Biological Variability of Cholesterol, Triglyceride, Low- and High-Density Lipoprotein Cholesterol, Lipoprotein(a), and Apolipoproteins A-I and B

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Biological variability is a major contributor to the inaccuracy of cardiovascular risk assessments based on measurement of lipids, lipoproteins, or apolipoproteins. We obtained estimates of biological variation (CV_b) for 20 healthy adults and calculated the percentiles of CV_b as an expression of the variability of CV_b among individuals for cholesterol, triglyceride, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, apolipoprotein (apo) A-I, apo B, and lipoprotein(a) [Lp(a)] by four biweekly measurements of these analytes. The CV_b for the group was 6–7% for cholesterol, HDL cholesterol, apo A-I, and apo B; 9% for LDL cholesterol; and 28% for triglyceride. However, for each analyte, there was a considerable variation of CV_b among individuals. For all analytes except Lp(a), there was no relation between the individual's CV_b and the analyte concentration. Lp(a) was inversely related to CV_b and there was a wide variation in the CV_b for Lp(a) among the participants, ranging from 1% to 51%. The number of independent analyses to perform to accurately assess an individual's risk for coronary artery disease should be determined on the basis of the individual CV_b for a given analyte rather than the average CV_b.

Indexing Terms: variation, source of cardiovascular risk factors

The variability of the measurement of a risk factor for premature coronary artery disease can have a significant impact on the degree of its association with the disease. Thus, those risk factors with high variability could have uncertain estimates of their relationship to coronary artery disease. The variability of repeated measurements depends on the analytical variability and the preanalytical or within-person biological variability, which includes variability of blood drawing and sample processing. Preanalytical sources of variation usually constitute a major proportion of the total variation in lipid and apolipoprotein measurements. Therefore, intraindividual biological variation may significantly contribute to misclassification of a person's risk for coronary artery disease. To minimize the effect of intraindividual variation on assessments of risk factor or therapeutic response, repeated independent measurements are required.

Over the past 25 years, numerous researchers have estimated the average intraindividual biological variability for plasma or serum lipids and lipoprotein cho-


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Laboratory Methods

Cholesterol and triglycerides were measured with an automated enzymic system (Abbott Spectrum Multichromatic Analyzer; Abbott Laboratories, N. Chicago, IL). HDL cholesterol was enzymatically quantified in the supernatant solution after precipitation of apo B-containing lipoproteins with dextran sulfate–magnesium (1). LDL cholesterol was estimated by subtracting the HDL cholesterol and one-fifth of the plasma triglyceride value from the total cholesterol value. Apo A-I and B were quantified with a nephelometer (Behring, Marburg, Germany), with calibrator and quality-control materials prepared in-house. The cholesterol method is standardized by the National Reference System for Cho-

2 Nonstandard abbreviations: Lp(a), lipoprotein(a); apo, apolipoprotein; LDL, HDL, low- and high-density lipoprotein, respectively.
cholesterol, and the apo A-I and apo B methods are standardized with the World Health Organization International Reference Materials for apo A-I and apo B.

Lp(a) protein concentration was measured by an in-house ELISA involving two monoclonal antibodies specific for apo(a), with no cross-reactivity with plasminogen, and directed to different epitopes on the apo(a) molecule (2). This direct-binding ELISA is based on the capture of apo(a)-containing particles by one monoclonal antibody coated on 96-well microtiter plates and the detection of apo(a) particles by a second monoclonal antibody conjugated to horseradish peroxidase (EC 1.11.1.7). Lp(a) isolated from a donor with an intermediate-size isofrom of apo(a), with total protein determined by a standardized sodium dodecyl sulfate-Lowry procedure, was used to calibrate the assay. The results are expressed in terms of total Lp(a) protein.

