Estimating Low-Density Lipoprotein Cholesterol by the Friedewald Equation Is Adequate for Classifying Patients on the Basis of Nationally Recommended Cutpoints

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We compared low-density lipoprotein cholesterol (LDL) values obtained by the Friedewald formula—i.e., total cholesterol minus high-density lipoprotein (HDL) cholesterol minus very-low-density lipoprotein (VLDL) cholesterol (estimated as triglyceride ÷ 5)—with those obtained by lipoprotein fractionation, using 4736 specimens. When triglycerides were <2.0 g/L, >90% of estimated LDL cholesterol values were acceptable, within ±10% of measured values. At triglyceride concentrations of 2.0–4.0 g/L and 4.0–6.0 g/L, only 72% and 39%, respectively, of the estimates were acceptable. LDL values derived from an alternative formula, estimating VLDL as triglycerides × 6, were even less accurate. Nevertheless, the use of estimated LDL for risk classification based on the National Cholesterol Education Program Adult Treatment Panel cutpoints of 1.30 and 1.60 g/L was considered acceptable. At triglyceride concentrations ≤5.0 g/L, 88% of classifications based on estimated LDL (using triglycerides ÷ 5) were concordant with those by measured LDL. Eleven percent of classifications were shifted across one cutpoint, evenly distributed between high and low. Fewer than 1% of classifications, all with Type III hyperlipoproteinemia, were misclassified two cutpoints high. Refinements in the estimation model did not substantially improve LDL estimation or concordance of risk classification.

The Expert Panel for Detection, Classification, and Treatment of High Blood Cholesterol, organized under the auspices of the National Cholesterol Education Program (NCEP), has established low-density lipoprotein (LDL) cholesterol as the primary decision analyze in identifying subjects for treatment, with recommended cutpoints of 1.30 and 1.60 g/L (1). In lipoprotein fractionation the widely accepted method for quantifying LDL cholesterol is the beta quantification procedure (2). Very-low-density lipoprotein (VLDL) is separated by ultracentrifugation and high-density lipoprotein (HDL) by precipitation. Because ultracentrifugation is unavailable in most routine laboratories and the procedure is expensive, time consuming, and technically demanding, the nearly universal approach in clinical laboratories (and that used commonly even in specialized lipid laboratories) has been to estimate LDL cholesterol from the formula of Friedewald et al. (3): after measurement of total cholesterol, triglycerides, and HDL cholesterol, LDL cholesterol is calculated as total cholesterol minus HDL cholesterol minus (triglyceride ÷ 5).

The Friedewald formula was used in the second National Health and Nutrition Examination Survey (4), from which the NCEP cutpoints were derived (1). This convenient model is based on the fact that most of the circulating triglyceride is carried in the VLDL fraction, the composition of which is relatively constant. The method is not without limitations; in cases of dysbetalipoproteinemia (Type III hyperlipoproteinemia), which are characterized by substantial cholesterol enrichment in the VLDL, the VLDL cholesterol is underestimated and hence LDL is overestimated (3). On the other hand, specimens with chylomicronemia or high concentrations of VLDL tend to have a lower proportion of cholesterol, so the formula overestimates VLDL and underestimates LDL cholesterol. Moreover, fasting is considered a requirement for LDL estimation, because nonfasting patients may exhibit chylomicronemia or increased concentrations of triglyceriderich particles, contributing to errors in estimation (5).

Since the Friedewald estimation procedure was published, evaluations in various populations (6–16) have demonstrated its limitations, and alternatives have been proposed involving different factors or more-sophisticated formulas (6, 9, 11, 12). In some cases specific models have been recommended for population subsets by categories of age, gender, and concentrations of triglyceride and cholesterol (13, 14).

In the alternative model that has attracted the most attention, that of DeLong et al. (11), VLDL cholesterol is estimated as triglyceride ÷ 6. Of the clinical laboratories measuring LDL in the College of American Pathologists' Chemistry Proficiency Survey in late 1988 (17), 95 (8%) of 1124 reporting laboratories used this formula; all other participating laboratories used the conventional Friedewald formula.

Now that LDL cholesterol has assumed increased importance in the NCEP guidelines, its accurate quantification is important. To determine the reliability of LDL estimation procedures, we examined data from referral patients and research subjects for which lipoprotein fractionations were completed at the Northwest Lipid Research Center. Estimates of LDL cholesterol by the conventional Friedewald equation (VLDL = triglyceride ÷ 5), the alternative De-Long equation (triglyceride ÷ 6), and another multiple regression model derived from our own data set were compared with measurements by lipoprotein fractionation. We also examined the concordance of classification in relation to the NCEP LDL cutpoints between measured LDL and LDL derived by the estimation models to determine whether different methods of estimation would affect a patient's classification for risk of heart disease.

