These data add to the evidence-based information necessary to use BV as a metric to evaluate important changes and perhaps a more refined definition of acute cardiovascular events (1).

<table>
<thead>
<tr>
<th>Table 1. Biologic and analytical variation metrics.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable</td>
</tr>
<tr>
<td>----------</td>
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<tr>
<td>Analytical variation*</td>
</tr>
<tr>
<td>CV&lt;sub&gt;A&lt;/sub&gt;,%</td>
</tr>
<tr>
<td>BV</td>
</tr>
<tr>
<td>CV&lt;sub&gt;B&lt;/sub&gt;,%</td>
</tr>
<tr>
<td>CV&lt;sub&gt;G&lt;/sub&gt;,%</td>
</tr>
<tr>
<td>Index of individuality</td>
</tr>
<tr>
<td>RCV (lognormal increase), %&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>RCV (lognormal decrease), %&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean δ increase, %&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean δ decrease, %&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* Based on duplicate results.
<sup>a</sup> CV<sub>A</sub>, analytical CV; CV<sub>B</sub>, intraindividual CV; CV<sub>G</sub>, interindividual CV.
<sup>b</sup> A CI of 95% was used to calculate the RCV for short- and long-term biologic variation at a z value of 0.84 (1-sided test).
<sup>c</sup> Mean δ increase/decrease: mean change (increase/decrease) in an individual’s troponin values compared with baseline (first time point); data are expressed as a percentage.
<sup>d</sup> Median (IQR), 44.7% (22.6%–68.3%).
<sup>e</sup> Median (IQR), 20.0% (10.5%–26.1%).
<sup>f</sup> Median (IQR), 24.2% (15.7%–67.9%).
<sup>g</sup> Median (IQR), 21.9% (14.7%–25.9%).

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Letters to the Editor

Limitations of Direct Methods and the Reference Method for Measuring HDL and LDL Cholesterol

To the Editor:

Miller and colleagues (1) compared the results obtained by direct measurements of HDL cholesterol (HDL-C) and LDL-C with those obtained with reference methods requiring ultracentrifugation. They used frozen pooled serum samples and fresh serum samples from 37 healthy controls and 138 individuals with dyslipidemia or heart disease that they had obtained from the NIH. Assay kits, calibrators, and controls were provided by 7 manufacturers. The authors reported CVs of <3.7% for HDL-C and <4.4% for LDL-C for the frozen serum pools, biases for fresh serum from the healthy controls of −5.4% to 4.4% for HDL-C and −6.8% to 1.1% for LDL-C. The biases for the patients with disease were −8.6% to 8.8% for HDL-C.
and −11.8% to 4.1% for LDL-C, compared with the reference method. The total error for the healthy controls ranged from −13.4% to 1.6% for HDL-C and from −13.3% to 13.5% for LDL-C. For the disease group, total errors ranged from −19.8% to 36.3% for HDL-C and from −26.6% to 31.9% for LDL-C. The authors concluded that 6 of 8 direct HDL-C methods and 5 of 8 direct LDL-C methods met the National Cholesterol Education Program total error goals for healthy individuals; however, all methods failed to meet these goals for individuals with disease, compared with results for the reference method (1).

The reference method used requires ultracentrifugation of serum (5 mL) at its own density (1.006 g/mL) for 16 h at 120,000g, with the removal of triglyceride-rich lipoproteins in the supernatant fraction, which is not feasible in clinical practice. The collected infranatant fraction is then brought back to its original volume, and the cholesterol content is then measured by the Abell–Kendall method. Heparin–manganese chloride precipitation of this infranatant fraction is used to remove LDL, and the cholesterol in the supernatant fraction is measured with the Abell–Kendall method to determine the HDL-C value. The LDL-C value is obtained by subtracting the HDL-C value from the total cholesterol value of the bottom fraction and is not measured directly. Therefore, in this study the reference methods for LDL-C and HDL-C were carried out on the infranatant fraction, whereas the direct methods were assessed with whole sera.

