Lipoprotein Profiles in Rhesus Monkeys with Divergent Responses to Dietary Cholesterol


From a group of 53 rhesus monkeys, we selected 12 animals, the six with the highest and the six with the lowest response to a high cholesterol diet, and we made detailed analyses of their cholesterol and apolipoprotein profile. The high responders differed from the low responders in several ways. During the high cholesterol diet period, the high responders had much higher plasma apolipoprotein B and E concentrations and much lower plasma apolipoprotein A-I concentrations than did the low responders. Nearly all the increase in plasma cholesterol and apolipoproteins B and E concentrations in the high responders occurred in the lower density fractions (d = 1.006–1.030 g/ml), while the decrease in plasma apolipoprotein A-I concentrations in the high responders was confined to the lower density fraction of the high density lipoproteins (HDL), i.e., HDL₂ (d = 1.063–1.125 g/ml). In the low responders, on the other hand, the slight increase in cholesterol concentrations was evenly distributed between the lower density fractions and HDL, and the increase in apolipoprotein A-I concentrations was confined to the HDL₃ fraction. We suggest that the increase in the concentration of the lower density fractions is related to the decrease in the concentration in the HDL₂ in the high responders. (Arteriosclerosis 3:223–232, May/June 1983)

Nonhuman primates are important animal models for the study of atherosclerosis because of their close phylogenetic relationship to humans. Serum cholesterol concentration varies significantly among various species of primates and among individual primates of the same species.¹⁻³ This variability is accentuated by consumption of a high cholesterol (HC) diet. We previously reported that rhesus monkeys that responded with the greatest increase in serum cholesterol concentrations to a HC diet (high responders) absorbed a higher percentage of dietary cholesterol than did monkeys that responded with only a small increase in serum cholesterol concentration (low responders).³ ⁴ We have also reported that cholesterol synthesis was less inhibited by dietary cholesterol in the low responders than in the high responders.⁵ Although these differences in cholesterol absorption and synthesis may be important factors in the variability of responsiveness in the rhesus monkey, additional factors such as lipoprotein and apolipoprotein metabolism, may also contribute to this variability.

Previous studies have provided data on the general characteristics of rhesus monkey lipoproteins and have shown that low density lipoproteins (LDL) increase and high density lipoproteins (HDL) decrease when these animals consume a diet containing cholesterol.⁶ ⁷ We used high and low responders for a comparison of the cholesterol changes; the changes of sera concentrations of apolipoprotein B (apo B), A-I (apo A-I) and E (apo E) and the changes of these apoproteins in several lipoprotein fractions are believed to be important in cholesterol metabolism and atherogenesis.
Methods

Animals and Diets

We acquired 53 male rhesus monkeys, 3 to 5 years of age, from an animal importer and quarantined them for 4 months. During the last 2 months of quarantine and for 2 additional months they were fed a high fat (38% cal), nonpurified, open formula diet described previously. Cholesterol (0.4 mg/cal) was then added to the basal diet for an additional 24 months. The animals were weighed and blood samples were obtained at 3- to 6-week intervals for an analysis of the total cholesterol, apo A-I, and apo B. Larger samples were obtained at greater intervals for an analysis of the lipoprotein profile (cholesterol, apo A-I, and apo B). Blood collected from the monkeys was allowed to clot and the serum was separated from the cells by centrifugation. Ethylene diamine tetraacetic acid (1 μg/ml serum) and sodium azide (1 μg/ml serum) were routinely added as preservatives.

Six of the 53 animals with the highest and six with the lowest response of serum cholesterol to dietary cholesterol were selected for more detailed analyses of their cholesterol and apolipoprotein profile. The bulk of the data reported here was obtained from these high- and low-responding groups; however, some results from all 53 animals are also presented.

Lipoprotein Isolation

Lipoproteins for quantitation were separated by ultracentrifugation in a 40.3 rotor at 39,000 rpm as described by Havel et al. The rotor was fitted with special adaptors (Beckman Instrument Company, Palo Alto, California) so that lipoprotein fractions could be isolated from 1 ml of serum. Individual 1 ml aliquots of serum were used for centrifugation at each of the following densities: d = 1.006, d = 1.020, d = 1.030, and d = 1.063 g/ml for 18 hours; d = 1.080 g/ml for 24 hours; and d = 1.125 and d = 1.21 g/ml for 48 hours.

