Estimation of LDL-Associated Apolipoprotein B from Measurements of Triglycerides and Total Apolipoprotein B

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BACKGROUND: VLDL and chylomicrons may interfere with measurements of apolipoprotein B (apo B) on LDL particles. Ultracentrifugation of samples enriched with measurements of triglycerides, total apo B, and LDL–apo B. Samples were ultracentrifuged, and 3 commercially available immunoassays that use different antibodies were used to measure LDL–apo B in the 1.006 infranate fraction.

RESULTS: After adjusting for triglyceride concentration, we found total apo B and LDL–apo B measurements to be strongly correlated. We derived a simple linear equation for calculating LDL–apo B concentration (in milligrams per deciliter) from measurements of triglycerides, total apo B, and LDL–apo B. This equation accurately predicts LDL–apo B values within ±12% of the measured value in 75% of cases.

CONCLUSIONS: Our equation provides a convenient means of estimating LDL–apo B from commonly available measurements of total apo B and triglycerides without the need for ultracentrifugation. LDL–apo B measurements were also independent of the different apo B antibodies in the 3 assays used in this study. An equation that predicts LDL–apo B particle number may be useful, regardless of the apo B assay used.

Measurements of plasma lipids (cholesterol, triglycerides) and HDL and LDL cholesterol subfractions (HDL-C, LDL-C) have been used to assess cardiovascular risk for several decades. Cholesterol, triglyceride, and HDL-C measurements have been readily available for quite some time, whereas LDL-C has been routinely estimated with the Friedewald equation. Although the reliability of this calculation decreases as the triglyceride concentration increases, the Friedewald equation is a useful clinical tool for estimating LDL-C in most cases, even with the current availability of assays that measure LDL-C directly.

Measurements of apolipoprotein B (apo B) are better for distinguishing and predicting cardiovascular disease risk than either measuring LDL-C directly (especially when triglycerides are high (1–6)) or calculating the LDL-C concentration via the Friedewald formula (7–11). Apo B measurement has been standardized, and current methods are highly precise and accurate. Apo B immunoassays use antibodies that recognize epitopes on LDL-C particles containing apo B-100, the main structural protein of these particles. Turbidity is a source of interference in apo B immunoassays of lipemic samples, and cross-reactivity of apo B-100 antibodies with apo B-48 on chylomicrons can also produce interference.

We propose that apo B values obtained for the 1.006 infranate fraction [density (d) = 1.006 kg/L] of a sample after ultracentrifugation (hereby termed LDL–apo B) accurately estimate LDL particle numbers. Furthermore, we provide a simple equation for estimating LDL–apo B from measurements of triglycerides and apo B that is independent of the antibody used in the immunoassay.

In the beta-quantification reference method by which LDL-C is measured, a sample is ultracentrifuged through a solution of d = 1.006 kg/L. The supranate contains chylomicrons, VLDL, and any beta-VLDL, whereas the infranate contains LDL, HDL, intermediate-density lipoprotein (IDL), and lipoprotein (a). Thus, ultracentrifugation can be used as a means to remove chylomicrons and VLDL from LDL and IDL. Apo B measured in the 1.006 infranate fraction is associated with LDL and IDL, which are considered atherogenic compared with VLDL particles. In addition, LDL–apo B has been associated with arteriographic changes in the FATS study and with decreased numbers of events and regression of coronary lesions in the SCRIP study (6, 12). LDL–apo B is likely to be a better marker of cardiovascular disease risk than total apo B.

