Lipid Concentrations in Serum and EDTA-Treated Plasma from Fasting and Nonfasting Normal Persons, with Particular Regard to High-Density Lipoprotein Cholesterol

Aaron R. Folsom, Kanta Kuba, Russell V. Luepker, David R. Jacobs, and Ivan D. Frantz, Jr.

Serum/plasma sample pairs, collected both during (12-h) fasting and nonfasting from 44 healthy subjects, were analyzed for total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides. Values for total cholesterol and triglycerides averaged 2.1% and 5.2%, respectively, higher for serum than for plasma. Values for HDL cholesterol were not different. Triglyceride concentrations in nonfasting subjects were 40% higher than concentrations during fasting, but total cholesterol and HDL cholesterol were not significantly affected by fasting. These findings suggest that concentrations of HDL cholesterol may be directly compared in clinical and epidemiologic settings without regard to whether serum or plasma was sampled or whether the subjects were fasting or nonfasting.

Additional Keyphrases: variation, source of, sample collection, lipids

Epidemiologists and clinicians frequently wish to compare blood lipids and lipoprotein concentrations within individuals and among different populations. Making such comparisons proves difficult if the methods of lipid analysis or the conditions of blood drawing vary. Two common sources of variation are whether serum or plasma is used for the lipid determinations and whether the subjects are fasting or nonfasting. Fortunately, the effects of these methodologic differences on total cholesterol and triglycerides are well documented. Total cholesterol and triglyceride values average 3% higher in serum than in plasma (1), and the difference between concentrations of total cholesterol measured during fasting vs nonfasting is negligible (2–5), whereas concentrations of triglycerides measured postprandially are markedly increased (3–5, 8–10).

No studies of serum/plasma differences in high-density lipoprotein (HDL) cholesterol have been reported, however, and the few studies of the effect of fasting vs nonfasting on concentrations of HDL cholesterol are inconclusive. For example, both a 4% decrease (6) and no change (6, 7) in HDL cholesterol have been observed postprandially. Clarification of these effects would facilitate comparisons among epidemiologic studies and other evaluations of HDL cholesterol. The purposes of our investigation were (a) to measure the difference between values for HDL cholesterol in serum and plasma and (b) to determine the difference between HDL cholesterol concentrations during fasting and nonfasting. We also sought to replicate previous findings for total cholesterol and triglycerides (1–10).

Materials and Methods

Blood was sampled on two consecutive mornings between November 30 and December 15, 1981, from 44 healthy men and women volunteers, ages 20–70 years. Twenty-nine were women; 15 were men. On one morning, the subjects had fasted for 12 h before the sampling. On the other morning, subjects had eaten an ad lib. breakfast about 2 h (range: 0.5–5 h) before the sampling. The order of the fasting/
nonfasting days was randomly assigned. Each morning, 30 mL of blood was collected from seated subjects from the antecubital vein through a 26-mm (1.0-in.) 21-gauge needle into a syringe that did not contain anticoagulant. The tourniquet was released before the sample was collected. Within 1 min of drawing, an aliquot of blood was transferred from the syringe to fill completely a 15-mL tube (for plasma), that contained 22.5 mg of disodium EDTA. The plasma tube was immediately inverted eight times to dissolve the anticoagulant and allowed to stand in an ice bath for 2 h. The remaining whole blood was transferred to a 15-mL serum-separating tube and was allowed to clot at room temperature for 2 h. The plasma and serum tubes were then centrifuged at 1500 × g for 30 min and the serum or plasma was withdrawn. Samples were used for total cholesterol, triglycerides, and HDL cholesterol by continuous-flow analysis (AutoAnalyzer II, Technicon Instruments Corp., Tarrytown, NY 10591), according to standardized Lipid Research Clinics protocol (11), at the Minnesota Lipid Research Clinic. The laboratory was kept uninformed throughout as to the identity of the matching serum/plasma, fasting/ nonfasting pairs.

