Lipoprotein Cholesterol Concentrations in the Plasma of Human Subjects asMeasured in the Fed and Fasted States

Jeffrey S. Cohn, Judith R. McNamara, and Ernst J. Schaefer

Lipoprotein cholesterol concentrations in plasma are routinely estimated by using the Friedewald formula, whereby very-low-density lipoprotein cholesterol (VLDL-C) is estimated to be one-fifth the plasma triglyceride concentration. Ordinarily, this formula is applied only to plasma sampled from patients in the fasted state. To determine whether lipoprotein cholesterol measurements are altered substantially in plasma sampled from nonfasting subjects, we obtained postprandial blood samples from 22 healthy subjects (nine men, 13 women, ages 22–79 years) fed a fat-rich meal (1 g fat per kilogram body wt.). The plasma triglyceride concentration increased postprandially in all subjects (233 ± 16% of baseline at 3 h). The mean cholesterol concentration in plasma was essentially unchanged. High-density lipoprotein cholesterol (HDL-C) was significantly decreased (94 ± 2% at 3 h, P < 0.001). VLDL-C and low-density lipoprotein cholesterol (LDL-C), estimated by the Friedewald formula, were compared with measurements obtained by modified Lipid Research Clinics (LRC) methodology. As measured by either method, VLDL-C increased and LDL-C decreased significantly after the fat-rich meal. These postprandial changes were significantly greater (P < 0.01) when estimated by the Friedewald formula than by LRC methodology. We conclude that (a) lipoprotein cholesterol concentrations measured in the fed subject differ significantly from those measured in the fasted subject, and (b) plasma must be obtained after at least a 12-h fast if an individual's risk of coronary heart disease is to be accurately assessed.

Additional Keyphrases: low-density lipoprotein cholesterol • high-density lipoprotein cholesterol • coronary heart disease risk assessment

Increased concentrations of total cholesterol [more specifically, low-density lipoprotein cholesterol (LDL-C)] and diminished concentrations of high-density lipoprotein cholesterol (HDL-C) are associated with increased risk of coronary heart disease (CHD) (1, 2).1 The Expert Panel of the National Cholesterol Education Program (3) has recently advised that all adults ages 20 years or older should have their total plasma cholesterol measured at least once every five years. It was recommended that further lipoprotein analysis (i.e., measurement of LDL-C and HDL-C) ought to be undertaken in individuals with high concentrations of plasma cholesterol (>240 mg/dL) and also in those with borderline-high concentrations of plasma cholesterol (200–239 mg/dL) who have definite CHD or at least two other CHD risk factors (i.e., male gender, family history of premature CHD, cigarette smoking, hypertension, diabetes mellitus, or obesity).

Concentrations of lipoprotein cholesterol in plasma are routinely assessed by using the Friedewald formula (4), whereby very-low-density lipoprotein cholesterol (VLDL-C) is estimated to be equal to one-fifth of the plasma triglyceride concentration; HDL-C is measured after precipitation of apolipoprotein (apo) B-containing lipoproteins, and LDL-C is calculated as total cholesterol – (VLDL-C + HDL-C). Ordinarily, this formula is applied only to plasma obtained in the fasted state, and patients are therefore advised to fast for at least 12 h before having blood drawn. However, sometimes a sample from a subject who has fasted <12 h is analyzed. To our knowledge, no data have been published showing what effect, if any, this will have on the estimation of lipoprotein cholesterol by the Friedewald formula.

We have recently described the lipoprotein changes that occur in the plasma of human subjects fed a fat-rich meal (5–7). Postprandial changes in the concentration of lipoprotein lipids in plasma were determined with a modified Lipid Research Clinics (LRC) methodology (8). This procedure requires the use of an ultracentrifuge, making it more expensive and time consuming than the method of Friedewald et al. (4), but it does provide a more nearly accurate measurement of LDL-C. VLDL is separated from plasma by ultracentrifugation for 18 h at d < 1.006. The VLDL supernate is isolated by tube slicing and cholesterol is determined in the infranate. VLDL-C is subsequently calculated as the difference between plasma cholesterol and infranate cholesterol. LDL-C is in turn calculated by subtracting HDL-C (measured after plasma precipitation) from infranate cholesterol.

In the present study, we reanalyzed data obtained from subjects fed a fat-rich meal, to assess the effect of feeding and fasting on lipoprotein cholesterol concentrations as estimated by the Friedewald formula. We compare these results with our previous data (5) obtained by LRC methodology.

