Variability in cholesterol measurements: comparison of calculated and direct LDL cholesterol determinations

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Calculated low-density lipoprotein cholesterol (LDL-C) concentrations determined from the Friedewald equation have a large intraindividual CV, in part because the calculation incorporates the variability of cholesterol, high-density lipoprotein cholesterol (HDL-C), and triglyceride measurements. We studied whether a new assay that measures LDL-C directly will reduce this variability and reduce the need for averaging serial specimens. Four blood samples were obtained 1 week apart from 35 mildly hypercholesterolemic subjects and analyzed for total cholesterol, triglycerides, and HDL-C. LDL-C was calculated by the Friedewald equation, and was also measured directly with a commercially available direct LDL-C assay. The intraindividual CV for the direct and calculated LDL-C assays were similar [CV of direct LDL-C assay (mean ± SE): 6.8 ± 0.5% vs calculated LDL-C: 7.3 ± 0.6%; difference 0.44%, 95% confidence interval: −0.7 to 1.5%]. For both assays, at least two blood tests were required from each subject to reduce total variability of LDL-C to ≤5%. We conclude that the direct LDL-C assay did not reduce the variability in LDL-C compared with the conventional LDL-C calculation. However, it may have a specific role in lipid disorder evaluation and (or) monitoring when triglycerides are increased or the LDL-C value alone is needed.

INDEXING TERMS: hypercholesterolemia • Friedewald equation • HDL cholesterol • triglycerides • VLDL cholesterol • analytic variability • biologic variability • methods comparison

Hypercholesterolemia is one of the most common risk factors for cardiovascular disease. By applying National Cholesterol Education Program (NCEP) guidelines [1] to the US population, >41% of American adults are estimated to have increased low-density lipoprotein cholesterol (LDL-C) concentrations requiring further evaluation, and 13 million are likely to require cholesterol-lowering drug therapy [2].4 These national guidelines stress the importance of utilizing both diet and drug therapy, if necessary, to achieve LDL-C target concentrations. Therefore, accurate and precise estimations of patients’ LDL-C concentrations are necessary to appropriately identify individuals with hypercholesterolemia and to monitor response to diet and drug treatment.

For clinical purposes, LDL-C is generally determined from the Friedewald equation, which assumes that the amount of cholesterol in very-low density lipoproteins (VLDL) can be estimated by dividing the blood triglyceride concentration by a factor of five [3]. The Friedewald equation correlates well with LDL-C concentrations determined by ultracentrifugation if blood triglyceride concentrations are <4.52 mmol/L (400 mg/dl) [3]. However, the reliability of the LDL-C calculation depends upon the accuracy and precision of total cholesterol, high-density lipoprotein cholesterol (HDL-C), and triglyceride measurements. Poor analytic precision in any or all of these measurements will contribute to the variability observed in LDL-C concentrations. The biologic variability inherent in each of the three lipid measurements will also contribute to the total variability of the LDL-C concentration when the Friedewald equation is used. In particular, the biologic CV of blood triglyceride concentrations may be ≥20% and may interfere with the reliability of the LDL-C calculation. Biologic variation is also a major component of the variability in HDL-C measurements, and is frequently in the range of 7–8%. Although analytic variability can often be reduced with methodological advances in the laboratory, sources of error introduced by excessive biologic variability are not easily overcome.

The total variability for LDL-C measurements calculated by the Friedewald equation has been reported to be as high as 9.6%

4 Nonstandard abbreviations: LDL-, HDL-, VLDL-C, LDL, HDL, and VLDL cholesterol, respectively; and NCEP, National Cholesterol Education Program.
which implies that individual LDL-C readings could vary by up to 40% (±2 SD) from one measurement to the next by chance alone. The usual approach to reduce this variability is to calculate the mean of several serial specimens [6, 7]. For example, to decrease the LDL-C CV to 5%, a level adequate to detect most LDL-C responses to diet and drug therapy, at least two, and as many as five, serial blood specimens may have to be analyzed and averaged [8].

To address these limitations of the Friedewald equation, a direct LDL-C assay has been developed. By using solid-phase immunocapture, HDL-C and VLDL-C are removed by centrifugation. The LDL-C remaining in the filtrate is quantified by an enzymatic cholesterol assay. The accuracy of this method compared with both standard ultracentrifugal techniques and the Friedewald calculation has been favorable, with an analytic imprecision of <3% [9]. Because this assay measures LDL-C independently of other lipid fractions, it may potentially reduce the variability introduced into the Friedewald equation from the cumulative analytic and biologic variability of triglyceride, HDL-C, and total cholesterol measurements. For this reason, a direct assay of LDL-C may be a more useful test even when triglyceride concentrations are not markedly increased. For example, decreasing the variability of LDL-C measurements may reduce the number of serial specimens necessary to accurately reflect LDL-C concentrations in a patient.

