Effect of Cholesterol Feeding on Lipolytic Activities in High- and Low-Responding Rhesus Monkeys


We compared the activities of lipoprotein lipase (LPL) and hepatic lipase (HL) in postheparin plasma in groups of high- and low-responding rhesus monkeys fed a low cholesterol diet followed by a high cholesterol diet. Cholesterol feeding resulted in a two-phase response in lipolytic activities: an initial phase lasting about 21 days that was similar in the two groups, followed by the second phase in which major differences became apparent between the groups. In the initial phase, LPL and HL activities increased along with plasma cholesterol and high density lipoprotein (HDL) cholesterol, but there was no change in plasma triglycerides or apolipoprotein (apo) A-I levels. These changes suggest that both high and low responders respond in a similar manner to metabolize an increased lipid load and that both groups continue to remove triglyceride efficiently while cholesterol accumulation begins. The second phase (between 21 and 42 days and thereafter) in high responders was characterized by a decrease in LPL and HL activities along with plasma HDL cholesterol and apo A-I levels, continued increase in plasma cholesterol, and a slow increase in plasma triglycerides. In low responders, LPL activity was maintained at a high level, HL activity decreased to the basal level, and plasma lipids were unchanged. All changes occurred simultaneously, suggesting metabolic relationships between plasma lipolytic activities, plasma lipids, and apolipoprotein.


The plasma cholesterol response to dietary cholesterol varies widely among various species of animals, including humans, and among individuals within the same species. Some animals (high responders) respond with a great increase in plasma cholesterol concentration (from a basal level of about 120 mg/dl to an average of about 750 mg/dl) when fed a high cholesterol diet, whereas others (low responders) respond with only a small increase (from the basal level of about 120 mg/dl to an average of about 250 mg/dl) when fed an identical high cholesterol diet. Over the years, we have studied various parameters of cholesterol metabolism in groups of high-responding and low-responding rhesus monkeys to define the mechanism(s) responsible for this differential response. We have reported that the high responders absorb significantly higher amounts of cholesterol than do the low responders.

In a previous study on lipoprotein profiles in the high and the low responders, we observed that cholesterol feeding resulted in a decrease in the high density lipoprotein (HDL) cholesterol concentration in the high responders but resulted in an increase in the low responders. We also reported that the level of plasma total apolipoprotein A-I (apo A-I), the major apolipoprotein of HDL, decreased in the high responders, whereas in the low responders, no change occurred. On separating the HDL fraction into HDL$_1$ (d = 1.063 to 1.125 g/ml) and HDL$_2$ (d = 1.125 to 1.21 g/ml) by ultracentrifugation, we found essentially no change in the apo A-I in HDL$_2$ in either high- or low-responding monkeys, but apo A-I in HDL$_2$ decreased significantly in the high responders when they were fed cholesterol.

HDL$_2$ and HDL$_3$ are interconvertible. The lipolytic enzymes, lipoprotein lipase (LPL) and hepatic lipase (HL), are intimately involved in this interconversion. LPL acting upon very low density lipoproteins (VLDL) removes triglycerides, thereby converting them to intermediate density lipoproteins (IDL). The excess surface components, apo C's, phospholipids, and cholesterol are transferred to HDL$_3$, thereby converting them to HDL$_3$-like particles. The mechanism of conversion of HDL$_2$ to HDL$_3$, however, is not clearly understood; it has been suggested that HL plays an important role in this conversion. In view of the roles played by LPL and HL in HDL metabolism, we compared the activities of both LPL and HL in postheparin plasma and also measured plasma cholesterol, triglycerides, plasma HDL cholesterol, and plasma apo A-I concentrations in a longitudinal study in groups of high- and
low-responding monkeys who were first fed a low cholesterol diet and then a high cholesterol diet.

**Methods**

**Animals**

The four high- and four low-responding rhesus monkeys (Macaca mulatta) used in the study were adult males, 10.5 to 14 years of age and weighing between 8 and 12 kg. They were identified as high or low responders from a group of 60 young adult monkeys fed a high cholesterol diet as previously described. These animals were taken off the high cholesterol diet and fed a low cholesterol diet of similar nutrient composition (see below). At the time of this study, these animals had been fed the low cholesterol diet for nearly 4 years.

**Diets**

The high and low cholesterol diets provided fat at 38% and protein at 15% of calories and cholesterol at levels of 0.35 or 0.02 mg/kcal, respectively. The two diets were identical except for the addition of dried egg yolk powder and crystalline cholesterol to the basic low cholesterol diet. The compositions of the diets have been reported. The animals were fed once daily an amount of diet (110 to 180 g) sufficient to maintain body weight.

