Response of Rhesus Serum High Density Lipoproteins to Cycles of Diet-Induced Hypercholesterolemia

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Two male rhesus monkeys underwent cyclical feeding of a hypercholesterolemic diet (2% cholesterol, 25% coconut oil) and a low-fat Purina monkey chow diet. During the latter diet, high density lipoprotein (HDL) exhibited two components with peak densities of $d = 1.081 \text{ g/ml}$ and $1.109 \text{ g/ml}$ named HDL$_1$ and HDL$_2$, respectively. During the initial hypercholesterolemic stage, except for apo A-II which remained unchanged, there was a transient rise in HDL (mainly HDL$_1$) as well as in HDL cholesterol and apo A-I, all reaching maximal values after about 2 weeks from the onset of the diet. The two HDL species changed neither in size nor density as compared to their baseline counterparts, but had a comparatively higher content in cholesteryl ester and lesser amounts of triglycerides and phospholipids as compared to the normocholesterolemic animal. With the development of overt hypercholesterolemia (plasma cholesterol levels above 400 mg/dl), both HDL particles increased in density due to the loss of surface components (phospholipids and unesterfied cholesterol) and core triglycerides with only minor changes in protein and cholesteryl ester contents. At this stage, the same two animals exhibited significant changes in the size and buoyant density of LDL. When returned to a normal Purina chow diet, the animals’ serum cholesterol levels declined rapidly to normal levels; normalization of the HDL distribution also occurred but at a comparatively later time (26 weeks).

Our studies indicate that the two HDL subsets characteristic of the normocholesterolemic rhesus monkey undergo significant changes in buoyant density as a function of the stage of hypercholesterolemia and that changes in concentration and size mainly affect the HDL$_1$ subspecies. At levels of plasma cholesterol below 400 mg/dl, this cholesteryl increment is reflected by a significant increase in the number of the HDL subspecies without the overt participation of the low density lipoprotein classes characteristic of the advanced hyperlipidemic stage. Since we previously reported that greatly increased levels of cholesteryl esters enriched low density lipoproteins, B-VLDL (very low density lipoprotein) and pre-B-VLDL during overt diet-induced hypercholesterolemia, it is apparent that cholesterol is distributed differently among lipoprotein particles containing either apo A-I, apo B, or apo E depending on its concentration in plasma. (Arteriosclerosis 4:154–164, March/April 1984)
mg/kcal), both HDL$_2$ and HDL$_3$ increased in concentration; but at a cholesterol intake of 2.0 mg/kcal, HDL$_2$ decreased and HDL$_3$ increased in concentration so that the ratio of HDL$_2$ to HDL$_3$ changed from 3.0 to 0.2.$^4$ However, in most studies where the concentrations of HDL$_2$ and HDL$_3$ were determined, the classical density cut of 1.125 g/ml for separating HDL$_2$ from HDL$_3$ was used although there is no guarantee that the density distribution of monkey HDL is similar to its human counterpart. We report here the results of a longitudinal study concerning the effects of a high cholesterol, high fat diet on HDL structure and density distribution in two rhesus monkeys. A report on dietary alteration of LDL over time in the same animals has appeared previously.$^5$

**Methods**

Two male rhesus monkeys were maintained either on regular Purina primate chow or a modified low fat Purina primate chow supplemented with 25% coconut oil and 2% cholesterol.$^3$ Blood was collected from the femoral vein of monkeys anesthetized with ketamine and the serum was separated by centrifuging the blood at 4°C for 30 minutes at 1000 g.

**Lipoprotein Preparation**

HDL was isolated by a combination of rate-zonal and isopycnic equilibrium density gradient ultracentrifugation. Total lipoproteins were floated by adjusting serum to $d = 1.21$ g/ml with solid NaBr and centrifuging 20 hours in the Ti-60 rotor (Beckman, Palo Alto, California) at 59,000 rpm. All solutions contained 0.01% disodium ethylenediaminetetraacetate (Na$_2$ EDTA) and 0.01% NaN$_3$ and were adjusted to pH 7.0. The background density of the isolated total lipoproteins was raised to 1.4 g/ml by adding more NaBr (0.29 g/ml lipoprotein solution), and the solution was layered under a linear 7.5% to 30% NaBr gradient in a SW-40 tube. Sample volumes were usually 2 ml or less. HDL was separated from LDL at 20°C by spinning the SW 40 rotor at either 20,000 rpm for 16 hours or at 35,000 rpm for 4 hours. Density gradient centrifugation of HDL was then carried out in a 7.5% to 20% NaBr gradient in the SW 40 rotor at 39,000 rpm, at 20°C for 48 hours, at which time isopycnic equilibrium was reached.

