Influence of Intermediate-Density Lipoproteins on the Accuracy of the Friedewald Formula

Mariano Sentí,† Juan Pedro-Botet,† Xavier Nogués,‡ and Juan Rubíes-Prat†,‡,†,‡

Values of low-density lipoprotein (LDL) cholesterol (C) according to the Friedewald formula (Clin Chem 1972;18:499–502) were compared with those obtained by lipoprotein fractionation in 98 healthy subjects (control group), 135 patients with peripheral vascular and cerebrovascular disease (atherosclerotic group), and 45 with chronic renal failure on hemodialysis (CRF group). All had concentrations of total cholesterol between 3.23 and 7.76 mmol/L (1.25–3.00 g/L) and triglycerides <3.39 mmol/L (<3.00 g/L). The percentage error of calculated LDL-C was 4% in controls with a cholesterol/triglycerides (C/TG) ratio for very-low-density lipoprotein (VLDL) of 0.20, but >50% in those with a (C/TG)/LDL ratio of 0.40. The percentage of error in sera of patients with atherosclerosis and chronic renal failure was higher than in controls with a similar mean (C/TG)/VLDL ratio. The percentage of error of calculated LDL-C increases progressively with the increase in the C/TG intermediate-density lipoprotein (IDL) ratio, both in controls and in the atherosclerotic and CRF groups. Similar findings are observed when the mean percentage of error of measured LDL-C is evaluated. The percentage of error from calculated LDL-C in the atherosclerotic and CRF groups is significantly lower than that obtained by comparison of LDL-C separated by ultracentrifugation when the "broad cut" LDL (IDL plus LDL, both by ultracentrifugation) was used. The high percentage of errors found in the groups of patients studied underlines the need for caution when assessing the reliability of the Friedewald formula, particularly in cases in which disturbances in IDL composition are suspected.

Additional Keyphrases: atherosclerosis · chronic renal failure · analytical error · hemodialysis

It is well established that the increase of plasma cholesterol, particularly cholesterol carried by low-density lipoproteins (LDL), is one of the major risk factors for coronary heart disease. The National Cholesterol Education Program (1) has recommended the determination of LDL-cholesterol (LDL-C) in individuals with total cholesterol values ≥6.21 mmol/L (≥2.40 g/L), as well as those with borderline-high values, 5.17–6.18 mmol/L (2.00–2.39 g/L), who are at high risk because they have definite coronary heart disease or two other cardiovascular risk factors. LDL-C concentrations then become the criterion for definitive diet and drug therapy. Because separation of LDL-C from plasma or serum by ultracentrifugation is generally unavailable in routine laboratories, its concentration is estimated by numerical calculation, with the formula of Friedewald et al. being the most extensively used (2, 3). It is generally accepted that the Friedewald formula is not reliable when triglyceride (TG) concentrations exceed 4.52 mmol/L (4.00 g/L) (4). However, several sources of inaccuracy have been described, and modifications or alternatives to the Friedewald formula have been proposed (4–18).

The inaccuracy of the Friedewald formula in patients with high or very high concentrations of total cholesterol is probably irrelevant in clinical practice. However, in individuals with "normal" or nearly normal concentrations of total cholesterol, errors in calculated LDL-C may lead to inappropriate decisions about therapy. On the other hand, the possible influence of intermediate-density lipoproteins (IDL), a well-known independent cardiovascular risk factor (19–22), is not taken into account by the proposed alternatives to the Friedewald formula. In this and other formulas (8–10, 14), the calculated LDL fraction includes the d >1.006 fraction cholesterol minus high-density lipoprotein cholesterol (HDL-C) obtained by precipitation. Thus a so-called "broad cut" LDL, which includes IDL (d = 1.006 to 1.019), is used as the basis for comparison.

The above discussion indicates the importance of accurate LDL-C quantification. Thus, with the aim of studying the accuracy of the Friedewald formula in individuals with serum cholesterol between 3.23 and 7.76 mmol/L (1.25 to 3.00 mg/L), we have compared the LDL-C values obtained by calculation with the formula and those obtained by ultracentrifugation. In addition, we evaluated the (C/TG)/IDL ratio to analyze the influence of IDL composition on LDL-C estimated by the Friedewald formula. We have also evaluated the errors in the calculated LDL-C by comparison with the values for the "broad cut" LDL (total IDL plus LDL, both obtained by ultracentrifugation).

Materials and Methods

Sera from 98 healthy subjects (control group), 135 patients with peripheral vascular and cerebrovascular disease (atherosclerotic group), and 45 with chronic renal failure undergoing hemodialysis (CRF group)
were analyzed because of the well-known IDL abnormalities in these patients (19–23). All the individuals included in the study were men, with total cholesterol concentrations of 3.23–7.76 mmol/L (1.25–3.00 g/L) and triglycerides <3.39 mmol/L (<3.00 g/L). The mean ± SD values were as follows: controls, total cholesterol 5.30 ± 0.98 mmol/L (2.05 ± 0.38 g/L) and triglycerides 1.26 ± 0.52 mmol/L (1.12 ± 0.46 g/L); atherosclerotic subjects, total cholesterol 5.43 ± 0.98 mmol/L (2.10 ± 0.38 g/L) and triglycerides 1.45 ± 0.62 mmol/L (1.28 ± 0.55 g/L); and CRF group, total cholesterol 4.47 ± 0.83 mmol/L (1.73 ± 0.32 g/L) and triglycerides 1.53 ± 0.59 mmol/L (1.36 ± 0.52 g/L).

