Comparative Study of Density Distribution of Plasma Lipoproteins of Normo- and Hypercholesterolemic Rhesus Monkeys and Humans

Gunther M. Fless and Angelo M. Scanu

Density gradient centrifugation was used to characterize the lipoprotein distribution of rhesus monkeys and human subjects with normal and elevated cholesterol levels. The lipoprotein profile of control monkeys differed from that of normal humans in that their density distribution as a whole was shifted to lower density. The most striking difference was that both rhesus high density lipoprotein (HDL) subspecies had densities lower than human HDL\textsubscript{3}, with one component having a lower, and the other a higher, density than human HDL\textsubscript{2}. Rhesus monkeys fed a diet supplemented with 0.5% cholesterol and 15% lard were divided into two groups. Those animals with cholesterol levels less than 435 mg/dl had a high apo A-I concentration and HDL subspecies similar to human HDL\textsubscript{2} and HDL\textsubscript{3}, whereas those with concentrations greater than 435 mg/dl had a low apo A-I concentration and HDL species with a density either similar to, or exceeding that, of human HDL\textsubscript{2}. Low density lipoprotein (LDL) density decreased in both groups of hypercholesterolemic monkeys and was particularly pronounced in animals with cholesterol levels above 435 mg/dl. When the lipoprotein profiles of normal humans were compared with those having hypercholesterolemia, increases in the density of both HDL\textsubscript{2} and HDL\textsubscript{3} in hypercholesterolemic men and to a lesser extent in the density of HDL\textsubscript{2} in women were detected. The results indicate that the overall density distribution of plasma lipoprotein is different between rhesus monkeys and humans, and may vary within each species as a function of the nutritional status. It follows that it is difficult to define lipoprotein classes of one species by using density intervals determined for another. Furthermore, these intervals can be inadequate in identifying lipoprotein classes within an individual before and after dietary manipulations. (Arteriosclerosis 6:88–97, January/February 1986)

Plasma lipoproteins are complex assemblies of lipids and proteins whose proportions vary according to the nutritional and metabolic state of the individual animal or human. In some individuals, this may lead to important alterations of the density distribution of lipoproteins within, and in some cases to density shifts outside, the conventional density class limits which have been developed for normolipidemic human subjects.

From the Departments of Medicine, Biochemistry and Molecular Biology, University of Chicago, Pritzker School of Medicine, Chicago, Illinois.

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Address for reprints: Gunther M. Fless, Ph.D., Department of Medicine, Box 81, University of Chicago, 5841 South Maryland Avenue, Chicago, Illinois 60637.

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During a recent study of the longitudinal changes in lipoproteins of two rhesus monkeys fed a high-fat, high-cholesterol diet, we observed important density shifts in their plasma lipoproteins as a function of the response of these animals to a high-fat diet.\textsuperscript{1,2} Furthermore, when these animals were normolipidemic during baseline studies, it appeared that the density of rhesus high density lipoprotein (HDL) was different from that of humans.\textsuperscript{2} The HDL class had bimodal behavior by density gradient centrifugation and banded in general at densities less than 1.125 g/ml, a normally accepted density cut for human HDL\textsubscript{2}.\textsuperscript{3} The issue of the structural relationships between plasma lipoproteins in rhesus monkeys and humans is yet unresolved, and to our knowledge no systematic study in this direction has been carried out, particularly in terms of comparing normo- and hypercholesterolemic states. In this report, we have
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addressed this question by studying a large group of normo- and hypercholesterolemic rhesus monkeys from a single colony and comparing them with a human population. We will show that there are important differences in the density distribution of the lipoproteins between these two species and that these differences are affected by the nutritional state of the animals and the levels of their cholesterol in plasma, thus emphasizing the inadequacy of the traditional density intervals used in defining lipoprotein classes.