**Single-Step Density Gradient Ultracentrifugation**

The lipoprotein profile of mesus serum was obtained by a “single-step” density gradient ultracentrifugation according to the slightly modified procedure of Foreman et al.$^6$ The discontinuous gradient was prepared by weighing 0.5 g of sucrose into an empty SW-40 tube, then layering in sequence 5 ml 4 M NaCl, 0.5 ml serum, and 0.46 M NaCl to the top of the tube. Centrifugation was carried out at 39,000 rpm for 66 hours at 20°C at which time isopycnic equilibrium was reached. The tubes were pumped out at a rate of 1 ml/min through an ISCO UA-5 monitor (Instrumentation Specialties Co., Lincoln, Nebraska) set at 280 nm. Densities of fractions from a control gradient were determined with a Precision Density Meter, DMA-02 (Anton Paar, Graz, Austria) as previously described.$^7$ Densities of HDL determined with this method are apparently less dense than the true buoyant density as measured in the analytical ultracentrifuge probably because of preferential binding of sucrose to HDL. The areas for HDL$_2$ and HDL$_3$ were determined digitally using an Apple II microcomputer by dividing the HDL species at the density representing the local minimum between the peak densities of HDL$_2$ and HDL$_3$.

**Analytical Ultracentrifugation**

The molecular weights and the buoyant densities ($\rho_b = 1/\rho$) of HDL fractions were determined simultaneously by high speed sedimentation and flotation at three different densities at 20°C in a Model E ultracentrifuge (Beckman, Palo Alto, California) equipped with a photoelectric scanner as previously described.$^6$ Before analysis, HDL samples were diazylized against three NaBr solutions of varying density and concentration (1.5%, 20%, and 30%) each containing 0.01% Na$_2$ EDTA (pH 7.0). The partial specific volume and therefore the molecular weight of HDL determined in sodium bromide is essentially anhydrous, because Patsch et al.$^9$ have shown that $\bar{v}$ measurements obtained in that salt are identical to those executed with a Mettler-Paar precision densimeter in conjunction with dry weight concentration measurements.

**Chemical Analysis**

Lipoprotein composition was determined by measuring protein,$^{10}$ phospholipid,$^{11}$ cholesterol, both free and esterified,$^{12,13}$ triglycerides$^{14}$ as previously described.$^9$ HDL cholesterol was quantitated after the precipitation of the apo B containing lipoproteins from 1 ml serum with 40 $\mu$l heparin (5 $\times$ 10$^5$ units/l) and 50 $\mu$l 2 M MnCl$_2$. The cholesterol concentration of the supernatant was determined with the Liebermann-Burchard reaction using the Auto Analyzer II.$^{16}$ Serum cholesterol was determined on the Auto Analyzer II.$^{16}$

**Immunoassays**

Rhesus serum apo A-I and apo A-II were measured in a double antibody radioimmunoassay using antisera to human LDL, antibodies and purified iodinated rhesus apo A-I and apo A-II. The method was based on a previously described procedure for human apo A-I$^{17}$ and human apo A-II.$^{18}$ Apo A-I and apo A-II were purified from rhesus HDL as described previously.$^{19}$ Apo E was determined by electroimmunoassay$^{20}$ using specific antisera raised in the goat against rhesus apo E which was prepared from rhesus VLDL as described previously.$^{21}$
Electrophoresis

The method of Weber and Osborn\textsuperscript{22} was used for sodium dodecyl sulfate gel electrophoresis with 10% polyacrylamide gels (SDS-PAGE).

