Decreased Postprandial Response to a Fat Meal in Normotriglyceridemic Men With Hypoalphalipoproteinemia

Michael Miller, Peter O. Kwiterovich Jr., Paul S. Bachorik, and Angeliki Georgopoulos

The plasma level of high density lipoprotein cholesterol (HDL-C) has been reported to be inversely correlated with the level of triglycerides (TGs) and the magnitude of postprandial lipemia because subjects with low HDL-C accompanying high TG levels often have an increased postprandial response to a fat load. However, information is limited regarding the postprandial response to a fat load in subjects with low HDL-C and normal fasting TG values (hypoalphalipoproteinemia [hypoalpha]). We administered an oral fat load (70 g/m² of body surface area) to six subjects with hypoalpha and six aged-matched control subjects. Plasma levels of lipids and lipoproteins and the mass of triglyceride-rich lipoproteins (TRLs: S<100 and S>20-100) were measured every 2 hours for 8 hours. The mass and chemical composition of S>100 but not S>20-100 TRLs was significantly lower in subjects with hypoalpha (200.4 ±64.8 mg/dL versus 110.6±50.9 mg/dL; analysis of variance, F test, p=0.04). In the hypoalpha subjects, the compositions of postprandial S>100 TRLs were TG poor and cholesterol and phospholipid enriched (p<0.001) while the S>20-100 TRLs were enriched in cholesterol and phospholipid but relatively protein depleted (p=0.002). When the hypoalpha subjects received gemfibrozil, the masses of both fasting (p=0.014) and postprandial (p=0.0004) S>20-100 TRLs were lower compared with baseline, a response that was accompanied by partial normalization of composition in postprandial hypoalpha S>20-100 TRLs. Our results indicate that in contrast to subjects with low plasma HDL-C and high TG levels, hypoalpha subjects with normal TG values did not display an increased postprandial response to fat feeding. Rather, the phenotype of hypoalpha appears to have a distinct metabolic component characterized by a reduced number of postprandial S>100 TRLs of abnormal composition. Gemfibrozil reduced the mass and altered the composition of S>20-100 TRLs in hypoalpha subjects. (Arteriosclerosis and Thrombosis 1993;13:385-392)

KEY WORDS • plasma triglycerides • plasma HDL cholesterol • postprandial response

The metabolic consequence of an oral fat load is the intestinal reesterification of long-chain free fatty acids to form triglycerides (TGs) that are packaged as chylomicrons and secreted into the lymph. These triglyceride-rich lipoproteins (TRLs) contain enterocyte-derived apolipoprotein (apo) A-I, apo A-IV, and apo B-48. Within minutes of entry into the venous circulation, surface phospholipids and apolipoproteins exchange between the chylomicrons and high density lipoproteins (HDLs), with chylomicrons receiving apo C-II, a cofactor for lipoprotein lipase (LPL), and apo E, the ligand for the chylomicron remnant receptor. As LPL and apo C-II hydrolyze TG, chylomicrons are converted into smaller remnant particles depleted in TG and relatively enriched in cholesteryl esters. The chylomicron remnants are rapidly taken up in the liver by the chylomicron remnant receptor. The catabolism of principally hepatically derived TG-rich particles (i.e., very low density lipoproteins [VLDLs]) is similar to that of chylomicrons. However, after hydrolysis of VLDL TG, there are two potential fates of the VLDL remnant particles: they may either be taken up directly by hepatocytes by the interaction of apo E with the low density lipoprotein (LDL) B.E receptor or be converted at the surface of the liver by hepatic triglyceride lipase into LDL.
glycemia and familial dysbeta lipoproteinemia). Little information, however, is available concerning the postprandial fat response in subjects with hypoalphalipoproteinemia (hypoalpha), defined as desirable total cholesterol (<200 mg/dL) and fasting TG (<150 mg/dL) levels but with HDL-C values in the lowest quintile. Previous studies have indicated that HDL-C is inversely related to postprandial lipemia and that this lipoprotein may be an important determinant of fat tolerance. We hypothesized that if HDL-C is a major determinant of fat tolerance, then subjects with hypoalpha might display delayed fat clearance in a manner similar to that described for patients with endogenous hypertriglyceridemia. We report here detailed studies of the postprandial response of hypoalpha subjects to a fat load and the effects of gemfibrozil on such a response.

