Atherogenic Hyperlipoproteinemia Induced by Cholesterol Feeding in the Patas Monkey†

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ABSTRACT: Patas monkeys were studied for 2 years on three dietary regimes: (1) commercial chow (control diet); (2) semipurified diet plus lard (fat-fed); and (3) semipurified diet plus lard and cholesterol (cholesterol-fed). The control and fat-fed animals had similar lipoproteins which were equivalent to the human very low density, low density (LDL), and high density lipoproteins. An additional lipoprotein referred to as LDL-II appeared to be equivalent to the human Lp(a). The cholesterol-fed animals developed accelerated atherosclerosis associated with a hypercholesterolemia which was characterized by (1) the appearance of β-migrating lipoproteins (B-VLDL) in the $d < 1.006$, (2) an increase in the intermediate lipoproteins and LDL, and (3) the appearance of LDL-II which contained a prominence of the arginine-rich apoprotein. The arginine-rich apoprotein was also a prominent component of the B-VLDL and intermediate lipoproteins. Characterization of this apoprotein revealed that it contained 11.5 mol % arginine, had a molecular weight of ~34 000, and coelectrophoresed with the arginine-rich apoprotein of man, dog, swine, rat, and rabbit.

The Patas monkey (*Erythrocebus patas*) is an old world monkey which shows considerable potential as a model for the study of plasma lipoprotein metabolism and atherosclerosis. Previously we reported that the Patas monkey on a control diet has plasma lipoproteins equivalent to the human VLDL,[1] LDL, and HDL with respect to physical and chemical properties (Mahley et al., 1976). In addition, there is a second low density lipoprotein referred to as LDL-II which occurs at a density of 1.05 to 1.085, ranges in size from 190 to 300 Å in diameter, contains the B, arginine-rich, and A-I apoproteins, and reacts with human Lp(a) antiserum. The monkey LDL-II have α_2 (pre-β) mobility and a high sialic acid content (2.8 times the typical LDL). It was proposed that this lipoprotein class is equivalent to the human Lp(a) (Mahley et al., 1976).

Cholesterol feeding studies in the Patas monkey were initiated to allow a characterization of the plasma lipoproteins associated with the production of accelerated atherosclerosis and to compare the hyperlipoproteinemia induced by cholesterol feeding in a primate with results obtained in lower species. Previously we reported that there are certain consistent features of the hyperlipoproteinemia induced by cholesterol feeding in the dog (Mahley et al., 1974), swine (Mahley et al., 1975), and rat (Mahley and Holcombe, 1976). The consistent features include: (a) the presence of the B-VLDL in the $d < 1.006$ fraction; (b) an increase in the intermediate lipoproteins ($d = 1.006$–1.02); (c) an increase in the LDL; and (d) the appearance of a lipoprotein referred to as HDL_c which becomes a prominent α_2-migrating lipoprotein primarily in the density range between 1.03 and 1.08. Associated with all of these cholesterol-induced lipoproteins is a prominence of the arginine-rich apoprotein. It will be shown that Patas monkeys develop many of the same changes in their plasma lipoproteins after cholesterol feeding.

Experimental Procedure

Patas monkeys were divided into three different dietary groups and maintained on diet for 2 years before necropsy. The control group was fed commercial monkey chow (Ralston Purina). The first experimental group was fed a semipurified diet containing 25% lard but no added cholesterol. The second experimental group was fed the same semipurified diet with 25% lard and 0.5% cholesterol. The semipurified diet contained 30% by weight dry milk solids, 20% flour, 13% casein, 7.3% applesauce, 2.2% vitamins, 2% mineral mix, and 25% lard with or without 0.5% cholesterol (formulation as described by Bullock et al., 1975). Plasma lipoproteins were analyzed when the animals had been on diet between 6 and 24 months. Plasma was obtained after an overnight fast.

Preparation of Plasma Lipoproteins. Plasma was fractionated by ultracentrifugation into various density classes (Havel et al., 1955), and the lipoproteins were purified by Geon-Pevikon block electrophoresis (Mahley and Weisgraber, 1974a). The purity of the lipoproteins was determined by paper and agarose electrophoresis and negative staining electron microscopy as previously described (Mahley and Weisgraber, 1974a,b).

Characterization of the Lipoproteins. Chemical analysis performed on the plasma and isolated lipoproteins included total (Abell et al., 1952) and esterified cholesterol (Sperry and Webb, 1950), triglyceride (Fletcher, 1968), phospholipid (Zilversmit and Davis, 1950), and protein (Lowry et al., 1951). Sialic acid was determined on native lipoproteins and values were corrected for interference from unsaturated lipids (Warren, 1959). The apoprotein content of the purified lipoproteins was analyzed by polyacrylamide gel electrophoresis on 10% gels in a Tris buffer system (pH 8.9) in 8 M urea (Reisfeld and Small, 1966) and on 10% gels in 0.1% sodium dodecyl sulfate at pH 8.2 (Weber and Osborn, 1969). Delipidation of the lipoproteins and solubilization of the apoproteins were performed as previously described (Mahley and Weisgraber, 1974b).