To reduce analytical variation, we used the following procedures:

- Serum/plasma sample pairs were analyzed in a single analytical run in the following sequence: serum–plasma, plasma–serum, serum–plasma, etc.
- For HDL cholesterol, the precipitation technique was performed in duplicate within 4 h of blood collection and an isopropanol extract of each HDL supernate was prepared.
- For total cholesterol and triglycerides, duplicate isopropanol extracts of the unfractionated serum and plasma were prepared.
- Extracts were analyzed in duplicate (and hence, samples in quadruplicate); the mean of the second and fourth analyses of each sample was the value reported in this paper, to minimize the effect of specimen carryover inherent in continuous-flow automated analytical systems (12).

Paired t-tests (13) were used for between-group comparisons. Significance tests were two-tailed. The coefficient of variation (CV) for the analyses was computed as the standard deviation of the differences between all duplicate samples, divided by the mean value.

**Results**

Table 1 shows the mean values for total cholesterol, HDL cholesterol, and triglycerides for serum and plasma specimens drawn from subjects, both fasting and nonfasting. The laboratory (analytical) CVs—determined on split samples—for total cholesterol, HDL cholesterol, and triglycerides, respectively, were 1.6%, 1.6%, and 2.9%. Fasting plasma values of these lipids ranged from 1210 to 2200 mg/L for total cholesterol, 270 to 940 mg/L for HDL cholesterol, and 340 to 3580 mg/L for triglycerides. Fasting HDL cholesterol was significantly negatively correlated with fasting triglycerides (r = -0.36, p < 0.02), but was not correlated with total cholesterol (p > 0.1).

Table 2 shows the mean differences between serum and plasma lipid values for fasting and nonfasting subjects. The differences between serum and plasma values were highly significant for total cholesterol and triglycerides, both fasting and nonfasting. The serum cholesterol values averaged 36 mg/L (2.1%) higher, and the serum triglycerides averaged 55 mg/L (5.2%) higher than corresponding plasma values. Serum HDL cholesterol values, on the other hand, were not different (p > 0.2) from plasma values for specimens collected during either fasting or nonfasting.

Table 3 shows the mean differences between fasting and nonfasting lipid values, determined on serum and plasma pairs. As expected, triglyceride concentrations were greater during nonfasting than fasting, averaging 357 mg/L (39.7%) higher 2 h postprandially. Concentrations of total cholesterol and HDL cholesterol from nonfasting subjects, however, were not significantly different (p > 0.2) from those during fasting for either serum or plasma specimens. In addition, there was no correlation (p > 0.2) between the increases in triglycerides within individuals and individual postprandial changes in HDL cholesterol.

**Discussion**

Previous investigators have reported that triglyceride concentrations increase substantially postprandially (3–5, 8–10), whereas total cholesterol does not change appreciably after a meal (2–6), and that values of total cholesterol and triglycerides average 3% higher for serum than for plasma (1). Our study corroborates these findings. We observed a 40% increase in triglycerides but none in total cholesterol postprandially. Using methods similar to a previous study (1), we also found that total cholesterol was 2.1% greater and triglycerides were 5.2% greater in serum than plasma. These serum/plasma differences fall within the ranges of differences reported for laboratories of the Lipid Research Clinics Program (1). Surprisingly, however, they contrast

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**Table 1. Values for Mean Total Cholesterol, HDL Cholesterol, and Triglyceride for Serum/Plasma Pairs from Fasting and Nonfasting Subjects**

<table>
<thead>
<tr>
<th></th>
<th>Total cholesterol</th>
<th>HDL cholesterol</th>
<th>Triglycerides</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD), mg/L (n=44 each)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting, serum</td>
<td>1746 (289)</td>
<td>551 (139)</td>
<td>921 (609)</td>
</tr>
<tr>
<td>Fasting, plasma</td>
<td>1707 (270)</td>
<td>547 (145)</td>
<td>876 (591)</td>
</tr>
<tr>
<td>Nonfasting, serum</td>
<td>1737 (294)</td>
<td>550 (144)</td>
<td>1288 (1061)</td>
</tr>
<tr>
<td>Nonfasting, plasma</td>
<td>1703 (278)</td>
<td>552 (137)</td>
<td>1223 (1005)</td>
</tr>
</tbody>
</table>

