Density gradient characterization of the high density lipoproteins in cholesterol-fed hyper- and hyporesponding patas monkeys (*Erythrocebus patas*)

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Abstract Diet-induced changes in high density lipoprotein (HDL) density and size were studied in patas monkeys. When the animals were switched from a moderate fat-low cholesterol diet to a high fat-high cholesterol (HFHC) diet, the plasma apoA-I levels increased initially in all of the animals. The apoA-I levels remained elevated in monkeys able to maintain their plasma cholesterol concentrations near basal levels (hyporesponders), but began to decrease in monkeys who became severely hypercholesterolemic (hyperresponders), reaching levels as low as 65–70% of their basal value by 24 weeks. The larger, lipid-rich HDL (HDL_2) was shown by density gradient ultracentrifugation and gradient-PAGE (polyacrylamide gel electrophoresis) to be the HDL fraction responsible for these changes in apoA-I, completely accounting for the increase in apoA-I in hyporesponders and the decrease in apoA-I in hyperresponders. The HDL_3 levels remained unchanged in hyporesponders but increased markedly in hyperresponders, partially compensating for the decrease of HDL_2 in those animals. Gradient-PAGE showed the HDL_3 to be heterogeneous, containing at least two populations of particles of the same density but differing significantly in size. The smaller of these HDL_3 were most prominent in the HFHC-fed hyperresponders. These data show that nonhuman primate HDL is both physically and metabolically heterogeneous, and indicate that a high fat-high cholesterol diet-induced hypercholesterolemia severely depresses the HDL_2 levels.—**Melchior, G. W., H. N. Baker, C. R. Abee, and P. S. Roheim.** Density gradient characterization of the high density lipoproteins in cholesterol-fed hyper- and hyporesponding patas monkeys (*Erythrocebus patas*). *J. Lipid Res.* 1984. **25:** 979–990.

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When nonhuman primates are fed a high fat-high cholesterol diet, their plasma cholesterol levels vary widely. Some animals, called hyporesponders, are able to maintain their plasma cholesterol concentrations at or near basal levels. Others, termed hyperresponders, react to the diet with sharply increased plasma cholesterol levels. The mechanisms governing this responsiveness have not been determined. Lofland et al. (1), using squirrel monkeys, studied the changes in cholesterol absorption, synthesis, and excretion during the period when the plasma levels of cholesterol were increasing. They observed a sharp increase in bile acid excretion in hyporesponders, a response that was considerably delayed in hyperresponders, and they concluded that this must have been partially responsible for the differences in the two groups. Eggen (2) and Eggen and Bhattacharyya (3), on the other hand, have consistently observed that hyperresponding rhesus monkeys absorb slightly more cholesterol than hyporesponders once they have equilibrated on the diet and feel that this also contributes to the responsiveness of these primates.

One thing that was clear from both sets of studies was that cholesterol absorption was markedly increased (tenfold or more) in all of the animals, including the hyporesponders. Thus, the amount of cholesterol fluxing through their liver and circulation was also markedly increased. Yet, few studies specifically designed to compare changes in lipoprotein chemistry and metabolism in hyper- and hyporesponding nonhuman primates have been reported.

Rudel and Lofland (4) reported that hyperresponding rhesus monkeys fed high cholesterol diets showed a steady decline in their HDL cholesterol levels with time, whereas hyporesponders showed an actual increase in HDL cholesterol. Those investigators also showed that when the cholesterol content of the animals' diets was increased from 0.05 mg/kilocalorie to 2 mg/kilocalorie,

Abbreviations: HDL (high density lipoproteins), those lipoproteins of d 1.080–1.225 g/ml which migrate with the α_1-globulins during agarose electrophoresis; HDL_2, lipoproteins of d 1.080–1.125 g/ml; HDL_{2B}, lipoproteins of d 1.080–1.100 g/ml; HDL_3, lipoproteins of d 1.125–1.225 g/ml; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; EIA, electroimmunoassay; HFHC diet, high fat-high cholesterol diet.

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both HDL_2 and HDL_3 increased; but at dietary cholesterol levels above 2 mg/kilocalorie, HDL_2 levels decreased, while HDL_3 levels increased. We recently reported similar changes in the rhesus monkey apoA-I levels (5). When hyperresponding rhesus monkeys were fed high fat-high cholesterol diets, their HDL_2 apoA-I levels had decreased significantly by 15 weeks and remained low for the duration of the study; whereas, the HDL_3 apoA-I levels appeared to have increased slightly. In hyporesponders, on the other hand, the HDL_2 apoA-I levels appeared to increase slightly in response to the dietary cholesterol, and little change was noted in the HDL_3 apoA-I levels. The mechanism responsible for these changes is not known; however, the hyper- and hyporesponding primate may offer a unique model for studying these changes, and thereby contribute to our understanding of HDL metabolism in general. The present study was designed to carefully characterize the HDL density distribution, as well as the effect of an atherogenic diet on that distribution in hyper- and hyporesponding primates.

METHODS

Animals and diets

Four mature patas monkeys, selected from a colony of twenty females on the basis of their response to a high cholesterol diet, were used for the present study. The four animals represented the two with the greatest increase in plasma cholesterol (hyperresponders) and the two with the least change from the basal state (hyporesponders) determined 7 weeks after they began consuming the challenge diet. Once the plasma cholesterol response hierarchy was established, the animals were switched to a "prudent diet" (**Table 1**) and maintained on that diet for 6 months. During that period, their "basal" apoA-I and HDL profiles were established. The prudent diet used here was modeled on the prudent diet recommended by the American Heart Association. The animals were then fed a high fat-high cholesterol diet (HFHC diet; Table 1) for the ensuing 24 weeks and their body weights, plasma cholesterol levels, and apoA-I levels were measured weekly. The mean body weight for the group was 8.55 kg at t = 0 and 8.58 kg at t = 24 weeks. No animal's weight fluctuated more than 5% during the study. The HDL distributions were determined twice during the HFHC diet period, at 1 and 24 weeks.