Results

HDL Density Gradient Profiles

Initially, when the animals were on the control diet, HDL consisted of two subspecies, one having a lower density (HDL\textsubscript{l}) than the other (HDL\textsubscript{h}). Both were located mainly in the HDL\textsubscript{h} density interval (Figure 1). No detectable peak, but only a trailing shoulder on the HDL\textsubscript{l} could be observed in the density region usually ascribed to human HDL\textsubscript{g}. The feeding of a high cholesterol-coconut oil-supplemented diet to two male rhesus monkeys resulted in progressive alterations of their HDL density gradient profiles. With the rise in serum cholesterol, there was an immediate change in the HDL profile caused by an increased concentration of HDL\textsubscript{l}. This increase was apparent in both monkeys after 3 to 4 days when serum cholesterol levels had risen to between 220 and 240 mg/dl. Maximal values of HDL\textsubscript{l} were reached in 10 to 11 days, at which time serum cholesterol in Monkey 6 was 410 mg/dl and in Monkey 24 was 332 mg/dl. During this time interval, the ratio of HDL\textsubscript{l} to HDL\textsubscript{h} changed from 0.25 to 0.39 in Monkey 6 and from 0.58 to 0.91 in Monkey 24 and represented a 56% increase for both. This ratio was determined by integration of the respective areas under the bimodal HDL peak. However, the increase was not permanent and returned to the basal values in both animals in 3 to 4 weeks while they were still on the high fat diet.

The increased concentration of light HDL\textsubscript{l} was not caused by the formation of an HDL\textsubscript{l}\textsubscript{-}like particle because electroimmunoassay of rhesus serum (Monkey 24) indicated that the concentration of apo E at

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

Figure 1. Alteration in the HDL density gradient profile of Monkeys 6 and 24 due to the feeding of monkey chow supplemented with 2% cholesterol and 25% coconut oil as a function of time. In each animal the data were collected during the last progression period. The lipoprotein profiles were recorded with an absorbance monitor set at 280 nm at a flow rate of 1 ml/min. The vertical line at d = 1.08 g/ml is included as a visual aid for easier observation of changes in HDL density.
baseline was only 0.045 mg/ml and in the hyperlipidemic state ranged between 0.15 and 0.25 mg/ml. Since apo E and apo A-I are the major apoproteins of HDL, the rise in concentration of apo E should have been similar to that of apo A-I, which was 10 to 20 times higher than apo E throughout the study. Furthermore, apo E was found mainly over a density interval ranging from Lp(a) to LDL with only minor quantities in the HDLc fraction (Figure 2), which indicated that the density distribution of HDLc differed from HDL.

After the concentration of HDLc had reached maximal values (in 10 to 11 days), the buoyant density of HDLc began to increase. In the 3 weeks during which the serum cholesterol had risen to over 500 mg/dl, both HDLc and HDLs were considerably denser than under control conditions. Although the serum total cholesterol values of Monkey 6 continued to rise to 745 mg/dl after 6 weeks on the hypercholesterolemic diet and the values for Monkey 24 rose to 600 mg/dl after 7 weeks, density values increased only slightly. HDLc reached a plateau at an apparent density of 1.082 g/ml and HDLs at densities ranging between 1.104 to 1.110 g/ml.

When the two monkeys were returned to a normal chow diet, their serum cholesterol regressed to normal values within 2.5 to 4 weeks. However, the HDL density gradient profile took much longer to normalize (Figure 3). In Monkey 6 a gradual decrease in HDL density was apparent 1 week after the regression started but did not become significant until the 26th week. In the case of Monkey 24, a permanent decrease in HDL density was evident after the fourth week. Normalization, however, was only reached after 26 weeks on the Purina Chow diet.

Effect of Diet on Serum Apo A-I and Apo A-II Levels

The concentration of serum apo A-I and apo A-II was determined by radioimmunoassay as a function of time over two cycles of progression and regression and is shown in Figures 4 and 5. These figures also include the serum cholesterol values taken from reference 5 to illustrate the induced cycles of hypercholesterolemia in these two animals and to serve as a point of reference. Serum apo A-I levels were generally much more variable than apo A-II. The latter appeared to be unaffected by the test diet and fluctuated approximately 30% about a value of 0.52 mg/ml for Monkey 6 and 0.46 for Monkey 24 over the course of the study.

Serum apo A-I levels were clearly altered by the administration of the test diet and its substitution with normal monkey chow during the regression period. Both monkeys exhibited similar behavior in that the density profile was

![Figure 2. SDS-PAGE (10%) of lipoprotein fractions obtained from Monkey 24 on the 8th day and 12th week after the start of the last progression. The numbers refer to fractions obtained from the "single-spin" gradients, which are indicated on the respective profiles in Figure 1. A. These gels are from the 8th day and are loaded with 20 μg protein per gel. B. This set is from the 12th week and is loaded with 40 μg protein per gel.](http://atvb.ahajournals.org/doi/fig/10.1161/HYPERTENSIONAHA.101.157022)
Figure 3. HDL density gradient profiles of Monkey 6 and Monkey 24 during the last regression phase. The experimental conditions are identical to those given in Figure 1.