Methods

Monkeys

Rhesus monkeys were housed at Litton Bionetics (Kensington, Maryland) while that Institution was serving as one of the national resource centers for nonhuman primates under the auspices of the National Blood and Heart Institute. All procedures used in these studies were in accordance with institutional guidelines concerning use of experimental animals. The animals were fed either a regular Purina primate chow diet or a diet of regular monkey chow supplemented with 15% lard and 0.5% cholesterol. Both diets were prepared by Ralston Purina (Richmond, Indiana). The singly caged animals were fed twice a day, and each feeding contained 12.5 g chow/kg body weight. Both the control and test groups had been fed their respective diets for 3 to 4 years at the time that the blood was taken. The age of the animals is given in Table 1. Blood was taken from nonanesthetized monkeys and placed into tubes containing at a final concentration 0.12% Na₂ EDTA and 0.01% NaN₃ in addition to gentamicin sulfate (0.8 mg/10 ml blood), chloramphenicol (0.8 mg/10 ml blood), and kallikrein inhibitors (80 units/10 ml blood). Plasma was immediately obtained from blood by low-speed centrifugation and shipped to Chicago within 24 hours on wet ice.

Human Subjects

Human subjects examined in this study were from a population of male and female normolipidemic donors recruited in relation to general studies on lipoprotein structure and function. They were students and staff of the University and ranged in age from 20 to 40 years. The hypercholesterolemic subjects were selected from a patient population referred to the Lipid Clinic of the University of Chicago; the men had a mean of 56.6 ± 18.1 years, and the women, 58.7 ± 17.8 years. They were not being treated with cholesterol-lowering agents at the time of examination. Blood was taken after overnight fasting from the antecubital vein and plasma was prepared as above. All human subjects gave informed consent in accordance with institutional guidelines.

Table 1. Weight, Age, Plasma Cholesterol, Triglyceride and Apoprotein Concentrations of Male and Female Rhesus Monkeys

<table>
<thead>
<tr>
<th>Monkey group</th>
<th>Gender</th>
<th>Weight (kg)</th>
<th>Age (yr)</th>
<th>Cholesterol (mg/dl)</th>
<th>Triglyceride (mg/dl)</th>
<th>Apo B (mg/dl)</th>
<th>Apo A-I (mg/dl)</th>
<th>Apo A-II (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Male</td>
<td>10.4±2.3</td>
<td>11.3±2.8</td>
<td>121.2±15.5</td>
<td>48.5±22.3</td>
<td>36.5±13.0</td>
<td>108.2±23.2</td>
<td>39.2±9.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(8.2-16.2)</td>
<td>(9-19)</td>
<td>(101-142)</td>
<td>(22-81)</td>
<td>(24-70)</td>
<td>(68-140)</td>
<td>(24-63)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>6.3±0.9</td>
<td>12.0±3.2</td>
<td>166.7±44.2†</td>
<td>56.1±30.0</td>
<td>39.8±15.5</td>
<td>152.9±38.2†</td>
<td>42.7±15.2</td>
</tr>
<tr>
<td>Group 1</td>
<td>Male</td>
<td>10.8±1.6</td>
<td>12.5±4.6</td>
<td>340.7±91.6</td>
<td>35.1±24.6</td>
<td>66.6±27.6†</td>
<td>162.8±40.4†</td>
<td>25.0±8.9†</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>6.0±0.8</td>
<td>11.4±3.6</td>
<td>299.4±75.8</td>
<td>51.0±57.1</td>
<td>70.7±26.4‡</td>
<td>158.7±75.7</td>
<td>24.4±12.2‡</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(4.9-8.1)</td>
<td>(7-19)</td>
<td>(183-435)</td>
<td>(5-269)</td>
<td>(24-114)</td>
<td>(65-340)</td>
<td>(4-51)</td>
</tr>
<tr>
<td>Group 2</td>
<td>Male</td>
<td>9.8±2.0</td>
<td>11.7±2.7</td>
<td>665.6±119.2</td>
<td>44.3±44.4</td>
<td>112.6±20.0§</td>
<td>74.9±30.3†</td>
<td>20.2±8.5‡</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>6.8±1.7</td>
<td>12.9±4.0</td>
<td>639.4±143.3</td>
<td>79.5±59.3</td>
<td>129.2±48.4‡</td>
<td>77.3±18.3‡</td>
<td>17.0±11.5‡</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(4.6-10.0)</td>
<td>(8-19)</td>
<td>(457-940)</td>
<td>(20-232)</td>
<td>(51-198)</td>
<td>(61-103)</td>
<td>(4-37)</td>
</tr>
</tbody>
</table>

Values are means ± sd, with the range in parenthesis. The values given below range are the number of animals.
* p 0.005, male different from female.
† p 0.05, different from respective control.
‡ p 0.005, different from respective control.
§ p 0.001, different from respective control.
Single-Step Density Gradient Ultracentrifugation