TABLE 1. *Composition of the experimental diets*

Ingredients	Prudent Diet	HFHC Diet
Wheat flour (g)	4170	2500
Cane sugar (g)	1070	1600
Soy flour, defatted (g)	390	200
Non-fat dry milk, fortified (g)	1260	1300
Wheat bran (g)	480	500
Beef suet (g)	480	1000
Soybean oil (g)	490	250
Butter (g)	150	150
Egg yolk, dried (g)	60	500
Plex-Sol-C vitamin mix (g)	50	50
Strained beef and chicken (g)	1440	2000
Crystalline cholesterol USP (g)	0	40
Total calories (per g of diet)	3.98	4.32
Protein calories (% of total)	13.9	13.0
Fat calories (% of total)	31.2	43.5
Carbohydrate calories (% of total)	54.9	43.5
P/S ratio	1.0	0.36

Apoprotein quantitation

The apoproteins were measured by electroimmunoassay as described by Laurell (6) and modified by Bar-On, Roheim, and Eder (7). Nonidet-P-40 (NP-40), a non-ionic detergent, was added to a final concentration of 1% in the samples and standards, and 0.05% in the agarose for the measurement of apoA-I (8). Antisera to apoA-I were prepared by injecting into goats apoA-I purified by polyacrylamide gel electrophoresis in sodium dodecyl sulfate (SDS-PAGE; 9). The antisera so obtained were tested for purity by double diffusion (10) and immunoelectrophoresis (11) against whole plasma and individual lipoprotein fractions. The apoprotein concentrations of the sera 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 apoA-I concentration. The control serum was standardized against apoA-I which was isolated by a modification of the method described by Weisgraber, Mahley, and Gerd (12) and Rall et al. (13). ApoHDL (50–75 mg), delipidated with chloroform–methanol 2:1 was solubilized in 6 M guanidine-HCl and passed through a 2.5 × 200 cm column of Sephacryl S-300 (Pharmacia, Uppsala, Sweden). The fractions containing apoA-I were identified by SDS-PAGE, pooled, dialyzed against 5 mM NH_4HCO_3, and lyophilized. The lyophilized sample was reconstituted in 0.1 M phosphate-buffered saline (pH 7.2) and an aliquot was taken to verify its purity by SDS-PAGE. No other apoproteins were visible on the gels. The purified apoA-I was diluted with serum from nonimmunized goats and the electroimmunoassays were carried out as described above. The rockets so obtained appeared morphologically identical to those obtained from the monkey serum standard, and the standard curves using purified apoA-I were parallel to those using dilutions of serum.

Lipoprotein isolation

HDL was isolated from 10 ml of serum by ultracentrifugation in an SW-41 rotor (Beckman Instruments,

Inc., Palo Alto, CA). The blood was collected in serum separator tubes (Becton-Dickinson, Rutherford, NJ) and centrifuged within an hour of collection. EDTA (1 mg/ml) and sodium azide (1 mg/ml) were added to the serum as preservatives. The d < 1.080 g/ml lipoprotein fraction was removed by adjusting the serum to d 1.080 g/ml with solid KBr and centrifuging for 22 hr at 40,000 rpm. The d 1.080–1.225 g/ml fraction was isolated from the infranatant by adjusting it to d 1.225 g/ml with solid KBr and spinning for 44 hr. In each instance, the top 2.0–2.2 ml was removed. The density distribution of the HDL was then determined by layering the d 1.080–1.225 g/ml lipoprotein fraction, previously adjusted to 2.4 ml, under 8.0 ml of d 1.125 g/ml KBr solution, which had, in turn, been layered under 3.0 ml of d 1.063 g/ml KBr solution, and centrifuging these preparations for 48 hr at 40,000 rpm. All centrifugations were carried out at 15°C. Preliminary studies in which the lipoproteins were centrifuged through the gradient for 24, 48, 72, and 96 hr indicated that an equilibrium was attained by 48 hr. Continued centrifugation beyond that time resulted in a slow but progressive movement of the KBr toward the bottom of the tube which, in turn, pushed the lipoproteins slightly higher in the tube and compressed somewhat the area over which they were distributed. Nonetheless, their density distribution appeared unchanged. Thus, 48 hr was chosen as the most ideal centrifugation interval. Salt blanks, prepared by substituting KBr solution of density d 1.225 g/ml for the lipoprotein fraction, were run with every set of lipoprotein fractions and were used to measure the gradient. After the final centrifugation, the tubes were removed from the rotor and placed in an ISCO Fractionator (ISCO, Lincoln, NE). The contents of each tube were displaced by pumping a high density solution (Fluoinert FC-43, 3M Co., St. Paul, MN) into the bottom of the tube at 1.5 ml/min. The contents of each tube, after passing through an ultraviolet monitor set at 280 nm, were collected in an ISCO fraction collector (0.75 ml/fraction). The density of each fraction was determined from a standard curve constructed by plotting the density of each salt blank fraction versus the volume displaced to that point, and assuming that the solvent density of the corresponding lipoprotein fraction was the same. The density of each salt blank fraction was determined by refractometry, using a standard curve that related refractive index to density. The total protein in each fraction was determined prior to dialysis by a modification of the Lowry method (14). The cholesterol content was determined by the enzymatic method of Allain et al. (15) and the triglycerides by the method of Bucolo and Davis (16). SDS-PAGE was performed using an LKB 2001 vertical electrophoresis apparatus (LKB Instruments, Inc., Gaithersburg, MD), essentially as described by Laemmli (17) but modified for the slab gels. The gels were fixed overnight in a mixture of 10% trichloroacetic acid and 3.5% sulfosalicylic acid dissolved in methanol–water 30:70. The gels were stained with Coomassie Blue R (0.1 g) dissolved in 100 ml of methanol–water–acetic acid 50:50:01 for 1 hr at room temperature and destained for several days in 10% acetic acid. Commercially available polyacrylamide gradient gels (4–30%) (Pharmacia Fine Chemicals, Division of Pharmacia Inc., Uppsala, Sweden) were used for the gradient PAGE, and were run exactly as suggested by Pharmacia, Inc. with the exception that specially made sample applicators, capable of holding 120 μl of sample, were used for sample application. The gels were calibrated using proteins of known molecular weight (Pharmacia electrophoresis calibration kit containing thyroglobulin [669,000], ferritin [440,000], catalase [232,000], lactate dehydrogenase [140,000] and bovine serum albumin [67,000]). The migration distances of the molecular weight standards were very reproducible from gel to gel and ranged from 18.3 ± 0.3 mm (mean ± SD) for thyroglobulin to 65.2 ± 0.3 mm for bovine serum albumin. In no instance did the standard deviation for a given protein exceed 0.5 mm.

RESULTS

Plasma cholesterol response

The plasma cholesterol concentration for each of the four monkeys (two hyperresponders and two hyporesponders) measured over the entire study are shown in **Fig. 1.** The basal cholesterol value (at zero time) was, in each instance, the mean of three samples taken over a 6-month period during which the animals were consuming the prudent diet. Hyperresponders showed a sharp increase in their plasma cholesterol levels within a week after they began consuming the HFHC diet, and their cholesterol concentrations continued to climb for 6 to 8 weeks. Thereafter, the plasma cholesterol, although variable from week to week, appeared to have plateaued at a mean of 641 mg/dl. The hyporesponders, on the other hand, showed no statistically significant deviations from the basal levels when followed for the entire HFHC diet interval. The plasma cholesterol for those animals averaged 121 mg/dl in the basal state, and the mean for the HFHC diet period was 140 mg/dl.