Blood samples were obtained in the morning after an overnight fast, and the subjects were seated during the phlebotomy. In dialysis patients, samples were taken before the dialysis session. The blood was allowed to clot for 1 h at room temperature; the serum was removed by centrifugation (1200 × g, 20 °C, 15 min), supplemented with a preservative (24), and stored at 4 °C for no more than four days before ultracentrifugation. Lipoprotein isolation was carried out by a double ultracentrifugation procedure as described recently (25), with minor modifications. Briefly, we overlaid 5 mL of serum with NaCl solution (density = 1.006 kg/L) in thick-wall polycarbonate tubes (Kontron Instruments, Milan, Italy) and centrifuged the samples in a fixed-angle 50.38 Ti rotor (Kontron Instruments) at 150 000 × g and 10 °C for 18 h. Very-low-density lipoprotein (VLDL) fractions were collected by aspiration from the top of the tube. The infranates (3 mL) were placed in cellulose nitrate tubes, adjusted to density = 1.25 kg/L with dried KBr and overlaid sequentially with a density = 1.21 kg/L salt solution and distilled water. The samples were then ultracentrifuged in a TST 41.14 rotor (Kontron Instruments) at 300 000 × g for 22 h, and the other lipoproteins—IDL 1.006 < d < 1.019, LDL 1.019 < d < 1.063, and HDL d > 1.063—were aspirated.

Cholesterol and triglycerides in serum and lipoprotein fractions were assayed by enzymatic methods with an ERIS selective multichannel analyzer (Eppendorf, Hamburg, F.R.G.), and standardized with control sera (Quailtol; Merck, Frankfurt, F.R.G.).

The mean intra- and interseries CVs for the double ultracentrifugation procedure never exceeded 10%. Lipoprotein recovery was verified by comparing the sum of cholesterol and triglycerides in the fractions with the total serum cholesterol and triglycerides. Quantification of HDL as cholesterol in the density >1.063 kg/L fraction obtained by ultracentrifugation has been considered a classical method that correlates well with precipitation methods for separating apoprotein B-containing lipoproteins (26, 27).

Results and Discussion

LDL-C calculated by the Friedewald formula differed by ±10% or more with respect to measured LDL-C in 14.2% of controls, 29.6% of the atherosclerotic group, and 42.2% of the CRF group. Figure 1 shows the percentage of samples in which calculated LDL-C differed from measured LDL-C by ±10% or more, according to the different triglyceride concentrations for each group. In the control group, the percentage of error exceeded 20% in samples with triglyceride values <0.26 mmol/L (<0.50 g/L). These data are similar to those described by others (4, 11) who considered hypertriglyceridemia to be one of the main causes of error in the estimation of LDL-C by the Friedewald equation. On the other hand, in the atherosclerotic group, and especially in the CRF group, the percentage of error was >20%, even in specimens with triglyceride values <1.13 mmol/L (<1.00 g/L), a fact recently described by González G²-Estrada et al. (18). Therefore, the choice of the Friedewald formula cannot be based confidently on triglyceride concentrations.

Another source of error attributed to the Friedewald formula lies in the variability of VLDL composition, which depends on the triglyceride turnover. In the present study, the mean (C/TG)VLDL ratio in the atherosclerotic and CRF groups did not differ significantly from that of controls. However, this ratio varies greatly in each group. In fact, in 41.8% of the control group, 44.9% of the atherosclerotic group, and 88.8% of the CRF group, the (C/TG)VLDL ratio was <0.20 or >0.30. The percentage of error was 4% in controls whose (C/TG)VLDL was <0.20, whereas with values ≥0.40, the percentage of error increased markedly, to >60%. In the atherosclerotic group and especially in the CRF group, the percentage of error of calculated LDL-C was much greater in sera with a higher (C/TG)VLDL ratio (Figure 2), in agreement with the observations of Ellefson (28). According to the total cholesterol concentration, two subsets of samples were selected: (a) total cholesterol <5.17 mmol/L and (b) total cholesterol between 5.17 and 6.46 mmol/L. The percentage of error was 19.1% for subset a in the control group, and 30.8% and 41.6% in the atherosclerotic and CRF groups, respectively. In subset b, the percentages of error were 9.8%, 28.9%, and 42.8%, respectively.