Methods

Study Subjects

Twelve men were studied. They were six age-matched control subjects and six subjects with normal TG and low HDL-C levels (fasting TG <150 mg/dL and HDL-C <40 mg/dL) whose total cholesterol level was <200 mg/dL. These individuals were representative of a controlled study evaluating the efficacy of the HDL-C-raiseing medication gemfibrozil. In this latter study, 14 subjects with hypoalpha were randomized to receive 3 months of placebo and 3 months of medication with a 1-month washout period and were asked to follow a National Cholesterol Education Program Step 1 diet. The study used a crossover design in which the order of administration of the drug and placebo was reversed: half of the subjects received placebo first, and half received the active medication first. After they enrolled in the study, 12 of the subjects (all of whom resided in the greater Baltimore area) were asked to participate in a postprandial study, and six agreed. An additional six normal control subjects (who were not treated with gemfibrozil) were also recruited for the postprandial study. All of the participants had fasting measurements of lipids and lipoproteins; the subjects with low HDL-C values had these measurements performed at the completion of each treatment phase. The average age (32 years) and body mass index (range, 19–25 kg/m²) were not significantly different between the groups. Baseline lipid and lipoprotein concentrations of hypoalpha and normal control subjects are depicted in Table 1. The only parameters differentiating the groups were the fasting levels of HDL-C, HDL-C subfractions, and apo A-I (p <0.001).

Study Protocol

Informed consent was obtained from the study subjects, and the study was approved by The Johns Hopkins Institutional Committee on Clinical Investigation. After a 12-hour fast (0 time), blood was drawn for measurements of lipids and lipoproteins. Subsequently, subjects were given a fat-rich meal consisting of 70 g corn oil per square meter of body surface area that was mixed with skim milk and Sweet and Low (Cumberland Packing Corp., Brooklyn, N.Y.) and flavored with vanilla or strawberry. The subjects were asked to drink this "milk shake" over a 10–15-minute period, after which time they were instructed to remain sedentary throughout the day and not to consume any food products; water was permitted ad libitum. Every 2 hours (for a total of 8 hours), blood was sampled by repeated phlebotomy and collected in tubes containing EDTA (1.5 mg/mL blood), mixed thoroughly, and immediately placed in wet ice. Blood was centrifuged at 2,000 rpm for 30 minutes at 4°C and the plasma removed for measurement of TRL and other lipids. Subjects tolerated the milk shake without difficulty, and there were no complications.

Lipid and Lipoprotein Analyses

For each time point, plasma was assessed for lipids, lipoproteins, and apolipoproteins. Enzymatic measurement of cholesterol and TGs was performed on a Hitachi 704 clinical chemistry analyzer (Boehringer Mannheim Diagnostics, Indianapolis, Ind.) using reagents supplied by the manufacturer. HDL-C was determined after precipitation of apo B–containing lipoproteins. Subfractions of HDL-C (i.e., HDL r C and HDL d C) were determined by ultracentrifugation of 5 mL plasma, after precipitation of apo B–containing lipoproteins as previously described. HDL-C subfraction was performed in 11 subjects; one hypoalpha patient did not have enough plasma to perform this test. LDL was estimated from the relation

\[ [\text{LDL-C}] = [\text{TC}] - [\text{HDL-C}] - ([\text{TG}] / 5) \]