Serum apoA-I concentrations

To follow the effects of diet and phenotype on HDL levels, serum apoA-I concentrations were measured periodically for the duration of the study. **Fig. 2** shows the changes in the apoA-I levels in the hyper- and hypo-

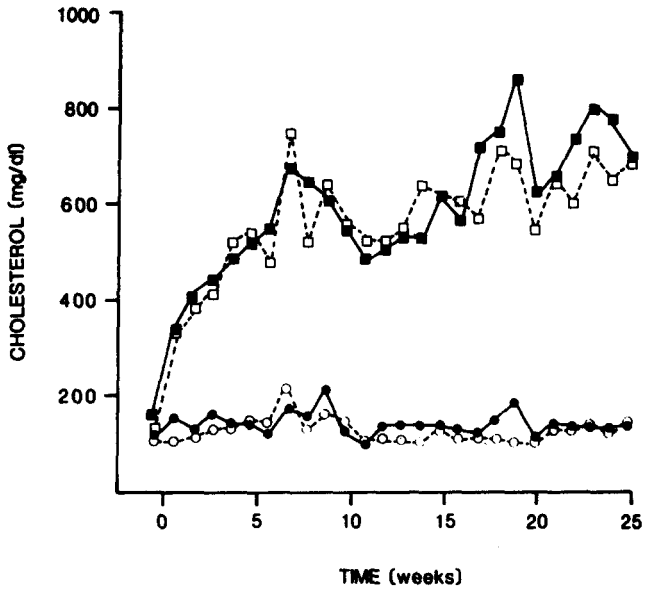

Fig. 1. Serum cholesterol concentration of two hyper- (□, ■) and two hyporesponders (○, ●) taken at weekly intervals for 25 weeks while they consumed a HFHC diet. The basal value (t = 0) was, in each instance, the mean of three samples taken over a 6-month period during which the animals were consuming the prudent diet.

responders over the course of the study. There was an increase in the apoA-I concentrations in all of the animals within 1 week after they began consuming the HFHC diet. This initial increase in apoA-I levels in response to the HFHC diet has been a consistent finding, not only in this study but in other related studies utilizing patas monkeys (18). The apoA-I levels, although variable, remained elevated in the hyporesponders for the ensuing 23 weeks, whereas, in the hyperresponders, they returned to basal levels within 6 to 7 weeks and continued to decrease slowly through 24 weeks. To confirm these changes in apoA-I levels, total HDL protein was measured in each animal twice during the prudent diet period and twice during the HFHC diet period, the latter at 20 and 24 weeks. **Table 2** shows that when the HFHC diet was fed for 5 to 6 months, HDL protein was increased 36% (as compared to a 43% increase in apoA-I) in hypo responders, whereas HDL protein decreased 42% (compared to a 30% decrease in apoA-I) in hyper responders. Thus, the changes in apoA-I shown in Fig. 2 apparently are reasonably representative of changes in total HDL protein. Only rarely was apoA-I detected at densities lower than 1.080 g/ml by SDS-PAGE in these monkeys, and when it was present, it was only in minute amounts. Furthermore, EIA showed that less than 5% of the apoA-I was present in the d > 1.225 g/ml fraction after the final isopycnic centrifugation. Thus, essentially all of the plasma apoA-I was contained in the d 1.080–1.225 g/ml density fraction of these monkeys. The changes in HDL cholesterol (data not shown) also closely paralleled the changes in HDL protein.

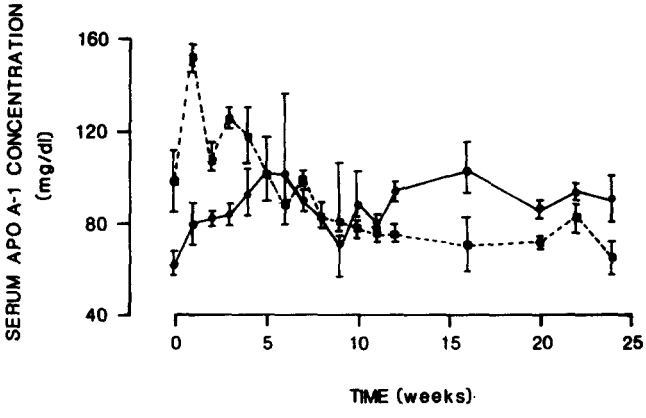

Fig. 2. The serum apoA-I concentration of the hyper- (■) and the hyporesponders (●) followed weekly for the duration of the study. The serum samples used for apoA-I measurements were the same used for cholesterol measurements (Fig. 1). Each point represents the mean ± half of the range (bars).

HDL density gradient distribution

The HDL distribution of the hyperresponders, the hyporesponders, and three midresponders, obtained while the animals were consuming the prudent diet, is shown in **Fig. 3.** It is immediately clear from that figure that during the prudent diet period, the HDL distribution was almost identical for the various phenotypes. Furthermore, with the exception of a distinct population of HDL between d 1.080–1.100 g/ml (seen here in the hyperresponders but also evident in other hypo- and midresponders), the patas HDL peak appeared fairly symmetrical, i.e., there were no HDL_2 and HDL_3 subfractions obvious from these data.

The HDL concentration peaked between d 1.120–1.130 g/ml and ranged in density up to d 1.22 g/ml;

TABLE 2. Comparison of diet-induced changes in apoA-I levels and total HDL protein in hyper- and hyporesponders[a]

Phenotype	Diet	ApoA-I Levels	HDL Protein
		mg/dl	
Hyporesponders	Prudent	63 ± 4	95 ± 16
	High cholesterol	90 ± 13	129 ± 20
Hyperresponders	Prudent	98 ± 14	121 ± 15
	High cholesterol	69 ± 7	70 ± 1

[a] The HDL was isolated for protein determination twice from each monkey during the prudent diet period and twice during the high cholesterol diet period, the latter at 20 and 24 weeks. The mean value for the hyperrresponders and the mean value for the hyporesponders were determined at each time, and the points contained here represent the mean and range of those means. The apoA-I levels during the high cholesterol diet period are the mean of the 20- and 24-week values shown in Fig. 2.