Materials and Methods

Subjects

Twenty-two healthy subjects (nine men, 13 women), ages 22–79 years, were studied, as previously described (5). They were of normal height and weight and had normal fasting triglyceride and cholesterol concentrations. These subjects had no history of ulcer disease, excessive alcohol intake, or bleeding tendency, and none was taking any medication known to affect plasma lipids. All studies were carried out in the Metabolic Unit of the USDA Human Nutrition Research Center on Aging at Tufts University. The experimental protocol was approved by the Human Investigation Review Committee of the New England Medical Center and Tufts University.

Study Design

The fat-feeding experiments were done as described previously (5). After a 14-h overnight fast, subjects were given a
fat-rich meal containing 1.0 g of fat per kilogram of body wt., in the form of soybean oil or as soybean oil plus cream, and 7.0 mg of cholesterol, in the form of egg-yolk powder. The amount of fat given was designed to be 1/3–1/2 as much as that which an average American ingests per day. The meal was prepared as a milkshake with added flavoring. Glucose polymer and egg-white protein were added so that the meal contained (by weight) 33.3% fat, 33.3% protein, and 33.3% carbohydrate or (in terms of energy) 53% fat, 23.5% protein, and 23.5% carbohydrate. Blood samples (20 mL) were obtained via a small forearm indwelling catheter, before the test meal and 3, 6, 9, and 12 h thereafter. Water, but no additional food, was allowed during the study.

Lipid Analysis

Blood was collected in tubes containing EDTA (final EDTA concentration, 1 g/L). Plasma was separated by centrifugation (1700 x g, 20 min, 4 °C). Triglyceride and cholesterol concentrations were measured with an Abbott Diagnostics ABA-200 bichromatic analyzer and enzymatic reagents (9). Our cholesterol and triglyceride assays have been standardized by participation in the Centers for Disease Control–National Heart, Lung, and Blood Institute Standardization Program. HDL-C was quantified by analyzing the infranate obtained after precipitation of a plasma aliquot with dextran sulfate–Mg²⁺ as described by Warnick et al. (10).

The CVs for our cholesterol assay are routinely <2.9%; for our triglyceride assay <4.2%; and for our HDL cholesterol assay <3.7% (9).

VLDL-C and LDL-C concentrations were determined by two methods. First, by the method of Friedewald et al. (4), VLDL-C was calculated as plasma triglyceride divided by five. LDL-C was in turn calculated as total cholesterol - (VLDL-C + HDL-C). In the second method, according to LRC methodology (8), VLDL and chylomicrons in the fed state were separated from 5 mL of plasma by a single ultracentrifugal spin (140 000 x g, 18 h, 4 °C) at d = 1.006, in a 50.3 Ti rotor (Beckman Instruments, Fullerton, CA). The infranate fraction was analyzed for cholesterol, and cholesterol in the VLDL (d <1.006) fraction was measured by subtracting results for the infranate from those for plasma. LDL-C was quantified by subtracting HDL-C from infranate cholesterol.

Statistics

The data were tabulated and stored in a VAX-11/780 computer (Digital Equipment Co., Maynard, MA), with use of the scientific software package RS/1 (BBN Research Systems, Cambridge, MA). Paired t-tests were used to compare the significance of mean differences.

Results

Plasma triglyceride concentration increased in all subjects after they ingested the fat-rich meal (Table 1). The response of individual subjects was variable and maximum increase in plasma triglyceride concentration ranged from 168% to 485% of baseline (0-h fasting concentration). Plasma triglyceride concentration was significantly increased at 3 h (233 ± 16% of baseline, P <0.001), 6 h (209 ± 16%, P <0.001), and 9 h (153 ± 12%, P <0.01) (Table 1).

The mean plasma cholesterol concentration did not change postprandially. However, the mean for the group as a whole disguised the fact that plasma cholesterol increased slightly in some subjects but decreased slightly in others. As previously described (5), we could categorize the subjects into three groups: seven subjects who showed a consistent postprandial increase in plasma cholesterol concentration, five subjects with essentially no change, and 10 subjects who showed a consistent postprandial decrease in plasma cholesterol. At 3 h postprandially, the mean (± SEM) increase in the first group was 3.6% (2.0%; not significant) and the mean decrease in the last group was 6.0% (0.8%; P <0.001). At 12 h postprandially, the mean increase in the former group was 6.0% (2.1%; P <0.05) and the mean decrease in the latter group was 7.1% (1.2%; P <0.001) (5).

The HDL-C concentration decreased in 20 of the 22 subjects, and the mean HDL-C concentration was significantly decreased at 3, 6, and 9 h after the fat-rich meal (Table 1).