The lipoprotein profiles of rhesus plasma and human plasma were obtained according to the method of Nilsson et al.4 The discontinuous gradient was prepared by weighing 0.5 g of sucrose into an empty SW-40 tube, then by layering in sequence 5 ml 4 M NaCl, 0.05% Na2 EDTA, 0.5 ml plasma, and 0.67 M NaCl, 0.05% Na2 EDTA to the top of the tube. Centrifugation was carried out at 39,000 rpm for 66 hours at 15° C, at which time isopycnic equilibrium was reached. The tubes were pumped out at a flow rate of 1 ml/min through an ISCO-UA-5 monitor (Instrumentation Specialties Company, Lincoln, Nebraska) which was set at 280 nm. Fractions (0.4 ml) were collected, and those corresponding to Lp(a) were dialyzed against saline and were analyzed electrophoretically. Densities of fractions from a control gradient were determined with a Precision Density Meter, DMA-02 (Anton Paar, Graz, Australia) as previously described. Densities of lipoproteins determined by this method deviate from the true values obtained in a two-component system at 20° C because of preferential binding of sucrose by lipoproteins and variation of the density of lipoproteins with temperature.5

Frequency Distribution

We evaluated the lipoprotein profiles by measuring the distance from the meniscus to each absorbance peak of every lipoprotein species irrespective of its concentration. In the case where lipoprotein components appeared as unresolved shoulders, we measured the distance from the meniscus to the peak maximum of the shoulder (inflection point of the first derivative of the curve) as was done by Blanche et al.6 To check the validity of this method, we isolated narrow cut fractions (tube containing peak center and one tube to either side of the peak) of HDLc and HDLs by single-spin density gradient centrifugation from the plasma of an individual whose lipoprotein profile is depicted in Figure 1D. Upon recentrifugation, these isolated HDLc and HDLs fractions banded in the same position on the gradient and underwent no change in density. These results indicated that the procedure used for determining lipoprotein density was satisfactory and that there was no redistribution of material from these fractions that could imply lipoprotein breakdown.

The chart length was usually 140 mm and did not vary by more than 1.5 mm. If the chart length deviated from 140 mm, the peak distances were normal-

Figure 1. Representative "single-spin" density gradient profiles of normolipidemic humans (A–D) and hypercholesterolemic humans (E–H). Two vertical lines were added to each lipoprotein profile as a visual aid. They were placed in the same location as in Figure 2. The arrows define peak lipoprotein densities.
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ized. The standard deviations of the peak distances of low density lipoprotein (LDL), Lp(a), HDL, and HDL subpopulations of human plasma from a single subject that was centrifuged in sextuplicate were 1.06 mm, 1.20 mm, 1.11 mm, and 0.97 mm, respectively. The peak distances from each individual were tabulated and used to construct the frequency distribution. Each lipoprotein profile was divided into 70 intervals of 2 mm each, and the number of observations falling into each interval was plotted in a histogram.

Since we were measuring the lipoprotein distribution of females, the possibility existed that lipoprotein densities could vary as a function of their menstrual cycles. To answer this question, we determined the lipoprotein profiles of one control and two hypercholesterolemic female monkeys on the test diet every sixth day during four consecutive menstrual cycles. We were unable to detect consistent cyclical changes in any of our three female monkeys. Based on the analysis of 24 density gradients per animal, the standard deviations for peak densities of LDL, Lp(a), low density HDL subspecies, and high density HDL subspecies ranged from 1.0 to 2.4 mm, 1.2 to 2.1 mm, 1.7 to 1.9 mm, and 1.2 to 1.4 mm, respectively. These standard deviations correspond to coefficients of variation of 2% to 8% for LDL and 1% to 3% for HDL. The coefficient of variation obtained for LDL is larger than that of HDL because the density gradient is more shallow at the top of the tube than at the bottom and therefore amplifies changes in peak position of LDL on the chart paper as a result of changes in peak density. Overall, the standard deviations took into account both biological and technical variance, and this indicates that the single-spin centrifugation is highly reproducible and that the lipoprotein densities are remarkably invariant with time.

