single subpopulation might emerge as the important HDL type, the HDL responsible for protection from CHD and the HDL that provides the clue about how HDL functions. Although some studies have identified associations of certain subpopulations with CHD risk, others have failed to find any associations. The discrepancies in findings may stem from the methods used to identify and quantify the three subpopulations (HDL-1, HDL-2, and HDL-3), since identity may be based on different densities as separated by ultracentrifugation, on different solubility characteristics as separated by differential precipitation, on different sizes as separated by gel filtration, or on different protein content as distinguished immunologically by antibodies to apo AI, AII, and E [13, 14]. However, HDL is likely to be even more heterogeneous than the existence of three subpopulation designations would suggest: High-resolution separation techniques have demonstrated as many as 14 subclasses of HDL [15].

Given that the mechanisms by which HDL provides protection have not been elucidated nor have the characteristics of desirable HDL subpopulations (those whose absence results in increased CHD risk), we cannot readily endorse a particular HDL method as providing the best measure of HDL-related risk of CHD. Until such time as we are able to target the measurement of the most “protective” HDL particles, we should strive to measure all HDL-C. The HPLC system described by Okazaki et al. [1] offers a useful tool for monitoring the ability of other HDL methods to do so.

References


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