Apoprotein Isolation and Characterization. The apopro-

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[1] Abbreviations used are: ARG, arginine-rich apoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein; HDL, high density liproprotein; Tris, tris(hydroxymethyl)aminomethane.

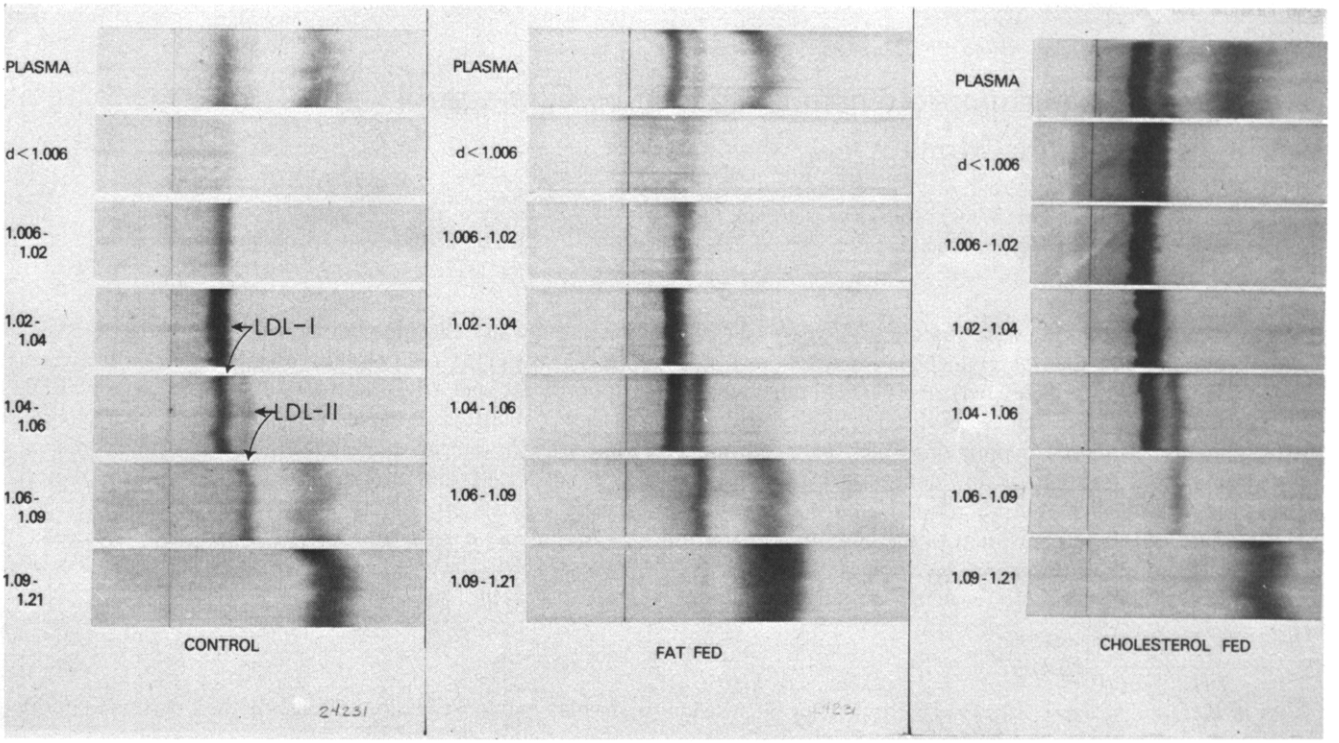

FIGURE 1: Paper electrophoretograms of the plasma lipoproteins from monkeys on the different diets.

TABLE I: Plasma Lipids.[a]

	Triglyceride	Total Cholesterol	Esterified Cholesterol	Phospholipid
Control[b]				
Mean	67	137	115	235
±SD	30.8	25.6	20.6	67.9
Fat-Fed[c]				
Mean	49	181	144	248
±SD	15.5	36.3	27	42.2
Cholesterol-Fed[c]				
Mean	39	681	502	405
±SD	32.9	288.4	211.8	76.2

[a] In units of mg/100 ml. Obtained after 6 months on experimental diets. [b] Values from 5 males and 5 females. [c] Values from 10 males and 10 females.

teins of B-VLDL ($d < 1.006$) and the intermediate fraction ($d = 1.006$–1.02) were solubilized in column buffer, 0.2 M Tris-Cl (pH 8.0) containing 4 M guanidine. Gel chromatography was performed at room temperature with Sephadex G-200 (Pharmacia) equilibrated with column buffer on 200 × 2.5 cm columns. Column fractions were dialyzed against 5 mM NH_4HCO_3 (pH 8.2) and lyophilized. The purity of the column fractions was judged by sodium dodecyl sulfate and Tris–urea polyacrylamide gel electrophoresis. Amino acid analysis was performed on pure apoproteins on a Phoenix amino acid analyzer after hydrolysis in 6 N HCl at 150 °C for 2 h in a nitrogen atmosphere.