Because feeding of the high fat-high cholesterol diet to the rhesus monkeys resulted in profoundly altered lipoprotein profiles, it was necessary to subdivide the animals to better describe the progressive changes seen with increasing hypercholesterolemia. However, to avoid making arbitrary divisions among the animals, we measured the plasma concentration of several apoproteins to see if one or more of these parameters could be used to segregate the monkeys into different groups. The levels of cholesterol, triglyceride, apo B, apo A-I, and apo A-II, along with the weight and age of male and female monkeys, are listed in Table 1. Because these determinations were done at a later time than the lipoprotein profiles, plasma from some of the male control animals was unavailable for chemical analysis, as was plasma from many females for apo A-I determinations.

The comparison of plasma cholesterol, triglyceride, and apoprotein concentrations of male and female monkeys revealed few sex-linked differences. Only the total cholesterol and apo A-I values were significantly higher in control females; the higher cholesterol values agreed with those in a previous report. The concentrations of apo B increased progressively in all monkeys, whereas those of apo A-II decreased with increasing cholesterol levels and were therefore not useful in dividing the animals. However, unlike the other two apoproteins, the concentration of apo A-I did not vary consistently with plasma cholesterol. Thus, at moderate cholesterol levels, apo A-I increased in male monkeys and remained high in females, but decreased below control values in both male and female monkeys with high cholesterol. Therefore, upon examination of the lipoprotein profiles in conjunction with the apo A-I values, we chose a cholesterol level of 435 mg/dl to divide the hypercholesterolemic monkeys into two groups.

Chemical Analysis

Plasma cholesterol and triglyceride were determined on the Auto Analyzer II. Rhesus apo B, apo A-I, and apo A-II were measured in double antibody radioimmunoassays as previously described.

Electrophoretic Methods

We performed SDS-PAGE using 3.5% gels in 6 mm tubes according to the method of Weber and Osborn. We carried out gradient gel electrophoresis in the presence of SDS according to the methods outlined by Pharmacia and described by us previously. By running the gels in the absence of reducing agents, we identified apo Lp(a) because of its characteristically lower mobility when compared to apo B-100.

Results

Normolipidemic Rhesus Monkeys and Humans

The control group consisted of 22 male and 27 female monkeys that were fed Purina Monkey Chow. Male monkeys had a mean plasma cholesterol level of 122 mg/dl, whereas female animals had a mean value of 162 mg/dl. By density gradient ultracentrifugation, their plasma lipoprotein profiles varied considerably, as indicated by the representative patterns in Figure 2 A-D. One of the two vertical lines in Figure 2 added as a visual aid marks the density below which no Lp(a) peaks were found in humans; the other line marks the mean density of the denser HDL subspecies of the control rhesus monkeys. Most animals had LDL peaks that were quite small when compared to HDL: this was particularly evident in monkeys with cholesterol levels less than 100 mg/dl. Thus, the lipoprotein profile of rhesus monkeys was dominated by a large HDL peak. Lipoprotein (a), on the other hand, was often relatively prominent in comparison to LDL, as indicated by the large absorbance peaks in some of the animals (Figure 2 A-C).

Figure 2 also shows that the lipoprotein profiles of control monkeys were notably different when they are compared to those of normolipidemic human subjects (Figure 1 A-D). Rhesus HDL was lower in density than human HDL. The average peak density...
of the heavier HDL subspecies was intermediate between human HDL₂ and HDL₃. Also, the absorbance peak due to LDL was smaller in monkeys than human subjects. Furthermore, rhesus Lp(a) was usually lower in density than human Lp(a).

There were obvious differences in the density absorbance profiles between rhesus and human lipoproteins. However, both monkeys and humans exhibited considerable individual variation. Thus, to properly compare the density profiles of the lipoproteins between these two species, we determined their frequency distribution by plotting the peak densities of all lipoprotein subspecies on the absorbance-density lipoprotein profiles against their frequency of observation (Figure 3). Since the number of rhesus monkeys was limited, we combined the data from both male and female animals in order to get a meaningful frequency distribution. To be consistent, we plotted the data from the human subjects in the same way, although the sample size was large enough to separate males from females. The frequency distribution of lipoprotein peak densities obtained for control rhesus monkeys with that of normolipidemic humans. The distribution for monkeys was determined from the density gradients of 22 male and 27 female monkeys. The human distribution was calculated from the profiles of 93 males and 47 females. Human (—) rhesus (-----) frequency distribution.