Apo A-I and apo B were measured by radial immunodiffusion (apo A-I, Tago, Burlingame, Calif.; apo B, Behring Diagnostics, Somerville, N.J.). These measurements were performed at The Johns Hopkins Lipoprotein Analytical Laboratory, which is standardized for lipid and lipoprotein measurements according to the criteria of the Centers for Disease Control–National Heart, Lung, and Blood Institute Lipid and Lipoprotein Standardization Programs. Postprandial TRL subfractions (S f >100 and S f 20–100) were isolated by salt-density gradient ultracentrifugation, as previously described. They were S f >100 (chylomicrons, chylomicron remnants, and large VLDL) and S f 20–100 (small VLDL). For each subfraction, analyses of protein, phospholipid, TG, and total

<table>
<thead>
<tr>
<th>Study Subjects</th>
<th>Low HDL-C and Low TG</th>
<th>HDL-C and Normal TG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>143 (12)</td>
<td>139 (10)</td>
</tr>
<tr>
<td>Hypoalpha</td>
<td>158 (13)</td>
<td>68 (14)</td>
</tr>
<tr>
<td>HDL-C</td>
<td>50 (9)</td>
<td>69 (11)</td>
</tr>
<tr>
<td>HDL-C subfraction 1</td>
<td>17 (8)</td>
<td>30 (3)</td>
</tr>
<tr>
<td>HDL-C subfraction 2</td>
<td>8 (6)</td>
<td>8 (2)</td>
</tr>
<tr>
<td>LDL-C</td>
<td>3 (4)</td>
<td>2 (2)</td>
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<tr>
<td>Apo B</td>
<td>78 (31)</td>
<td>94 (12)</td>
</tr>
<tr>
<td>Apo A-I</td>
<td>102 (21)</td>
<td>106 (17)</td>
</tr>
</tbody>
</table>

HDL, high density lipoprotein; TC, total cholesterol; TG, triglyceride; LDL, low density lipoprotein; apo, apolipoprotein; NL, normal control; H, hypoalphalipoproteinemia; p, placebo; d, drug. Values are mean in milligrams per deciliter and (SD).

TABLE 1. Lipid and Lipoprotein Levels in Normal Control and Normotriglyceridemic/Low HDL Subjects on Placebo and Drug
cholesterol were performed, and at each time point the total lipoprotein mass was calculated in milligrams per deciliter from the sum of the measurements of TG, protein, total cholesterol, and phospholipids.

Statistical Analysis

TRL measurements. We measured the plasma TRL response to a fat meal by analyzing subfractions Sf > 100 and Sf 20–100 with a repeated-measures analysis of variance (ANOVA). The lipoprotein masses of 1) the placebo phase in the hypoalpha patients and normal control subjects and 2) the placebo phase with drug phase in the hypoalpha patients were compared. All reported significant probability levels refer to the F test. Because we measured only total cholesterol, the mass of fatty acids that was esterified with cholesterol was not used in the calculation of lipoprotein mass.

The mean differences in lipid and lipoprotein measurements between the groups were assessed using Student's t test. Significance was expressed as p<0.05.

Compositional analysis. Statistical significance of the group differences in lipoprotein mass composition between the fasting (hour 0) and postprandial (hours 2, 4, 6, and 8) state was assessed by log-ratio analysis. In both subfractions Sf > 100 and Sf 20–100, compositional pairs (total cholesterol and phospholipid, total cholesterol and protein, total cholesterol and TG, TG and protein, and phospholipid and protein) were analyzed as previously described. The CODA statistical package was employed for these analyses.

Results

Table 1 lists the mean fasting plasma lipid and lipoprotein levels in the control and normotriglyceridemic/low HDL-C subjects. As expected, HDL-C and apo A-I values were significantly lower in subjects with reduced HDL-C. When these six subjects were receiving gemfibrozil, TG levels were lower (p=0.08), but there were no increases in HDL-C or apo A-I; this was most likely due to the small number of study subjects because significant HDL-C elevations were noted in the main study. Table 2 includes the mean±SD of lipid profiles and apolipoprotein levels of the hypoalpha subjects based on three separate fasting measurements. None of the subjects were taking medications. One half of the study subjects had a strong family history of premature coronary disease (myocardial infarction in father <50 years old).

