

PLASMA LIPOPROTEINS OF FREE-RANGING HOWLING MONKEYS (*ALOUATTA PALLIATA*)

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Abstract—1. Plasma lipids and lipoproteins of free-ranging howling monkeys from Costa Rica (*Alouatta palliata*), aged 5 months to 23 years, were characterized.

2. High density lipoproteins were lipid-rich, similar to HDL₂ of human plasma.

3. Fatty acid compositions of major lipid classes of very low, low and high density lipoproteins differed among social groups, possibly due to both dietary and genetic factors.

4. Low and high density lipoprotein phospholipids were enriched in phosphatidylethanolamine.

5. Howler plasma cross reacted with antihuman apoA-I antibodies but not with antihuman LDL antibodies.

6. No dimeric form of apoA-II was present, unlike human apoA-II.

INTRODUCTION

Primates such as apes, Old and New World monkeys are considered good animal models for human physiology and pathology because of their close phylogenetic relationships to humans (*Homo sapiens*). In particular, non-human primates are used frequently to study induced hyper- and hypolipidemias and the mechanisms for the development and regression of atherosclerotic lesions. Without exception, these animals are maintained in captive colonies on defined diets. However, because of the probable confounding effects of artificial confinement and of the ingestion of a constant, unvaried diet on the development of cardiovascular disease, we believe it is important to determine the plasma lipid and lipoprotein patterns of free-living monkeys eating a self-selected diet. The present report represents, to our knowledge, the first such determinations, which were conducted on a group of New World monkeys (*Alouatta palliata*, howling monkeys) captured on their foraging ranges as part of a long-term study of their behavior, ecology and demography (Glander, 1980, 1981; Clarke and Glander, 1984). This is also the first report on howling monkey lipoproteins under any condition, primarily because this species has never been maintained in captive colonies unlike other New World primates such as squirrel monkeys (*Saimiri*), capuchin (*Cebus*), spider (*Ateles*), night monkeys (*Aotus*) and marmosets (*Callithrix*) (Srinivasan *et al.*, 1974; Chapman *et al.*, 1979; Chapman, 1980).

MATERIALS AND METHODS

The study group comprised 11 free-ranging howling monkeys from four different social groups inhabiting 990 acres of forest located on Hacienda La Pacifica in north-

western Costa Rica (see Glander, 1980, for description of study area). The monkeys' ages, estimated by tooth wear, ranged from five months to about 23 years. There were nine males and two females, one lactating. The animals were caught while foraging and blood was obtained from a femoral vein within 3 hr of capture. The animals were anesthetized on the range with Telezol® (Warner-Lambert, Ann Arbor, MI) to facilitate capture and, when necessary, received a supplementary dose of ketamine prior to venipuncture.

Between 4–10 ml of blood was collected from each animal into EDTA-containing vacutainer tubes and the formed elements were separated immediately by centrifugation. The plasmas were transferred to plastic tubes containing Na azide (0.01%), EDTA* and PMSF (1 mM) final concentrations and were kept frozen until analyzed eight weeks later.

Plasmas (1–4 ml) were subjected to density gradient equilibrium ultracentrifugation in SW41 swinging bucket rotors (Redgrave *et al.*, 1975) to separate high, low and very low density lipoproteins (HDL, LDL, VLDL). The three fractions, which were visibly distinguishable as two yellow bands (HDL and LDL) and a creamy top layer (VLDL) separated by clear zones, were collected by serial aspiration at densities (g/ml), respectively, of <1.01 (VLDL), 1.048–1.063 (LDL), 1.089–1.145 (HDL) and a bottom fraction ($d > 1.21$ g/ml). To define the lipoprotein (LP) density ranges, the densities of aliquots collected from identical regions of a blank gradient tube containing no plasma, which was included with the centrifugal run, were determined by picnometry in calibrated Lang-Levy pipettes (100 μ l). The separated LPs were lyophilized and their component lipids and apolipoproteins were determined. Other aliquots of whole plasma were used for agarose gel electrophoresis to separate LP classes (Corning electrophoresis system, Palo Alto, CA), for apolipoprotein determinations by immunoassay (A-I and B) and for quantitation of component lipids.

Lipid analyses

Plasma (200 μ l) was extracted according to Folch *et al.* (1957), the solvent was removed under nitrogen and the lipids were dissolved in chloroform-methanol (2:1) (1 ml).

*Abbreviations used: EDTA, disodiumethylenetetraacetic acid; PMSF, paramethylsulphonyl fluoride.

Replicate aliquots of the hexane solutions were taken for determination of total lipid wt (10 μ l), total phospholipids (PL; 100 μ l; Bartlett, 1959), glyceride glycerol (TG; 100 μ l; Sigma Kit #320-UV, Sigma Chemical Co., St Louis, MO) and total and unesterified cholesterol by gas-liquid chromatography (TC and UC; 50 μ l; Bennett Clark and Norum, 1977). Esterified cholesterol (CE) was calculated from the difference, TC minus UC.