Figure 2. Representative "single-spin" density gradient profiles of normolipidemic rhesus monkeys (A–D) and hypercholesterolemic monkeys (E–H). Two vertical lines were added to each lipoprotein profile to act as a visual aid (see text) in comparing the lipoprotein profiles of rhesus monkeys and humans (Figure 1). HDL₃ and HDL₄ refer to the HDL subspecies of low and high density, respectively. The arrows define peak lipoprotein densities.

Figure 3. Comparison of the frequency distribution of lipoprotein peak densities obtained for control rhesus monkeys with that of normolipidemic humans. The distribution for monkeys was determined from the density gradients of 22 male and 27 female monkeys. The human distribution was calculated from the profiles of 93 males and 47 females. Human (—) rhesus (-----) frequency distribution.
quency distribution of rhesus LDL was broad, ranging from \( d = 1.032 \) g/ml to \( d = 1.057 \) g/ml, and did not segregate into well-resolved peaks. On the other hand, the distribution of human LDL was characterized by four maxima that had densities of 1.042, 1.047, 1.050, and 1.055 g/ml, respectively. The densest human LDL subfraction (\( d = 1.055 \) g/ml) did not appear to have an equivalent in the rhesus monkeys because it was located at a density that was the minimum in frequency distribution between rhesus LDL and Lp(a). Overall, the frequency distribution of rhesus LDL peaks was situated at a lower density than LDL species of humans. Most of rhesus Lp(a) was distributed in one major peak at \( d = 1.061 \) g/ml and a possible minor peak at \( d = 1.074 \) g/ml. The distribution of human Lp(a) was broad and consisted of several species, most of which were denser than their rhesus counterpart.

The frequency distribution of rhesus HDL showed two well-resolved peaks located at \( d = 1.061 \) g/ml and \( d = 1.117 \) g/ml. On the other hand, the distribution of human HDL showed three maxima; HDL\(_{L2}\) peaked at \( d = 1.107 \) g/ml and thus had a density intermediate between the two rhesus HDL subspecies. HDL\(_{L1}\) was denser than either of the two rhesus HDL subspecies and its frequency distribution had two maxima: one at \( d = 1.139 \) g/ml and the other at \( d = 1.150 \) g/ml.

### Hyperlipidemic Rhesus Monkeys

The 32 male and 41 female monkeys that were fed Purina Monkey Chow supplemented with 15% lard and 0.5% cholesterol had profoundly altered lipoprotein profiles. As shown by the representative lipoprotein patterns in Figure 2 E–H, progressive alterations of the absorbance-density profiles were observed with increasing plasma cholesterol. At moderate plasma cholesterol levels, many of the profiles were characterized by elevated levels of the low density HDL subspecies (Figure 2 E and F). This was especially true in female monkeys. In numerous monkeys, LDL was shifted to lower density, while HDL species denser than control HDL became apparent (Figure 2 E). Animals with high cholesterol levels had generally a reduced concentration of HDL species of an increased density (Figure 2 G and H). LDL was usually elevated and banded at a lower density. In some cases, LDL was shifted into the VLDL and LDL density region; in turn, the HDL density had increased to such an extent that it banded with the bottom fraction, thus creating a lipoprotein profile that was essentially devoid of peaks (Figure 2 G).

The frequency distribution of the lipoprotein peak densities from the animals given the high fat diet was determined after dividing the animals into two groups: those with low-to-moderate cholesterol levels were placed in Group 1, and those with high cholesterol levels in Group 2. A plasma cholesterol level of 435 mg/dl was chosen as the dividing line between these two groups because above this cholesterol concentration there was no gross elevation of plasma apo A-I and no increased concentrations of the low density relative to the high density HDL subspecies. Although the number of monkeys was limited, the frequency distribution for those in Group 1 clearly show that as a result of the test diet, HDL subspecies shifted substantially to a higher density, while LDL became less dense (Figure 2 B). These shifts were even more pronounced in Group 2, as indicated by the frequency distribution of their lipoprotein peak densities (Figure 2 C). These data, which are given in Table 2, show that only 14% of male and 11% of female control animals had LDL species that banded in the density range of 1.027 to 1.035 g/ml as compared to 29% and 36% observed in the male or female monkeys of Group 1. Furthermore, in Group 2, 84% of the males, but only 56% of the females, exhibited these lower density LDL spe-