**Experimental Protocol**

The animals were fasted overnight for 14 to 16 hours, and all experimental procedures were done at about the same time (9:00 A.M.) on all occasions. The animals were anesthetized with Ketaset (Bristol Laboratories, Syracuse, NY), and a blood sample to be used as a control was obtained in a heparinized Vacutainer tube (Becton Dickinson Labware, Lincoln Park, NJ). Immediately afterward, heparin (The Upjohn Co., Kalamazoo, MI), 100 units/kg body weight, was injected intravenously into a leg vein. A blood sample was obtained 15 minutes after the intravenous heparin injection because during the standardization of the methods for LPL and HL, we noted that maximum activities for both enzymes occurred at 15 minutes after the intravenous injection of heparin. All blood samples were put on ice, and the plasma was separated by centrifugation at 2000 rpm at 4°C and was stored at −20°C. During the final 3-month period in which the monkeys were fed the low cholesterol diet, the plasma concentrations of cholesterol, triglycerides, HDL cholesterol, apo A-I, and the lipolytic activities in the 15-minute postheparin plasma were measured four times. The animals were then fed the high cholesterol (0.35 mg/kcal) diet, and the plasma lipids and lipolytic activities were measured at intervals described in the Results.

**Analytical Methods**

Triolein (Sigma grade), gum arabic, and fatty acid free bovine serum albumin (BSA) (Sigma Chemical Company, St. Louis, MO), lecithin (Supelco Incorporated, Bellefonte, PA), glycerol, reagent grade (Mallickdrot, St. Louis, MO) were used in all assays. Tri-1-14C-oleyl glycerol and 14C-oleic acid (New England Nuclear, Boston, MA) were purified before use on silica gel H plates developed in the solvent system, hexane/diethyl ether/acetic acid, 80:20:1 (vol/vol/vol). All reagents used in the assay were of reagent grade.

Stock solutions of triolein (10 mg/ml) and lecithin (5 mg/ml) were prepared in chloroform and stored at −20°C. Purified radioactive trioleoyl glycerol and oleic acid were dissolved in toluene and stored at −20°C. Tris buffers were prepared in 0.15 M NaCl and adjusted to pH 8.6 with 0.3 N HCl. BSA (5%) solution was prepared in 0.62 M Tris-HCl (pH 8.6) made up in 0.15 M NaCl and stored at −20°C in aliquots of 5 ml.

**Determination of Lipoprotein Lipase Activity**

The LPL activity was measured by the method described by Corey and Zilversmit. One day before the assay was done, the glycerol-based emulsion (enough for 50 tubes) was prepared as follows: About 15 μCi of 14C-trioleoyl glycerol in toluene was evaporated to dryness under nitrogen and was redissolved in chloroform in a 10-ml screw-cap vial. To this, appropriate volumes of trioleoyl glycerol (10 mg/ml solution in chloroform) to provide 41.4 mg and lecithin (5 mg/ml solution in chloroform) to provide 2 mg were added. The mixture was evaporated to dryness under nitrogen. Then, 3 ml of glycerol was added, and the mixture was homogenized with a Polytron PT-10 ST homogenizer (Brinkman Instruments Inc., Westbury, NY) for four 1-minute intervals at setting 4. This emulsion was kept at room temperature and used the next day.

To the above emulsion, 3 ml of 5% BSA solution and 1.5 ml of heated monkey plasma (providing the activating peptides for LPL) were added, and the mixture was gently and thoroughly mixed by shaking. This was the working radioactive emulsion. During the standardization of the method, we found that 250 μl of the heated plasma (citrated plasma from an animal fasted 14 to 16 hours was heated at 56°C for 60 minutes, was centrifuged, and was stored at −70°C in 2-ml batches) from either a high-responding or a low-responding monkey gave the maximal activation of the enzyme.

An aliquot of 0.1 ml of the working radioactive emulsion per 0.250 ml total assay volume gave a final concentration of 0.1 M Tris, 2.5 mM trioleoyl glycerol, 0.8% BSA exclusive of added plasma, 8% plasma, and 16% glycerol.

For the assay of enzyme activity, 0.1 ml of 0.15 M NaCl was added to 0.05 ml of the postheparin plasma in a glass tube 16×100 mm and was preincubated at 30°C for 15 minutes. The reaction was started by the addition of 0.1 ml of the radioactive working emulsion, and the incubation was continued at 30°C for 60 minutes. The reaction was stopped by the addition of 3.25 ml of solvent mixture (methanol/chloroform/heptane, 1.45:1:25:1; vol/vol/vol). This was followed by the addition of 1.05 ml of 0.1 M potassium carbonate-potassium borate buffer (pH 10.5). The tubes were vortexed for 30 seconds and centrifuged at 3000 rpm for 15 minutes. The radioactivity in 1-ml aliquot of the upper phase was counted by using liquid scintillation cocktail (Ready-solv HP, Beckman Instruments Co., Palo Alto, CA) in a liquid scintillation spectrometer (LS 7000, Beckman Instruments Co.).

Blank assays were run simultaneously with preheparin plasma samples from each animal to correct for sponta-
neous hydrolysis. Total radioactivity in 0.1 ml of the radioactive working emulsion was also determined. The efficiency of extraction of free fatty acids was monitored by using 14C-oleic acid carried through the entire procedure.

The percent inhibition of LPL activity by 1 M NaCl was determined on three different occasions (once while the animals were fed the basal low cholesterol diet and twice when the animals were fed the high cholesterol diet) during the entire study.