Lipoprotein fractions are defined as follows:

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>Density (g/ml)</th>
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<tbody>
<tr>
<td>VLDL</td>
<td>&lt; 1.006</td>
</tr>
<tr>
<td>IDL</td>
<td>1.006–1.020</td>
</tr>
<tr>
<td>LDL</td>
<td>1.020–1.063</td>
</tr>
<tr>
<td>LDL$_1$</td>
<td>1.020–1.030</td>
</tr>
<tr>
<td>LDL$_2$</td>
<td>1.030–1.063</td>
</tr>
<tr>
<td>HDL</td>
<td>1.063–1.21</td>
</tr>
<tr>
<td>HDL$_1$</td>
<td>1.063–1.125</td>
</tr>
<tr>
<td>HDL$_2$</td>
<td>1.125–1.21</td>
</tr>
</tbody>
</table>

We have chosen to subfractionate LDL at d = 1.030 g/ml based on preliminary observations with these density cuts. We have chosen to subfractionate HDL into particles of d = 1.063–1.125 g/ml (HDL$_1$) and d = 1.125–1.21 g/ml (HDL$_2$) based upon previous studies on humans and on rhesus monkeys which suggested that these particles at these densities are distinct subfractions of HDL.

Individual lipoprotein fractions were isolated sequentially by centrifugation in a SW41 rotor (Beckman Instrument Company, Palo Alto, California) for characterization, isolation of apoproteins, and antibody preparation as follows: d < 1.030 g/ml (containing VLDL, IDL and LDL$_1$); d = 1.030–1.050 g/ml (containing LDL$_1$); d = 1.050–1.080 g/ml (containing LP(a)); and d = 1.080–1.21 g/ml (containing HDL$_2$).

Apoprotein Quantitation

The apoproteins were measured by electroimmunoassay as described by Laurell and modified by Bar-On et al. Nonidet-P-40 (NP-40), a nonionic detergent, was added to a final concentration of 1% in the samples and standards, and 0.05% in the agarose for the measurement of apo A-I. Antiser to apo A-I was prepared by injecting rhesus monkey LDL (d = 1.030–1.050 g/ml) into goats. Antiser to apo A-I was prepared by injecting into goats apo A-I purified by polyacrylamide gel electrophoresis in sodium dodecyl sulfate (PAGE-SDS). The antisera so obtained were tested for purity by double diffusion and immunoelectrophoresis against whole plasma and individual lipoprotein fractions.

The apo B and apo A-I concentrations were measured in the serum and in the infranatant from each fraction isolated by ultracentrifugation as described above. The apoprotein concentrations of the serum or infranatant fractions were determined by comparing their rocket heights with those of rockets produced from a series of dilutions of a standard pool of control rhesus monkey serum of known apo B and apo A-I concentrations.

The control serum was standardized for apo B concentration against LDL (d = 1.030–1.050 g/ml) isolated from noncholesterol-fed rhesus monkeys and which was shown by PAGE-SDS to contain only apo B. The standardization for apo B was performed with LDL in the presence of goat d = 1.21 g/ml infranatant. The control serum was also standardized against apo A-I which was isolated from HDL (d = 1.080–1.21 g/ml) by chromatofocusing. The HDL preparation was shown by PAGE-SDS to contain predominantly apo A-I with some apo C's. The preparation of HDL was also used to standardize the control serum for apo A-I. The measures of apo A-I in the control serum derived from apo A-I and HDL were in good agreement.

The data reported for the concentrations of apo B, apo A-I and cholesterol in the lipoprotein fractions were obtained by differences: e.g., the concentration of apo B or cholesterol in VLDL was obtained by subtracting the concentration of apo B or cholesterol in the d = 1.006 g/ml infranatant from that in whole serum; the concentration of apo A-I in HDL$_2$ was obtained by subtracting the concentration of apo A-I in the d = 1.125 g/ml infranatant from that in the d = 1.063 g/ml infranatant. Since, theoretically, all the albumin should be present in the infranatant, albumin was measured in the infranatant to verify recovery and to use as a correction factor.
**Other Methods**

The cholesterol concentrations of the serum and the ultracentrifugally derived infranatant fractions were analyzed on a Technicon Auto Analyzer II (Technicon Instruments Corporation, Tarrytown, New York). The protein concentrations were measured by the Lowry procedure using bovine serum albumin as the standard. Agarose electrophoresis of the serum and lipoprotein fractions was performed as described by Noble. The apoproteins from the supernatant of single density centrifugation or from isolated lipoprotein fractions were separated by PAGE-SDS using a Hoefer slab gel apparatus (Hoefer Scientific Instruments, San Francisco, California). The aliquots were delipidated before reduction with mercaptoethanol in the presence of SDS. The apoproteins were stained with Coomassie brilliant blue for 2 hours at 60°C. The background was removed by destaining in 10% acetic acid for 72 hours at room temperature.