Methods and Materials

Study Database

Results for all specimens received for analysis at the NWLRC Lipoprotein Laboratory from July 1987 to the present are contained in a computerized database. From this database we selected all records for human specimens

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for which complete lipoprotein fractionation (beta-quantification) was performed between July 1987 and January 1989, inclusive. Characteristics for the 4736 subjects (in g/L) were as follows: total cholesterol mean 2.31, median 2.21, standard deviation 0.7, range 0.53 to 15.66; triglyceride mean 2.10, median 1.22, standard deviation 3.88, range 0.03 to 78.80; and HDL cholesterol mean 0.50, median 0.47, standard deviation 0.16, range 0.06 to 1.48.

Because this laboratory receives many referrals for diagnosis of Type III hyperlipoproteinemia, the data set was sought that would predict VLDL cholesterol, and thus LDL cholesterol, more accurately than the Friedewald equation. The SPSS/PC+ (20) regression procedure was used to compare models in which only triglycerides was a predictor variable with models including HDL cholesterol, total cholesterol, and triglycerides, all as predictor variables.

The mean and median VLDL cholesterol to triglyceride ratios were calculated for various subsets selected by values of triglycerides and cholesterol. With cholesterol values >2.22 g/L, the ratio of VLDL cholesterol to triglycerides increased from 0.16 to 0.22 as the triglyceride measurements increased from 1.0 to 5.0 g/L, implying a synergistic effect between cholesterol and triglycerides. Therefore all possible interaction terms among triglycerides, total cholesterol, and HDL cholesterol were considered as possible predictor variables in the SPSS/PC+ stepwise regression procedure. The best interaction regression model selected from the stepwise procedure was as follows: VLDL cholesterol (in g/L) = 0.21 + 0.00033 TG - 0.00077 CH - 0.0014 HDLCH + 0.000008 CH · TG - 0.000007 HDLCH · TG + 0.000005 CH · HDLCH.

LDL predictions based on VLDL estimation by the interaction regression model were compared with that by the traditional Friedewald equation and the DeLong (triglycerides + 6) model. Based on the NCEP LDL cutpoints of 1.30 and 1.60 g/L, the agreement in classification of 4444 subjects with net triglycerides ≤5.0 g/L according to their estimated and measured LDL was compared for the three models. Using the SPSS/PC+ crosstables procedure, a 3 by 3 grid was produced (Figure 3, below). The squares running diagonally from top left to bottom right represent cases in which the estimated and measured LDL cholesterol concurred. Off-diagonal squares represent cases with misclassification by the estimation method.

Results and Discussion

Formulas for estimation of VLDL cholesterol are based on an assumption of uniform specimen composition, with a relatively consistent relationship with triglyceride. Figure 1 shows the actual relationship between VLDL cholesterol and net triglycerides (≤10 g/L) in this database. The line corresponding to VLDL cholesterol equals triglyceride + 5 (Friedewald formula) is illustrated. The actual relationship determined as the slope of the linear regression between VLDL cholesterol and net triglyceride in this data set is 0.23. For the subset with triglycerides ≤5.0 g/L the slope is 0.22. The latter relationship is consistent with a triglyceride denominator of 4.5, suggesting that, on the average, in this data set the Friedewald denominator of 5 should slightly underestimate VLDL cholesterol and overestimate LDL cholesterol.

Our database is enriched with specimens with Type III hyperlipoproteinemia, characterized by cholesterol enrichment of VLDL, which tends to make the slope higher than
might be observed in a more heterogeneous population. There are 328 cases with a VLDL cholesterol/triglyceride ratio >0.3 (the usual diagnostic cutpoint for Type III) in the subset with triglycerides ≤5 g/L and 350 cases in the subset with triglycerides ≤10 g/L. In addition, there are a few cases with negative values for VLDLs, an artifact of the analytical procedure. VLDL cholesterol is calculated as the difference between cholesterol in the plasma and in the bottom fraction obtained by ultracentrifugation. Because VLDL cholesterol is generally small, with analytical variability in both of the measured indices, the calculated difference is occasionally negative. Deleting specimens with negative VLDL cholesterol values and those with a VLDL cholesterol/triglyceride ratio >0.3 gives a slope of the relationship by linear regression of 0.21 for the subset with triglycerides ≤5 g/L and 0.20 for the subset with triglycerides ≤10 g/L, consistent with the Friedewald denominator of 5.