Determination of Hepatic Lipase Activity

The HL activity was determined by the method described by Kinnunen and Thuren.17 The radioactive substrate (enough for 50 tubes) used for the measurement of the HL activity was prepared on the day of the assay as follows: About 3.5 μCi of 14C-trioleoyl glycerol was purified by thin layer chromatography as described above and was stored in toluene in a 50-ml screw-cap vial. The mixture was evaporated to dryness under a gentle stream of nitrogen. The dried lipids were washed three times with 0.5 ml heptane and were evaporated to dryness under nitrogen.

To the dried lipids, 10 ml of gum arabic solution (5% in 0.2 mol/liter Tris buffer [pH 8.6] stored at −20°C in 11-ml batches) was added, and the mixture was placed in an ice-bath and sonicated (Branson Sonifier-Cell Disruptor, Branson Instruments Co., Danbury, CT) at setting 4 for 4 minutes. To this sonicated mixture, 6.25 ml of BSA solution (10% in 1 mol/liter Tris, pH 8.6 stored at −20°C in 6.5-ml aliquots), 6.25 ml of NaCl/Tris solution (NaCl, 4 mol/liter and Tris, 0.4 mol/liter, adjusted to pH 8.6 with HCl), and 2.5 ml of water were added; the mixture was vortexed thoroughly.

An aliquot of 0.49 ml of the radioactive working emulsion per 0.500 ml assay volume gave a final concentration of trioleoyl glycerol (3.16×10^-3 mol/liter), Tris buffer (pH 8.6, 0.24 mol/liter), NaCl (1.0 mol/liter), albumin (25 g/liter), and gum arabic (20 g/liter).

For the assay of enzyme activity, 0.01 ml of the postheparin plasma was added to 0.49 ml of the radioactive working substrate in a glass test tube (16×100 mm); this was vortexed and incubated in a shaking water bath at 28°C for 1 hour. The reaction was stopped by the addition of 3.25 ml of methanol/chloroform/heptane (1.41:1.25:1, vol/vol/vol) followed by the addition of 0.75 ml of potassium carbonate/boric acid buffer (0.14 mol/liter, pH 10.5, adjusted with KOH, 2.0 mol/liter). The tubes were vortexed and centrifuged at 3000 rpm for 15 minutes. The radioactivity in 1 ml of the supernatant obtained after precipitating VLDL and low density lipoprotein (LDL) with heparin/MnCl₂ (BioRad, EBS Division, Anaheim, CA) was measured using the Technicon Auto Analyzer II (Technicon Instruments Corp., Tarrytown, NY).18 Plasma HDL cholesterol was determined enzymatically (cholesterol reagent kit, Ciba-Cominng, Gilford Systems, Oberlin, OH) in the supernatant obtained after precipitating VLDL and low density lipoprotein (LDL) with heparin/MnCl₂ (BioRad, EBS Division, Anaheim, CA). Plasma total apo A-I was measured by the method of Laurell19 as modified by Roheim and Vega.20

Statistical Analysis

Standard descriptive statistics, t test of difference between means, t test of paired observations, and analysis of variance for repeated measurements to compare between groups were performed. All measured variables were analyzed for variation with time by linear and quadratic regression with or without logarithmic transformations. The analyses indicated that the quadratic regression without logarithmic transformations best described the time trend for all variables. Inspection of the data suggested that with cholesterol feeding, plasma HDL cholesterol, LPL, and HL activities increased for about 21 days and then decreased; quadratic regression analyses for all variables were, therefore, carried out separately for the time periods between 0 to 21 and 42 to 518 days of cholesterol feeding. Stepwise multiple regression analyses were also carried out with LPL and HL activities as the independent variables and plasma cholesterol, triglycerides, HDL cholesterol, and apo A-I as the dependent variables. All analyses were done using the SAS statistical package (SAS Institute, Cary, NC).

Results

The coefficients of variation (CV) for the determination of LPL and HL activities measured in quadruplicate six different times during the entire study (i.e., the intra-assay variations), were 2.7%±0.6 (SD) and 3.6%±1.7 (SD) respectively. The variations were statistically insignificant (p>0.05). The specificity of LPL assay was checked by determining the percent inhibition of the enzyme activity by preincubating postheparin plasma with 1 M NaCl in the incubation medium. As stated above, the percent inhibition of LPL activity by 1 M NaCl was determined in all animals three times during the entire study. We observed no significant differences in percent inhibition of LPL activity by 1 M NaCl among the three determinations. The mean percent inhibition was 95.7%±0.4 (SD) in the high responders and 95.6%±0.6 (SD) in the low responders. On one
The animals did not gain or lose weight during the study.

For the high responders were 9.11 ± 0.4 kg (SEM) and age between groups was not significant (p>0.05). The 9.43 ± 0.2 kg, respectively, and those for the low responders 9.82 ± 0.2 kg and 10.08 ± 0.8 kg, respectively. The animals did not gain or lose weight during the study.