Figure 4. The response of serum apo A-I, apo A-II, and cholesterol over two cycles of diet-induced hypercholesterolemia with subsequent regressions in Monkey 6. Arrow P indicates the start of the progression period and Arrow R, the regression period.

The concentration of apo A-I immediately increased 30% to 70% and reached maximal values in about 1.5 weeks upon the start of each progression period. The increase in apo A-I was temporary and lasted 6 weeks and 4 weeks, respectively, during the two progression periods that were initiated in Monkey 6, and 3 weeks for the progression periods of Monkey 24 before returning to preprogression apoprotein levels. After the initial transient rise, apo A-I levels increased at a slower rate over 10 to 20 weeks. The middle progression period of Monkey 24 differed in that apo A-I continued to decline in concentration for 8 weeks until the animal was regressed with monkey chow. The animal was not bled during this regression period because of treatment for a severe shoulder injury.

At the start of each regression period there was an immediate drop of 40% to 70% in the concentration of apo A-I that reached a minimum within 1 week in
both monkeys. However, the decrease was only transient and the apo A-I concentration returned within 1 to 2 weeks to preregression values. With the decrease in apo A-I, there was also a parallel drop in the ratio of HDL_L to HDL_H, especially in Monkey 24. Again the decrease was only transient; however, instead of returning to normal values, the ratio rose above preregression values between 2 to 3 weeks after regression. Stable ratios were achieved after 3 to 5 weeks.

HDL Cholesterol

The sera of the two test monkeys (6 and 24) were also analyzed for HDL cholesterol over an 18–25 week period at the start of the experiment (Figure 6). In Monkey 6, at the start of the progression period, there was a close association between the concentration of apo A-I and the level of HDL cholesterol. Both underwent a transient increase which occurred at the same time of the increase in the light HDL by density gradient centrifugation (Figure 1). In Monkey 24, these parameters also followed a parallel behavior during the first regression period. Both the apo A-I and HDL cholesterol decreased immediately after the return to normal monkey chow. Thus, HDL cholesterol values closely correlate with serum apo A-I levels in both monkeys so that the observed changes in apo A-I probably reflect alterations in the concentrations of HDL.

Figure 5. The response of serum apo A-I, apo A-II, and cholesterol over three cycles of diet-induced hypercholesterolemia with subsequent regressions in Monkey 24. Arrow P indicates the start of the progression period and Arrow R, the regression period.

Figure 6. A comparison of serum apo A-I concentrations to HDL cholesterol during the first progression and regression period in Monkey 24 and the first progression period in Monkey 6.

Chemical and Physical Properties of HDL

The chemical composition of the two forms of HDL_L and HDL_H of Monkey 24 were analyzed before, after 2 weeks, and after 3 months of the test diet during the last progression cycle (Table 1). In addition, molecular weights and buoyant densities were determined simultaneously by equilibrium centrifugation in solutions of NaBr (1.5% NaBr, 20% NaBr and 30% NaBr). The particle size and density of HDL_L was 3.73 x 10^5 and 1.081 g/ml respectively while HDL_H had a molecular weight of 2.47 x 10^5 and density of 1.109 g/ml. Two weeks of feeding the hypercholesterolemic diet to the animal did not change these values significantly, although there appeared to be a small increase in the buoyant density of the two HDL particles. After 3 months of the regimen, the size of HDL_L was significantly reduced to 2.80 x 10^5 with a concomitant increase in density to 1.101 g/ml. On the other hand, the molecular weight of HDL_H was not altered in spite of a significant rise in particle density to 1.129 g/ml.

Although HDL particle size and buoyant density were not greatly affected after 2 weeks of the test diet, the chemical composition was significantly changed and serum HDL cholesterol and apo A-I were maximal. Both HDLs lost phospholipids and triglycerides and gained cholesteryl esters. Free cholesterol increased only in HDL_L whereas the HDL_H maintained its allotment of free cholesterol. The protein content remained relatively unchanged with just a slight increase for the HDL_L particle.

The sum of free and esterified cholesterol molecules increased 31% in HDL_L but only 6% in the denser HDL_H. The greater cholesterol content of the HDL_L particle accounted for only part of the increased serum HDL cholesterol. A further 33% increment was due to a 56% rise in the number of HDL_L particles.