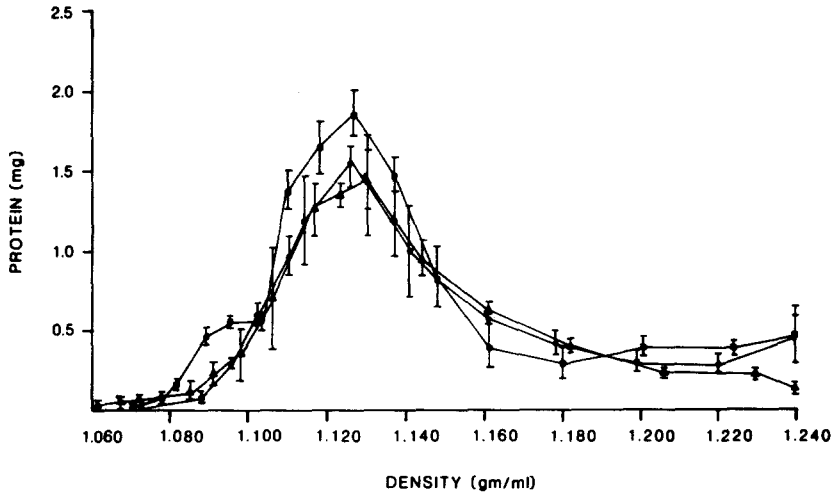

Fig. 3. The HDL density distribution as measured by density gradient ultracentrifugation of two hyper- (■), two mid- (▲), and two hyporesponders (●) consuming the prudent diet. Each point represents the mean ± half the range (bars).

however, by far the majority of the HDL was contained in the interval d 1.080–1.180 g/ml. The only notable difference between the various phenotypes during the prudent diet period was the somewhat higher concentration of HDL in the hyperresponders, an observation which compares favorably with the higher apoA-I levels in these animals as shown in Fig. 2.

The HDL distribution of both phenotypes during the HFHC diet period is shown in **Fig. 4.** It is clear from that figure that the HFHC diet had a definite effect on the HDL distribution of all animals, but the diet-induced changes were quite different in hyporesponders than in hyperresponders. In hyperresponders, consumption of the HFHC diet for 1 week caused two distinct HDL subfractions to increase: HDL of d 1.080–1.125 g/ml and HDL of d 1.140–1.21 g/ml. The substantial increase in these two subfractions apparently accounts for the corresponding increase in apoA-I, shown in Fig. 2. By 24 weeks, however, the HDL of d < 1.125 g/ml had decreased considerably in concentration to well below even the prudent diet levels, whereas the d 1.140–1.21 g/ml fraction increased even further. Thus, there was a clear shift in HDL density as the animals' hypercholesterolemia progressed, to primarily particles of d > 1.125 g/ml. Although these latter HDL increased substantially, it was not sufficient to compensate for the loss of HDL of d < 1.125 g/ml and thus it was loss of the latter fraction that accounted for the decreased apoA-I levels seen in hyper responders at 24 weeks (Fig. 2).

The effect of the HFHC diet on the HDL distribution in hyporesponders is also shown in Fig. 4. The initial response (at 1 week) was very similar to the initial response of the hyperresponders, that being a distinct increase in the d 1.080–1.125 g/ml HDL. However, their HDL distribution at 24 weeks was exactly the opposite of that of the hyperresponders. Rather than a decrease of the d 1.080–1.125 g/ml HDL, this fraction had increased still further. This increase was especially pronounced in the d 1.080–1.100 g/ml fraction; and when the animals were switched back to the prudent diet after 24 weeks, the d 1.080–1.100 g/ml fraction had decreased almost to basal levels within 1 week (data not shown), suggesting that this might be an especially rapidly turning-over subfraction of the HDL.

To examine how these various density subfractions differ in size, the d 1.080–1.225 g/ml fraction and each of the density gradient subfractions was analyzed by 4–30% gradient gel electrophoresis. **Figs. 5** and **6** show patterns typical of those obtained. The samples shown are from hyper- and hyporesponders consuming the HFHC diet. Note that the HDL from hyperresponders are much smaller than those from hyporesponders and both appear heterogeneous (Fig. 5). This heterogeneity was also evident in the density fractions (Fig. 6), especially those of d > 1.125 g/ml. Thus, HDL of different size but of overlapping density existed in those animals. **Fig. 7** shows the relationship between patas monkey HDL molecular weight and density. The patas monkey HDL appear to range in molecular weight from 350,000 (at densities near 1.080 g/ml) to 125,000 (at densities greater than 1.18 g/ml). The molecular weight of HDL_{2B} averaged 325,000 and that of the major HDL peak (d 1.125 g/ml) averaged 200,000. The molecular weight of the HDL_2 decreased linearly, 3355 daltons for every 0.001 g/ml increment in density ($r^2 = 0.97$) up to approximately d 1.125 g/ml. Thereafter, the rate of change per increment in density was much smaller,