An indication of the relative changes in the apo E found in rhesus monkeys was determined by scanning 10% PAGE-SDS gels of the d < 1.080 g/ml fraction from those monkeys with an E-C densitometer (E-C Apparatus Corporation, Indianapolis, Indiana). This fraction was used because it contained the majority of the apo E, but only a small fraction of the plasma apo A-I. Thus, apo E was a major component of the total protein loaded onto the gel.

**Statistics**

Changes in serum concentration on addition of cholesterol to the diet were tested for statistical significance by t-test of paired differences; differences in concentration between high- and low-responding groups were tested by t test of differences between means.

**Results**

**Plasma Cholesterol Concentration**

After cholesterol was added to the diet of the 53 monkeys, plasma cholesterol concentrations rose from a mean basal value of 150 mg/dl to a mean steady-state level of 394 mg/dl at 18 weeks (Figure 1). The plasma cholesterol concentration of the six low responders had reached steady-state levels at 3 weeks, while in the high responders, steady-state levels were not attained until the 15th to 18th week after the addition of cholesterol. As a measure of the steady-state level, we computed the mean for each animal using the 19 data points between 19 and 102 weeks. The mean ± sd and range of these steady-state values are given in Table 1. The low responders differed from the high responders by more than twice the standard deviation for the group of 53 animals.

![Figure 1](http://atvb.ahajournals.org/)

**Plasma Apolipoprotein Concentrations**

The divergent responses of the two groups of monkeys were also evident from changes in their plasma apo B, apo E and apo A-I levels. Plasma apo B levels closely paralleled the changes in cholesterol concentrations in both groups. In the high responders, plasma apo B levels increased considerably (Table 1). The low responders, on the other hand, had only minimal increases in their plasma apo B levels. The correlation between cholesterol concentration and apo B levels at 36 weeks was high (r = 0.97, p < 0.001) (Figure 2 A). It was also high (r =

**Table 1. Mean, Standard Deviation, and Range of the Steady-State Plasma Cholesterol and Apo B Concentrations**

<table>
<thead>
<tr>
<th></th>
<th>No.</th>
<th>Mean</th>
<th>SD</th>
<th>Range</th>
</tr>
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<tbody>
<tr>
<td>Plasma cholesterol (mg/dl)</td>
<td>53</td>
<td>394</td>
<td>127</td>
<td>158–820</td>
</tr>
<tr>
<td>Total sample</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High responders</td>
<td>6</td>
<td>632</td>
<td>103</td>
<td>529–820</td>
</tr>
<tr>
<td>Low responders</td>
<td>6</td>
<td>216</td>
<td>35</td>
<td>156–246</td>
</tr>
<tr>
<td>Plasma apo B (mg/dl)</td>
<td>53</td>
<td>122</td>
<td>45</td>
<td>48–250</td>
</tr>
<tr>
<td>Total sample</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High responders</td>
<td>6</td>
<td>200</td>
<td>33</td>
<td>159–250</td>
</tr>
<tr>
<td>Low responders</td>
<td>6</td>
<td>56</td>
<td>6</td>
<td>48–64</td>
</tr>
</tbody>
</table>

The values used are the averages of 19 cholesterol measurements and eight apo B measurements obtained on each animal from 18 to 102 weeks after beginning the high cholesterol diet.
0.94, p < 0.001) when measured using data from the entire 2-year period in which 53 monkeys were used. The slope of the line in Figure 2A was 0.35, indicating that each molar increment in plasma apo B concentration was accompanied by a 4100 molar increment in cholesterol (see Discussion).