Currently, serum or plasma cholesterol concentrations are measured via automated enzymatic analyses that provide results that are very comparable with those obtained with the Abell–Kendall method. The CDC uses the ultracentrifugation step because heparin–manganese chloride precipitation does not always remove all lipoproteins containing apolipoprotein B, especially in samples from patients with marked hypertriglyceridemia. Therefore, the way this study was carried out is biased and puts the direct measurements at a considerable disadvantage, especially for samples obtained from individuals with disease, who are more likely to have marked hypertriglyceridemia or a variety of other rare inborn errors of lipoprotein metabolism often seen at the NIH. Other methods of precipitating lipoproteins containing apolipoprotein B, such as the dextran sulfate–Mg2+ method, have been developed because of the limitations of the heparin–manganese chloride precipitation procedure. For some patients in our experience, only ultracentrifugation can be used for accurate lipoprotein assessment.

Our laboratory has measured HDL-C and LDL-C directly in frozen plasma with the kits from one of the manufacturers (Kyowa Medex) and compared the results with those obtained with the dextran sulfate–Mg2+ method for HDL-C and calculated LDL-C, for fresh plasma from participants in cycle 6 of the Framingham Offspring Study (3182 participants: 1335 male controls, 1601 female controls, and 246 heart disease cases) (2).

Values for HDL-C and LDL-C obtained by these methods were very highly correlated ($r^2 = 0.94$ for both; $P < 0.001$), with >10% bias being observed in only 8.5% of the samples for HDL-C and 7.7% of the samples for LDL-C. Compared with the dextran sulfate–Mg2+–HDL-C method, we observed negative biases of −2% for healthy participants and −7% for heart disease cases (2). Our conclusions were that direct measurements of HDL-C and calculated LDL-C values provide a reasonable guide to lipid management and that direct measurement of LDL-C is not generally required. We came to a similar conclusion when we compared direct and standard methods in a study of diabetics and control individuals (3). Direct online measurements of HDL-C provide considerable advantages over older methods. Moreover, calculated non–HDL-C is superior to calculated LDL-C as a heart disease risk parameter (4).

Our own studies also indicate that these assays can be used in both the fasting and fed state in healthy, obese, and dyslipidemic individuals (5). Additional comparisons of such methods with reference methods need to be carried out with the same samples, and the precise lipid abnormalities present in the patients studied need to be provided.

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Ernst J. Schaefer*
Seiko Otokozawa
Masumi Ai

Lipid Metabolism Laboratory
Human Nutrition Research Center on Aging
Tufts University and
Tufts University School of Medicine
Boston, MA

* Address correspondence to this author at:
Human Nutrition Research Center on Aging
711 Washington St.
Boston, MA 02111
Fax 617-556-3103
E-mail ernst.schaefer@tufts.edu

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In Reply

We disagree with the statement of Schaefer et al. that our study design (1) was “biased and puts the direct measurements at a considerable disadvantage” because we used the β-quantification reference measurement procedure as the basis for evaluating the performance of the direct HDL cholesterol (HDL-C) and LDL-C methods. It is misleading for these authors to suggest that “in this study [referring to our previous report (1)] the reference methods for LDL-C and HDL-C were carried out on the infranatant fraction, whereas the direct methods were assessed with whole sera.” The reference measurement procedure starts with ultracentrifugation of whole sera, with subsequent measurements carried out on the infranatant fraction to obtain accurate quantification of the HDL-C and LDL-C components. Schaefer et al. agree with this point in their statement, “For some patients in our experience, only ultracentrifugation can be used for accurate lipoprotein assessment.” The cholesterol content in HDL and LDL lipoprotein density intervals defined by ultracentrifugation is the basis for the current National Cholesterol Education Program Adult Treatment Panel III interpretive guidelines. It is important to note that direct HDL-C and LDL-C methods have been used with the same Adult Treatment Panel III decision points as the older assays. Consequently, in our study design, the direct HDL-C and LDL-C methods were evaluated for their ability to produce results that agreed with the best-available reference measurement procedure.

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Reference


W. Greg Miller1*
Gary L. Myers2
Alan T. Remaley3

1 Department of Pathology
Virginia Commonwealth University
Richmond, VA
2 AACC
Washington, DC
3 Lipoprotein Metabolism Section
Pulmonary and Vascular Medicine Branch
National Heart, Lung and Blood Institute
NIH
Bethesda, MD

* Address correspondence to this author at:
Virginia Commonwealth University
P.O. Box 980286
Richmond, VA 23298-0286
Fax 804-828-0353
E-mail gmiller@vcu.edu

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