Table 1 shows the basal and final concentrations of plasma cholesterol, triglycerides, HDL cholesterol, and apo A-I in rhesus monkeys fed low and high cholesterol diets.

Table 1. Concentrations of Plasma Cholesterol, Triglycerides, HDL Cholesterol, and Apo A-I in Rhesus Monkeys Fed Low and High Cholesterol Diets

<table>
<thead>
<tr>
<th>Cholesterol</th>
<th>plasma concentrations</th>
</tr>
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<tbody>
<tr>
<td>LC diet</td>
<td>HDL cholesterol</td>
</tr>
<tr>
<td>Basal†</td>
<td>Final</td>
</tr>
<tr>
<td>High responders</td>
<td></td>
</tr>
<tr>
<td>7373</td>
<td>7340</td>
</tr>
<tr>
<td>168±15</td>
<td>185±6</td>
</tr>
<tr>
<td>656</td>
<td>683</td>
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<td>45±3</td>
<td>38±3</td>
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<td>103</td>
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<td>76±6</td>
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<tr>
<td>149±3</td>
<td>161±1</td>
</tr>
<tr>
<td>103</td>
<td>68</td>
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<tr>
<td>Mean±SEM</td>
<td>162±6</td>
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<tr>
<td></td>
<td>87±5</td>
</tr>
<tr>
<td>Low responders</td>
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<tr>
<td>7350</td>
<td>7364</td>
</tr>
<tr>
<td>139±8</td>
<td>165±5</td>
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<td>164</td>
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<td>Mean±SEM</td>
<td>165±10</td>
</tr>
<tr>
<td></td>
<td>85±6</td>
</tr>
<tr>
<td></td>
<td>164±8</td>
</tr>
</tbody>
</table>

Values are means±SEM and are given in mg/dl. Monkeys were fed for 518 days.

*On the 238th day of cholesterol feeding. †The values are means±SEM of four values determined during the final 3-month period in which the animals were fed the basal low cholesterol diet.

By Student's t test, during the basal period for all variables, the differences between the means for the high and low responders are not significant (p>0.05). By Student's t test, the significance of the difference between means between the high and low responders on the high cholesterol diet at the end of the study (final values) are as follows: plasma cholesterol, p<0.001; plasma triglycerides, not significant, p>0.05; plasma HDL cholesterol, p<0.001; plasma apo A-I, p<0.005.

By paired t test, the significance of the difference in means between basal and final values within animal groups are:

<table>
<thead>
<tr>
<th></th>
<th>High responders</th>
<th>Low responders</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma cholesterol</td>
<td>p&lt;0.005</td>
<td>p&lt;0.025</td>
</tr>
<tr>
<td>Plasma triglycerides</td>
<td>p&lt;0.05</td>
<td>not significant, p&gt;0.05</td>
</tr>
<tr>
<td>Plasma HDL-cholesterol</td>
<td>p&lt;0.001</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Plasma apo A-I</td>
<td>p&lt;0.01</td>
<td>not significant, p&gt;0.05</td>
</tr>
</tbody>
</table>

LC diet = low cholesterol diet, HC diet = high cholesterol diet, HDL = high density lipoprotein.

 occasion, the mean percent inhibition of LPL activity in all animals was also determined in the presence of 1.25 M NaCl because Goldberg et al. reported that in cynomolgus monkeys, 1 M NaCl could inhibit only about 80% of the LPL activity. The mean percent inhibition was 95.2±0.9 (SD) and 94.2±0.3 (SD), respectively, in the high and low responders. These were not significantly different from those found with 1 M NaCl.

Similarly, the specificity of HL assay was checked by determining the enzyme activity in the presence of additional 1 M NaCl in the incubation medium. No inhibition of the lipolytic activity was observed.

The age of the animals was estimated on the basis of the dental eruption sequence according to the method of Hurme22 when the animals were received from the animal importer in 1978. At the time of this study, the mean age in years was 11.7±0.8 years (SEM) for the high and 12.3±0.6 years for the low responders. This difference in age between groups was not significant (p>0.05). The mean body weight at the beginning and end of the study for the high responders were 9.11±0.4 kg (SEM) and 9.43±0.2 kg, respectively, and those for the low responders were 9.82±0.2 kg and 10.08±0.8 kg, respectively. The animals did not gain or lose weight during the study.

Table 1 shows the basal and final concentrations of plasma cholesterol, triglycerides, HDL cholesterol, and apo A-I in the high- and the low-responding rhesus monkeys fed the basal low cholesterol diet and the high cholesterol diet, respectively. The basal values in the two groups of monkeys represent the mean (±SEM) of four determinations during the final 3-month control period on the low cholesterol diet. The plasma lipids and apo A-I concentrations are similar (p>0.05) in the high- and the low-responding monkeys while they were fed the basal low cholesterol diet. Cholesterol feeding increased the plasma cholesterol level to an average final value of 1670 mg/dl (270% increase over the basal level) in the high responders but only to 232 mg/dl (41% increase over basal) in the low responders (p<0.001). The final plasma triglyceride levels were similar in the two groups fed the high cholesterol diet. The final plasma HDL cholesterol level in the cholesterol-fed high responders was 31% of that in the cholesterol-fed low responders; this difference was highly significant (p<0.001). Similarly, the final plasma apo A-I level in the cholesterol-fed high responders was 59% of that in the cholesterol-fed low responders; the difference was also highly significant (p<0.005).