Figure 2 illustrates the accuracy of LDL estimation by the Friedewald (triglyceride = 5), the DeLong (triglyceride = 6), and the interaction regression equations as a function of increasing triglyceride concentrations. In this instance the derived LDL cholesterol values are compared with the measured LDL values determined in the beta-quantification procedure. The reliability of the LDL cholesterol estimations decrease considerably with increasing triglyceride concentrations. For the 3526 specimens with triglyceride concentrations <2.0 g/L, the triglyceride + 5 and triglyceride + 6 formulas were reasonably accurate, with 93% and 92%, respectively, of estimated values considered acceptable, within ±10% of the measured values. For specimens with triglyceride concentrations between 2.0 and 3.0 g/L, 3.0 and 4.0 g/L, and 4.0 and 5.0 g/L, the percentage of acceptable values decreased to 75% and 76%, 61% and 44%, and 41% and 40%, respectively. The percentage of acceptable values decreased to less than 20% and 30% when triglyceride concentrations were >5.0 g/L.

The Friedewald (triglyceride = 5) model demonstrates a slightly better comparison between estimated and measured LDL than does the DeLong (triglyceride = 6) model. More importantly in the Friedewald model, the distribution of error is more symmetrical, with approximately equal proportions of positive and negative errors (Figure 2). VLDL cholesterol is systematically underestimated when the DeLong formula is used, giving rise to more positive errors in LDL estimation.

When triglyceride alone was included in a regression model, the correlation coefficient between the predicted and observed VLDL cholesterol values was 0.86. Because the interaction regression model, selected by the SPSS/PC+ stepwise procedure, was fitted to our particular data and included total and HDL cholesterols, it should theoretically improve our prediction of LDL cholesterol. In fact, by several criteria the improvement was modest. The correlation coefficient increased from 0.86 when we used triglycerides + 5 only in the regression model to 0.89 for the interaction regression model. The standard error of the predicted value decreased modestly, from 0.126 to 0.111 g/L.

The improvement in LDL estimation by the interaction regression model was primarily for those specimens with triglyceride concentrations between 2.0 and 4.0 g/L (Figure 2). For triglyceride concentration values <2.0, 94% (vs 93% for triglycerides + 5) of the LDL cholesterol values estimated by the interaction regression model were considered acceptable, i.e., within 10% of the measured value. For triglyceride concentrations between 2.0 and 3.0 g/L, 80% of the values (vs 75%) were acceptable, and between 3.0 and 4.0 g/L, 4.0 and 5.0 g/L, and >5.0 g/L, 64% (vs 61%), 42% (vs 41%), and 17% (vs 20%) of the values were acceptable.

Thus, use of the more complex model did not substantially improve the prediction. Furthermore, in our database a denominator of five better approximates the average relationship between VLDL cholesterol and net triglycerides than does six, suggesting five is more appropriate, certainly so with a blanking triglyceride method. Most clinical laboratories use non-blanking methods, so derivation of the relationship from total or unblanked triglyceride values would be more appropriate. The contribution of free
glycerol averages approximately 0.15 g of triolein equivalent per liter by our ultraviolet-type method and is even lower by the increasingly common glycerol phosphate oxidase (Trinder) methods (unpublished observation). The inclusion of free glycerol would be expected to give a slightly smaller slope coefficient, hence a larger triglyceride denominator in the estimation equation. In fact, in our database the slope coefficient of 0.21 (for the subset with triglycerides ≤5, VLDL cholesterol ≥0, and VLDL cholesterol to total triglyceride ratio ≥0.3) decreased to only 0.20 when we used total or unblanked triglyceride values.

The DeLong (triglyceride + 6) formula (11) was derived from the Lipid Research Clinics Prevalence Study, in which the non-enzymic Keslerier method was used in the AutoAnalyzer II (Technicon Corp., Tarrytown, NY) (2). Specimens were extracted into isopropyl alcohol and zeolite, which removed a variable amount but not all of the free glycerol. Although the non-enzymic method was capable of correcting for the remaining free glycerol, this was not done in the results from the LRC Prevalence Study. Thus the triglyceride values from which the DeLong formula was derived were only partly corrected for free glycerol, perhaps contributing to the larger denominator. Another factor might be method biases from the non-enzymic LRC methods for cholesterol and triglycerides.

Under the NCEP Adult Treatment Panel guidelines, LDL cholesterol has become the major lipid variable determining classification and treatment. In practice the primary concern will usually be correct classification in relation to the NCEP cutpoints of 1.30 and 1.60 g/L for borderline-high and high LDL cholesterol, respectively.

The categories for which the formula incorrectly estimates VLDL cholesterol—those with Type III hyperlipoproteinemia and those with chylomicrons or cholesterol-depleted VLDL—usually have low LDL cholesterol, in most cases below the lower cutpoint of 1.30 g/L. Thus, even though LDL cholesterol may be estimated incorrectly, classification could still be reliable.