Figure 1 compares the postprandial change in plasma TG level after the fat meal in control and hypoalpha subjects while receiving placebo. Mean fasting TG levels were similar in both groups, but the control subjects exhibited a higher postprandial TG peak and significantly greater changes from baseline (fasting) at 4 and 8 hours. Administration of gemfibrozil to the hypoalpha subjects did not significantly alter the postprandial plasma TG response (data not shown).

Table 2. Lipid and Apolipoprotein Levels in Hypoalphalipoproteinemic Subjects From Three Fasting Samples

<table>
<thead>
<tr>
<th>Subject</th>
<th>TC</th>
<th>TG</th>
<th>HDL</th>
<th>Apo B</th>
<th>Apo A-I</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>140 (11)</td>
<td>61 (13)</td>
<td>27 (1)</td>
<td>103 (10)</td>
<td>99 (5)</td>
</tr>
<tr>
<td>2</td>
<td>134 (3)</td>
<td>110 (19)</td>
<td>31 (4)</td>
<td>95 (6)</td>
<td>118 (12)</td>
</tr>
<tr>
<td>3</td>
<td>139 (11)</td>
<td>101 (11)</td>
<td>27 (5)</td>
<td>115 (7)</td>
<td>121 (8)</td>
</tr>
<tr>
<td>4</td>
<td>137 (14)</td>
<td>105 (22)</td>
<td>28 (3)</td>
<td>107 (6)</td>
<td>108 (11)</td>
</tr>
<tr>
<td>5</td>
<td>156 (7)</td>
<td>65 (6)</td>
<td>33 (7)</td>
<td>109 (5)</td>
<td>124 (14)</td>
</tr>
<tr>
<td>6</td>
<td>157 (11)</td>
<td>60 (3)</td>
<td>35 (3)</td>
<td>115 (7)</td>
<td>120 (7)</td>
</tr>
</tbody>
</table>

TC, total cholesterol; TG, triglyceride; HDL, high density lipoprotein; apo, apolipoprotein. Values are mean in milligrams per deciliter and (SD).

None of the subjects were taking medications. One half of the study subjects had a strong family history of premature coronary disease (myocardial infarction in father <50 years old).

Figure 1. Line plot of change in postprandial plasma triglyceride (TG) levels in milligrams per deciliter. Increases over baseline (0 time) in control and hypoalphalipoproteinemia (hypoalpha) subjects are shown. Significantly different from baseline at *p=0.02, **p=0.03.
FIGURE 2. Line plots of total lipoprotein mass of $S_f >100$ and $S_f 20-100$ triglyceride-rich lipoproteins (TRLs) in control and hypoalphalipoproteinemia (hypoalpha) subjects during placebo and gemfibrozil treatment phases. Total mass was calculated from values at 2, 4, 6, and 8 hours. Significance was determined by calculating the mean total lipoprotein mass (in milligrams per deciliter) within each group analyzed by analysis of variance. Significant differences were found between 1) postprandial $S_f >100$ TRLs in control and hypoalpha subjects during placebo (p=0.04) and gemfibrozil (p<0.02) administration; 2) fasting $S_f 20-100$ TRLs in hypoalpha subjects (placebo versus gemfibrozil) (p=0.014); and 3) postprandial $S_f 20-100$ TRLs in hypoalpha subjects (placebo versus gemfibrozil) (p=0.0004).

Hypoalpha patients were relatively enriched in cholesterol and phospholipid, whereas $S_f 20-100$ TRLs from hypoalpha patients were depleted in protein compared with control subjects (p<0.001 and p=0.002, respectively). Similar compositional changes were seen in the fasting state but did not reach statistical significance. There were no differences in the composition of fasting or postprandial $S_f >100$ TRLs in hypoalpha subjects after administration of gemfibrozil, but the drug affected the composition of postprandial $S_f 20-100$ TRLs (p=0.017) (Figure 5). Gemfibrozil appeared to correct some of the abnormalities (i.e., the protein depletion and cholesterol enrichment) but did not affect phospholipid enrichment. As a result, the postprandial $S_f 20-100$ TRLs were still significantly different from control particles after gemfibrozil treatment (p=0.001). Similar changes were observed in the fasting state but did not attain statistical significance.