<table>
<thead>
<tr>
<th>Lipoprotein fraction</th>
<th>Density range (g/ml)</th>
<th>Control monkeys</th>
<th>Hypercholesterolemic monkeys</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL</td>
<td>1.027–1.035</td>
<td>Male (%)</td>
<td>Female (%)</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>57</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>1.036–1.046</td>
<td>95</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td>1.047–1.056</td>
<td>55</td>
<td>63</td>
</tr>
<tr>
<td>Lp(a)</td>
<td>1.052–1.056</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>1.057–1.065</td>
<td>64</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>1.066–1.085</td>
<td>28</td>
<td>15</td>
</tr>
<tr>
<td>HDL</td>
<td>1.086–1.103</td>
<td>69</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>1.104–1.122</td>
<td>100</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>1.123–1.161</td>
<td>100</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>1.162–</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

TC = total cholesterol.
In the case of HDL, none of the male and 7% of the female animals had HDL subspecies with a peak density between 1.123 and 1.161 g/ml. However, in Group I, all male monkeys and 80% of the female monkeys had HDL subspecies in this density range. A further increase in density of HDL subspecies above 1.162 g/ml occurred in 44% of the male and 38% of the female animals of Group 2. Only 4% to 8% of either male or female monkeys exhibited HDL that still banded in the density range of control HDL. In the case of Lp(a), we did not have enough animals to clearly show whether there was a shift in density as a function of diet (Figure 4). Furthermore, the spreading of band width of the Lp(a) peak in many of the hypercholesterolemic animals (see Figure 2 G) made it difficult to accurately measure the Lp(a) peak density. Nevertheless, there appeared to be a shift of Lp(a) toward lower density in the females of Group 2. In the case of the male animals, however, the results were inconclusive.

Hypercholesterolemic Humans

The frequency distribution of lipoprotein peak densities was also determined in a population of human donors with elevated plasma cholesterol levels and the results were compared to normolipidemic humans. The plasma total cholesterol and triglyceride concentrations of normolipidemic male and female subjects, including those with hypercholesterolemia, are given in Table 3. A value of 210 mg/dl for total cholesterol and 150 mg/dl for triglyceride was chosen as the upper limit for the normal subjects. Cholesterol and triglyceride values were similar in males

<table>
<thead>
<tr>
<th>Group</th>
<th>Total cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normolipidemic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>166.4 ± 28.1 (92-210)</td>
<td>86.9 ± 33.8 (32-150)</td>
</tr>
<tr>
<td>Females</td>
<td>171.6 ± 26.1 (96-210)</td>
<td>79.0 ± 31.9 (18-150)</td>
</tr>
<tr>
<td>Total</td>
<td>168.1 ± 27.4</td>
<td>84.2 ± 33.2</td>
</tr>
<tr>
<td>Hypercholesterolemic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>257.6 ± 49.7 (211-417)</td>
<td>104.4 ± 29.6 (26-150)</td>
</tr>
<tr>
<td>Females</td>
<td>259.3 ± 53.8 (211-460)</td>
<td>94.1 ± 25.1 (39-150)</td>
</tr>
<tr>
<td>Total</td>
<td>258.3 ± 51.5</td>
<td>99.9 ± 27.7</td>
</tr>
</tbody>
</table>

Values are means ± sd, with the range given in parentheses. The number of subjects is given below the range.

Hypercholesterolemic Humans

The frequency distribution of lipoprotein peak densities of human populations: A. Comparison of normolipidemic humans (93 males and 47 females) with hypercholesterolemic humans (49 males and 38 females). The normal distribution is indicated by the solid line; that for hypercholesterolemic humans is indicated by the dotted line. B. Comparison of 93 normocholesterolemic males (solid line) with 49 hypercholesterolemic males (dotted line). C. Comparison of 47 normocholesterolemic females (solid line) with 38 hypercholesterolemic females (dotted line).
and females as well as in the normolipidemic and hyperlipidemic groups. Neither LDL nor Lp(a) subclasses as a whole appeared to be shifted in density in the hypercholesterolemic subjects (Figure 5 A), although it appears that the LDL subspecies located at 

\[ d = 1.047 \text{ g/ml} \]

in normal humans may have decreased in density to 

\[ d = 1.045 \text{ g/ml} \]

in the hypercholesterolemic subjects. However, the density distribution of HDL was clearly shifted to higher values. The density of HDL increased from 

\[ d = 1.107 \text{ g/ml} \]

to 

\[ d = 1.111 \text{ g/ml} \]

and the density of the heavier HDL sub-subspecies, from 

\[ d = 1.148 \text{ g/ml} \]

to 

\[ d = 1.155 \text{ g/ml} \].