No significant difference was observed between the mean 0-h and 12-h concentrations of triglyceride, cholesterol, or HDL-C in plasma for the group as a whole. In the fasted state, the mean (± SEM) VLDL-C concentration, as measured by the modified LRC methodology, was 29 (3) mg/dL, and the mean LDL-C concentration was 106 (6) mg/dL. VLDL-C increased postprandially and differed significantly from the mean 0-h (fasting) concentration at 3 h (37 ± 4 mg/dL, P <0.01) and 6 h (38 ± 4 mg/dL, P <0.01). LDL-C decreased postprandially and was also significantly different at 3 h (96 ± 6 mg/dL, P <0.05) and at 6 h (100 ± 6 mg/dL, P <0.05).

As estimated by the formula of Friedewald et al. (4), the concentration of VLDL-C in plasma was significantly increased and that of LDL-C was significantly decreased at 3, 6, and 9 h after the meal (Table 2). By 12 h after the meal, both had returned to their 0-h (fasting) concentrations.

VLDL-C and LDL-C plasma concentrations in the fed and fasted state, as estimated by the method of Friedewald et al. (4), were compared with VLDL-C and LDL-C measured by LRC methodology. By both methods, VLDL-C increased and LDL-C decreased after ingestion of the fat-rich meal (Figure 1). In the fasted state (0 h), VLDL-C measurements obtained by use of the Friedewald formula were significantly less (P <0.01) and LDL-C measurements were significantly greater (P <0.01) than those obtained by using LRC methodology. Conversely, in the fed state (3 h), VLDL-C measurements obtained by using the Friedewald formula were significantly greater (P <0.01) and LDL-C measurements were significantly less (P <0.01). Postprandial changes in the plasma

<table>
<thead>
<tr>
<th>Table 1. Plasma Concentration of Triglyceride, Total Cholesterol, and HDL Cholesterol in Human Subjects Fed a Fat-Rich Meal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time after the meal, h</td>
</tr>
<tr>
<td>Triglyceride</td>
</tr>
<tr>
<td>Cholesterol</td>
</tr>
<tr>
<td>HDL cholesterol</td>
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*Means ± SEM (mg/dL) for 22 subjects. Significantly different from fasting (0-h) concentration by paired t-test: ** P <0.01; *** P <0.001.
Table 2. Plasma Concentration of VLDL and LDL Cholesterol before and after Ingestion of the Fat-Rich Meal, Estimated by the Method of Friedewald et al. (4)

<table>
<thead>
<tr>
<th>Time after meal, h</th>
<th>VLDL cholesterol (mg/dL)</th>
<th>LDL cholesterol (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>20 ± 2*</td>
<td>114 ± 6</td>
</tr>
<tr>
<td>3</td>
<td>46 ± 4***</td>
<td>95 ± 6**</td>
</tr>
<tr>
<td>6</td>
<td>43 ± 5***</td>
<td>95 ± 6**</td>
</tr>
<tr>
<td>9</td>
<td>30 ± 4**</td>
<td>105 ± 6***</td>
</tr>
<tr>
<td>12</td>
<td>19 ± 3</td>
<td>112 ± 7</td>
</tr>
</tbody>
</table>

Values represent means ± SEM (mg/dL) for 22 subjects. Significantly different from fasting (0-h) concentration by paired t-test: *P < 0.01, ***P < 0.001.

The postprandial changes in lipoprotein cholesterol were most pronounced 3 h after the fat-rich meal, but even at 6 h the concentrations in plasma differed significantly from the fasting concentrations (Table 1, Figure 1). By 9 h after the fat-rich meal, plasma triglyceride concentrations were still increased above baseline, and VLDL-C and LDL-C concentrations, as estimated by the Friedewald formula, were still significantly different from their values during fasting. These results support the concept that plasma lipoprotein cholesterol must be measured in plasma from patients who have fasted for at least 12 h if results are to reflect accurately the risk of cardiovascular disease.

The increase in VLDL-C after fat-feeding reflects the intestinal secretion of chylomicrons containing cholesterol of dietary origin (11, 12). Increased secretion of VLDL from the liver also contributes to this increase (6, 7). The decrease in LDL-C was probably caused by (a) diminished conversion of VLDL to LDL, owing to saturation of the lipolytic process (13) and increased direct uptake of VLDL remnants; (b) alteration in the cholesterol ester content of LDL, owing to postprandial changes in the activity of cholesterol ester transfer protein (14); and (c) increased receptor-mediated uptake of LDL (15).