Changes in apo E levels also appeared to parallel the changes in apo B and cholesterol concentrations in cholesterol-fed monkeys. A distinct apo E band appeared in the PAGE-SDS gels of the d < 1.080 g/ml lipoprotein fraction in high responders, which was consistently much more intense than that in the low responders (Figure 3). Examination of isolated lipoprotein fractions from the high-responders showed that apo E was mainly in the d < 1.030 g/ml lipoproteins (Figure 4). The results of scanning gels of the d < 1.080 g/ml fraction from a subgroup of monkeys whose plasma cholesterol levels ranged from 245 to 658 mg/dl indicate that the apo E band intensity increases in proportion to the plasma cholesterol concentration (Figure 2C, r = 0.91, p < 0.01).

Plasma apo A-I levels also responded differently in the two groups of monkeys (Figure 5). A significant reduction in the mean plasma apo A-I levels occurred in the high responders within 3 weeks after they began to consume the HC diet, and the mean apo A-I level continued to decrease through 36 weeks. After 36 weeks, the apo A-I levels appeared to plateau and to fluctuate about a mean that was roughly 60% of the basal value. The plasma apo A-I concentrations showed a different response in the low responders. After a slight decrease at 3 weeks (p < 0.1), the apo A-I levels rose to slightly above the basal value by 18 weeks and remained at or above the basal values for the remainder of the HC diet period. We observed a negative correlation (r = −0.5, p < 0.001) in 53 animals between apo A-I and plasma cholesterol concentration.

**Cholesterol and Apo B Distribution**

Because the distribution of cholesterol among the fractions was nearly identical in the two groups during the basal diet period, the data for the two groups were combined for comparison with data obtained 18 weeks after the beginning of the HC diet (Figure 6). During the basal diet period, about one-half of the cholesterol was carried in the HDL with most of the remainder in the LDL. Addition of cholesterol to the diet caused a marked difference between the two groups in the distribution of cholesterol among the lipoprotein fractions. In the high responders, all the increase in plasma cholesterol concentrations appeared in lipoproteins of d < 1.030 g/ml, while LDL cholesterol did not change and the HDL cholesterol decreased. In the low responders, only about two-thirds of the increase in plasma cholesterol occurred in the d < 1.030 g/ml region, with the remaining one-third contributed by the d > 1.063 g/ml (HDL) region. This increase in HDL cholesterol was accompanied by an increase in HDL apo A-I. Thus, dietary

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**Figure 2.** A. Apo B vs cholesterol concentration in 53 rhesus monkeys fed the high cholesterol diet for 36 weeks. B. Apo A-I vs cholesterol concentration from the same 53 monkeys. C. Apo E vs cholesterol concentration in a separate group of 16 rhesus monkeys fed the high cholesterol diet for 52 weeks.
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Figure 3. PAGE-SDS gels (10%) of supernatant fractions from typical high (A) and low (B) responding rhesus monkeys fed the high cholesterol diet for 1 year. Lane 8 in A and Lane 1 in B contain 50 μg rat apolipoproteins isolated at d < 1.21 g/ml. The other lanes (left to right) contain rhesus monkey apolipoproteins of: d < 1.006 g/ml, d < 1.020 g/ml, d < 1.030 g/ml, d < 1.063 g/ml, d < 1.080 g/ml, d < 1.125 g/ml and d < 1.21 g/ml respectively. In every instance, 1 ml of serum was used for lipoprotein isolation and thus the same plasma volume equivalent was loaded into each well. This ranged from 2 to 75 μg of protein.

Figure 4. PAGE-SDS gels (10%) of isolated lipoprotein fractions from a high-responding rhesus monkey. Lane 5 contains rat apolipoproteins isolated at d < 1.21 g/ml. Lanes 1 to 4 contain rhesus apolipoproteins of d < 1.030, d = 1.030–1.050, d = 1.050–1.080 and d = 1.080–1.21 g/ml respectively. Each sample contained 50 μg protein.

Figure 5. Apo A-I concentrations (mean ± sem) of six high responders (−−−) and six low responders (−Δ−) for 2 years following start of the high cholesterol diet. The zero time point represents the mean ± sem of the average of four basal diet period values for each animal. Each point thereafter represents the mean ± sem of a single value for each animal.
cholesterol caused a slight increase in the HDL of low responders, and a marked decrease in HDL of high responders. These differences in HDL profile are examined later in more detail.