On the other hand, the lower density HDL subspecies of the hypercholesterolemic subjects appeared to be unaffected. When the frequency distributions were analyzed according to sex, it became evident that the changes in HDL density of the hypercholesterolemic population as a whole were due primarily to a shift in the HDL peak densities of the male population (Figure 5 B). No apparent differences were noted in the frequency distribution of normo- and hypercholesterolemic females (Figure 5 C).

### Discussion

In the present study, we utilized the technique of density gradient ultracentrifugation to characterize the plasma lipoproteins of monkeys fed normal and hypercholesterolemic diets and compared the results with a group of normal and hypercholesterolemic human subjects. Analysis of the frequency distribution indicated that the densities of rhesus lipoproteins were generally lower than those of humans. Perhaps the most striking difference was that both rhesus LDL subspecies had mean peak densities less than 

\[ 1.125 \text{ g/ml} \]

which were either lower or higher than the density of human HDL. Using our "single-spin" density gradient centrifugation to analyze a normocholesterolemic and a hypercholesterolemic human population, we detected only one HDL component with a mean density of 

\[ 1.107 \text{ g/ml} \].

However, a second HDL component with a density of 

\[ 1.101 \text{ g/ml} \]

became prominent in hypercholesterolemic subjects. This would agree with the results of a study of 12 normal subjects by Krauss and Burke, who identified multiple distinct density intervals.

The evaluation of the lipoprotein profiles of the hypercholesterolemic monkeys indicates that with increasing serum cholesterol concentrations, these profiles deviated more and more from those of the control monkeys. In addition, it was quite apparent that the profiles of monkeys with moderate plasma cholesterol levels and exhibiting increased levels of low density LDL subspecies were different from those monkeys with high cholesterol levels. In these animals, the lipoprotein profile was often flat in the middle, having only a large LDL peak at the top and a small HDL peak at the bottom of the gradient (Figure 2 G). By dividing the monkeys on the high-fat high-cholesterol diet into two groups, we were able to obtain a better frequency distribution of lipoprotein

Nelson and co-workers also found that rhesus HDL (\( d = 1.109 \text{ g/ml} \)) is lighter in density than human HDL (\( d = 1.129 \text{ g/ml} \)) but that it exhibits a unimodal flotation peak in the analytical ultracentrifuge. However, this is not in conflict with our findings, since flotation analyses are less sensitive detectors of heterogeneity than density gradient centrifugation. In fact, Melchior et al. found a bimodal distribution for the HDL of two rhesus monkeys with densities that correspond to our two HDL subspecies, respectively. Contrary to our present study, however, their findings suggested that the HDLs of primates are similar in all species. We consider this conclusion to be only partially correct because we show here that the density distribution of lipoproteins of rhesus monkeys is clearly different from that of humans.

This difference in distribution between rhesus monkeys and humans also applies to Lp(a) in that the rhesus species had two maxima located at 

\[ d = 1.061 \text{ g/ml} \]

and 

\[ d = 1.073 \text{ g/ml} \],

whereas the human particles had several maxima at higher densities. These observations also agree with previous studies indicating that in the rhesus monkey and in humans, Lp(a) is heterogeneous. In those studies, the frequency distribution of rhesus LDL peak densities was not sufficiently resolved to allow for a precise assignment of densities of the various LDL sub-subspecies. However, the broad distribution of LDL peak densities implies that LDL was heterogenous, which is in keeping with our present findings (Figure 2) and previous findings showing that rhesus LDL contains at least two different subspecies. Overall, rhesus LDL appears to deviate in several respects from human LDL. We found no rhesus equivalent for the densest human LDL subspecies which floats at 