This study assesses the variability in serial lipid measurements from hypercholesterolemic subjects obtained under basal conditions, and examines whether the analytic, biologic, and total variability of LDL-C will be reduced by using the direct LDL-C assay compared with LDL-C calculated from the Friedewald equation. Decreased test variability will improve clinical decision making, and may reduce the frequency with which the test must be repeated to accurately assess the response to an intervention.

Subjects and Methods

Subjects

Subjects were recruited from employees and patients of the Milwaukee Veterans Affairs Medical Center. Subjects were included if they met the following conditions: (a) age <70 years, (b) total cholesterol >5.17 but <7.76 mmol/L, and (c) triglycerides <3.39 mmol/L. To be included, dietary habits, exercise patterns, and body weight had to be stable for 3 months before study entry. Volunteers were excluded for taking any lipid-lowering medication, hormones, or immunosuppressive medications for 3 months before the study, and for the presence of hypothyroidism, diabetes, nephrotic syndrome, or renal insufficiency. Medication changes were not permitted during the study period, and subjects were encouraged to continue their customary diet or exercise patterns.

Between May and June 1994, 125 men <70 years old were screened for participation. Of the volunteers screened, 63 were excluded because their cholesterol fell outside the required range, and 24 were ineligible because of hypertriglyceridemia. Three additional subjects were excluded because of fasting hyperglycemia. Two subjects were included only after lipid-lowering drugs had been discontinued for >3 months. A total of 35 subjects successfully completed the screening protocol, signed informed consent, and were included in the study.

Methods

Study design. Subjects were instructed to have four fasting blood tests 1 week apart performed at the Milwaukee Veterans Affairs Medical Center clinical laboratory. Before each blood test, subjects were weighed and queried concerning any changes in diet or exercise patterns. The Medifacts Dietary Assessment Questionnaire [11] was administered at each study visit, and a dietary adherence score determined. The study was concluded after the fourth visit. The total duration of the study, including an initial screening visit, was 6 weeks. The study was approved by the Human Research Review Committees of the Medical College of Wisconsin and the Milwaukee Veterans Affairs Medical Center.

Blood collection. Blood was obtained with a tourniquet applying gentle pressure, with the subject in the seated position after a 12-h fast. All laboratory tests were performed on serum at the Clinical Chemistry Laboratory at the Milwaukee Veterans Affairs Medical Center, using identical procedures for all patients.

Friedewald calculation. For total cholesterol, HDL-C, and triglyceride measurements, a Beckman Synchron CX7 automated chemistry analyzer with Beckman reagents was utilized (Beckman Instruments, Brea, CA). This instrument system involves standard enzymatic assays for cholesterol and triglycerides, as previously described [10, 11]. HDL-C was measured as described for total cholesterol after the precipitation of VLDL-C and HDL-C with phosphotungstate and magnesium [12]. Calibration of the cholesterol assay is referenced to Veterans Affairs National Clinical Laboratory Standardization Program, which is part of the National Reference Method Laboratory Network established by the Centers for Disease Control as part of the National Reference System for cholesterol. This certification requires accuracy and precision of ±3%.

To calculate LDL-C, the Friedewald equation was used, with VLDL-C estimated by dividing blood triglycerides by five [3].

Direct LDL-C assay. The direct LDL-C measurement was performed by personnel in the Clinical Chemistry Laboratory at the Milwaukee Veterans Affairs Medical Center by using the direct LDL-C assay kit provided by Sigma Diagnostics (St. Louis, MO). This kit consists of an LDL-C reagent, two controls, and LDL-C separation tubes. The LDL-C reagent is composed of latex beads coated with affinity-purified goat polyclonal antiserum to specific human apolipoproteins. The assay was performed as recommended by Sigma Diagnostics, and includes the following steps: (a) placing the LDL-C reagent into the inner compartment of a separation tube, (b) adding 30 µL of a specimen into the inner compartment containing the LDL-C reagent, (c) mixing and then incubating at room temperature for 5–10 min, (d) centrifuging at 2200g for 5 min, and (e) discarding the inner compartment and assaying the filtrate solution re-
remaining in the outer compartment with the cholesterol enzymatic assay.