Results

Patas monkeys (24 males and 24 females) divided among three different dietary groups were maintained on diet for 2 years prior to necropsy. During the period of time between 6 and 24 months on diet, the plasma lipoproteins from representative animals were characterized. All animals underwent a complete necropsy which included a detailed study of the topographic distribution and morphologic characterization of the cholesterol-induced atherosclerosis (results to be presented elsewhere).

The three dietary protocols included 10 animals (5 males and 5 females) on monkey laboratory chow (to be referred to as control diet), 20 monkeys (10 males and 10 females) on a semipurified diet which contained 25% lard but no added cholesterol (to be referred to as the fat-fed group), and 20 monkeys (10 males and 10 females) on the same semipurified diet with 25% lard and 0.5% cholesterol (to be referred to as the cholesterol-fed group). The monkeys fed the laboratory chow and the semipurified diet plus lard served as the controls for the cholesterol-fed animals.

Plasma lipid levels of the monkeys obtained after 6 months on the respective diets are presented in Table I. The fat-fed animals consistently had plasma cholesterol levels slightly higher than those of the monkeys on the control laboratory chow. However, there was a marked hypercholesterolemia in the cholesterol-fed group.

Previously, we have reported that the control Patas monkey had lipoproteins equivalent to the human VLDL, LDL, and HDL. In addition, the Patas monkey had an α_2-migrating lipoprotein in the density range from 1.05 to 1.085 referred to as LDL-II. Paper electrophoretograms of various ultracentrifugal density fractions revealed that the fat-fed animals had similar lipoproteins (Figure 1). However, following cholesterol feeding, there were several changes in the lipoprotein type and distribution (Figure 1). The $d < 1.006$ fraction of the cholesterol-fed animals contained a β-migrating lipoprotein (B-VLDL) as well as a pre-β VLDL. There was also an increased prominence of lipoproteins in the intermediate fraction ($d = 1.006$–1.02) and an increase in the LDL. Concomitant with these increased lipoproteins was a reduction in the HDL ($d = 1.09$–1.21). The LDL-II were present in the ultracentrifugal density fractions from $d = 1.04$ to 1.09 (Figure 1).

TABLE II: Percent Distribution of Plasma Cholesterol and Lipoprotein Protein among the Ultracentrifugal Fractions.

Plasma Cholesterol:[a]	Control			Fat-Fed	Cholesterol-Fed		
	92	98	140	141	488	648	850
			Cholesterol Distribution				
$d < 1.006$	1.0	1.1	2.8	1.2	15.7	19.7	20.0
1.006–1.02	0.6	4.0	1.8	1.8	19.3	25.9	46.0
1.02–1.04	2.6	27.9	22.3	23.8	55.0	45.9	23.6
1.04–1.06	41.5	11.8	35.2	24.6	6.5	3.2	3.6
1.06–1.09	14.0	8.8	9.4	7.9	0.4	1.3	3.9
1.09–1.21	40.3	46.3	28.4	40.8	4.1	3.9	2.9
			Protein Distribution				
$d < 1.006$	6.2	0.6	5.6	3.2	8.9	9.9	10.6
1.006–1.02	0.3	2.1	0.9	1.4	10.0	14.3	23.1
1.02–1.04	1.7	7.2	9.0	7.3	45.0	37.9	20.9
1.04–1.06	14.6	5.4	18.5	9.8	6.4	4.2	4.2
1.06–1.09	9.0	6.2	7.1	7.4	2.3	5.7	14.2
1.09–1.21	68.2	78.4	58.9	70.8	27.3	28.0	26.6

[a] mg/100 ml.

The qualitative impressions from paper electrophoresis of changes in the plasma lipoproteins following cholesterol feeding were supported by a comparison of the percent distribution of the plasma cholesterol and lipoprotein protein among the ultracentrifugal fractions (Table II). Animals on the control or fat-fed chow had a similar distribution of the plasma cholesterol with approximately 50% of the cholesterol transported by lipoproteins with a density greater than 1.06. Following cholesterol feeding, less than 7% of the total plasma cholesterol was associated with the d = 1.06–1.21 fractions. The elevated plasma cholesterol was principally associated with the B-VLDL and VLDL ($d < 1.006$), the intermediate lipoproteins (d = 1.006–1.02), and LDL (d = 1.02–1.04). A comparison of the percent distribution of the total lipoprotein protein revealed a similar shift to lipoproteins of lower densities (Table II). The prominence of lipoproteins at the lower densities was greater as the plasma cholesterol levels increased. Following cholesterol feeding, there was an absolute increase in total lipoprotein protein which ranged from 1.5 to 2 times the values obtained for the control animals. However, there was an absolute decrease in total HDL protein (d = 1.09–1.21). The mean total HDL protein level for five control animals was 92 mg % (range: 83–104) and for three fat-fed animals 108 mg % (range: 100–113). In eight monkeys fed the cholesterol diet, the mean HDL protein was 56 mg % (range: 45–74).