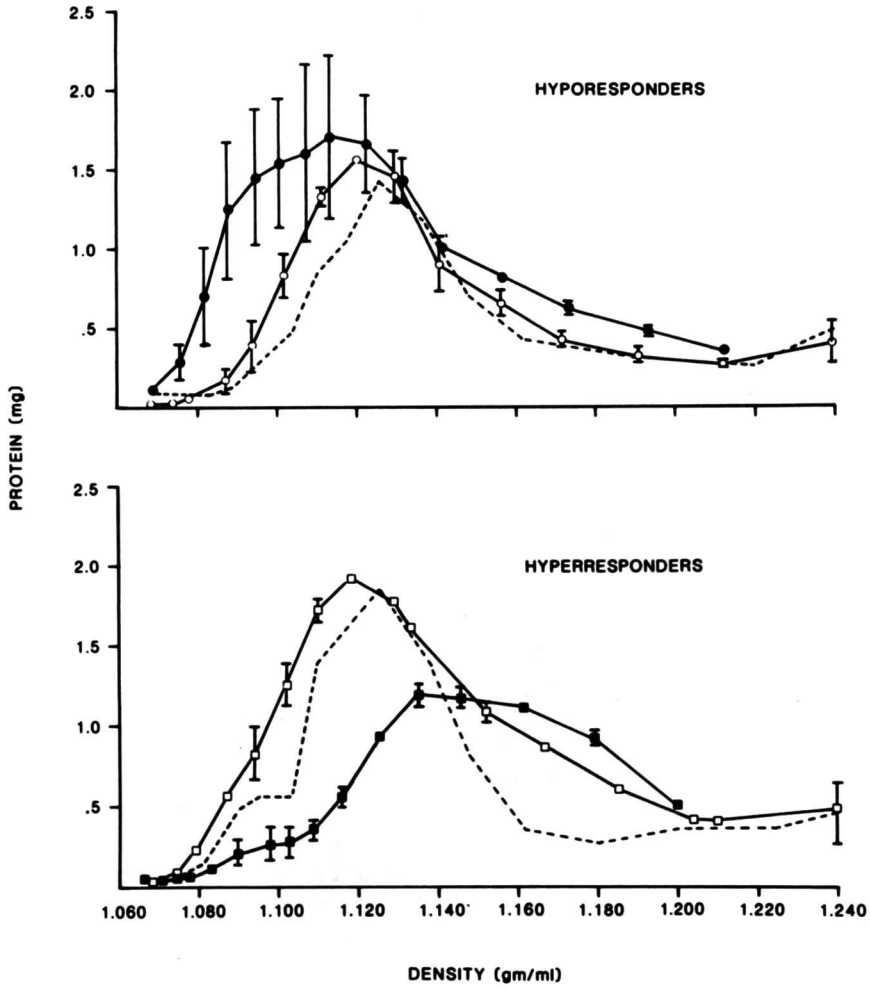

Fig. 4. The HDL density distribution in the hyper- and hyporesponders during the prudent diet period (– – –) and after they had consumed the HFHC diet for 1 week (○, □) and 24 weeks (●, ■). Each point represents the mean ± half the range (bars).

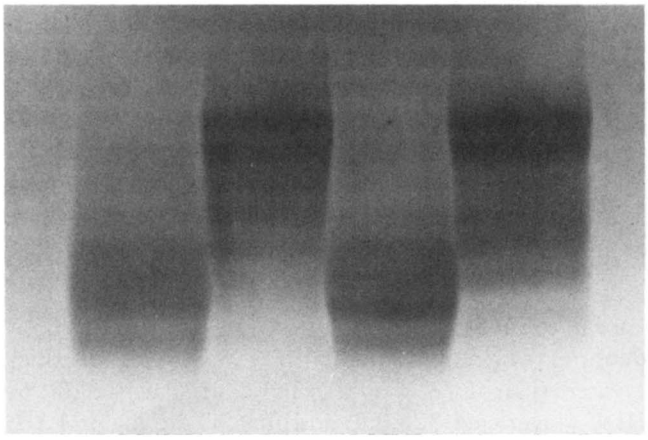

Fig. 5. The migration patterns of the HDL from two hyper- (lanes 1 and 3, L–R) and two hyporesponders (lanes 2 and 4) which had consumed a HFHC diet for 24 weeks. Whole HDL (20 μg protein) isolated by isopycnic ultracentrifugation were loaded on each lane, electrophoresed into a 4–30% gradient gel, and stained with Coomassie Blue. The direction of migration is from top to bottom.

and the doublet appeared in the gels (Fig. 6). The larger HDL_3 ranged in apparent molecular weight from 160,000–200,000, whereas the smaller HDL_3 ranged from 125,000–140,000. This heterogeneity was evident to varying degrees in all of the fractions collected between d 1.125 and 1.21 g/ml, regardless of diet or phenotype; however, this heterogeneity was much more obvious in hyperresponders consuming the HFHC diet, a change which appears to coincide with the marked increase in HDL_3 in those animals (Fig. 4). To determine if this HDL heterogeneity was unique to patas monkeys, we examined the HDL of hyper- and hyporesponding rhesus monkeys and found gel patterns very similar to those described here, suggesting that this HDL_3 heterogeneity might be a characteristic common among non-human primate species.

We also examined the chemical composition of each of the 18 density gradient subfractions from each monkey during both dietary periods to determine what effect

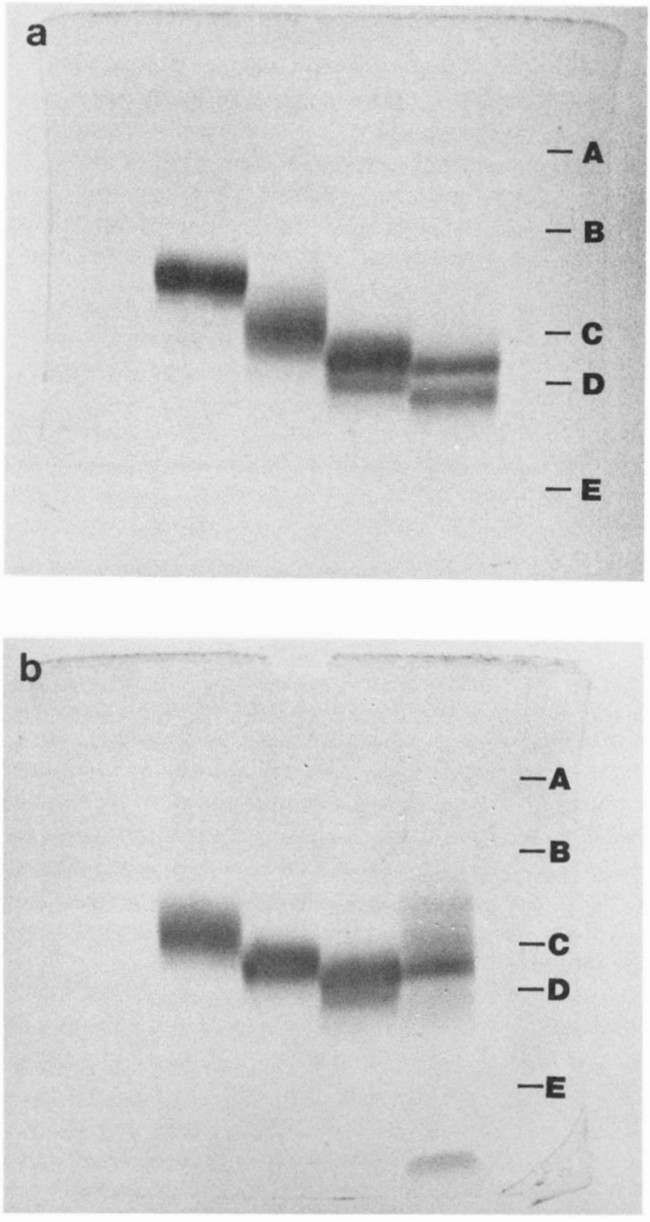

Fig. 6. The migration patterns of HDL subfractions isolated by density gradient ultracentrifugation from a hyperresponder (a) and a hyporesponder (b) consuming the HFHC diet. The densities (g/ml) of the fractions shown in a were (L–R) 1.098, 1.116, 1.135, and 1.162 g/ml. Those in b were 1.098, 1.118, 1.136, and 1.165 g/ml. The lines along the right side of the gels show where the protein standards migrated. The standards were: A, thyroglobulin (669,000); B, ferritin (440,000); C, catalase (232,000); D, lactate dehydrogenase (140,000); and E, bovine serum albumin (67,000). The electrophoresis conditions were the same as in Fig. 5.

diet and phenotype had on the components of the HDL. **Table 3** contains the chemical composition of selected subfractions across the gradient. With the exception of slight differences in apoprotein composition, we did not detect a significant effect of diet or phenotype on the HDL chemical composition measured across the gradient.