**Determination of components of analytic, biologic, and total test variability.** Analytic variance ($V_a$) was determined by dividing serum obtained from two subjects into 31 aliquots, storing at $-20^\circ$C, and measuring lipids (total cholesterol, HDL-C, triglycerides, calculated LDL-C, and direct LDL-C) daily (Monday through Friday) without replications on freshly thawed aliquots over 6 weeks. Although a consistent negative bias increasing with storage time has been reported when samples frozen at $-70^\circ$C are analyzed with the direct LDL-C assay [9], we report an interassay CV of 1.5% using specimens frozen at $-20^\circ$C, which compares favorably with that reported by others using fresh specimens [9]. However, it is possible that the analytic variability may have been slightly overestimated by performing serial measurements on thawed, rather than fresh, samples.

For each subject, biologic variation ($V_b$) was determined for total cholesterol, HDL-C, triglycerides, and LDL-C by using the formula: $V_b = V_t - V_a$ [13], where $V_t$ is the intraindividual variance of the test observed in the four serial specimens obtained for each subject, and $V_a$ is the analytic (interassay) variability. The CVs of the biologic and total variability ($V_b$ and $V_t$, respectively) were determined by taking the square root of the variance for each test, dividing by the patient mean, and multiplying by 100. The mean CV, and $V_b$, for all subjects are presented in Table 1.

On the basis of the $V_t$ determined for a single specimen, the potential for the mean value derived from serial specimens to reduce total variability of LDL-C was estimated by dividing the observed test variability of a single specimen by the number of serial specimens obtained [14, 15].

**Statistical analysis.** The biologic and total variability between calculated and direct LDL-C were compared by using the paired t-test. Correlations between lipid measurements were assessed by using the Pearson correlation coefficient. One-way ANOVA for repeated measures was used to detect differences in dietary adherence scores and weights between visits. In the text, the term “variability” refers to the CV unless otherwise stated.

To detect a difference in total variability $>1.5\%$, we determined that a sample of 35 subjects should be sufficient to achieve an $\alpha$ and $\beta$ error of $<0.05$ and $<0.10$, respectively. We estimated that smaller differences were unlikely to be clinically relevant for decision making at the clinical level.

### Table 1. Mean concentrations and analytic, biologic, and total variability (CV) of lipid measurements derived from four specimens obtained weekly from each of 35 subjects.

<table>
<thead>
<tr>
<th>Lipid measurement</th>
<th>Mean $\pm$ SD, mmol/L</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Analytic</td>
<td>Biologic</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>6.0 $\pm$ 0.7</td>
<td>2.0</td>
</tr>
<tr>
<td>HDL-C</td>
<td>1.1 $\pm$ 0.2</td>
<td>4.7 $\pm$ 2.5</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>1.4 $\pm$ 0.7</td>
<td>6.4 $\pm$ 4.3</td>
</tr>
<tr>
<td>LDL-C (Friedewald)</td>
<td>4.3 $\pm$ 0.6</td>
<td>19.6 $\pm$ 7.4</td>
</tr>
<tr>
<td>LDL-C (direct)</td>
<td>4.2 $\pm$ 0.6</td>
<td>6.6 $\pm$ 3.6</td>
</tr>
</tbody>
</table>

**Results**

The mean age of study subjects was 52 $\pm$ 13 years (mean $\pm$ SD). Of the study population, 40% were hypertensive, 14% smoked, 3% had coronary heart disease, and 46% were taking at least one medication (range one to six: mean $\pm$ SD 2.5 $\pm$ 1.2). The mean body mass index was 27.7 $\pm$ 3.2. Food scores and weights were unchanged between study visits.

Mean total cholesterol, HDL-C, triglycerides, and LDL-C values for the 35 subjects are shown in Table 1 and reflect mild hypercholesterolemia. The calculated LDL-C showed a strong correlation with the direct LDL-C assay ($r = 0.95, P <0.001$), but was, on average, 2.5% lower than the LDL-C measured directly (mean $\pm$ SE 4.30 $\pm$ 0.10 mmol/L vs 4.20 $\pm$ 0.10, $P = 0.002$). The CV$_b$ for total cholesterol, triglyceride, and HDL-C measurements was <3%, and for the LDL-C calculation was 2.4%. The CV$_b$ for the direct LDL-C assay was 1.5%.