Isolation and Characterization of Lipoproteins Associated with Hypercholesterolemia. Detailed characterization of the plasma lipoproteins of monkeys fed control laboratory chow has been presented elsewhere (Mahley et al., 1976) and only pertinent findings for control animals necessary to demonstrate changes following cholesterol feeding will be shown here. There were no significant differences in the chemical composition, particle size by electron microscopy, or apoprotein content between the laboratory chow and fat-fed control animals. However, all results to be shown will be from the animals fed laboratory chow.

When more than one lipoprotein class was present within a particular ultracentrifugal density fraction, the lipoproteins were isolated by the Geon-Pevikon block electrophoretic procedure. Recovery of the lipoprotein protein applied to the electrophoretic block was usually greater than 80%. The purified lipoproteins were subjected to chemical analysis, electron microscopy by negative staining, and polyacrylamide gel electrophoresis. The lipoproteins from eight hypercholesterolemic monkeys were analyzed with comparable results. Data from representative experiments are presented.

Geon-Pevikon electrophoresis of the $d < 1.006$ fraction from the cholesterol-fed group resulted in two zones which were removed separately from the block. The band which migrated approximately 6 cm from the origin with mobility similar to LDL was referred to as the B-VLDL. The VLDL migrated ahead of the B-VLDL (~11 cm from the origin). The chemical composition of the B-VLDL revealed 60% cholesterol, of which ~75% was esterified, and 6% triglyceride (Table III). The VLDL were also cholesterol rich (45%) compared with the control VLDL (9%) (Table III). The B-VLDL ranged in size from 270 to 600 Å in diameter by negative staining electron microscopy as compared with 320 to 900 Å for the VLDL (Table III). Control VLDL were similar in size to the VLDL of the hypercholesterolemic animals.

The d = 1.006–1.02 and d = 1.02–1.04 ultracentrifugal fractions contained only a β-migrating lipoprotein which was not further purified. The chemical composition and size by negative staining of the intermediate lipoproteins (d = 1.006–1.02) and the LDL (d = 1.02–1.04) are presented in Table III.

There were two prominent lipoprotein bands visualized in the d = 1.04–1.06 and d = 1.06–1.09 fraction which were isolated by the Geon-Pevikon electrophoretic procedure. The lipoprotein with β mobility which migrated ~6 cm from the origin was referred to as LDL-I and the lipoprotein with α_2 mobility (migrated ~9.5 cm) was referred to as LDL-II as previously described for the control monkey (Mahley et al., 1976). An intermediate zone 0.5 to 1.0 cm in width between the LDL-I and LDL-II on the electrophoretic block contained a variable mixture of these two lipoproteins which accounted for 10–20% of the total protein applied to the block. The chemical composition of the LDL-I and LDL-II from the cholesterol-fed animals revealed that they were more cholesterol rich than the corresponding lipoproteins of the control animals (Table III). The LDL-II usually contained 5–10% triglyceride. Particle sizes by electron microscopy are shown in Table III. Quantitation of the total plasma LDL-II was difficult because of the variable mixture of LDL-I and LDL-II in the intermediate zone between the two purified lipoproteins

TABLE III: Percent Composition and Particle Size of the Lipoproteins.

	Triglyceride	Cholesterol	Phospholipid	Protein	Particle Size (Å)[a]
Control Monkey					
$d < 1.006$					
VLDL	40.0	8.6	37.1	14.3	300–900
1.02–1.09					
LDL-I	4.7	45.5	27.9	21.9	190–250
LDL-II	6.5	36.5	29.7	27.0	200–300
1.09–1.21					
HDL	2.2	18.0	36.6	43.2	70–100
Cholesterol-Fed					
$d < 1.006$					
B-VLDL	6.4	60.0	25.2	8.4	270–600
VLDL	24.8	44.6	22.9	7.7	320–900
1.006–1.02					
ILDL	0.8	56.8	29.9	12.4	270–400
1.02–1.04					
LDL-I	1.9	53.4	26.3	18.4	
1.04–1.06					
LDL-I	2.1	51.2	28.5	18.3	200–300
LDL-II	10.0	43.3	28.3	18.3	175–300
1.06–1.09					
LDL-II	7.9	35.0	35.7	21.4	150–275
HDL					100–150
1.09–1.21					
HDL	0.8	17.7	31.6	49.7	70–100

[a] The diameters of approximately 200 particles for each lipoprotein class were measured. Each range represents more than 95% of the particles measured excluding the occasional particles at either end of the spectrum of sizes.

on the electrophoretic block. However, with cholesterol feeding there did not appear to be a significant increase in the total LDL-II protein in comparison with the control animals. Based on results from the eight studies of hypercholesterolemic animals, the total LDL-II protein was approximately 6 mg/100 ml, essentially the same as determined in the plasma of the control monkeys (Mahley et al., 1976).