However, when the data from all of the animals during both dietary periods were pooled, some interesting changes in the relationships among the HDL components measured across the gradient were observed. A plot of protein (expressed as a percent of total mass) was found to increase linearly with density (r^2 = 0.98) over the entire HDL density range. Cholesteryl ester content, on the other hand, dropped rapidly from greater than 25% of the total mass at d 1.080 g/ml to approximately 15% of total mass at d 1.125 g/ml, but changed little thereafter, ranging from 12 to 15% of the total mass over the HDL_3 density range. Of special interest was the relationship between cholesteryl ester and phospholipid. When these two entities, expressed as nanomoles in each gradient subfraction, were plotted versus one another, a strong linear relationship was observed (**Fig. 8**) with a slope of 0.571 (r^2 = 0.98). This indicates that, regardless of diet or phenotype, for every molar increment in cholesteryl ester in the HDL, there was a 1.75 molar increment in phospholipid.

The apoprotein content of each of the density gradient subfractions was also determined for each monkey by SDS-PAGE. A typical apoprotein distribution is shown in **Fig. 9.** In every instance, apoA-I was the dominant apoprotein, accounting for at least 80% of the protein as determined by gel scanning. Traces of a high molecular weight protein are seen at the top of the gel and probably represent a slight contamination of the HDL fraction with the patas monkey Lp(a) analogue (19). There were no apparent phenotypic or dietary-induced changes in the apoprotein pattern. There were, however, changes in the distribution of the small peptides across

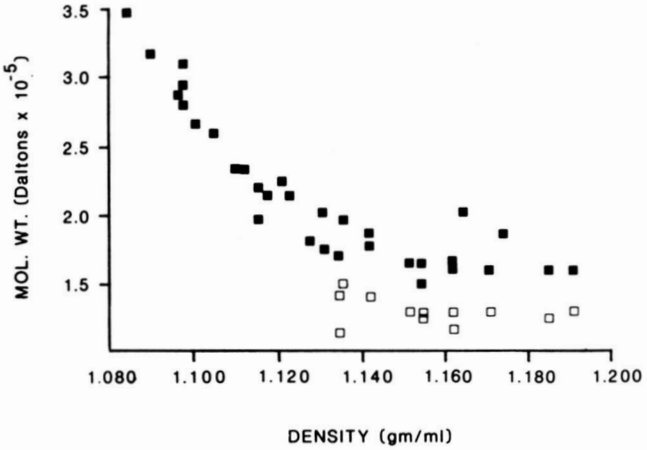

Fig. 7. The change in HDL molecular weight with change in density. The molecular weight was estimated by comparing the migration distance into the gel of a given density fraction to that of protein of known molecular weight (see Methods). The HDL_3 (d 1.125–1.225) often appeared as a doublet in the gel (Fig. 6), with the larger HDL_3 ranging in molecular weight from 160,000–200,000 (■) and the smaller HDL_3 from 125,000–140,000 (□).

TABLE 3. Change in HDL molecular weight and composition with increasing density

	Density (g/ml)					
	1.090	1.110	1.130	1.150	1.170	1.190
Molecular weight (daltons × 10^{-5})	3.22[a]	2.41	1.85 1.42	1.74 1.35	1.66 1.29	1.59 1.25
Composition (mol/mol HDL)						
Amino acid[b]	1157 ± 141[c]	936 ± 98	801 ± 69 612 ± 53[d]	833 ± 72 646 ± 56	868 ± 102 671 ± 79	914 ± 72 703 ± 56
Cholesteryl ester	98 ± 17	63 ± 9	42 ± 8 32 ± 6	37 ± 7 28 ± 5	32 ± 7 25 ± 5	28 ± 6 22 ± 5
Cholesterol	33 ± 5	21 ± 3	14 ± 3 11 ± 2	12 ± 2 9 ± 2	11 ± 2 8 ± 2	10 ± 2 8 ± 2
Phospholipid	149 ± 4	112 ± 5	80 ± 3 61 ± 2	70 ± 5 54 ± 4	58 ± 8 45 ± 6	45 ± 5 35 ± 4

[a] Mean, derived from the curve shown in Fig. 7.
[b] The average molecular weight of an amino acid was assumed to be 110 daltons.
[c] Mean and standard deviation.
[d] In some instances, more than one HDL was present in a given density fraction (Fig. 6). In those instances, the chemical composition of the mixture was determined and, for the purposes of this table, the ratios of the various components to one another were assumed to be the same in both bands (see text).

the density gradient as determined by isoelectric focusing (data not shown). Those gels indicated that apoA-II, or a band with the same apparent pI, was of higher concentration in the HDL_2 than in the HDL_3 of all animals; however, this latter observation has not been confirmed immunochemically. In any case, as pointed out above, apoA-I was by far the dominant apoprotein in each instance.

DISCUSSION

We have reported previously that a diet-induced hypercholesterolemia was associated with a decreased plasma apoA-I concentration in hyperresponding rhesus monkeys (5). Sequential isolation of the lipoprotein fractions by isopycnic centrifugation indicated that the majority of the decrease in apoA-I could be accounted for as a decrease in the HDL_2 (arbitrarily defined as HDL of d < 1.125 g/ml) in those animals. The purpose of the present study was to characterize more carefully this diet-induced change, which appears to be a common response of nonhuman primates (4, 5, 19–22). Prelimi-

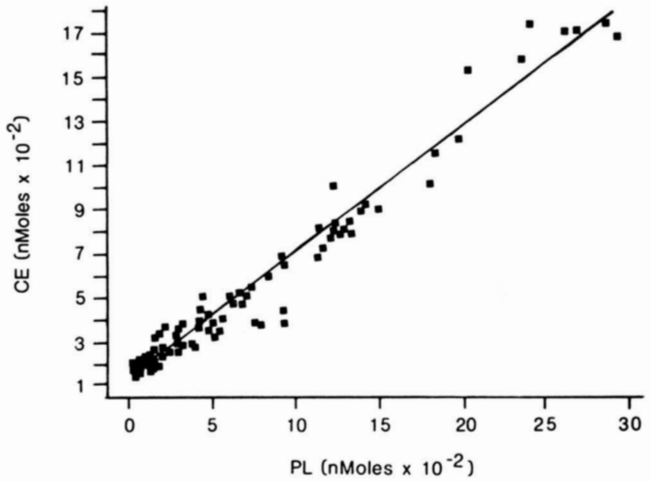

Fig. 8. The relationship between HDL cholesteryl ester and phospholipid. The line represents a least squares fit, $r^2 = 0.98$.

