Effect of Serum Lipoprotein(a) on Estimation of Low-Density Lipoprotein Cholesterol by the Friedewald Formula

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The calculation of serum low-density lipoprotein cholesterol (LDL-C) by the Friedewald formula does not account for the cholesterol associated with lipoprotein(a) [Lp(a)]. To quantify the contribution of Lp(a) cholesterol to total serum cholesterol, we measured concentrations of serum Lp(a) by an ELISA and concentrations of other serum lipids and lipoproteins by standard assays in 23 normolipemic women, ages 50–60 years. In measuring serum high-density lipoprotein cholesterol, we found that polyethylene glycol 6000 precipitated >99.8% of all Lp(a). When serum Lp(a) concentrations were ≤300 mg/dL, 301–600 mg/dL, and >600 mg/dL, the uncorrected serum LDL-C was overestimated, respectively, by a mean of 4.1% (n = 7), 8.5% (n = 8), and 21.4% (n = 8). Serum Lp(a) concentrations were positively correlated with percentage overestimation (P < 0.001), but were not correlated with either corrected or uncorrected serum LDL-C. We conclude that the Friedewald formula should be modified to take into account the contribution of Lp(a) cholesterol to total serum cholesterol.

Indexing Terms: atherogenesis/triglycerides/apolipoproteins

Direct measurement of low-density lipoprotein cholesterol (LDL-C) usually requires the use of a preparative ultracentrifuge, which is time-consuming, labor intensive, and not well suited to routine use in the clinical chemistry laboratory. However, LDL-C can be fairly accurately estimated from the Friedewald formula (1):

\[ \text{LDL-C} = \text{TC} - (\text{HDL-C} + \text{VLDL-C}) \]

where TC is total serum cholesterol, HDL-C is high-density lipoprotein cholesterol, and VLDL-C is very-low-density lipoprotein cholesterol. This widely used formula is based on the assumption that the mass of VLDL-C present in serum is equivalent to one fifth of the mass of triglycerides in serum VLDL (2). At higher triglyceride concentrations (>4.5 mmol/L), inconsistencies in the value of the triglycerides:cholesterol ratio in VLDL occur so that the formula becomes inaccurate and should not be used.

Methods for measuring serum cholesterol (3), separating and quantifying serum HDL-C (4), and measuring serum triglycerides (2) have all been studied extensively. Less information is available about the possible contribution from serum lipoprotein(a) [Lp(a)] to LDL-C as calculated by the Friedewald equation. Lp(a) is an atherogenic lipoprotein particle independently associated with cardiovascular risk, in which apolipoprotein(a) [apo(a)] is disulfide-bound to apo B-100. Although Lp(a) is cholesterol rich, it contributes <5% of total plasma cholesterol; the atherogenicity of Lp(a) is related more to apo(a) than to its cholesterol content (5). When LDL-C concentration is calculated from the Friedewald formula by using the HDL-C concentration determined after precipitating VLDL, LDL, and Lp(a), the calculated LDL-C would be expected to comprise some or all of the cholesterol originally present in Lp(a).

Thus, in distinguishing the relative atherogenicity of LDL-C and Lp(a), it is important to correct the Friedewald formula for the cholesterol contributed by Lp(a), as already emphasized by Dahlen (6). In the present study, we compared the serum Lp(a) concentration with the estimated LDL-C, the corrected LDL-C, and the supernatant concentration of Lp(a) in the HDL-C assay. We also examined the effect of serum Lp(a) concentration on the accuracy of the Friedewald equation for estimating LDL-C.

Subjects and Methods

Subjects

We recruited 23 normolipemic women, ages 50–60 years, who were attending the menopause clinic, Sydney Royal Hospital for Women, for the study. All of the women were apparently healthy and had not taken medication for the previous 3 months. Blood was drawn after an overnight fast by venipuncture and allowed to clot for 30 min at room temperature. After centrifugation for 10 min at 1000g, the serum was pipetted off and stored at −70°C until analyzed.

Quantitation of Serum Lipids and Lp(a)

Total serum cholesterol (7) and triglycerides (8) were measured enzymatically on a Cobas-Bio centrifugal analyzer (Roche Diagnostic Systems, Montclair, NJ) with the appropriate enzymatic kit (Boehringer Mannheim Diagnostics, Indianapolis, IN). HDL-C was determined by measuring cholesterol in the supernatant after removing LDL, VLDL, and Lp(a) lipoprotein fractions by polyethylene glycol 6000 precipitation (9). HDL-C assays were standardized with QChem HDL-CholKits from Sigma Pharmaceuticals, Melbourne, Australia. The kit was initially calibrated against frozen serum.
standards prepared for standardization of HDL-C determinations and was purchased from G. R. Warnick (Northwest Lipid Research Clinic, University of Washington, Seattle) as part of the Australian Lipid Standardisation Programme, 1984. The assays are now referenced to a cholesterol standard, SRM 911 (National Institute of Standards and Technology, Gaithersburg, MD). LDL-C was calculated according to the Friedewald formula (1). Corrected LDL-C was calculated by subtracting the cholesterol portion of Lp(a) [estimated as 0.3 × Lp(a) mass; ref. 10] from the Friedewald formula estimation.

The concentrations of Lp(a) in serum and in the supernatant obtained in the HDL-C assay were determined by an ELISA previously developed in this laboratory (11).