LDL–apo B has been measured >60 000 times at Berkeley HeartLab using 3 distinct commercially available assays with different specificities for apo B. In each measurement, 2 parts of an aqueous NaCl solution (11.5 g NaCl plus 0.1 g EDTA per 1 L water; d = 1.006) are added to 1 part patient serum, and the procedure is followed by ultracentrifugation in a Beckman 50.4 Ti fixed-angle rotor with a Beckman Coulter L8–80MR or L80–XP ultracentrifuge (Beckman Coulter) at 45 000 rpm for 11 h at 10 °C, resulting in 218 068 g. The top third of the centrifuged sample contains chylomicrons and VLDL and is removed. The apo B content in the bottom fraction (d > 1.006) is then measured with commercially available reagents in au-
tomated chemistry analyzers. We have used the following 3 methods to measure apo B in the 1.006 fraction: (a) Abbott reagents (cat. no. 9D93) with the Abbott Aeroset, (b) Roche reagents (cat. no. 03032639) with the Roche/Hitachi Module P-800, and (c) Kamiya K-Assay reagents (cat. no. KAI-024) with the Roche/Hitachi Module P-800.

We used deidentified data from 64,895 samples in our laboratory information system in a retrospective analysis in which we retrieved triglyceride, total apo B, and LDL–apo B data for each sample. LDL–apo B was measured with one of the 3 methods mentioned above. The data set was limited to samples with triglyceride concentrations >200 mg/dL, the threshold above which LDL–apo B was measured. This threshold was chosen because it is near the 95th percentile of triglyceride values for our laboratory’s patient population and because our laboratory can accommodate routine measurement of LDL–apo B for this quantity of samples. Total apo B and LDL–apo B were strongly correlated, especially after adjusting for triglyceride concentration (Fig. 1, A and B). Plotting the difference between apo B and LDL–apo B values against triglyceride concentration revealed a linear relationship until the triglyceride concentration exceeded approximately 800 mg/dL (Fig. 1C). The following linear equation fits the data obtained with any of the 3 methods:

$$LDL–apo\ B = apo\ B - 10 \text{ mg/dL} - \frac{\text{triglycerides}}{32},$$

where LDL–apo B, apo B, and triglycerides are in milligrams per deciliter and apo B is the concentration of total apo B.

1 Editor’s footnote: Although this journal has a policy of using SI nomenclature and expressing triglyceride and apo B concentrations in milligrams per liter, consideration of the likely clinical application of the equation presented herein requires these concentrations to be left expressed in milligrams per deciliter, the units in common clinical usage.
To demonstrate that this equation accurately predicts measured LDL–apo B values, we compared LDL–apo B values calculated for subsets of data (n = 1200) with measured values obtained with each of the 3 methods (Table 1). The equation predicted measured LDL–apo B values to within ±15% for all 3 methods in >85% of the cases (n = 3600). A comparison of all the LDL–apo B measurements (n = 64 895) with calculated LDL–apo B values demonstrated that the equation predicted the measured value to within ±15% in 84% of the cases. The correlations between measured and calculated LDL–apo B values were high (R² = 0.88; Fig. 1D) and were independent of the method used. Thus, our equation appears to correctly predict measured LDL–apo B values. Although the performance criteria for apo B measurements have not been formally established, the total error for LDL–C has been established by the National Cholesterol Education Program to be ±12% (13). Our equation was able to meet this 12% performance criterion with respect to measured LDL–apo B values in 75% of the cases (n = 64 895, all data for the 3 methods).

Other, more complex equations fit the data slightly better; however, the simplicity of our equation does not appear to compromise its predictive power compared with the more complex equations. Furthermore, its simplicity facilitates its use because it is easier for a clinician to remember. The triglycerides/32 term takes into consideration the VLDL contribution to apo B and is reminiscent of the triglycerides/5 term in the Friedewald equation. Because ultracentrifugation is not readily available in most clinical laboratories, our equation conveniently estimates LDL–apo B from commonly available measurements for total apo B and triglycerides. Furthermore, because our data suggest that the LDL–apo B values obtained with the 3 apo B assays are similar, this equation is likely to be broadly applicable and independent of the particular apo B assay used. Clinically, this equation can be used to estimate atherogenic apo B particle number without requiring specialized laboratory measurements. Further investigation is needed to determine whether this or a similar equation can be used universally for calculating LDL–apo B.

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References


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