**Discussion**

The most important finding of this study was that hypoalpha subjects exhibited neither higher postprandial TG (or TRL) peaks nor delayed postprandial response to a fat load (Figures 1 and 2) compared with age-matched control subjects, in contrast to the response characteristic of endogenous hypertriglyceridemia, a condition that is also associated with reduced HDL-C levels.23,25 These results suggest that a low HDL-C level is not necessarily a marker of abnormal fat clearance, as previously suggested,25 if accompanied by a normal fasting TG value.

Kinetic studies have demonstrated that hypoalpha is a heterogenous disorder consisting of both enhanced catabolism and reduced synthesis of apo A-I.24,25 While the mechanisms accounting for the observations in the present study are not known, the reduced postprandial TRL value observed may have been secondary to reduced intestinal TRL production that resulted from diminished apo A-I synthesis. Moreover, subjects with apo A-I deficiency exhibit fasting TG levels within the low-normal range,26-28 and one such subject displayed low TG peaks and had TRLs of abnormal composition (similar to our subjects) after a postprandial fat challenge.29 Reduced apo A-I synthesis may, therefore, affect intestinal TRL production because this apolipoprotein appears to be an important mediator of TRL formation.30 Consistent with this hypothesis is that hypoalpha subjects in this study followed a low saturated-fat diet, which has been reported to reduce apo A-I production in normal subjects,31 although to our knowledge, no studies to date have specifically focused on apo A-I production in hypoalpha subjects on a low-fat diet.

One additional explanation for the present observation is reduced intestinal absorption of fat resulting in
**Figure 3.** Pie charts showing mean percentage composition of lipoprotein subfractions in fasting and postprandial $S_f >100$ triglyceride-rich lipoproteins (TRLs) from normal control and hypoalphalipoproteinemic (low high density lipoprotein cholesterol [HDL-C]) subjects. There were significant compositional differences between normal and low HDL-C postprandial TRLs ($p<0.001$). PL, phospholipid; chol, cholesterol; TG, triglyceride.

**Figure 4.** Pie charts showing mean percentage composition of fasting and postprandial lipoprotein subfractions ($S_f 20–100$ triglyceride-rich lipoproteins [TRLs]) in normal control and hypoalphalipoproteinemic (low high density lipoprotein cholesterol [HDL-C]) subjects. There were significant compositional differences between normal and low HDL-C postprandial TRLs ($p=0.002$). PL, phospholipid; chol, cholesterol; TG, triglyceride.
Associated with a reduced mass of postprandial $S_t > 100$ TRLs were differences in hypoalpha particle composition as well (Figures 3 and 4). While the differences in particle composition between fasting control and hypoalpha $S_t > 100$ TRLs did not reach significance, the particles of the subjects with reduced HDL-C levels were enriched in phospholipid and cholesterol. As expected after a fat meal, the proportion of TG was increased in $S_t > 100$ TRLs, although this was observed to a greater extent in the control subjects. The increase in TG was coupled to a relative decrease in protein and phospholipid composition in the control $S_t > 100$ TRLs, an effect that was far less pronounced in postprandial $S_t > 100$ TRLs of hypoalpha subjects. In the hepatically derived ($S_t 20-100$) TRLs, fasting particles from subjects with reduced HDL-C levels were protein depleted and cholesterol enriched but did not differ significantly compared with control particles ($p=0.10$). The postprandial response did not change the composition of heptatically synthesized particles in either group, confirming similar findings in normolipidemic individuals.33