Analysis of the relations between concentrations of serum Lp(a), estimated LDL-C, corrected LDL-C, percentage overestimation of LDL-C, and supernatant Lp(a) was done by the least-squares linear regression method. The mean difference between estimated LDL-C and corrected LDL-C was assessed by the Student’s paired t-test. A value of $P < 0.05$ was considered significant.

**Results**

Concentrations of the lipids and lipoproteins measured in the fasting sera of the 23 subjects in the study are shown in Table 1. There was, not surprisingly, a gradual increase in percentage of overestimated LDL-C with increasing Lp(a) concentration. For serum Lp(a) concentrations ≤300 mg/L (mean 160 mg/L), LDL-C was overestimated by a mean of 4.1% (range 1.1–9.1%; $n = 7$). With Lp(a) between 301 and 600 mg/L (mean 423 mg/L), there was an 8.5% (range 5.7–11.4%; $n = 8$) overestimation. There was a greater overestimation (21.4%; range 12.2–27.5%; $n = 8$) when Lp(a) concentrations were >600 mg/L (mean 875 mg/L). The overestimation of LDL-C was significant ($P < 0.001$) at all concentrations of serum Lp(a).

As shown in Fig. 1, there was a significant positive correlation between serum Lp(a) and percentage overestimation of LDL-C ($r = 0.95, P < 0.001$). There was no correlation between Lp(a) and either the corrected or uncorrected LDL-C concentration. To investigate the accuracy of the precipitation method in the HDL-C assay, we measured the Lp(a) remaining in the supernatant of the HDL-C assay. For the women in the three Lp(a) subgroups (i.e., ≤300, 301–600, and >600 mg/L), the residual Lp(a) in their serum supernatants and its proportion as a percentage of unprecipitated Lp(a) (mean ± SEM) were, respectively: 0.30 ± 0.04 mg/L (0.19% ± 0.02%), 0.45 ± 0.03 mg/L (0.11% ± 0.01%), and 0.68 ± 0.04 mg/L (0.09% ± 0.01%). Thus virtually all Lp(a) was precipitated by polyethylene glycol 6000, and the cholesterol in the residual Lp(a) did not significantly affect the accuracy of the HDL-C measurement. There was also a high positive correlation between serum Lp(a) concentration and Lp(a) concentrations in the supernatants ($r = 0.94, P < 0.001$).

**Discussion**

When LDL-C is calculated according to the Friedewald formula, the calculated LDL-C also contains a contribution from the cholesterol present in Lp(a). The present study clearly shows a strong positive correlation between serum Lp(a) concentration and overestimation of LDL-C concentration. Thus the error in calculating LDL-C will be small at Lp(a) concentrations ≤300 mg/L, moderate when Lp(a) is between 301 and 600 mg/L, and severe at >600 mg/L, a serum Lp(a) concentration found in ~5% of the community (10). In evaluating the relative importance of Lp(a) and LDL-C as atherogenic factors, the present findings indicate that Lp(a) cholesterol should also be subtracted from total serum cholesterol for a more accurate estimation of LDL-C and its contribution to cardiovascular risk. This could be done by using Dahlen’s modification of the Friedewald formula (ref. 10):

![Fig. 1. Correlation between serum concentration of Lp(a) and % overestimation of serum LDL-cholesterol.](image)

$r = 0.948; P < 0.001; n = 23.$
LDL-C = TC - HDL-C - VLDL-C - Lp(a)-C

or, with concentrations expressed in mg/L and the concentrations of VLDL-C and Lp(a)-C calculated as above:

LDL-C = TC - HDL-C - 0.2TG - 0.3Lp(a)

where TG is triglycerides.

Since the accuracy of HDL-C is also one of the key factors affecting the Friedewald calculation, we investigated the possible influence of precipitation of Lp(a) in measurement of HDL-C. Polyethylene glycol 6000 precipitated >99.8% of Lp(a). Thus the cholesterol content in HDL was unaffected by unprecipitated Lp(a).

The present study also confirms an absence of any correlation between serum Lp(a) concentrations and either the uncorrected or the corrected LDL-C concentrations. This finding supports the contention that Lp(a) is an independent cardiovascular risk factor, and implies that LDL and Lp(a) are not strongly linked metabolically. Thus the apportioning of risk associated with increased concentrations of each requires the adjustment of calculated LDL-C concentrations for concentrations of Lp(a).

The separation of Lp(a)-C from LDL-C as we have discussed here clearly allows a more complete analysis of dyslipidemia as a potential coronary risk. Since the adjustment of LDL-C values will decrease their calculated concentrations, often by several percentage points, LDL-C values obtained in this way may not be readily comparable with uncorrected LDL-C values. This follows because reduction in serum cholesterol concentrations of this order are known to be tracked by reduced coronary risk of comparable magnitude. On the other hand, since LDL and Lp(a) appear to be independent vascular risk factors, measurement of uncorrected LDL-C may continue to provide a sensitive and useful practical gauge of the sum of these two atherogens. In the longer term, studies of the independent contributions of these two serum components to coronary risk may lead to a fresh appraisal of the clinical value of measuring serum concentrations of one or both of these analytes, and perhaps to standardized measurements for the various serum lipids involved.

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References