**Table 2. Mean Difference between Serum and Plasma Values for Total Cholesterol, HDL Cholesterol, and Triglycerides from 44 Fasting and Nonfasting Subjects**

<table>
<thead>
<tr>
<th></th>
<th>Total cholesterol</th>
<th>HDL cholesterol</th>
<th>Triglycerides</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/L*</td>
<td>%*</td>
<td>mg/L*</td>
</tr>
<tr>
<td>Fasting</td>
<td>30 (9)*</td>
<td>2.3</td>
<td>4 (4)</td>
</tr>
<tr>
<td>Nonfasting</td>
<td>34 (7)*</td>
<td>2.0</td>
<td>-2 (5)</td>
</tr>
</tbody>
</table>

*Mean (and SE).

**p < 0.001 for difference between values for serum and plasma.

*p < 0.002.**

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with the serum/plasma differences of 3.9% for total cholesterol and 0.0% for triglycerides previously measured by our laboratory (Minnesota Lipid Research Clinics laboratory) in 1975 (1). The reason for this intra-laboratory difference is unclear, because the 1975 and 1981 laboratory methods were identical and were standardized by the Center for Disease Control in Atlanta.

The present investigation provides additional information on HDL cholesterol. We found that values for HDL cholesterol in serum and plasma were virtually identical; moreover, nonfasting HDL concentrations did not differ significantly from fasting concentrations.

Total cholesterol and triglyceride concentrations are lower in plasma than in serum, apparently because the anticoagulant, disodium EDTA, causes an osmotic redistribution of water between cells and plasma, a redistribution reflected by changes in hematocrit produced by the anticoagulant (14). It is unclear, however, why HDL cholesterol values were not also lower in plasma than in serum. Our sample size (n = 44) was probably adequate [the probability that we failed to observe a serum/plasma HDL cholesterol difference of 3% if one truly existed (i.e., a Type II statistical error) was only 2%]. Even if the serum/plasma HDL cholesterol difference were as low as 2%, the probability that we made a Type II error was only 20%. The absence of a serum/plasma difference in HDL cholesterol was more probably due to differences between the serum and plasma assays in the heparin-Mn²⁺ precipitation step. Burstein and Samaille's original method (15) of heparin-Mn²⁺ precipitation (with 46 mmol of Mn²⁺ per liter) quantified HDL cholesterol in serum. This same concentration of Mn²⁺ is used for assays of both serum and plasma in the Lipid Research Clinics Program. Warnick and Albers (16) have shown that for serum this concentration gives complete precipitation of apoprotein B-containing lipoproteins, but for plasma, the Mn²⁺ concentration gives incomplete precipitation, owing to chelation of Mn²⁺ by disodium EDTA. Thus, plasma HDL cholesterol may be somewhat overestimated by this method, thereby obscuring the expected serum/plasma HDL cholesterol difference.

Knowing that postprandial HDL concentrations do not differ from fasting values is useful, because having subjects fast before clinical or epidemiological studies is often convenient. In this study, we tried to simulate conditions that might occur in "fasting" and "nonfasting" lipid studies by drawing blood from subjects after a 12-h fast and after their typical breakfasts. Our findings (Table 3) suggest, as do those of two previous studies (6, 7), that it does not matter whether or not specimens are drawn for HDL cholesterol analysis during fasting. Why HDL cholesterol decreased 4% postprandially in another study (6) remains unexplained but is probably related to the very high fat content of the meal consumed then.

A negative correlation between fasting HDL cholesterol and triglycerides has been observed in this and other studies (17-19). This association probably exists because HDL apoproteins participate in the esterification and transfer of cholesterol to very-low-density lipoproteins (20, 21). HDL apoprotein synthesis and turnover, however, appears to be relatively slow (21), which may explain why we observed no correlation between the change in triglyceride just a few hours after a meal and the HDL cholesterol change within individuals. Future investigations of the effects of diet on HDL cholesterol and its metabolism may explore this relationship more fully.