Table 2 shows the mean LPL and HL activities in the high and low responders during the basal low cholesterol diet period and at the end of the high cholesterol diet period. The animals were fed two different 1 M NaCl in the incubation medium. No inhibition of the lipolytic activity was observed.
Table 2. Lipoprotein Lipase and Hepatic Lipase Activities in High and Low Responders Fed Low and High Cholesterol Diets

<table>
<thead>
<tr>
<th></th>
<th>Lipolytic activities (nmol FFA released /ml plasma/hour)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>LC Diet Basal*</td>
</tr>
<tr>
<td></td>
<td>HC Diet</td>
</tr>
<tr>
<td>High responders</td>
<td></td>
</tr>
<tr>
<td>7373</td>
<td>6288±739</td>
</tr>
<tr>
<td>7340</td>
<td>6952±575</td>
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<tr>
<td>7367</td>
<td>6288±508</td>
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<tr>
<td>7369</td>
<td>5899±536</td>
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<tr>
<td>Mean±SEM</td>
<td>6357±218</td>
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<tr>
<td>Low responders</td>
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<tr>
<td>7350</td>
<td>6159±185</td>
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<tr>
<td>7364</td>
<td>6155±516</td>
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<tr>
<td>7334</td>
<td>5439±765</td>
</tr>
<tr>
<td>7374</td>
<td>5441±412</td>
</tr>
<tr>
<td>Mean±SEM</td>
<td>5799±207</td>
</tr>
</tbody>
</table>

FFA=free fatty acids, LPL=lipoprotein lipase, HL=hepatic lipase, LC diet=low cholesterol diet, HC diet=high cholesterol diet. Animals were on diets for 518 days.

Values are means±SEM of four determinations during the final 3-month period when the animals were fed the basal low cholesterol diet.

By Student's t test, during the basal period the differences between the means for the high and low responders for both enzyme activities are not significant (p>0.05). By Student's t test, the differences between the means between the high and low responders fed the high cholesterol diet at the end of the study (final values) are not significant (p>0.05).

By paired t test, the significance of the differences in means between basal and final values within animal groups are:

<table>
<thead>
<tr>
<th></th>
<th>High responders</th>
<th>Low responders</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipoprotein lipase (LPL)</td>
<td>not significant, p&gt;0.05</td>
<td>p=0.05</td>
</tr>
<tr>
<td>Hepatic lipase (HL)</td>
<td>p&lt;0.05</td>
<td>p&lt;0.005</td>
</tr>
</tbody>
</table>

period. Both enzyme activities are similar in the two groups while the basal low cholesterol diet was fed. The mean final LPL activity in the high responders fed the high cholesterol diet is similar to the basal level of activity on the low cholesterol diet period, but in the cholesterol-fed low responders, it was significantly higher by about 37% than that during the basal period (p<0.05). In both groups of cholesterol-fed animals, the final level of activity of HL was 57% and 61% higher than that during the basal period of low cholesterol diet feeding (p<0.005).

Figure 1 shows the time course for plasma total cholesterol concentration in the high and the low responders fed the high cholesterol diet. With cholesterol feeding, plasma cholesterol increased, with the major increase occurring after 21 days in both groups of animals. The increase in the high responders was much greater than that in the low responders. By 238 days of cholesterol feeding, plasma cholesterol had increased more than fourfold in high responders and less than twofold in the low responders.

Figure 2 shows the time course for plasma triglycerides in the high and the low responders fed the high cholesterol diet. Upon cholesterol feeding, both groups showed decreasing trends in the first 2 weeks, but quadratic regression analyses indicated that these slopes were not significant (p>0.05). With continued feeding of cholesterol in the high responders, plasma triglycerides slowly increased (p<0.01) and by 126 days had increased twofold over the basal value (p<0.025), whereas in the low responders, the level was not significantly different from the basal value at any time during the cholesterol feeding period.