Mean biologic and total variability computed for each subject are presented in Table 1. The total CVs for the direct and calculated LDL-C were 6.8% and 7.3%, respectively, and were not significantly different when evaluated by either the CV [direct LDL-C (mean $\pm$ SE): 6.8 $\pm$ 0.5% vs calculated LDL-C: 7.3 $\pm$ 0.6%; difference 0.44%, 95% confidence interval: $0.7-1.5\%$] or the variance (direct LDL-C: 0.12 $\pm$ 0.02 mmol/L vs calculated LDL-C: 0.09 $\pm$ 0.01 mmol/L, difference 0.03, 95% confidence interval: $-0.01-0.06$ mmol/L). The biologic variability was also not significantly different between direct and calculated LDL-C as assessed by comparing either the CV (6.5 $\pm$ 0.6 vs 6.6 $\pm$ 0.6%; difference 0.07%, 95th confidence interval: $-1.0-1.2\%$) or the variance (0.11 $\pm$ 0.02 mmol/L vs 0.09 $\pm$ 0.01 mmol/L; difference 0.019, 95th confidence interval: $-0.02-0.05$ mmol/L). The biologic variability was higher than the analytic variability for all lipid measurements, and contributed to most of the total variability. This was particularly true for triglycerides, where both the biologic and total variability were $\sim20\%$.

To assess the relative contribution of total cholesterol, triglycerides, and HDL-C variability to the variability of LDL-C, we determined the correlation between total variability in LDL-C with the variability in these other lipid measurements (Table 2). The total variability of total cholesterol was significantly associated with the variability of calculated LDL-C ($r = 0.76, P <0.001$). However, the variability in either triglycerides or HDL-C did not contribute significantly to the observed variability in calculated LDL-C determinations. For the direct LDL-C assay, the variability in total cholesterol was associated with the variability in direct LDL-C measurements, but to a
lesser extent than for the calculated LDL-C value \(r = 0.42, P = 0.02\). The mean triglyceride concentration for each patient was also significantly associated with the total CV of the calculated \(r = 0.43, P = 0.009\), but not the direct \(r = 0.11, P = 0.41\), LDL-C.

On the basis of the test variability observed for the calculated and the direct LDL-C, we estimated the ability of serial specimens to reduce total variability (Fig. 1). Averaging the concentration of two LDL-C specimens rather than one reduced the total variability from ~7 to 5%. Obtaining additional specimens produced diminishing reductions in LDL-C variability. The total variability of the direct and calculated LDL-C value were similar for any number of specimens obtained.

Because of the large variability in calculated LDL-C measurements, current NCEP guidelines suggest that decision making be based on the mean of two LDL-C concentrations, and recommend obtaining a third LDL-C measurement if the first two concentrations differ by >0.78 mmol/L [11]. Among our subjects, 9% (3 of 35) would have required a third measurement with the calculated LDL-C, and 6% (2 of 35) required a third measurement with the direct assay (\(P = \text{not significant, } \chi^2\)).

**Discussion**

Because the calculated LDL-C is derived from total cholesterol, triglycerides, and HDL-C measurements, one may expect that the considerable variability of these three measurements would contribute directly to the observed variability in the calculated LDL-C value. By determining LDL-C directly, the dependence upon three separate and relatively variable lipid measurements, total cholesterol, HDL-C, and triglycerides, is eliminated. In spite of this, however, we found that the direct LDL-C assay did not have significantly lower biologic or total variability of LDL-C compared with the calculated LDL-C. The analytic precision of the direct LDL-C assay was excellent, meeting the precision criteria set for total cholesterol of ±3% and performing better than reported for other direct LDL-C assays involving chemical precipitation methods [16]. The analytic variability for total cholesterol, HDL-C, triglycerides, and the calculated LDL-C measurements were also <3%, comparing favorably with most previously published reports [3]. On the other hand, the high biologic variability present in LDL-C measurements increased the total variability of both the calculated and direct LDL-C estimations. The total variability (CV) for either measurement was ~7%. To reduce this CV for the test to ≤5%, at least two specimens would be required. Because the direct LDL-C assay did not reduce the total variability for LDL-C, serial specimens remain necessary to reliably determine an individual's LDL-C concentration even when using the direct LDL-C assay.