Gemfibrozil, a fibrin acid derivative, is a hypolipidemic agent with TG-lowering and HDL-C-raising effects.34 Despite the lack of significant differences in the TG and HDL-C levels within the hypoalpha subjects during drug and placebo phases (which may in part have reflected the small number of study subjects), partial reversal of the baseline $S_t 20–100$ TRL response was observed in hypoalpha subjects during gemfibrozil administration as the particles became protein enriched, approximating the composition of TRLs found in the normal control subjects (Figure 5). No significant differences in $S_t > 100$ TRL mass were noted (Figure 2) during the drug phase, which is not surprising, in view of the low postprandial peak exhibited by hypoalpha subjects in this study. Had higher peaks of TG and/or a delayed postprandial response been evident in this group, then it is possible that gemfibrozil may have had such an effect, as was recently noted when another fibrin acid derivative, fenofibrate, decreased postprandial lipemia in a group of fasting subjects with TG >150 mg/dL and low HDL-C levels.35

In addition to reduced apo A-I synthesis, other possibilities for the lack of delayed postprandial TRL mass in hypoalpha subjects include enhanced cholesterol ester transfer protein (CETP) or hepatic lipase or LPL activity. Elevated CETP activity may reduce HDL-C by accelerating transfer of cholesteryl esters from HDL-C to LDL-C and VLDL-C in exchange for TG and phospholipid.36 Although free cholesterol mass measurements were not performed, if elevated CETP activity were the principal etiology in the low HDL-C group, relatively phospholipid- and TG-depleted $S_t 20–
100 TRLs should have resulted. However, in the hypoalpha group, both S₁ > 100 and S₁ 20–100 TRLs were phospholipid enriched. Elevated hepatic lipase activity would be expected to reduce HDL₃-C by conversion to HDL₂-C. 37 However, in five hypoalpha subjects for whom HDL-C subfractions were measured, no differences were found in postprandial HDL₃-C or HDL₂-C values compared with baseline. In fact, we found greater decreases in HDL₃-C in control subjects than in the low HDL-C group after a fat meal (data not shown). Elevations in LPL activity might also account for the differences in postprandial TRL levels between the groups.

However, in a recent study by Brinton et al., 24 no differences were observed in fasting LPL or hepatic lipase in the low HDL-C/normal TG group compared with normal control subjects. LPL levels were significantly lower, however, in the low HDL-C/low TG group compared with the normal TG groups. 24 Therefore, one would not anticipate high LPL activity in hypoalpha subjects. Our results do not rule out the possibility that our normo- triglyceridemic/low HDL-C subjects had enhanced CETP or LPL or hepatic lipase activity because these levels were not directly measured. However, the compositional data and theoretical considerations make these possibilities unlikely.

In summary, while many subjects with endogenous hypertriglyceridemia and low HDL-C manifest delayed clearance of TRLs because of abnormalities in chylomicron and/or chylomicron remnant metabolism, hypoalpha appears to be a distinct phenotype with a reduced rather than an increased number of postprandial TRLs of abnormal composition. In patients with low HDL-C and high TG, increased numbers of chylomicron remnant particles after a fat-enriched meal may be avidly internalized by scavenger cells, with the subsequent conversion to foam cells and accelerated atherogenesis. 2,3 Although an association between hypoalpha and atherosclerotic heart disease also exists, 30,40 not all subjects with isolated low HDL-C appear to be equally at risk. 41 The mechanism(s) of enhanced atherogenesis in hypoalpha subjects is unknown but may be similar to that operative in apo A-I deficiency.

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References

23. Patsch JR, Prasad S, Goto AM, Patsch W: High density lipoprotein concentration of plasma levels of this lipoprotein species to its composition, to the magnitude of postprandial lipemia, and to the activities of lipoprotein lipase and hepatic lipase. J Clin Invest 1987;80:341–347
28. Matsuno T, Hisa Y, Yanagi H, Aida T, Hamauchi H: Apolipoprotein A-I deficiency due to a codon 84 nonsense mutation of the


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