The time course for plasma HDL cholesterol concentration in the two groups of animals fed cholesterol is shown in Figure 3. By 3 days of cholesterol feeding, there was a significant increase in plasma HDL cholesterol concentration in the two groups, i.e., from 84 to 109 mg/dl (30%, p<0.01) in the low responders and from 86 to 118 mg/dl (37%, p<0.005) in the high responders. This increase in HDL cholesterol was maintained until 21 days of cholesterol feeding in both groups of monkeys. Quadratic regression analyses indicated that in both groups of animals, the regression slopes during this initial phase of cholesterol feeding were significant; however, the slopes were not different (p>0.05) between the two groups of animals. With continued cholesterol feeding in the high responders, there was a nearly 17% decrease in HDL cholesterol by the 42nd day of cholesterol feeding. This decrease continued; by 126 days, it had decreased by 50% (p<0.005) and by 518 days of cholesterol feeding, it had reached nearly 35% (p<0.001) of the basal value. In contrast, with continued cholesterol feeding in the low responders, the plasma HDL cholesterol concentration remained at a level significantly higher than the basal level on the low cholesterol diet and the level attained in the high responders.
Figure 1. Mean (±SEM) plasma cholesterol concentrations (mg/dl) in high-responding rhesus monkeys (●) and low-responding rhesus monkeys (○) fed cholesterol. The solid lines are the predicted lines obtained by quadratic regression analyses of the data in the high- and the low-responding rhesus monkeys, respectively. In the high responders, the regression slopes during the initial phase (between 0 and 21 days of cholesterol feeding) and the second phase (between 42 and 518 days of cholesterol feeding) were significant, p<0.05 and p<0.01, respectively. Between the high and the low responders, both initial and final regression slopes were significantly different, p<0.03 and p<0.01, respectively.

Figure 2. Mean (±SEM) plasma triglyceride (TG) concentrations (mg/dl) in high-responding and low-responding rhesus monkeys fed cholesterol. For other details, please see the legend for Figure 1. In the high responders, the regression slope during the second phase (between 42 and 238 days of cholesterol feeding) was significant, p<0.05. The regression slopes between the high and the low responders during the second phase was significant, p<0.05.

Figure 4 shows the time course of plasma apo A-I in high and low responders upon feeding high cholesterol diet. Cholesterol feeding produced a significant (p<0.025) increase in apo A-I levels by about 22% in both groups of animals at 3 days. This increase was short-lived. The apo A-I level returned to the basal level by 7 days of cholesterol feeding and was maintained until 21 days of cholesterol feeding in the two groups. During the second phase of cholesterol feeding, the regression slopes were significant (p<0.05) in both groups of animals. In the high responders, the apo A-I concentration decreased to about 80% (p<0.01) of the basal value by 42 days. The decline in apo A-I continued steadily and finally stabilized at about 43% (p<0.01) of the basal level. In contrast, the plasma apo A-I level in the low responders remained at basal level throughout the cholesterol feeding period.

The time course of LPL activity in the two groups of animals consuming the high cholesterol diet is shown in
The activity began to increase rapidly in both groups of animals consuming the high cholesterol diet. In both groups of animals, the regression slopes during the initial phase of cholesterol feeding were significant (p<0.05 and p<0.01, respectively). This increase continued in both groups, reaching a level about twofold greater than basal (p<0.001) in both groups. After this initial rise, the HL activity declined to basal levels by 42 days and thereafter remained at that level until 238 days of cholesterol feeding in both groups. The final HL activity measured at 518 days after cholesterol feeding was begun is significantly higher than the basal value on the low cholesterol diet (p<0.005) in both groups (Table 2).

Discussion

It is well established that HDL is a negative risk factor for coronary heart disease, and this effect is likely associated with the HDL2 subtraction. We reported that, in the high-responding rhesus monkeys, cholesterol feeding decreases the HDL2 level drastically. It was, therefore, important to understand the mechanism for this decrease. Since lipolytic enzymes play important roles in HDL metabolism,8-14 we measured by the method of Nilsson-Ehle and Schotz23 the postheparin lipolytic activity (PHLA) in different groups of cholesterol-fed high and low responders (n=4 in each group). In every instance, when one high- and one low-responding monkey was studied in pair, the PHLA activity was markedly lower in the high responder than in the low responder.24 These observations suggested that the lipolytic activities are different to a greater extent in cholesterol-fed high responders than in cholesterol-fed low responders; this led us to design the present longitudinal study to explore the possible causal relationships between plasma lipids, apo A-I level, and the LDL and HL activities in high- and low-responding rhesus monkeys fed a high fat, high cholesterol diet. The results showed that, in both groups of animals, cholesterol feeding resulted in a two-phase response: an initial phase lasting about 21 days that was similar in both high and low responders, and a second phase (starting between 21 and 42 days of cholesterol and continuing thereafter) when major differences became apparent between the two groups.

Because cholesterol feeding produces changes in the composition and concentrations of plasma lipoproteins that might affect the measurements of the activities of the enzymes, we measured the activities of the enzymes in the postheparin plasma of the two groups once after 2 weeks and a second time after 18 weeks of cholesterol feeding in the presence of preheparin plasma of either the cholesterol-fed high- or the cholesterol-fed low-responding monkey. The results from the two mixing experiments (not presented) showed that the activities of either LPL or HL were essentially the same with or without added preheparin plasma, suggesting that they were not affected by changes in the composition and concentrations of the plasma lipoproteins during cholesterol feeding.

The HL activity in the two groups of rhesus monkeys fed either the basal low cholesterol or the high cholesterol diet was between 13% and 19% of the LPL activity. These results are similar to those reported by Goldberg et al.22 but are higher than those reported by Wang et al.25 in cynomolgus monkeys. In rats, Kuusi et al.26 also found that LPL activity is much higher than HL activity.
HL ratio was about 2). In contrast, in humans, Stalenhoef et al.27 have reported that HL activity is much higher than LPL activity. It appears that LPL and HL activities differ between species.