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Compositional Analysis of HDL

The precipitous decline in the content of phospholipid, and to a lesser degree of free cholesterol, per HDL particle with increasing severity of hypercholesterolemia raised the question whether these HDL species still conformed with the common lipoprotein structure in which a spherical core of cholesteryl esters and triglycerides is surrounded by a monolayer of cholesterol and phospholipid fatty acyl chains with protein and phospholipid head groups occupying the outer surface of the particle. The compositional data of the various rhesus HDLs (Table 1) was analyzed according to Shen et al.\textsuperscript{23} and was compared to the correlations between size and chemical composition of normal human lipoproteins obtained by these authors.

The space and surface fitting of the hydrophobic core of rhesus HDL are shown in Figure 7 A and B and conform reasonably well with those determined for human lipoproteins. This indicates that the variation in the total number of hydrophobic cholesteryl esters and triglycerides, on the one hand, and that of phospholipid and free cholesterol on the other, is not arbitrary but is related to the size of the particle. The packing of phospholipid and protein at the lipoprotein-water interface for rhesus HDL is shown in Figure 7 C. The slope of a line through the experimental points is identical to that obtained for human lipoproteins and indicates that the packing of phospholipid in HDL is identical in both species. However, the intercept of this line gave a molecular area of 17.6 Å\textsuperscript{2} per amino acid instead of 15.6 Å\textsuperscript{2} for human lipoproteins, which indicates that the apoproteins in rhesus HDL may be packed somewhat more loosely than

### Table 1. Physicochemical Parameters of HDL Subfractions as a Function of Time

<table>
<thead>
<tr>
<th></th>
<th>HDL&lt;sub&gt;L&lt;/sub&gt;</th>
<th></th>
<th>HDL&lt;sub&gt;H&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>2 weeks</td>
<td>3 months</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>3.73</td>
<td>3.78</td>
<td>2.80</td>
</tr>
<tr>
<td>Buoyant density</td>
<td>1.081</td>
<td>1.087</td>
<td>1.101</td>
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<tr>
<td>Equivalent radius</td>
<td>51.7</td>
<td>51.7</td>
<td>46.6</td>
</tr>
<tr>
<td>Protein (g/mol)</td>
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<td>129000</td>
<td>113000</td>
</tr>
<tr>
<td>(33.1)&lt;sup&gt;†&lt;/sup&gt;</td>
<td></td>
<td>(34.1)</td>
<td>(40.5)</td>
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<tr>
<td>Phospholipid (mol/mol)</td>
<td>183</td>
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<td>111</td>
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<tr>
<td>Free cholesterol (mol/mol)</td>
<td>50</td>
<td>56</td>
<td>30</td>
</tr>
<tr>
<td>Cholesteryl ester (mol/mol)</td>
<td>98</td>
<td>138</td>
<td>98</td>
</tr>
<tr>
<td>Triglycerides (mol/mol)</td>
<td>29</td>
<td>11</td>
<td>6.6</td>
</tr>
</tbody>
</table>

<sup>*The radius of an equivalent sphere r was calculated from the molecular weight and the density of HDL based on the assumption that these particles are spherical.</sup>

<sup>†Numbers in parentheses refer to percentage composition.</sup>

<sup>‡The following molecular weights were used in calculating the number of lipid molecules per mole HDL: phospholipid 775; free cholesterol 387; cholesteryl ester 650; triglycerides 850.</sup>

particles as determined from the area of the HDL species on the density gradient (Figure 1) whereas the concentration of the HDL<sub>L</sub> was constant in this time period. Therefore, the increase in HDL cholesterol during the transient rise was accounted for by two roughly equal processes: an expansion of the free and esterified cholesterol pool per HDL<sub>L</sub> particle and an increased number of HDL<sub>L</sub> molecules. Further feeding of the test diet resulted in additional changes in composition of HDL. Thus, HDL<sub>L</sub> underwent a small loss in protein and large decreases of all lipid components relative to the 2-week composition. When compared to baseline values, only the cholesteryl ester content was unchanged, whereas phospholipid and free cholesterol decreased by 40%, triglyceride by 77% and protein by 8%. HDL<sub>H</sub> also underwent further changes after 3 months of the test regimen. Phospholipid, free cholesterol, and triglycerides were reduced relative to control values by 28%, 23%, and 46% respectively, whereas the contents of cholesteryl ester and protein increased by 15% and 10%, respectively. The total cholesterol content of HDL<sub>H</sub> was therefore slightly higher and that of HDL<sub>L</sub>, lower when compared to the baseline composition. Although the total cholesterol carried by the two HDL species was slightly less than during the period when normal monkey chow was fed, HDL cholesterol did not fall in most cycles due to increased concentrations of serum apo A-I. We were able to confirm this for only one cycle. Because the design of this experiment required too much serum volume that was committed to the analysis of other parameters, we could not afford to continue measuring HDL cholesterol.
Discussion