The postprandial changes that we observed in HDL cholesterol need to be discussed in light of previous studies, in which ultracentrifugal techniques were used to study postprandial changes in HDL. Tall et al. (16), using density gradient ultracentrifugation, and Groot and Schek (17), using rate-zonal ultracentrifugation, both have shown that the total mass of HDL constituents increases after fat-feeding. The postprandial cholesterol concentration of HDL increased predominantly owing to an increase in cholesterol of larger lipoproteins in the HDL2 subfraction. Havel et al. (18) also observed a postprandial increase in the cholesterol concentration of HDL2, but not HDL3 (isolated by sequential ultracentrifugation). In contrast, Kay et al. (19), using heparin–Mg2⁺ precipitation of apoB-containing lipoproteins (VLDL and LDL), found that HDL-C decreased postprandially. A similar decrease (5–10%) was observed in the present study in which dextran–Mg2⁺ was used for precipitation. These results, taken together, suggest the possibility that HDL-C in plasma of the nonfasted subject is underestimated when determined after precipitation from the plasma. We can offer two possible explanations. It has been previously shown (8, 20) that the tendency of lipoproteins to form insoluble complexes, and hence to precipitate, depends on the size of the sulfated polysaccharide used in the precipitation reaction and on the size of the lipoproteins themselves. Thus chylomicrons precipitate more readily than smaller VLDLs or LDLs. Fat-feeding causes an increase in the size (increase in the lipid:protein ratio) of triglyceride-rich lipoproteins (21) and of HDL (16, 17), so it is possible that lipoprotein precipitation is increased and less cholesterol is thus measured in the supernate as HDL-C. A second possible explanation is that postprandial changes in lipoprotein distribution of the C apolipoproteins (18, 22) and (or) apoE (23) could cause the precipitation characteristics of the lipoproteins to be altered. In the fed state, the C apolipoproteins and apoE transfer from lipoproteins of high density to those containing apoB of lower density. Because these apolipoproteins are polar molecules, and because the precipitation reaction is affected by charge, increased precipitation possibly could occur, resulting in less cholesterol being measured in HDL. The apparent underestimation of HDL cholesterol in nonfasted plasma (containing increased amounts of triglyceride) is in stark contrast to the well-documented overestimation of HDL cholesterol in hypertriglyceridemic specimens, caused by turbidity of the supernate resulting from incomplete sedimentation of insoluble lipoproteins (20, 24).

Our results for VLDL-C in the fasting state can be
compared with those of Wilson et al. (25), who studied a larger population. In more than 6000 subjects, ranging in age from 20 to 79 years, VLDL-C (calculated as plasma triglyceride concentration divided by five) was 81% to 89% of the VLDL-C measured after ultracentrifugation (a 11% to 19% underestimate). A larger underestimation (28%) was observed in the present study, probably owing to the fact that a relatively small, selected group of subjects was studied, who had low-normal plasma triglyceride concentrations in the fasted state. LDL-C was in turn overestimated by 7.5% in our subjects, when determined by the Friedewald formula. In contrast, the data of Delong et al. (26) suggest that in the fasting state LDL-C tends to be underestimated by the Friedewald formula. The reason for this disagreement is unclear.

Of critical significance to the present study is the apparent decrease in LDL-C in the fed state. At 3 h postprandially, LDL-C (as estimated by the Friedewald formula) was decreased by 22%, and at 9 h it was still significantly lower (9%, P < 0.001) than its mean fasting value. The postprandial decrease in LDL-C was considerably greater when measured by the Friedewald formula than when measured by LRC methodology (Figure 1). Assessment of cardiovascular risk based on LDL-C (3) will therefore be underestimated if a nonfasted subject's sample is obtained for analysis, particularly if the Friedewald formula is used (as is usual clinical practice).

Previous studies have given rise to the concept that fat feeding causes no change in plasma cholesterol concentration (16, 17, 21, 22, 27) and, indeed, in the present study no mean change was observed (Table 1). Some change was observed in individual subjects, but this was relatively small, about 5% in most subjects. For initial screening purposes, it is therefore valid to measure plasma cholesterol in nonfasted plasma. However, follow-up measurements, especially in those individuals with hypercholesterolemia, need to be made on plasma from fasted subjects, as recommended by the National Cholesterol Education Program (3). The results of the present study clearly reinforce the concept necessity of analyzing plasma obtained after at least 12 h of fasting, for accurate assessment of an individual's risk for coronary heart disease.

References
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