As stated above, cholesterol feeding resulted in a two-phase response in the lipolytic activities in both high- and low-responding rhesus monkeys. In the initial phase (the first 21 days of cholesterol feeding), an increase in LPL and HL activities (Figures 5 and 6) was observed, together with an increase in plasma total cholesterol (Figure 1) and HDL cholesterol (Figure 3) and practically no change in plasma triglyceride (Figure 2) or apo A-I concentrations, except at 3 days of cholesterol feeding (Figure 4). The increased activities of the two enzymes immediately after cholesterol feeding suggest that both high and low responders respond in a similar manner to metabolize an increased lipid load and continue to remove triglycerides efficiently. While cholesterol accumulation begins. Wang et al.25 have also reported increased LPL activity in cynomolgus monkeys fed a high cholesterol, high fat diet. Our study suggests that dietary cholesterol may have a role in the increase in lipolytic activities, but the mechanism is not clearly understood.

In the initial phase of cholesterol feeding, the percentage increase in LPL activity (about 16% and 13%, respectively, over the basal level in the high and the low responders) was much lower than the increase in HL activities (141% and 86%, respectively, over the basal level in the high and low responders). During this phase, plasma HDL cholesterol also increased in the two groups of animals (Figure 3). In addition, the increase in plasma apo A-I level at 3 days of cholesterol feeding was short-lived. By 7 days of cholesterol feeding, the level returned to the basal level in both groups of animals (Figure 4). These results suggest that, during the initial phase of cholesterol feeding, the composition of HDL is altered. The reason for the increase in HDL cholesterol in particular in the face of increased HL activities is not clearly understood. It is possible that the increase in HDL cholesterol (hence, altered HDL composition) made this lipoprotein a somewhat unsuitable substrate for HL. The study by Baker et al.9 had shown that apo A-I concentration in HDL2 was unchanged, whereas it decreased in HDL2 with cholesterol feeding. These results taken together would imply that, in the early stage of cholesterol feeding, the composition of HDL2 is changed in both groups of animals. Since both groups of animals respond in similar fashion, it appears that, in the early stage of cholesterol feeding, the body attempts to adapt to an increased lipid load by metabolizing triglycerides efficiently. This is in agreement with the observation that in the initial phase of cholesterol feeding (up to 21 days), the plasma triglyceride concentration in both groups of animals remained essentially at the basal level (Figure 2).

In the second phase, there was a divergence of response between the high- and the low-responding rhesus monkeys. In high responders, but not in the low responders, HDL cholesterol and apo A-I concentrations in plasma began to decrease (possibly reflecting a change in HDL2 concentration9), plasma cholesterol continued to rise, and plasma triglyceride also started to increase slowly, while the LPL activity decreased. All of these changes in the high responders occurred simultaneously between 21 and 42 days of cholesterol feeding. These temporal associations suggest metabolic interrelationships between these events, although a causal relationship could not be established.

Patsch and colleagues26 explored the interrelationships between lipolytic activities, triglyceride content of HDL2, and postprandial lipemia in normolipidemic humans and proposed that: 1) individuals with a high HDL2 level were able to clear alimentary fat faster than individuals with low HDL2; and 2) postheparin LPL activity correlated positively with HDL2 level.26,27 They suggested that HDL cholesterol (hence, HDL2 and HDL3 level) is not an independent parameter of lipid transport but is closely linked to triglyceride metabolism.26

Our results can be explained in part by this proposed mechanism. We found by stepwise multiple regression analyses that LPL activity is significantly associated with plasma triglyceride level only in the high responders, suggesting that triglyceride metabolism is linked to this process. We suggest that, in individuals with high LPL activity (as in the low responders), there is no accumulation of triglyceride-rich lipoproteins. Surface components are transferred to HDL3, and they become "postprandial HDL2". HL removes the added phospholipid from the "postprandial HDL2" particles, and they convert to regular HDL2. In this situation, HDL3 can continue to accept surface components from triglyceride-rich lipoproteins, and HDL2 will not be reduced. On the other hand, with LPL activity returning to basal level with a continuing high lipid load due to high absorption of dietary cholesterol (as in the high responders), triglyceride-rich lipoproteins increase in plasma as indicated by the increase in plasma triglyceride level (Figure 2). In this situation, as a result of lipid transfer protein activity, more triglyceride may be transferred to the core of HDL2; at the same time, cholesterol esters are moved to the lower density lipoprotein fractions. HL removes from these "postprandial HDL2" particles not only the surface phospholipids, but also the triglycerides in the core of the HDL2, resulting in a smaller HDL3 particle. In this respect, it should be noted that Melchior et al.30 reported that in the high-responding patas monkeys there was an increase in the smaller HDL3.