Feeding diets rich in fat and cholesterol to nonhuman primates changes not only the concentration, but also the density distribution of the serum lipoproteins.1-5 Although alterations in the ratio of HDL2 to HDL3 were reported in rhesus monkeys,6-8 these observations were based on the use of density 1.125 g/ml to separate human HDL2 from HDL3. However, the extension of this operational definition to nonhuman primates has not been documented and could lead to misleading estimations of HDL subspecies.6-8 In the present report, we attempted to circumvent this problem by subjecting the sera to isopycnic density gradient ultracentrifugation. In this way we were able to sequentially follow the changes in the HDL profile occurring in two rhesus monkeys before, during, and after a hypercholesterolemic diet. However, we encountered a problem in the nomenclature of the HDL subspecies because they both floated at a density less than 1.125 g/ml and exhibited large density shifts as a result of the diet-induced hypercholesterolemia. Since HDL consisted of two subspecies throughout the study, we resorted to naming the one having a lower density, HDL-L, and the other with higher density, HDL-H. The two rhesus HDL subfractions may be the rhesus equivalent of human HDL2 and HDL3, even though our particles contained less protein and more cholesteryl esters and triglycerides than the human species and have lower buoyant densities and molecular weights. In a previous study28 we examined 22 male and 27 female normal rhesus monkeys by density gradient centrifugation and found that their HDL exhibited a bimodal behavior similar to that described in the present study. In a recent report, Morris et al.29 found a unimodal, rather than a bimodal, distribution for HDL in chow-fed monkeys. The reason for this difference in results is unclear; however, their HDL had a mean density (1.1105 g/ml) and a molecular weight (207,000) which were similar to those of the HDL-L subspecies observed in our studies. During the more advanced stage of the hyperlipidemia (e.g., after 3 months of feeding the test diet), the physical and chemical properties of our two HDL species also began to approach those of human HDL2 and HDL3 (see Table 1). Relative to the control HDL species, the increased density observed in both particles and the reduction of the HDL-L size were caused by an...
almost parallel loss of surface components, namely, phospholipid and free cholesterol and core triglycerides and by only minor changes in the content of cholesteryl ester and protein. Although there were great differences in the chemical makeup of the HDL species, depending on the severity of the hypercholesterolemia, compositional analysis according to Shen et al.23 indicated that accepted lipoprotein structure was maintained.

A transient rise in HDL concentration was previously reported on feeding high fat, high cholesterol diets to rhesus,1 sooty mangabey,2 and spider monkeys.30 The increase in HDL that we observed was not due to the generation of HDLc particles, which have been well described by the work of Mahley et al.31 but was caused by increased HDLr and was attended by a parallel rise of apo A-I, but not apo A-II. Thus, serum apo A-I is much more sensitive to dietary manipulation than apo A-II. This conclusion is in keeping with previous human studies32 and the work of Parks and Rudel33 who found that neither synthesis nor degradation of serum apo A-II in vervets was influenced by diet. Egg consumption in humans was shown to lead to increased plasma HDL cholesterol which is entirely due to increased HDLr accompanied by an increase in apo A-I, but not apo A-II.34

One possible explanation for the rise in apo A-I in our rhesus monkeys is an increased synthesis (intestinal, hepatic, or other). With regard to the intestine, chylomicrons contribute a significant portion of apo A-I to plasma HDL.35,36 Since Parks and Rudel33 found that chylomicrons of vervets fed a high fat (either saturated or unsaturated), high cholesterol diet contained apo A-I, an increased chylomicron influx into the circulation could account for the plasma elevation in apo A-I. However, these authors also found that chylomicrons contained equivalent amounts of apo A-II. Thus, chylomicron metabolism alone may not explain the divergent behavior of apo A-I and apo A-II that we observed in our animals. Obviously, we need to assess the contributions by both liver and intestine.