Because the calculated LDL-C value incorporates the analytic and biologic variability inherent in three separate lipid measurements, it is surprising that the direct LDL-C assay was not more precise than the calculated LDL-C determination. In particular, the total variability for both HDL-C and triglyceride

<table>
<thead>
<tr>
<th>Lipid measurement</th>
<th>Friedewald</th>
<th>Direct</th>
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<tbody>
<tr>
<td>Total cholesterol</td>
<td>0.12</td>
<td>-0.32</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>0.06</td>
<td>0.19</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.43(^b)</td>
<td>0.11</td>
</tr>
</tbody>
</table>

\(^{a}P < 0.05.\)

\(^{b}P < 0.01.\)
measurements was greater than the total variability present for the direct LDL-C assay. However, despite the larger total variability present in HDL-C and triglyceride measurements, only the variability derived from total cholesterol determination was significantly associated with the variability of the calculated LDL-C. This suggests that an accurate and reliable total cholesterol measurement is of primary importance to ensure the accuracy and precision of LDL-C estimations with the Friedewald formula. Recommendations to improve both accuracy and precision of the total cholesterol concentration to CVs of <3% have been formulated [17], and will help to ensure the reliability of the calculated LDL-C determination.

An examination of the Friedewald equation suggests why neither triglyceride nor HDL-C variability is an important determinant of LDL-C variability. By dividing blood triglyceride concentrations by 5 to estimate VLDL-C, the equation limits the impact of triglyceride variability on the LDL-C measurement. Further, both HDL-C and VLDL-C concentrations are usually less than half of LDL-C concentrations, also diminishing their impact on calculated LDL-C variability. Therefore, increasing triglyceride concentrations are likely to add progressively more variability to the calculated LDL-C measurement. Consistent with this explanation was the presence of a significant positive correlation between blood triglyceride concentration and calculated LDL-C variability ($r = 0.43, P = 0.009$), but not between triglyceride concentration and direct LDL-C variability ($r = 0.11, P = 0.41$).

When should the direct LDL-C measurement be ordered? In patients with triglycerides $>4.52$ mmol/L, VLDL-C cannot be estimated accurately, and the calculated LDL-C becomes less accurate and precise [18]. The calculated LDL-C may also be less reliable in patients with type II diabetes [19] and liver disease [20], possibly because of the propensity towards increased blood triglycerides in these illnesses. On the other hand, the direct LDL-C assay accurately measures LDL-C with triglyceride concentrations to 9.03 mmol/L or higher [21]. Patients requiring lipid determinations while not fasting may have increased triglyceride concentrations and may also benefit from a direct LDL-C measurement.

Because the performance of the direct LDL-C assay was comparable with the calculated LDL-C determination, cost may be an important factor determining which assay to use. An informal survey of charges in five commercial laboratories in Wisconsin that have used the direct LDL-C assay for $>6$ months show a range for the direct LDL-C assay from $30$ to $45$ (mean $36$), and for a lipid “panel” (total cholesterol, HDL-C, triglyceride measurements, and calculated LDL-C) of $40$ to $68$ (mean $57$). Although the direct LDL-C assay is less expensive than the calculated LDL-C value, it does not provide the additional information of triglyceride and HDL-C measurements. When this additional information is important, and the triglyceride concentrations are $<4.52$ mmol/L, a routine lipid panel and calculated LDL-C are probably sufficient (Fig. 2). However, if the triglycerides may potentially increase above this concentration, or if only the LDL-C concentration is required, then the direct LDL-C assay should be the test of choice.

According to current recommendations for lipid monitoring in the hypercholesterolemic patient, triglyceride and HDL-C determinations, in addition to LDL-C, should be obtained yearly, whereas total cholesterol can be used to assess therapeutic effectiveness at interim visits [1]. The direct LDL-C assay is less expensive than the standard lipid panel, yet provides a more accurate assessment of LDL-C than does the total cholesterol alone. Therefore, the direct LDL-C assay may have a role in routine monitoring of hypercholesterolemia therapy when triglyceride and HDL-C values are not required.

A potential limitation of these recommendations is that our findings may not extend to lipid abnormalities not evaluated in this study. In particular, patients with triglyceride concentrations between 3.39 and 4.52 mmol/L may have sufficiently large biologic variability to significantly influence the variation of calculated LDL-C to a greater extent than suggested by our results. Similarly, inclusion of more subjects with higher HDL-C concentrations may also increase the variability of the calculated LDL-C value, rendering the direct LDL-C assay a better test. In these settings, further study will be required to determine the comparability of the two assays.

In conclusion, the direct LDL-C assay does not reduce the variation in LDL-C compared with the conventional LDL-C calculation. Therefore, serial specimens are still necessary to accurately assess LDL-C values and gauge response to therapy. However, because the direct assay is accurate even when triglycerides are increased, and because it allows a less expensive assessment of LDL-C (as an isolated test) than does the standard lipid panel, it appears to have a potentially useful role in lipid disorder management.

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References