The plasma apo B distribution in high and low responders before the HC diet and 18 weeks after beginning the HC diet is shown in Figure 7. Again, during the basal diet, the distribution of apo B among the lipoproteins did not significantly differ between the high and low responders. However, 18 weeks after the start of the HC diet, the apo B of the IDL + LDL$_2$ fraction in high responders increased considerably, whereas the increase in low responders was much smaller. The apo B content of the LDL$_2$ was not changed in either group at 18 weeks, which indicated that nearly all the increase in the total plasma apo B levels noted earlier was due to an increase in the apo B content of IDL + LDL$_2$. The addition of cholesterol to the diet had no significant effect on the very low levels of apo B detected in the HDL of both groups. Similar analyses of samples obtained 36 and 102 weeks after cholesterol was added to the diet indicated that the distributions shown in Figures 6 and 7 did not change appreciably. A slight increase in the LDL$_2$ cholesterol and apo B for both groups was observed beyond 18 weeks; however, this increase accounted for less than 5% of the total increment in plasma apo B or cholesterol, with the remaining 95% in the d < 1.030 g/ml fraction.

**Apolipoprotein A-I Distribution**

To gain further insight into the difference in the effect of dietary cholesterol on high- and low-responding groups, we examined the effect on HDL in greater detail. We observed that more than 90% of the total plasma apo A-I was found in the HDL region on both diets and that the apo A-I composition of the fraction observed at d < 1.063 g/ml was not significantly affected by dietary cholesterol. Thus, most of the decrease in total apo A-I in the high responders was due to an absolute decrease in the HDL and not to a shift of apo A-I to lipoproteins of lower density.

The changes in HDL cholesterol concentration after the addition of cholesterol are shown for the two groups in Figure 8. In high responders the mean HDL cholesterol concentration had decreased by 50% at 18 weeks and remained low for the remainder of the study. In low responders the HDL cholesterol concentration had increased by about 30% at 18 weeks and remained elevated thereafter. The changes in HDL (or total) apo A-I concentrations (Figure 5) were similar to those of HDL cholesterol (Figure 8).

Previous studies in humans have suggested that there are two density subfractions$^a$ that are metaboli-
Figure 8. Mean HDL cholesterol concentration of six high responders (---) and six low responders (---) for 2 years after the start of the high cholesterol diet. The zero time point represents the mean ± SEM of the average of four basal diet period values for each animal. Each point thereafter represents the mean ± SEM of a single value for each animal.

Figure 9. Mean HDL₂ apo A-I (---) and HDL₃ apo A-I (---) concentrations of six high responders for 2 years after the start of the high cholesterol diet. The zero time point represents the mean ± SEM of the average of four basal diet period values for each animal. Each point thereafter represents the mean ± SEM of a single value for each animal.

Figure 10. Mean HDL₂ apo A-I (---) and HDL₃ apo A-I (---) concentrations of six low responders for 2 years after the start of the high cholesterol diet. The zero time point represents the mean ± SEM of the average of four basal diet period values for each animal. Each point thereafter represents the mean ± SEM of a single value for each animal.

Discussion

Clarkson and Eggan have reported that certain nonhuman primates are fed a diet containing cholesterol, the plasma cholesterol concentrations within the group vary over a relatively wide range. Some animals, low responders, are able to maintain nearly normal plasma cholesterol concentrations, whereas others, high responders, develop a severe hypercholesterolemia. In studies on other groups of high- and low-responding rhesus monkeys, we observed that high responders absorb a significantly greater fraction of intestinal luminal cholesterol than low responders, and that this difference is reflected in the degree of inhibition of cholesterol biosynthesis. We have also shown that the increment in total body cholesterol caused by an increase in dietary cholesterol is distributed among major body pools similarly in the two groups. Jones et al. have also shown that the percentage of cholesterol absorbed was greater for hyperresponding than hyporesponding squirrel monkeys although in an earlier study in this species no significant difference in absorption was observed between hyper- and hyporesponders. Parks et al. observed that in the Afri- can green monkey a larger fraction of plasma cholesterol was derived from dietary sources in the hyperresponding than in the hyporesponding animals.

The present study was carried out to define differences in the lipoprotein and apolipoprotein profiles in these two groups in order to further our understanding of the mechanism involved in cholesterol homeostasis. High-responding monkeys differed from their low-responding counterparts in several ways: their
steady-state plasma cholesterol concentrations were much higher; their steady-state apo B and apo E concentrations were much higher; their plasma apo A-I levels were only about one-half those of the low-responding group; and, their HDL2 levels (apo A-I and cholesterol) were much lower than those of low responders.