This is demonstrated in Figure 3. Cross tabulations comparing classification based on measured LDL to that based on estimated LDL by the three models for subjects with triglycerides ≤5.0 g/L demonstrated reasonable concordance. Eighty-eight percent of the classifications based on estimated LDL cholesterol were in concordance with those based on measured LDL when the Friedewald (triglyceride + 5) model was used. An additional 5% were classified one cutpoint low, and 6% were classified one cutpoint high. Only 18 subjects (0.4%) were classified two cutpoints high, and all of these were diagnosed as Type III, appropriate candidates for treatment irrespective of their LDL cholesterol values. Limiting the comparison to only those cases with triglyceride concentrations ≤4.0 g/L increased the concordant proportion only from 88% to 89%.

When we used the DeLong (triglyceride + 6) model, 96% of the subjects were classified in concordance with that based on measured LDL cholesterol. As might be expected, the misclassification was skewed high. Only 2% were misclassified one cutpoint low compared with 11% misclassified one cutpoint high. A total of 26 subjects (0.6%) were misclassified two cutpoints high.

The interaction regression model gave results quite similar to those for the Friedewald (triglyceride + 5) model, with 99% classified in concordance, 6% classified one cutpoint low, 6% classified one cutpoint high, and 0.3% classified two cutpoints high. Thus this model, which modestly improves the estimation of LDL cholesterol, demonstrated a very slight improvement in the concordance of classification.

Reliable classification of patients according to the NCEP Adult Treatment Program guidelines requires accurate quantitation not only of total cholesterol but also of LDL cholesterol, and concern has arisen regarding the Friedewald procedure commonly used to estimate LDL cholesterol in clinical laboratories. In the original report, estimation was considered invalid in subjects with triglycerides >4.0 g/L, chylomicrons, or with Type III hyperlipoproteinemia. Subsequent evaluations have concluded that LDL cholesterol estimation is often unreliable in other instances.

Attempts have been made to derive better formulas, either by refining the estimation factor, by deriving more sophisticated equations including other variables, or by segmenting the population into subsets by gender, age, and lipid concentrations. Because none of these formulas has been substantially effective at modeling the heterogeneity of VLDL cholesterol in the population, error in prediction has not been reduced.

Our interaction regression model, in which not only triglycerides but also total and HDL cholesterol were used,

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<th>Estimated LDL</th>
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slightly improved the estimation of LDL cholesterol and the concordance of classification compared with the Friedewald equation in our data set. However, the improvement was modest and probably does not justify use of such a complicated formula. Furthermore, our interaction regression equation may not be appropriate for other methods and data sets, and we therefore do not recommend its general use.

Based on this analysis, the Friedewald (triglyceride + 5) equation, which has been used to estimate LDL for over 15 years, appears to be adequate for patient classification. The DeLong (triglyceride + 6) equation did not improve the estimation, and in fact produced an upward skewed estimation of LDL. Because our triglyceride values were corrected for the free glycerol blank, a slightly larger denominator might be appropriate for use in clinical laboratories that generally do not perform a blanking correction. Nevertheless, in our experience the contribution of free glycerol is too small to justify using the triglyceride + 6 formula.

The NCEP Adult Treatment Panel recommended that triglyceride concentrations >5.0 g/L be considered abnormally high. Patients with triglyceride concentrations exceeding this cutpoint should be treated at least for the hypertriglyceridemia, regardless of the LDL cholesterol value. Many subjects with triglyceride concentrations >5.0 g/L will have low LDL, often <1.30 g/L. In addition, patients with high values for both triglycerides and cholesterol will often be referred for complete lipoprotein quantification, which provides an accurate LDL cholesterol measurement and allows for detection of Type III hyperlipidemia. Thus, the cutpoint of 5.0 g/L would appear to be a value above which reliable estimation of LDL cholesterol might be considered less important. Of particular concern is that classification based on LDL cholesterol be reliable in the majority of patients with triglycerides ≤5.0 g/L. Our results suggest that changing the accepted upper triglyceride limit for the Friedewald estimation from 4.0 to 5.0 g/L would not significantly impair classification.

The presence of chylomicrons is considered an exclusion for using the Friedewald formula. We did not evaluate this source of interference. However, chylomicrons in a specimen with triglycerides ≤5.0 g/L would almost always be a result of not fasting, and would lead to an error in estimation (5). Thus, the presence of chylomicrons, which can easily be detected visually, signals an inappropriate specimen.

In summary, while the use of the Friedewald formula for estimating VLDL cholesterol and LDL cholesterol becomes less reliable as the triglyceride concentration increases, the concordance of classification based on the National Cholesterol Education Program Adult Treatment Panel cutpoints is quite good. The Friedewald formula would appear to be adequate for continued use in patient classification.

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