\[ d = 1.056 \text{ g/ml} \]; in addition we found a high percentage of monkeys with LDL species having densities lower than those of humans. The LDL of the normal human subjects examined in this study had a frequency distribution that exhibited four maxima as did the LDL from hypercholesterolemic subjects. This would agree with the results of a study of 12 normal subjects by Krauss and Burke, who identified multiple subclasses of LDL that could be assigned to four distinct density intervals.
peak densities for each group because the combining of all animals into one group obscured and averaged out the progressive changes in lipoprotein densities seen with increasing cholesterol levels. An examination of Figure 2 E and Table 2 reveals the dramatic shift of HDL to higher density and, to a lesser extent, of LDL to lower density in monkeys with moderate plasma cholesterol elevation. These changes became even more pronounced in monkeys that had higher cholesterol levels. The frequency distribution of HDL of Group 1 monkeys coincided with that of human HDL2 and HDL3. However, Group 2 animals had a HDL subspecies with a density of 1.170 g/ml, which is substantially higher than the denser of the two human HDL3 subspecies (d = 1.150 g/ml). LDL, on the other hand, was considerably less dense in Group 1 monkeys than in humans since the frequency of LDL species with densities higher than 1.047 g/ml was very low. The LDL species from Group 2 monkeys had maxima at densities of 1.033 g/ml and 1.037 g/ml which are lower than the density range in which human LDL is found (d = 1.040 g/ml to d = 1.060 g/ml).

The alteration of lipoprotein density of cholesterol-fed rhesus monkeys is real and is caused by changes in the number of components making up the lipoprotein particles. Thus, the physicochemical characterization of LDL subspecies obtained from the plasma of monkeys receiving a high-fat-cholesterol diet indicated that their decreased density and larger size were due to an increased content of free cholesterol and cholesteryl ester and reduced amounts of phospholipid and triglyceride. Similar chemical changes occurred in Lp(a), although without the increase in size and concentration seen with LDL. We have also shown that higher HDL density with concomitant smaller particle size of rhesus monkeys receiving the same diet was caused by a lower content of phospholipid, free cholesterol, and triglyceride.

The increase in HDL density found when rhesus monkeys were fed a high-cholesterol high-fat diet was not immediate and, depending on the animal under study, might take from a few weeks to several months. Thus, two monkeys receiving a 2% cholesterol, 25% coconut oil supplemented diet showed no change in either the density or size of their HDL during the first 2 weeks of the diet, although there was a marked increase in the concentration of the lower density HDL subspecies. This response was transient and was essentially over in 4 weeks, at which time the HDL concentration had normalized, although the density of HDL started to increase. We have evidence indicating that when the cholesterol load of the diet is decreased to 0.25%, the animals with a moderate response (cholesterol <400 mg%) have a much longer delay that lasts up to 24 weeks, during which time there is no change in HDL density (unpublished observations). In turn, the low respondents (cholesterol <250 mg%) have an HDL of constant density, which is independent of the length of cholesterol feeding. In the present study, our animals were fed a high-fat high-cholesterol diet for approximately 5 years, a time interval that allowed plasma lipoprotein densities to stabilize.

When the frequency distribution of normal human subjects was compared to that of hypercholesterolemic patients, changes in lipoprotein density became apparent. Both HDLc and the dense component of HDLc increased in density; however, these changes were minor in comparison to the density shifts seen in rhesus monkeys and appeared to affect mainly the male population. More pronounced changes were seen in patients with familial hypercholesterolemia who had decreased HDL concentrations and dense HDL and thus exhibited lipoprotein profiles that resembled those of monkeys with cholesterol levels above 435 mg/dl.

Finally, these studies demonstrate that classical density cuts used to isolate human lipoproteins should not be used to prepare plasma lipoproteins of rhesus monkeys, because these two species have lipoproteins with differing density distributions. Not only are there variations in lipoprotein density among individuals consuming the same diet, but there are even greater density changes upon dietary manipulation, as exemplified by the rhesus monkeys receiving the high-fat high-cholesterol diet. Therefore, if isolation of homogenous lipoprotein classes is the objective, then the method of choice would be density gradient centrifugation rather than sequential flotation at fixed densities. We routinely used "single-spin" isopycnic density gradient centrifugation to obtain the lipoprotein profile of potential blood donors (human or animal). This gives the density information necessary for us to determine the proper protocol for the isolation of most lipoprotein species or subspecies.

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References
Lipoprotein Density in Rhesus Monkeys and Humans

Fless and Scanu


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Comparative study of density distribution of plasma lipoproteins of normo- and hypercholesterolemic rhesus monkeys and humans.

G M Fless and A M Scanu

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