Nearly all the increment in cholesterol, apo B, and apo E concentrations was contained in lipoproteins of d = 1.006 to 1.030 g/ml in the high responders. This increase was evident not only from analyses of lipoprotein fractions obtained by sequential ultracentrifugation (Figures 4, 7, and 8), but also from changes in the plasma apo B/cholesterol ratio, (i.e., the relationship between the plasma apo B levels and the cholesterol concentrations of the d < 1.063 g/ml fraction appeared to be linear through cholesterol concentrations of 870 mg/dl (r = 0.97, p < 0.001) with a slope of 0.35. See Figure 2 A). If the molecular weight of apo B is assumed to be 550,000 daltons,27 this relationship would indicate that 4100 moles of cholesterol accumulated per mole of apo B. The molar ratio of cholesterol to apo B in the IDL + LDL (regardless of whether or not the animals were hypercholesterolemic) was 4100, whereas in the HDL2, it averaged 2800. Had LDL2 made a significant contribution to the diet-induced hyperlipoproteinemia, one would have expected a lower molar ratio of cholesterol to apo B than that obtained.

It is not clear why the increment in cholesterol and apo B was confined to the IDL + LDL, fraction in the high responders. One explanation may be that the precursors of these lipoproteins (VLDL) were enriched in cholesteryl esters as a result of the HC diet, and thus the remnants produced after hydrolysis of their triglycerides by lipoprotein lipase were of lower than normal density (d = 1.006 to 1.030 g/ml rather than d = 1.030 to 1.063 g/ml). Alternatively, the cholesteryl ester-rich lipoproteins that accumulate in the high responders may be secreted, as such, by the liver or intestine and undergo no major alterations in the circulation. Liver perfusion studies have suggested that the latter may be the case in the hypercholesterolemic rat,28 but similar information is not yet available for nonhuman primates.

Studies of cholesterol-fed rabbits29 have suggested that lipoproteins of intestinal origin (chylomicron remnants) accumulate in the plasma of that species and make a major contribution to the diet-induced hypercholesterolemia; however, analogous studies using hypercholesterolemic dogs30 have shown that intestinal lipoproteins do not make a significant contribution to the plasma cholesterol pool in those animals. The time required for pronounced hypercholesterolemia to develop in the rhesus monkey is considerably longer than in the rabbit, and the resulting lipoprotein profile is more like that of the dog than the rabbit, (i.e., at cholesterol concentrations below 750 mg/dl, only a small fraction of the plasma cholesterol of the hypercholesterolemic rhesus monkey is associated with particles of d < 1.006 g/ml, the fraction from which chylomicron remnants are usually recovered). Furthermore, we were unable to detect the "intestinally derived" low molecular weight apo B in the plasma of these high responders.27 Thus, the indication is that lipoproteins of intestinal origin probably do not make a significant contribution to the plasma cholesterol pool in these monkeys. Nonetheless, experiments specifically designed to answer this question are necessary before the possibility can be eliminated.

The diet-induced changes in the d < 1.063 g/ml lipoproteins in the high responders are similar to those described by Rudel et al.7 even though the diets in the two studies are different. The cholesterol distribution among the lipoprotein fractions separated by ultracentrifugation, as reported by Rudel et al.7 (see their Table 2) showed somewhat less of the total cholesterol in the d = 1.006–1.020 g/ml fraction (16% vs 33% in the present study) and more in the d = 1.020–1.063 g/ml fraction (72% vs 48%) than we observed. Rudel and coworkers did not examine the d = 1.020–1.030 g/ml fraction specifically; however, their data are consistent with our observations. Furthermore, we would suggest that the "large LDL" observed by these researchers is equivalent to our d = 1.020–1.030 g/ml fraction.

In this regard, it is noteworthy that Melchior and Rudel31 showed that LDL from hypercholesterolemic African green monkeys contained at least two metabolically distinct entities. This finding suggests that in this species the particles accumulating as a result of cholesterol feeding are not metabolized in the same way as native LDL. The same is probably true of various cholesterol-rich fractions that accumulate in the high-responding rhesus monkey.