The ultracentrifugal fraction $d = 1.09–1.21$ from the cholesterol-fed animals contained a single α-migrating lipoprotein with a chemical composition of 50% protein and 32% phospholipid. The composition was similar to that of the HDL of the control monkey (Table III). The HDL from both the cholesterol-fed and control animals ranged in size from 70 to 100 Å in diameter (Table III).

The apoprotein patterns by sodium dodecyl sulfate–polyacrylamide gel electrophoresis revealed several changes in the various lipoproteins after cholesterol feeding (Figure 2). A prominent apoprotein appeared in the B-VLDL, the intermediate lipoproteins ($d = 1.006–1.02$), and the LDL-II. This protein (labeled b in Figure 2) has been identified as the arginine-rich apoprotein (characterization to follow). The arginine-rich apoprotein (ARG) purified from the $d < 1.006$ fraction of the cholesterol-fed monkey is shown in Figure 2 for comparison. This apoprotein was present in small amounts in the VLDL, intermediate lipoproteins, and LDL-II in the laboratory chow or fat-fed animals (Mahley et al., 1976). The A-I apoprotein isolated from the control monkey HDL and previously shown to be homologous to the human apo-A-I was present in the VLDL, LDL-II, and HDL of the cholesterol-fed animals (labeled c in Figure 2). The purified A-I apoprotein is shown for comparison.

The apoprotein pattern for the LDL-II from the cholesterol-fed monkeys revealed an increase in the arginine-rich and the A-I apoproteins as compared with the LDL-II of the control animals (Figure 2). In addition, the LDL-II contained a major apoprotein which did not enter the acrylamide gel and which appeared to be equivalent to the B apoprotein. As previously reported for the LDL-II of the control monkeys, the B apoprotein appeared to be an apoprotein constituent, not secondary to LDL contamination, because further subfractionation of the LDL-II class by Geon-Pevikon electrophoresis or A-5m agarose column chromatography failed to remove the B apoprotein. The apoprotein content of this class which included apo-B, the arginine-rich apoprotein, apo-A-I, and the low-molecular-weight C apoproteins remained qualitatively the same for the various subfractions isolated by the electrophoretic or chromatographic procedures (Mahley et al., 1976).

Polyacrylamide gel electrophoresis on the Tris-urea (pH 8.9) system confirmed and extended the above impressions. The arginine-rich apoprotein (labeled b in Figure 3) was a major component of the several lipoproteins of the cholesterol-fed monkey. In addition to the arginine-rich apoprotein, the B-VLDL and VLDL contained apoprotein bands equivalent to the B (labeled a), the A-I (labeled c), and a variable amount of the fast-migrating C apoproteins (labeled d). The higher the plasma cholesterol, the less visible were the C apoproteins. Monkey M-1 had a plasma cholesterol of 488 mg/100 ml as compared with M-2 which had a cholesterol of 648 mg/100 ml. The presence of the arginine-rich apoprotein and a decrease in the C apoproteins have been reported in the B-VLDL of human type III hyperlipoproteinemic patients (Havel and Kane, 1973). The LDL-I contained primarily the B apoprotein but also small amounts of the arginine-rich and A-I apoproteins. The LDL-II contained the B apoprotein, a large amount of the arginine-rich apoprotein, and a variable amount of the A-I and C apoproteins. The major apoproteins of the HDL of the cholesterol-fed monkey were apo-A-I and apo-A-II (labeled e). In addition, the HDL in this particular experiment