Gross abnormalities in IDL composition are characteristic of dysbetaIipoproteinemia, although changes
may also occur in patients with other types of hyperlipoproteinemia and even in apparently normolipidemic individuals (29). Alterations in IDL composition have been reported in diabetes mellitus (30), chronic renal failure (23), coronary heart disease (19–21), peripheral vascular disease (22), and cerebrovascular disease (unpublished data). To assess the influence of IDL composition on calculated LDL-C by the Friedewald formula, we evaluated the (C/TG)IDL ratio. The percentage of samples with erroneous calculated LDL-C in the three groups studied according to this ratio is shown in Figure 3. The percentage of error observed increased progressively with the increase in the (C/TG)IDL ratio in both the controls and the atherosclerotic and CRF groups. On the other hand, similar findings were seen in the evaluation of the mean percentage of error of measured LDL-C (Table 1). With the "broad cut" LDL, the percentage of errors from calculated LDL were 13.2% in controls, and 15.5% and 28.8% in the atherosclerotic and CRF groups, respectively. These percentages of errors, for calculations based on the "broad cut" LDL, were significantly lower in the former two groups than those obtained by comparison of LDL-C separated by ultracentrifugation. Because the Friedewald and most subsequent equations (8–10, 14) include IDL in the "broad cut" LDL, our findings are in the expected directions.

Twenty percent of the sera from the atherosclerotic group and 24.4% of those from the CRF group showed an IDL-C concentration >0.44 mmol/L (>0.17 g/L), which corresponds to the 0.975 percentile of the control group.

In sera of the atherosclerotic group with IDL-C >0.44 mmol/L, the percentage of error for calculated LDL-C was 87.5% in those with total cholesterol <5.17 mmol/L and 63% with total cholesterol ≥5.17 mmol/L. In samples from the CRF group whose IDL-C concentrations exceeded 0.44 mmol/L, the percent error was 100%, regardless of the total cholesterol concentration. In all of these cases, the LDL-C calculated by the Friedewald formula was overestimated.

As has been established, the Friedewald formula is not applicable for estimation of LDL-C in dysbeta-lipoproteinemia (4). Furthermore, the high percentages of error found in the groups of patients we studied suggest that the formula also is not reliable in other IDL alterations, e.g., those observed in peripheral vascular disease, cerebrovascular disease, chronic renal failure with hemodialysis, and perhaps in diabetes mellitus. We underline the need for caution when assessing the reliability of the Friedewald formula, particularly in cases in which disturbances of IDL composition are suspected.

This study was supported by a grant from the Fondo de Investigaciones Sanitarias de la Seguridad Social (0469/89).

References

Table 1. Percentage Deviation of Calculated LDL-Cholesterol from Measured LDL-Cholesterol

<table>
<thead>
<tr>
<th>(C/TG)IDL ratio</th>
<th>Control group</th>
<th>Atherosclerotic group</th>
<th>Chronic renal failure group</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.30</td>
<td>1 (6)</td>
<td>2 (4)</td>
<td>7 (12)</td>
</tr>
<tr>
<td>0.30–0.60</td>
<td>6 (8)</td>
<td>11 (8)</td>
<td>12 (16)</td>
</tr>
<tr>
<td>0.61–0.90</td>
<td>10 (7)</td>
<td>11 (10)</td>
<td>20 (4)</td>
</tr>
<tr>
<td>&gt;0.91</td>
<td>10 (12)</td>
<td>36 (28)</td>
<td>34 (7)</td>
</tr>
</tbody>
</table>

Mean (SD)% difference, calculated vs measured.
Interference in Thyroid-Function Tests in Postpartum Thyroiditis


Three women are described from a study of patients with postpartum thyroiditis whose sera gave spuriously increased concentrations of free thyroid hormone because of antibody binding of radiolabeled thyroid hormone (T) and triiodothyronine (T) analogs. All of the women showed increased serum concentrations of thyroid autoantibodies. The antibody binding of radiolabeled analogs and its effect on free T and free T assays disappeared by 48 weeks postpartum. Postpartum women who develop thyroid autoantibodies have ~2% prevalence of increased binding of radiolabeled analogs, which can result in an interference in thyroid hormone assays involving T and T analogs.

Additional Keyphrases: autoantibodies, radiolabeled thyroid hormone analogs, analytical error

Since the first report of a patient whose serum contained a thyroid (T)-binding immunoglobulin (I), many patients have been identified with T or triiodothyronine (T)-binding immunoglobulins that caused interference in radioimmunoassays of T or T (2). Spuriously high or low estimates of total thyroid hormone were observed, depending on the method used to separate antibody-bound and free hormone concentrations in the RIA technique. The reported prevalence of T or T-binding antibodies varies widely, depending on the patients studied and the methods for detection. Thus, although the interference in total T and total T RIAs is low (3), the incidence of interference is much higher in assays for free thyroid (FT) and free triiodothyronine (FT) involving radiolabeled T or T analogs. In samples submitted to a routine clinical chemistry department for thyroid-function testing, the incidence of antibody interference in an

CLIN. CHEM. 37/8, 1397–1400 (1991)


Kraus RM. Relationship of intermediate and low-density lipoprotein subspecies to risk of coronary artery disease. Am Heart J 1987;113:57–82.