The observation that the apo E levels are increased in the high responders agrees with that reported for hypercholesterolemic patas monkeys32 and rhesus monkeys.7 Most of the increase in the plasma apo E appeared to be in the d < 1.030 g/ml lipoproteins (Figure 4), the same fractions in which the cholesterol and apo B increased. The question then arises as to whether the apo E is a component of the apo B-containing particles, or whether a separate family of apo E-containing lipoproteins exists in those animals.33 There is no obvious lipoprotein subfraction analogous, for example, to the apo E-rich LDL-II from patas monkeys,32 or the apo E HDL from dogs,34 which might account for the increased apo E levels seen in these rhesus monkeys. Furthermore, the apo E levels appeared to increase in direct proportion to the cholesterol and apo B levels. These observations suggest that the apo E was a component of the apo B-containing particle.

The function of apo E in the high-responding monkeys has not been determined. Hepatic apo E receptors have been shown to exist in dogs, swine, and in humans35,36 and they are thought to be responsible for chylomicron remnant clearance; however, these receptors also recognize other apo E-containing lipoproteins (apo E HDL¢, for example38). We might
postulate that one function of this apo E is to aid in the clearance of these cholesteryl ester-rich lipoproteins. If this is the case, apo E receptor number, or capacity might be a determinant of high and low responsiveness.

**HDL Metabolism**

An additional potentially important observation was that a decrease in HDL, principally HDL₂, occurred with the increase in IDL + LDL in high responders. It has been reported that diet-induced hypercholesterolemia is associated with a reduction in HDL in several species. ³⁷ During a period of extreme cholesterol overload, why would a cholesterol-carrying species of lipoprotein decrease in concentration? This change did not occur in low responders. One possibility might be that HDL₂ became enriched in cholesteryl esters and that this enrichment reduced the HDL₂ hydrated density. It has been proposed that this occurs with HDL in the dog. ³⁴ In these monkeys, however, a reduction in the density of particles containing apo A-I did not occur. While total plasma apo A-I (the principal protein component of rhesus monkey HDL) decreased in high responders, there was no increase in apo A-I in the d < 1.063 g/ml fraction. Furthermore, subfractionation of the d = 1.063–1.21 g/ml fraction into HDL₂ and HDL₃ showed that the decrease in plasma apo A-I was associated with a decrease in HDL₂ levels.

It is not clear how this decrease in HDL₂ is related to the animal’s response to dietary cholesterol. One theory³⁸ of HDL metabolism suggests that during VLDL conversion to LDL some of the surface components are transferred to HDL₂, converting it to HDL₂. If a cholesteryl ester-enriched VLDL were secreted by the livers of the high responders, the remnants resulting from triglyceride hydrolysis by lipoprotein lipase would also be enriched in cholesteryl esters. This increased retention of “core lipids” in the remnant should require a greater retention of surface components for thermodynamic stability, (i.e., the larger the particle, the greater the surface area). This, in turn, could result in decreased plasma HDL₂ levels.

Although an altered HDL₃ to HDL₂ interconversion could explain the observed decrease in plasma HDL₂ levels, it does not explain why total HDL or total apo A-I levels were decreased since, presumably, apo A-I could have been conserved as HDL₂. It is possible that at some point during the development of hypercholesterolemia in high responders, the apo A-I catabolic rate exceeded the apo A-I synthesis rate, with a resulting decrease in plasma apo A-I concentration. It is not known whether a decreased apo A-I synthesis rate or an increased apo A-I catabolic rate was the primary defect, or why a similar change did not occur in low responders.

In view of the negative correlation between HDL and coronary heart disease, ³⁶–⁴¹ it is essential to determine whether a metabolic link exists between HDL (or HDL₂) levels and coronary atherosclerosis, or whether these changes simply coincide but are not functionally related. It would, for example, be important to differentiate between total plasma cholesterol concentration and HDL₂ concentration in the development and regression of atherosclerosis. A recent paper by Holme et al.⁴² has presented evidence for an inverse relationship between raised atherosclerotic lesions of the coronary arteries as determined at autopsy and antemortem HDL cholesterol concentrations.

In a subgroup of 15 animals whose cholesterol concentrations were within the narrow range between 350 and 400 mg/dl, we found that plasma HDL₂ apo A-I levels ranged from 26 to 317 mg/dl. The range for HDL₂ apo A-I in the entire group of 53 animals was from 26 to 330 mg/dl. This finding suggests that HDL₂ can vary independently of cholesterol level. Studies are currently in progress to evaluate the relationship between plasma HDL₂ levels and the evolution and regression of atherosclerotic lesions in these animals.

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**References**


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