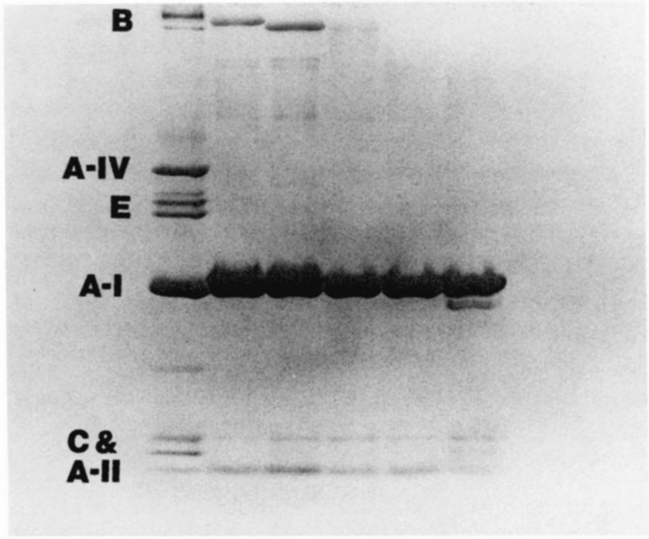

Fig. 9. SDS-PAGE of the apoproteins of HDL isolated by density gradient ultracentrifugation from a hyperresponder consuming the HFHC diet. Lane 1 (L–R) contains rat apoproteins of d < 1.21 g/ml. The densities of the HDL samples (lanes 2–6) were: 1.084, 1.103, 1.116, 1.135, 1.162 g/ml.

nary studies indicated that patas monkeys responded to an HFHC diet in a manner similar to that observed in rhesus monkeys. Because we had available a colony of 20 patas monkeys not previously fed any HFHC diets, we chose to evaluate this diet-induced change in HDL in those animals. The changes reported here are very compatible with the observations reported previously using large groups of rhesus monkeys.

The plasma apoA-I concentrations of these patas monkeys were somewhat lower than those reported for rhesus monkeys (80 versus 160 mg/dl (5)), but the diet-induced changes in apoA-I were quite similar to those reported previously, i.e., a slight but sustained increase in apoA-I levels in hyporesponders, and a transient increase followed by a slow, progressive decrease in apoA-I levels in hyperresponders. The only major difference in the apoA-I response between this and the previous study was a sharp increase in apoA-I levels observed in the hyperresponders 1 week after they began consuming the HFHC diet. As discussed above, this has been an absolutely consistent finding in patas monkeys fed the HFHC diet. It is possible that this also occurred in the rhesus monkeys but went unobserved, since the first plasma samples were not taken from those animals until they had eaten the diet for 3 weeks (5). The mechanism for this increase in apoA-I levels (whether the result of a stimulated synthesis or an inhibited catabolism) has not been determined, although it is clearly a consequence of the high fat-high cholesterol diet in those animals.

Density gradient analysis indicated that patas monkey HDL is very similar in many respects to the human HDL when the animals are consuming the prudent diet. The patas HDL range in density from d 1.080 to 1.21 g/ml, with the peak very near d 1.125 g/ml (Fig. 3). SDS-PAGE indicated that little or no apoA-I was present at densities less than d 1.080 g/ml, and very small quantities (<5%) were detected by electroimmunoassay in the d > 1.225 g/ml fraction. Furthermore, there was no accumulation of apoA-I in the bottom of the tube after density gradient analysis. Thus, it did not appear that the ultracentrifugation procedures were shearing appreciable quantities of apoA-I from the HDL. The patas monkey HDL ranged in molecular weight from 350,000 to 125,000. The HDL of d 1.125 g/ml was present in highest concentration and had an average molecular weight of 200,000.

Our measurements of patas monkey HDL density distribution differ considerably from those reported recently by Fless et al. (22) for rhesus monkeys. To determine if these marked differences were the result of differences in methodology or true species differences, we analyzed the HDL distribution of two chow-fed, male rhesus monkeys exactly as we did for the patas monkeys (data not shown). The distributions that we obtained were bimodal, appearing almost identical in shape to those reported by Fless et al. (22). However, the mean densities of the two peaks were considerably higher than reported by Fless. Our HDL_L analogues had mean densities ranging from d 1.080 to 1.100 g/ml and the HDL_H analogues had mean densities ranging from d 1.115 to 1.125 g/ml. We also analyzed one hypercholesterolemic rhesus monkey by rate zonal ultracentrifugation and found that the HDL_3 fraction (the dominant HDL fraction in that monkey) floated at a significantly slower rate than did human HDL_3 run simultaneously, i.e., that the rhesus HDL_3 was of a density greater than 1.125 g/ml. Thus, rate zonal ultracentrifugation also indicated that the rhesus HDL was of higher density than reported by Fless et al. (22). Finally, to eliminate the possibility that our method, which requires only a 48-hr centrifugation (as opposed to that of Fless et al. which, in most instances, was of 66 hr duration) failed to allow the HDL to reach equilibrium, we centrifuged two patas monkey samples for 48 and 72 hr and saw only minor shifts in the HDL_3 and no change in the HDL_2 after the additional 24 hr. Furthermore, as indicated in the methods, we found that these subtle changes were due to the slow redistribution of salt rather than an incomplete equilibrium of the HDL. Thus, the reasons for the differences in the apparent densities of the HDL between this report and that of Fless et al. (22) appear to be due to differences in the methods used to evaluate the HDL density distribution. As Fless et al. (22) point out, their method of measuring the density may underestimate the true density of the HDL. On the other hand, our estimates are based on the salt distribution in "salt blank tubes," and we assumed that the salt distribution in the tube containing the lipoproteins was identical. What effect the movement of lipoproteins through this medium has on the true salt distribution is unknown. Thus, our estimates of the HDL density may also be incorrect to some extent. Nonetheless, our estimates of the HDL density distribution agree well with those reported for humans (23), rhesus monkeys (24), gorillas (24), and African green monkeys (25), all of which may indicate that the HDL density range in primates is similar from species to species.