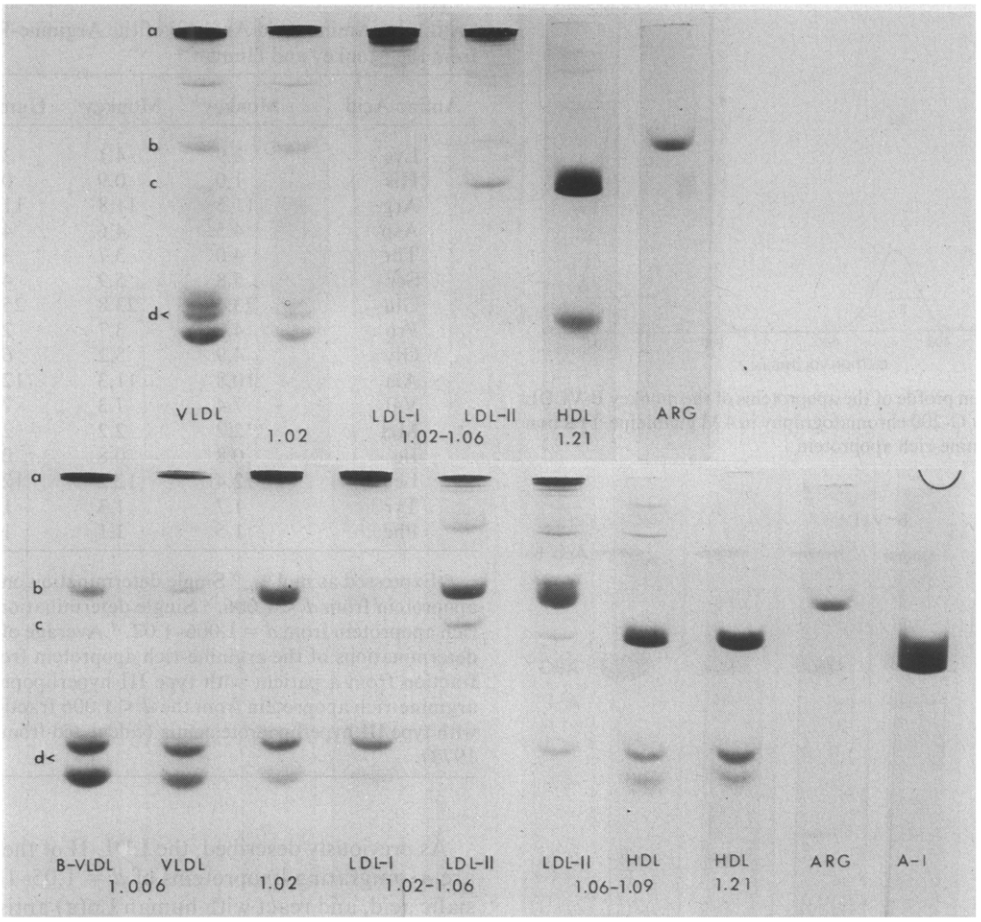

FIGURE 2: Sodium dodecyl sulfate polyacrylamide gel electrophoresis of the apolipoproteins of control (top panel) and cholesterol-fed monkeys (bottom panel) (8–12 μg of protein/gel). The bands labeled a–d correspond to the B, the arginine-rich, the A-I, and the low-molecular-weight C apoproteins, respectively. The purified A-I and arginine-rich (ARG) apoproteins are shown for comparison.

contained the arginine-rich apoprotein.

Apoprotein Isolation and Characterization. Sephadex gel filtration with guanidine of the lipoproteins at $d < 1.006$, B-VLDL, and $d = 1.006$–1.02 (intermediate fraction) from the cholesterol-fed monkey resolved three main fractions (Figure 4). Fraction I occurred near the void volume of the column and on polyacrylamide gel electrophoresis the protein did not enter the gel. Fraction II was obtained from the first portion of the second peak and was found to be the arginine-rich apoprotein. It migrated as a single band on sodium dodecyl sulfate and Tris–urea polyacrylamide gel electrophoresis. The second portion of the second peak contained the A-I apoprotein. The third fraction contained the fast-migrating proteins equivalent to the C apoproteins.

The arginine-rich apoprotein from the $d < 1.006$ (B-VLDL and VLDL) and $d = 1.006$–1.02 (intermediate) fractions had the same migration on polyacrylamide gel electrophoresis and an apparent molecular weight of approximately 34 000. In addition, the arginine-rich apoprotein from the monkey had the same migration as the arginine-rich apoprotein from B-VLDL of the human type III and the cholesterol-fed swine, dog, rabbit, and rat (Figure 5). The amino acid analyses of the monkey arginine-rich apoprotein as compared with the arginine-rich apoprotein from type III B-VLDL are presented in Table IV.

Sialic Acid Content of LDL-I and LDL-II. Previously, we had shown that LDL-I and LDL-II of the control monkey differed with respect to their sialic acid content. LDL-I from the control monkey contained 11.7 μg of sialic acid/mg of protein as compared with 33.4 in LDL-II (Mahley et al., 1976). Likewise, after cholesterol feeding, the LDL-II revealed a higher sialic acid content than the LDL-I. The values obtained from the cholesterol-fed animals were as follows (μg of sialic acid/mg of protein): LDL-I from $d = 1.02$–1.06, 12.5; LDL-II from $d = 1.02$–1.06, 24.6; LDL-II from $d = 1.06$–1.09, 30.7.

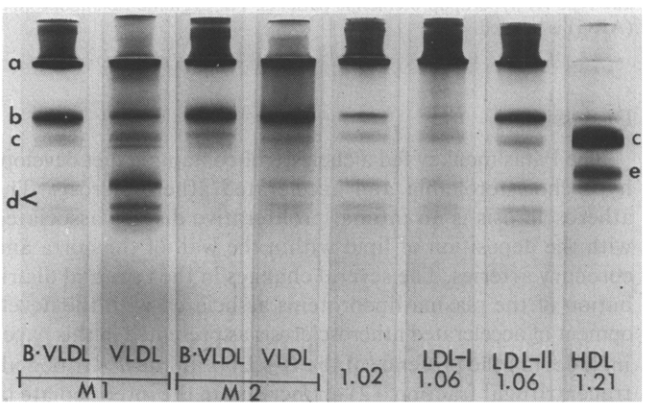

FIGURE 3: Tris–urea polyacrylamide gel electrophoresis of the apoproteins from cholesterol-fed monkeys (50 μg of protein/gel). The bands labeled a–e correspond to the B, arginine-rich, A-I, C, and A-II apoproteins, respectively.