An alternative explanation for our findings might be that the changes in LPL activity upon cholesterol feeding may be mediated by changes in the hormonal status of the animal. For example, for some time it has been suggested that insulin is the predominant hormonal regulator of adipose tissue LPL.31 Recently, Modan et al.32 from their population-based study, reported that hyperinsulinemia was characterized by elevated VLDL and LDL and reduced HDL. Whether the reverse observation would be true is a matter of conjecture. At the present time, therefore, we can only speculate that at later times (after about 21 days of cholesterol feeding) in high responders, insulin resistance may develop, resulting in a decrease in LPL activity, which in turn will decrease HDL2 concentration. The speculation is based on the observation that, in the high responders fed cholesterol between 42 and 238 days, plasma triglycerides show a slow but significant (p<0.01)
increasing trend, while LPL activity decreased to basal level (Figure 5), suggesting a possible increase in VLDL production due to a continued high lipid load. In this respect, it is worth noting that Steiner and Vranic have suggested that hypertriglyceridemia can lead to insulin resistance even without concomitant obesity or noninsulin-dependent diabetes. In any event, this testable hypothesis would bring into focus the importance of assessing lipoprotein metabolism as a complex system not separated from cholesterol and triglyceride metabolism. In fact, based upon metabolic studies, Eisenberg suggested that the overall plasma lipoprotein profile needs to be considered to evaluate the concomitant changes in various lipoprotein fractions.

The sharp increase in plasma apo A-I concentration by 3 days of cholesterol feeding (Figure 3) is similar to that observed by Melchior et al. in the high-responding, but not in the low-responding, patas monkeys (Erythrocebus patas) 1 week after the animals began consuming a high fat, high cholesterol diet. This early increase in plasma apo A-I in the two groups of rhesus monkeys (present study), however, did not last long. By 7 days of cholesterol feeding, the levels returned to near basal level in the two groups of monkeys (Figure 4). The early increase in plasma apo A-I was not observed in the study by Baker et al. because the first measurement was made 15 weeks after the cholesterol-containing diet was begun. The reason for this short-lived increase in apo A-I level is not clear, although it suggests that it is a consequence of feeding the high cholesterol diet.

The difference that distinguishes these two groups of rhesus monkeys as high and low responders is the response of plasma cholesterol level to the feeding of cholesterol. We have shown that the high responders consistently absorb significantly higher amounts of cholesterol. We have also shown that the high responders have a greater degree of feedback inhibition of cholesterol biosynthesis than do low responders. Although these differences in cholesterol absorption and synthesis are important factors in the variability of responsiveness in plasma cholesterol to dietary cholesterol, it is clear that plasma lipoprotein and apolipoprotein metabolism also contribute significantly to this variability. We have observed that in both groups of animals cholesterol feeding increased the concentrations of plasma VLDL (d=1.006 mg/dl) and IDL (d=1.006 to 1.030 mg/dl) but not that of LDL (d=1.030 to 1.063 mg/dl); the increases in VLDL and IDL were significantly higher in high responders than in low responders.

In addition, the composition of VLDL and IDL, but not that of LDL, were changed in the two groups of animals: again the degree of change in the high responders was greater than in low responders. It is possible that these composition changes in VLDL and IDL in cholesterol-fed high responders are so severe that these lipoproteins become somewhat, but not completely, unsuitable as substrates for LPL and HL activities. Further, due to decreased LPL activity in the high responders, it is possible that HL cannot adequately interact with these lipoproteins.

Previous studies have shown that hepatic intracellular cholesterol concentration is higher in cholesterol-fed high responders than the cholesterol-fed low responders. It was also observed that the cholesterol-fed high responders have lower 7α-hydroxylase activity than cholesterol-fed low responders. These results suggest that, when consuming a high cholesterol diet, the high responders accumulate higher levels of intracellular cholesterol, possibly due to low 7α-hydroxylase activity and may thus down-regulate hepatic LDL receptor to a greater extent than the cholesterol-fed low responders. Whether there is any relationship between lipase activities and the hepatic receptor activities is not known. But in view of the suggestion by Steiner and Vranic that LDL can impair insulin receptor function, we can only speculate again that, in the high responders, the increasing levels of plasma triglycerides that were observed between 42 and 238 days of cholesterol feeding (Figure 2) suggesting increased VLDL production (see above) may interact with hepatic LDL receptors and, thus, may be responsible for accumulation of LDL.

In summary, this study shows that, in the high- and low-responding rhesus monkeys, cholesterol feeding resulted in a two-phase response in lipolytic activities: an initial phase lasting about 21 days that was similar in the two groups, followed by a second phase in which major differences became apparent between the groups. Thus, we find further distinctions in response of LPL and HL activities, plasma HDL cholesterol, triglyceride, and apo A-I metabolism between the high and the low responders when they are fed cholesterol. In regard to apo A-I, it is possible that at some point during the development of hypercholesterolemia in the high responders, the apo A-I catabolic rate exceeds that of apo A-I synthesis, resulting in a decrease in plasma apo A-I concentration. In this context, it is worthy of note that recently Melchior et al. suggested that the decreased level of plasma apo A-I results from an increased catabolism of apo A-I in hypercholesterolemic cynomolgus monkeys.

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