Our results indicate that it makes little difference whether HDL cholesterol determinations are made on serum or plasma or whether subjects are fasting or non fasting. Serum/plasma differences, however, do exist for total cholesterol and triglycerides, and the state of fasting clearly influences triglycerides. These conditions should, therefore, be considered in the design of epidemiologic and clinical studies for measuring these lipids.

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References
Analytical, intra-individual, and inter-individual components of variation were estimated from duplicate analyses of 11 plasma analytes in an average of 13 specimens obtained, over a two-day period, from nine patients with impaired renal function. Analytical variance was 17.3% of the total variance for sodium; <5% of the total variance for potassium, chloride, bicarbonate, albumin, and calcium; and <1% for urea, creatinine, glucose, creatinine kinase (EC 2.7.3.2), and alanine aminotransferase (EC 2.6.1.2). Average intra-individual variations were of the same order as those found in healthy individuals. All analytes except glucose showed strong individuality. We postulate that, in nonacute pathological processes where new homeostatic steady states are reached, biological variations around the new set points are of the same order as those found in healthy individuals.

Additional Keyphrases: intra- and inter-individual variation - reference interval

The biological variation of certain commonly assayed analytes of serum has been investigated by several groups (1-6). As is also true for biological variation of analytes in urine (7), these have been studies of ostensibly healthy individuals. Much useful information has been generated, but the clinical chemist is usually dealing with diseased persons in hospital. It has therefore been suggested that the data of most value would be on the biological variation in specific disease states (8).

We have described the short-term biological variation in eight analytes in sera from a group of 20 patients who had uncomplicated myocardial infarction (9). The average intra-individual biological variation for these analytes was larger, but of the same order, as for healthy individuals. This was expected, because pre-analytical variance was not minimized and the particular analytes were not likely to have been disturbed by the disease process. We concluded that the extensive published data on biological variation could be validly used more often in routine laboratory practice. To assess whether these findings were unique to patients with myocardial infarction, and in an attempt—for the first time, to our knowledge—to assess the components of variation of certain plasma analytes significantly affected by a pathological process, we investigated the short-term biological variation of 11 commonly assayed analytes in nine patients with various degrees of renal failure. They were inpatients of the Repatriation General Hospital, Daw Park, South Australia 5041. Three of the patients were said to have had mild renal impairment, three moderate impairment, and three severe impairment. The subjects were adults who had given informed consent to participate in a trial to determine the bioavailability of ranitidine, an H2 antagonist. Patients were given a single 150-mg dose of ranitidine, orally.

**Procedures**

Samples were collected during 48 h via an intravenous catheter (31.8 mm, 20 gauge; Jelco Laboratories, Raritan, NJ 08869) at 0, 0.5, 1, 2, 4, 6, 8, 10, 12, 16, 24, 36, and 48 h after the administration of ranitidine. Each sample was immediately transferred to a 10-mL heparinized plastic container and promptly centrifuged. An aliquot was frozen at −20 °C, in a screw-capped 2.5-mL container. The samples were then stored at −70 °C until analysis. On the day of analysis, all samples from one patient were allowed to thaw at room temperature, mixed thoroughly, and centrifuged briefly in a Sorvall centrifuge (Du Pont Instruments, Wilmington, DE 19898) to precipitate fibrin. Two aliquots of each sample were pipetted into 0.5-mL sample cups and one aliquot into a 0.8-mL container.

Analyses for Na+, K+, Cl−, HCO3−, urea, creatinine, glucose, Ca2+, and albumin were performed in an Astra 8 discrete analyzer (Beckman Instruments, Inc., Fullerton, CA 92634). Assays of creatine kinase (CK; EC 2.7.3.2) and alanine aminotransferase (ALT; EC 2.6.1.2) activity were...