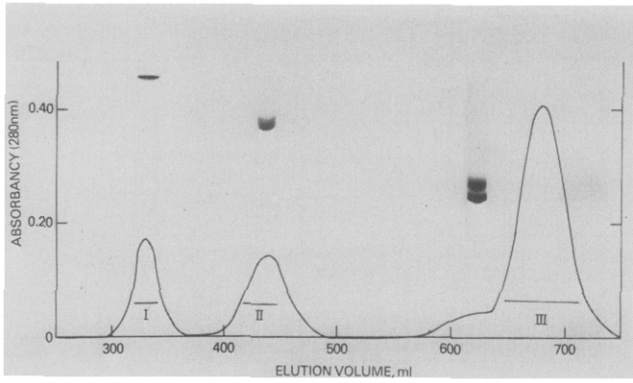

FIGURE 4: The elution profile of the apoproteins of the monkey B-VLDL obtained by Sephadex G-200 chromatography in 4 M guanidine. Fraction II contained the arginine-rich apoprotein.

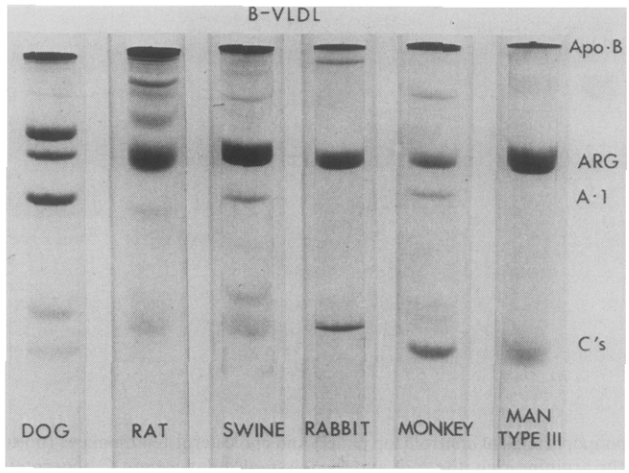

FIGURE 5: Sodium dodecyl sulfate polyacrylamide gel electrophoresis of the β-migrating lipoproteins (B-VLDL) obtained from the $d < 1.006$ fraction following cholesterol feeding in the dog, rat, swine, rabbit, monkey, and type III hyperlipoproteinemic patients. The arginine-rich apoprotein (ARG) is labeled.

TABLE IV: Amino Acid Analysis of the Arginine-Rich Apoprotein from the Monkey and Human.[a]

Amino Acid	Monkey[b]	Monkey[c]	Human[d]	Human[e]
Lys	3.9	4.1	3.7	5.5
His	1.0	0.9	0.6	1.1
Arg	11.5	11.8	11.0	10.4
Asp	4.5	4.6	4.3	4.7
Thr	4.0	3.7	3.6	3.7
Ser	5.8	5.9	4.9	5.1
Glu	23.4	23.8	25.8	23.6
Pro	4.2	3.7	2.4	2.1
Gly	4.9	5.2	6.8	6.8
Ala	10.8	11.3	12.2	12.5
Val	7.4	7.3	7.5	5.9
Met	2.2	2.2	2.1	2.0
Ile	0.8	0.8	0.8	0.6
Leu	12.4	12.2	12.8	12.5
Tyr	1.7	1.3	1.6	1.7
Phe	1.5	1.1	1.2	1.7

[a] Expressed as mol %. [b] Single determination on the arginine-rich apoprotein from $d < 1.006$. [c] Single determination on the arginine-rich apoprotein from $d = 1.006$–1.02. [d] Average of two independent determinations of the arginine-rich apoprotein from the $d < 1.006$ fraction from a patient with type III hyperlipoproteinemia. [e] The arginine-rich apoprotein from the $d < 1.006$ fraction from a patient with type III hyperlipoproteinemia (calculated from Havel and Kane, 1973).