Statistical Methods

The total measurement variability (SD\(\text{total}^2\)) is equal to the sum of analytical variability (SD\(\text{anal}^2\)) and the natural biological variability (SD\(\text{bio}^2\)). Variability values can also be expressed in terms of the coefficient of variation (CV) around the test mean. The total intraindividual test variability (CV\(\text{t}^2\)) is defined as \(\left(\text{SD}_{\text{t}}^2 + \text{SD}_{\text{a}}^2\right)^{1/2}\), that is, \(\text{CV}_{\text{t}} = \left(\text{SD}_{\text{t}}^2 + \text{SD}_{\text{a}}^2\right)^{1/2}\), where CV\(\text{t}\) is the coefficient of biological variation and CV\(\text{a}\) is the coefficient of analytical variation. Because CV\(\text{t}\) is computed from CV\(\text{a}\) and CV\(\text{a}\), it is important that CV\(\text{a}\) be accurately estimated. We computed CV\(\text{a}\) from the variation of the measurements on the participants' samples rather than on quality-control samples and minimized the CV\(\text{a}\) by analyzing the frozen samples from each participant in the same analytical run. From analysis of the frozen plasma, we computed the SD\(\text{a}\) for each subject from the formula SD\(\text{a}\) = \(\left(\sum d^2/n - 1\right)^{1/2}\), where \(d\) is the difference between the number of duplicate measurements, \(n\); \(n\) = 4; and the CV\(\text{a}\) = SD\(\text{a}\)/(mean of all values for a given subject). From the analyses of the fresh samples, the SD\(\text{a}\) for each participant was computed from the three values measured in the morning and the three values measured in the afternoon and from the CV\(\text{a}\) for each individual. Because preliminary analyses indicated that the CV\(\text{a}\) computed for each subject was independent of the analyte concentration for all analytes except Lp(a), the CV\(\text{a}\) for each analyte for all participants was computed from the CV\(\text{a}\) of the analyte values of each subject as \(\left(\sum \text{CV}_\text{a}^2/n\right)^{1/2}\), where \(n\) = 20. This CV\(\text{a}\) was then used to compute CV\(\text{b}\) for each subject for each analyte, and the CV\(\text{b}\) for all subjects was computed as \(\left(\sum \text{CV}_\text{b}^2/n\right)^{1/2}\). The CV\(\text{b}\) of an analyte for a participant is equal to SD\(\text{b}^2/\text{mean of all measurements for a given participant}, and the CV\(\text{b}\) for all participants is computed as \(\left(\sum \text{CV}_\text{b}^2/n\right)^{1/2}\). Because preliminary analyses indicated that CV\(\text{a}\) and CV\(\text{b}\) varied as a function of Lp(a) concentration, we calculated CV\(\text{a}\) and CV\(\text{b}\) for each of the following Lp(a) protein concentration ranges: 0–30, >30–60, >60–150, and >150 mg/L.

Results

Table 1 provides the overall mean values and the range for each analyte, and the CV components. The calculated CV\(\text{b}\), from the data obtained on fresh plasma was nearly identical to that obtained with frozen plasma. By analyzing the frozen samples in the same run, however, we eliminated the between-assay variability. Therefore, unless otherwise indicated, all results are computed from the analyses on frozen plasma. As a consequence, the analytical variability was very low for each analyte, ranging from 0.7% for total cholesterol to 2.2% for apo A-I (Table 1). The overall biological variation was 6–7% for cholesterol, HDL cholesterol, apo A-I, and apo B; ~9% for LDL cholesterol; and 28% for triglycerides. The biological variation in men was not significantly different from that in women.

Each analyte displayed a fairly broad range of values and biological CVs (Table 1). For example, although the computed CV\(\text{b}\) for the 20 participants was ~7% for cholesterol, HDL cholesterol, apo A-I, and apo B, the CV\(\text{b}\) in the participant subjects varied widely, from as low as 0–3% to as high as 12–14%. The CV\(\text{b}\) for LDL cholesterol varied from 2% to 15%. The biological CV\(\text{b}\) was much higher for triglycerides, 28%, and ranged widely among the subjects: one participant, with a mean triglyceride value of 0.61 g/L, had a CV\(\text{b}\) of 5%; another, with 2.28 g/L triglyceride, had a CV\(\text{b}\) of 75%. For all analytes except Lp(a) there was no relation between the CV\(\text{b}\) and the analyte concentration (Fig. 1).