In no instance did we observe an inflection in HDL protein distribution near d 1.125 g/ml which would suggest that this is a point of demarcation for HDL_2 and HDL_3 in the patas monkey. That density was chosen arbitrarily, based on previous studies in monkeys (26) and man (23, 27). We did, however, make three separate observations which indicated that that density was in fact a pivotal point in the HDL-density spectrum in the patas monkey. First, this appeared to be the best point

of demarcation for the diet-induced changes in the HDL, i.e., the density below which the HDL decreased in cholesterol-fed hyperresponders and accumulated in cholesterol-fed hyporesponders. Second, it was at this point that the HDL heterogeneity, shown in Fig. 6, was first observed, i.e., the doublet was never present at densities below d 1.125 g/ml. Finally, inflections in the curves relating HDL size to density (Fig. 7) and HDL cholesterol content to density (not shown) occurred near d 1.125 g/ml. Thus, it appears that the density d 1.125 g/ml is a critical point in the density spectrum of patas monkeys. Nonetheless, as used here, the terms HDL_2 and HDL_3 refer to density subfractions and not necessarily to metabolically distinct entities.

Although the patas monkey HDL appeared relatively homogeneous in the control state, we often observed an inflection in the HDL protein distribution near d 1.100 g/ml, suggesting that the d 1.080–1.100 g/ml fraction was a distinct subpopulation of HDL in the patas monkey, just as in the human (22). Preliminary studies have indicated that this HDL_{2B} fraction is quite variable from animal to animal and is especially sensitive to dietary manipulations. More careful studies characterizing that HDL subfraction are in progress.

When the density gradient analyses were repeated after the animals had consumed the HFHC diet for 5–6 months, the HDL appeared considerably more heterogeneous with regard to density distribution, and the hyper- and hyporesponders were found to have responded in an opposite fashion. The HFHC diet caused a marked increase in the HDL_2 of hyporesponders, with an especially prominent change in the HDL_{2B}; but there was little or no effect on the HDL_3. In hyperresponders, on the other hand, the hypercholesterolemia was associated with a marked decrease in the HDL_2 and a significant increase in the HDL_3 fraction.

Measurements of the chemical composition of each density subfraction indicated that there was no effect of phenotype on the HDL composition at a given density, and SDS-PAGE showed that no major changes in apoproteins occurred across the peak, apoA-I being by far the most prominent apoprotein in all subfractions. Thus, the major difference between the various HDL density fractions did not appear to be their apoprotein content but rather their phospholipid-cholesteryl ester content. That observation suggests that the HDL_3 of both hyper- and hyporesponders has "reserve phospholipid/cholesteryl ester-carrying capacity," i.e., that under the proper circumstances, the HDL_3 is capable of accepting more phospholipid and cholesteryl ester. Table 3 shows, for example, that HDL of d 1.090 carries 85 mmol of cholesteryl ester and 129 mmol of phospholipid per mol of amino acid, whereas HDL of d 1.170 carries only 37 mmol of cholesteryl ester and 67 mmol of phospholipid per mol of amino acid. Therefore, if we assume that the protein content of the two HDL subfractions does not differ qualitatively, then these data would indicate that the HDL of d 1.170 have a "reserve cholesteryl ester-carrying capacity" of at least 48 mmol per mol of amino acid, and a reserve phospholipid-carrying capacity of 62 mmol per mol of amino acid. However, Fig. 8 shows that a strong correlation exists between cholesteryl ester (a core component) and phospholipid (a surface component). This suggests that the core volume to surface area relationship remains constant as the HDL changes in size. This may indicate that, regardless of their size, the HDL is saturated with cholesteryl ester, i.e., that no additional cholesteryl ester can be incorporated into the core of either the large or small HDL without the addition of surface components. Therefore, the concept that any HDL has "reserve cholesteryl ester-carrying capacity" may be misleading, and the availability of phospholipid to the HDL may be the dominant factor regulating its ability to function as an effective cholesterol transport vehicle. Furthermore, phospholipid availability may also play a prominent role in the reduced levels of large HDL and the accumulation of small HDL in hyperresponders.

Current theory holds that a principal source of phospholipid appearing in the HDL is surface remnants produced during the lipolysis of chylomicrons and VLDL (28, 29). During the process of core hydrolysis, the redundant surface area so produced pinches off of the chylomicrons and VLDL and exists transiently as phospholipid vesicles (30). Patsch et al. (31) proposes that these surface remnants condense with HDL_3, thereby converting it to HDL_2. HDL_3 is thought to be regenerated by the loss of phospholipid and cholesteryl ester, processes presumably catalyzed by the hepatic lipase (32–36) and possibly the cholesteryl ester transfer proteins (37–39). Thus, if surface remnant production were impaired (as might occur if cholesteryl ester-rich VLDL and chylomicron core remnants retained a larger fraction of their surface components), or if surface remnant production were normal but their partition coefficients between the lipoproteins were altered (such that they partitioned into lipoproteins other than HDL in hypercholesterolemic animals), small, more dense HDL such as those observed in the hyperresponders might result. This, of course, is pure speculation, and there are other explanations that could explain our results. Nonetheless, it is interesting that such large quantities of cholesteryl ester and phospholipid exist in the plasma of the hypercholesterolemic hyperresponder, and yet the relatively cholesterol-poor, phospholipid-poor HDL predominate.

One interesting new observation arising from these studies was the heterogeneity of the nonhuman primate HDL_3. A similar HDL_3 heterogeneity has been observed

in humans (40), but it was not clear from those studies that the various HDL_3 subfractions were of identical density, as apparently is the case in the patas monkey. Preliminary studies, designed to separate the HDL_3 subfractions, have indicated that apoA-I is the principal apoprotein of both entities, but how these HDL_3 differ, other than in size, has not been determined. We find it interesting, however, that the relative increase in the 125,000–140,000 molecular weight entity corresponds with the increase in total HDL_3 and the decrease in HDL_2 in the high responders, indicating that the diet-induced increase of HDL_3 in those animals may not be a simple increase of preexisting particles; i.e., it appears from these two animals that the 125,000–140,000 HDL of d 1.125–1.22 g/ml increase out of proportion to the 160,000–200,000 molecular weight HDL of d 1.125–1.22 g/ml. However, this observation is based on a small number of animals (two hyperresponders and two hyporesponders) and additional studies will be necessary to establish that this is a consistent response in hyperresponders.

In summary, these data show that the HDL of patas monkeys is similar to that of humans in its density distribution and composition as well as in its physical and metabolic heterogeneity. Furthermore, they show that an HFHC diet, similar in many respects to that consumed by North Americans, can have a marked effect on the HDL distribution in these primates, even (as in the case of hyporesponders) in the absence of changes in their plasma cholesterol level. Why the changes reported here occur remains to be determined.

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