Discussion

The Patas monkey fed a cholesterol-containing diet develops hypercholesterolemia and accelerated atherosclerosis. The atherosclerosis is an intimal proliferative disease associated with the deposition of lipid within the wall of the aorta and coronary arteries. The several changes in the type and distribution of the plasma lipoproteins associated with the development of accelerated atherosclerosis as presented in this paper include: (1) the presence of the B-VLDL in the $d < 1.006$ ultracentrifugal fraction; (2) an increase in the intermediate lipoproteins; (3) an increase in the LDL referred to as LDL-I; and (4) the occurrence of LDL-II which are α_2-migrating lipoproteins in the ultracentrifugal fraction $d = 1.05$–1.085 and which contain an abundance of the arginine-rich apoprotein. The B-VLDL induced by cholesterol feeding resemble the B-VLDL described in patients with type III hyperlipoproteinemia (Havel and Kane, 1973) and in several lower species fed cholesterol-containing diets (Mahley et al., 1974; Mahley et al., 1975; Mahley and Holcombe, 1976; Shore et al., 1974). The B-VLDL are rich in cholesterol, contain an abundance of the arginine-rich apoprotein, and may represent remnants of chylomicrons or VLDL catabolism. The intermediate lipoproteins ($d = 1.006$–1.02) are also rich in cholesterol and contain a prominence of the arginine-rich apoprotein.

As previously described, the LDL-II of the control monkey are α_2-migrating lipoproteins of $d = 1.05$–1.085, are rich in sialic acid, and react with human Lp(a) antiserum. The apoprotein content includes the B apoprotein and small amounts of the arginine-rich and A-I apoproteins. Following cholesterol feeding, the LDL-II have the same mobility, occur at the same density, and retain the high sialic acid content. However, the apoprotein content reveals a prominence of the arginine-rich and A-I apoproteins along with the B apoprotein. The LDL-II resemble the HDL_c of the lower species with respect to α_2 mobility and an abundance of the arginine-rich apoprotein. However, the HDL_c of the lower species lack the B apoprotein and, unlike HDL_c, the LDL-II do not appear to increase in concentration in the plasma with cholesterol feeding. The occurrence of the arginine-rich apoprotein may reflect a heterogeneity of this class of lipoproteins which is not resolved by the electrophoretic or chromatographic methods employed. There may be an arginine-rich apolipoprotein which appears in response to cholesterol feeding distinct from the LDL-II.

Lipoproteins with properties similar to the monkey LDL-II are present in human plasma and referred to by a variety of names, e.g., "sinking" pre-β (Rider et al., 1970), pre-β_1 (Dahlén, 1974), and Lp(a) (Berg, 1963; Albers and Hazzard, 1974). It appears likely that these are the same lipoproteins (for review, see Dahlén, 1974). These lipoproteins migrate in the α_2 position, occur at a density of approximately 1.05–1.08, and have chemical properties which distinguish them from LDL. Little is known about the origin of this class of lipoproteins, but it has been suggested that they may arise from VLDL (Ehnholm et al., 1972). Detection of Lp(a) immunologic reactivity in association with VLDL ($d < 1.006$) led to further speculation that Lp(a) may arise from VLDL in a manner similar to LDL production (Walton et al., 1974). Likewise, little is known about the metabolic significance of the Lp(a). Plasma Lp(a) levels do not correlate with age, sex, total cholesterol, glyceride, or the total B apoprotein (Albers et al.,

1974). In addition, Lp(a) levels are refractory to pharmacological and dietary manipulation, but have been shown to be elevated in type IIa patients (Albers et al., 1975). There is a correlation between plasma Lp(a) reactivity and coronary artery disease (Berg et al., 1973) and between sinking pre-β lipoprotein and myocardial infarction and intermittent claudication (Eriksson and Carlson, 1973). The Lp(a) antigen is detectable by immunofluorescence in atherosclerotic lesions with a topographic distribution similar to that of LDL (Walton et al., 1974). The precise identity and role of these lipoproteins await more vigorous characterization of the purified lipoprotein. The Patas monkey may serve as a useful model in this regard.

Associated with cholesterol feeding and accelerated atherosclerosis in various species including the monkey is the appearance of several different cholesterol-rich lipoproteins all of which contain the arginine-rich apoprotein. The mechanism of transport of plasma lipoproteins across the endothelial surface and the accumulation of their cholesterol in arterial cells (principally smooth muscle cells) is a critical factor in understanding the cellular basis of atherosclerosis. The recognition that cell surface receptors on fibroblasts bind plasma lipoproteins and initiate a series of events which culminates in the accumulation of cholesteryl esters within the cells greatly advanced our understanding of cellular metabolism (Brown and Goldstein, 1974). Goldstein and Brown (1974) demonstrated that LDL and VLDL which contain the B apoprotein are bound to the cell surface receptors and postulated that there was a specific LDL apoprotein receptor. However, in addition to the B apoprotein, the arginine-rich apoprotein may also function as a cholesterol-transporting protein important in the transfer of cholesterol to cells. Tissue culture studies using aortic smooth muscle cells (Assmann et al., 1975; Mahley, 1976) and fibroblasts (Bersot et al., 1976) have demonstrated that HDL_c, a cholesterol-induced lipoprotein which lacks the B apoprotein and contains primarily the arginine-rich apoprotein, can bind at the "LDL" receptor site and lead to cellular cholesteryl ester accumulation. Comparative studies of the types of plasma lipoproteins and the morphologic characteristics of the arterial disease in experimental models such as the monkey provide insight into common mechanisms associated with the induction of atherosclerosis.

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