<table>
<thead>
<tr>
<th>Analyte conc. g/L</th>
<th>CH</th>
<th>TG</th>
<th>LDL</th>
<th>HDL</th>
<th>Apo B</th>
<th>Apo A-I</th>
<th>CV(\text{a})</th>
<th>CV(\text{b})</th>
<th>CV(\text{b})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>1.86</td>
<td>0.98</td>
<td>1.15</td>
<td>0.51</td>
<td>0.87</td>
<td>1.42</td>
<td>6.7</td>
<td>28.3</td>
<td>9.3</td>
</tr>
<tr>
<td>Range</td>
<td>1.34–2.45</td>
<td>0.42–3.34</td>
<td>0.76–1.85</td>
<td>0.29–0.74</td>
<td>0.57–1.26</td>
<td>0.96–2.00</td>
<td>0.7</td>
<td>7.6</td>
<td>7.6</td>
</tr>
<tr>
<td>CV(\text{b})</td>
<td>6.7</td>
<td>28.3</td>
<td>9.3</td>
<td>7.6</td>
<td>7.7</td>
<td>6.6</td>
<td>6.7</td>
<td>28.2</td>
<td>9.2</td>
</tr>
<tr>
<td>CV(\text{b})</td>
<td>6.7</td>
<td>9.3</td>
<td>7.5</td>
<td>7.4</td>
<td>6.2</td>
<td>6.2</td>
<td>6.7</td>
<td>26.4</td>
<td>9.2</td>
</tr>
</tbody>
</table>

Table 1. Mean concentrations and coefficients of variation components for cholesterol, triglyceride, HDL and LDL cholesterol, and apo A-I and B.

CH, cholesterol; TG, triglyceride.

* Numbers in parentheses computed from analyses of fresh plasma. All other data obtained from analyses of frozen plasma.

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For all the evaluated analytes the values obtained on fresh plasma and those obtained on plasma stored at −70°C correlated well, with r ranging from 0.957 to 0.999 (Table 2). The average absolute bias between the values obtained on fresh and frozen plasma was <5% for all analytes (Table 2).

The CV_b for Lp(a) varied widely among subjects, from 0.9% to 51%, with the participants with the higher Lp(a) concentrations having a lower CV_b (Fig. 2). Therefore, we did not compute the overall CV_b for all participants but rather computed CV_b for participants in each of four Lp(a) concentration ranges (Table 3). For the 12 participants with Lp(a) protein <30 mg/L, the CV_b estimated from frozen plasma specimens was 27% and the SD_b was 4 mg/L, with the CV_b among individuals ranging from

Table 2. Correlation between values obtained from fresh (y) and frozen (x) plasma.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Slope</th>
<th>y-intercept, g/L</th>
<th>r</th>
<th>Average absolute % bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>1.00</td>
<td>0.17</td>
<td>0.997</td>
<td>1.2</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>1.03</td>
<td>-3.01</td>
<td>0.999</td>
<td>4.6</td>
</tr>
<tr>
<td>LDL chol</td>
<td>1.00</td>
<td>2.53</td>
<td>0.993</td>
<td>3.5</td>
</tr>
<tr>
<td>HDL chol</td>
<td>0.91</td>
<td>2.11</td>
<td>0.991</td>
<td>4.7</td>
</tr>
<tr>
<td>Apo B</td>
<td>1.01</td>
<td>0.19</td>
<td>0.982</td>
<td>4.0</td>
</tr>
<tr>
<td>Apo A-I</td>
<td>1.02</td>
<td>0.09</td>
<td>0.957</td>
<td>4.8</td>
</tr>
</tbody>
</table>

Frozen plasma was stored at -70°C for 1-4 weeks.
3% to 38%. For the three subjects with Lp(a) between 30 and 60 mg/L, the CV_b was 22% and the SD_b 10 mg/L. For the subjects with Lp(a) between 60 and 150 mg/L, the CV_b was 16% and the SD_b 17 mg/L, whereas for participants with Lp(a) > 150 mg/L, the CV_b was only 9% and SD_b 22 mg/L. The correlation between the Lp(a) values obtained on fresh plasma and those obtained on plasma stored at –70°C was very high (r = 0.988), with the average bias between the values being only 3 mg/L.

Discussion

Intraindividual biological variability for lipids and lipoproteins varies among published studies. However, metaanalysis of 30 studies published between 1970 and 1992 (3) yielded estimates of CV_b of 6.1% for total cholesterol, 7.4% for HDL cholesterol, 9.5% for LDL cholesterol, and 22.6% for triglycerides—very similar to the CV_b estimates in the present study. However, in addition, we documented the relatively wide variation in the CV_b for each of these analytes. For example, although the group CV_b for HDL cholesterol was estimated to be 7.5%, 3 of the 20 study subjects had a CV_b < 4% and 3 had a CV_b > 10%. Similarly, the CV_b for LDL cholesterol for the total group was estimated to be ~9%, but the 5 participants had CV_b < 4% and four had CV_b > 12%.