Alternatively, the rise in serum apo A-I level or HDLr could be caused by a decreased degradation of apo A-I. Schaefer et al.37 established recently that the residence time of apo A-I in humans was positively correlated with the level of HDLr which approaches the size and density of HDLc in our rhesus monkey. Another factor which favors this concept is the fact that HDLr contains particles that have apo A-I as the main apolipoprotein with very little or no apo A-II.38,39 We may speculate that the removal of these HDLs occurs either wholly or in part via the apo A-I receptor that have been described in the liver,40 lymphoblastoid cells,41 and steroidogenic tissues.40 This may no longer be true when with continuation of the high fat and cholesterol-enriched diet serum cholesterol levels approach 400 mg/dl. If these HDL species, and especially HDLr, become enriched with cholesterol to the degree that they acquire apo E, these particles would be removed by the HDLr-apo E receptor, which is a relatively fast process compared to that ensured by the apo A-I receptor, and lower levels of HDLr may be expected.

It has been shown that pronounced hypercholesterolemia with serum cholesterol levels above 400 mg/dl is marked by the appearance of significant amounts of cholesteryl ester enriched β-VLDL (very low density lipoprotein) and pre-β-VLDL42 and the end of the transformation of LDL into the larger and less dense cholesteryl ester-enriched hyperlipidemic LDL5. These particles are structurally suited for uptake by B, and E receptors. At this stage, the HDL species finished their progressive shift to higher density and are relatively unimportant in comparison to LDL, β-VLDL, and pre-β-VLDL as vehicles for cholesterol transport. Why denser HDLs accumulate in the plasma during overt hypercholesterolemia is unclear. Cholesteryl esters may now be synthesized in increased amounts by either the liver or intestine and appear in the circulation as members of β-VLDL and pre-β-VLDL.42,43 As a consequence, there is less generation of surface components (apolipoproteins, phospholipids, free cholesterol) through the action of lipoprotein lipase on these relatively triglyceride-poor particles to insure the transformation of dense to light HDL particles.

Based on the above and previous observations, we envisage that animals exhibiting a progressive elevation of plasma cholesterol as a response to a hypercholesterolemic diet undergo an early HDL stage where HDL particles assume a major role in the transport of LCAT-produced cholesteryl esters and their removal from plasma (total cholesterol below 400 mg/dl). With the continuing influx into the circulation of cholesteryl esters from liver and intestine, apo B and apo E containing particles take care of the increased cholesterol mass. In animals that are low responders, we would expect to see only the early HDL stage. Recent studies conducted in this laboratory on rhesus monkeys fed a 15% lard, 0.25% cholesterol, diet support this conclusion (unpublished observations). Thus, it is apparent that there is a close interplay between levels of plasma cholesterol and functional participation of plasma lipoproteins in cholesterol transport. Rudel et al.44 investigated the effect of dietary cholesterol and saturated fat on the HDL cholesterol concentration in two subspecies of African green monkeys. They found that animals responding with a moderate hypercholesterolemic serum (200–400 mg/dl) often exhibited a significant elevation of HDL cholesterol. In turn, animals that were maximally responsive to their dietary regime had most of their plasma cholesterol transported in the low density lipoprotein class.

Our observation that serum cholesterol levels return to baseline before normalization of the HDL profile deserves a comment. Either the factors controlling the interconversion among plasma lipoproteins are not fully operative in the immediate reversal stage of the diet-induced hypercholesterolemia or a
complete equilibration between plasma and tissue cholesterol pool has not yet taken place. A more detailed investigation of this stage of regression is worth undertaking.

As a final comment, we realize that the changes induced in these animals were the consequence of a diet containing both saturated fat and cholesterol. Thus, we are not in a position to assess whether the effects are due to either of these components or due to their synergistic interaction. We tend to favor the latter hypothesis based on data by Ershow et al. who showed that neither coconut oil nor cholesterol substantially altered rhesus plasma cholesterol concentration or lipoprotein profile. Studies to answer these questions unequivocally are now being conducted in this laboratory.

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References

32. Zech LA, Schaefer EJ, Bronzert TJ, Aamodt RL, Brewer
42. Lusk L, Chung J, Scanu AM. Properties and metabolic fate of two very low density lipoprotein subfractions from rhesus monkey serum. Biochim Biophys Acta 1982;710:134–142

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