Relatively few studies have evaluated the biological variability of apo A-I and apo B. Our estimate of CV_b of 6.2% and 7.4%, respectively, is similar to that recently reported by Kafonek et al. (4), who found a CV_b of 7.1% for apo A-I and 6.4% for apo B, but lower than the 9–10% CV_b reported by Wasenius et al. (5). We found that not only is the overall CV_b lower for apo A-I than for HDL cholesterol, but also that this was true individually for 17 of 20 study subjects; this strongly suggests that apo A-I is less sensitive to environmental or physiological changes than is HDL cholesterol. Similarly, the overall CV_b for apo B of 7.4% was less than the 9.2% observed for LDL cholesterol, with 17 of 20 participants having a lower CV_b for apo B than for LDL cholesterol. Thus, the apolipoprotein measurements appear to be less susceptible to environmental influences than are the measurements of lipoprotein cholesterol.

To minimize the impact of intraindividual variation on the accuracy of the coronary artery disease risk assessment, repeated measurements are required. The National Cholesterol Education Program (6) recommends that at least two specimens, collected at least one week apart, be analyzed for lipid or lipoprotein cholesterol to estimate an individual's risk for heart disease. The number of repeated measurements needed depends on the magnitude of the difference in the serial measurements and whether or not the mean value is near the target cutoff limit. The total intraindividual variability is a function of the intraindividual CV_b, the CV_a of the laboratory, the number of specimens analyzed, and the number of analytical determinations per specimen. For laboratories using standardized lipid and apolipoprotein measurements, where CV_a < CV_b, replicate analyses on the same specimen are not required. For those participants whose CV_b is significantly higher than the median CV_b for a given analyte, measurement of more than two specimens is required. Cooper et al. (7) suggest that the magnitude of the differences on two or more specimens, or the relative range, can be used to estimate the magnitude of the CV_b and can thereby serve as a guideline for the number of specimens needed to accurately estimate a person's risk of heart disease.

Because the major sources of preanalytical variation, such as lifestyle, are not readily controlled for, measuring serial specimens is usually the most practical approach to control for the effects of preanalytical variation. A potentially significant source of preanalytical variation is posture. The results of a recent study (8) suggest that the requirement for subjects to sit for 5 min before venipuncture may not be adequate to eliminate the effect of posture on preanalytical variation.

Another potentially significant contributor to error in the estimate of a patient's mean value of an analyte is analytical bias. For analytes such as total cholesterol and HDL and LDL cholesterol, this bias should be <5% for standardized laboratories. For HDL cholesterol, apo A-I, and apo B, much greater among-laboratory biases are often observed (9, 10), although recent international efforts to standardize apo A-I and apo B measurements suggest that an average bias of <5% from an accuracy-based target value is possible (11). The situation for Lp(a) is quite different. The structural peculiarity of this lipoprotein particle and the resulting immunochemical problems (12) lead to a lack of comparability of Lp(a) values among laboratories, a lack of established performance criteria by which laboratories can monitor the reliability of their measurements, and a lack of common reference values and cutoff points for clinical use. Obviously, standardization of Lp(a) measurements is of foremost importance if the measurement of this analyte is to reach its full clinical potential.

Our earlier investigations of the CV_b of Lp(a) showed a 7% week-to-week variation in Lp(a) in the few subjects studied (13). In a recent study of 40 subjects, in which Lp(a) was measured three times with a 1- to 2-week interval between each visit, the CV_b for Lp(a) was estimated to be only 2.9% (14). The reasons for the differences in the estimates of the biological variation in these two studies and the present study are unclear, but may in part relate to the concentration of Lp(a) in the participants, in view of our finding here that the variation in Lp(a) depends significantly on Lp(a) concentration. Although >90% of the variability of apo(a) is deter-
determined by the apo(a) gene (15) and Lp(a) concentrations do not change in response to changes in cholesterol and most other dietary perturbations (12, 13, 16), apo(a) has been shown to change in response to anabolic steroids (17), estrogen, (18, 19), and thyroid hormone (20). Thus, the biological variability of Lp(a) may be greater than previously believed. Our study suggests that the CVb of Lp(a) for participants with Lp(a) protein <60 mg/L is ~26% but is highly variable, ranging from 3% to 51%. For individuals with Lp(a) protein >60 mg/L, the CVb is considerably lower, ranging from 1% to 16% (mean, ~11%). Because Lp(a) concentrations vary more than 1000-fold among subjects, the CVb of Lp(a) is not likely to be a major contributor to the misclassification of a person's risk on the basis of the mean Lp(a) value, unless the value is near the cutoff point.

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References