Plasma lipoprotein fractions (VLDL, LDL, HDL) and the $d > 1.21$ g/ml fraction were combined within social groups for determination of PL class composition and of CE, TG and lecithin (PC) fatty acid (FA) profiles. All animals contributed equal lipid masses to the pools. Neutral lipids were separated by silicic acid thin layer chromatography, the CE and TG bands were scraped from the plates and the lipids were eluted. The component FAs were hydrolyzed and methylated in the presence of boron trifluoride. Individual FA methyl esters were separated by gas-liquid chromatography (GLC) with flame ionization detection using a 6 ft, 5% DEGS on Chromosorb W column and were identified by their relative retention times. PL classes were separated by high performance liquid chromatography (HPLC) on a silica column, using a hexane-isopropanol-water-ethanol (32:50:8:16.7) mobile phase. The major PL fractions were collected and were quantitated by phosphorus determination (Bartlett, 1959). Fatty acid methyl esters were prepared from the PC fractions and were analyzed by GLC as for TG and CE fatty acids.

Radioimmunoassay of apolipoproteins A-I and B in whole plasma

Plasmas (50 μ l) were diluted 21-fold and apoA-I and apoB concentrations were determined by radioimmunoassay (RIA) using monoclonal antihuman apoprotein A-I and polyclonal antihuman LDL antibodies, respectively (Ventrex Laboratories, Portland, ME). ApoA-I is the major apoprotein of HDL and apoB is the sole apoprotein of LDL.

Apoprotein compositions of lipoproteins

Lipoprotein fractions (LDL, HDL) obtained by gradient ultracentrifugation of plasma from the lactating female monkey (351) were dialyzed, concentrated and the proteins were separated by electrophoresis in SDS-polyacrylamide gradient gels (3–24%) containing 18% glycerol, in the presence and absence of 2-mercaptoethanol, and were stained with Coomassie Blue.

RESULTS

Plasma

The concentrations of the major lipid classes (TG, PL, UC, CE) in non-fasting howling monkey plasma, ranked according to the animals' ages, are shown in Table 1. Within the limits of the method used to estimate age, namely, tooth wear, there appear to be no trends in the plasma lipid profiles. There was one aberrant animal (251) in the sample population which had extremely low plasma lipid and apoA-I levels, possibly due to an intrinsic lipoprotein abnormality. Except for monkey 251, the apparent apoA-I concentrations, determined with monoclonal antibodies directed against human apoA-I were 90 ± 11 mg/dl, quite similar to human plasma levels of 120–130 mg/dl. ApoB on the other hand, determined with polyclonal antibodies directed against human LDL, revealed very little cross-reactivity (monkey plasma apoB equivalent to < 12 mg/dl compared with a human range of 80–130 mg/dl).

Lipoproteins

Electrophoretic separation of the major LP classes in agarose gels showed both α and β LPs (respectively HDL and LDL) of howlers to be more negatively charged (faster migrating) than the equivalent LPs of normal human plasma. Figure 1 shows the densitometric scans of representative developed gels; all individual monkeys showed identical electrophoretic patterns. Equilibrium density gradient ultracentrifugation, performed to separate the LP classes according to their absolute densities, also revealed differences in the monkey and the human LPs. In particular, howler HDL appeared to contain only the lighter, more lipid-rich, fraction similar to human HDL₂, which floats at a density range of 1.063–1.125 g/dl. No LP band was visible in the density range 1.125–1.21 g/dl, equivalent to human HDL₃.

Of the total lipid found in plasma $27.4 \pm 3.0\%$ was recovered in VLDL, $33.0 \pm 3.1\%$ in LDL and $23.9 \pm 3.2\%$ in HDL, amounting to a total recovery of $84.4 \pm 4.5\%$ in the three LP fractions. When lipid

Table 1. Plasma lipids and estimated age

Males	Group	Age (years)	TG*	PL†	UC (mg/dl)	CE‡	Total lipids§
355	19	5 months	101	177	42	160	495
258	11	4	111	178	30	127	475
385	3	7	180	178	40	131	540
254	11	11	70	159	33	123	430
250	11	18	123	157	31	99	400
252	11	18	56	181	35	127	450
180	18	20	102	188	34	105	470
387	3	22	159	160	33	116	420
251	11	23	0	11	tr	tr	35
Females							
351	19	lactating	114	193	40	158	520
181	18	15	96	212	48	180	555
$\bar{x} \pm SE¶$			111 \pm 37	178 \pm 17	37 \pm 2	133 \pm 26	476 \pm 16

*An average mol. wt of 885 was assumed in converting moles glycerol to TG mass.

†A factor of 25 was used to convert phosphorus mass to PL mass.

‡A factor of 1.68 was used to convert esterified cholesterol as sterol to cholesterol ester (CE).

§By direct weighing of extracted total lipids. Recoveries of individual lipids assayed (TG + PL + UC + CE) = 97.8 ± 2.6 (SE) of total lipids extracted.

||Trace amount present.

¶Excluding aberrant animal 251.

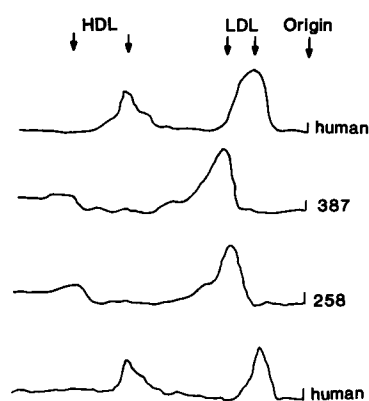


Fig. 1. Densitometric scan traces of representative agarose gel electrophoretograms of human and howling monkey plasma. All plasmas were run simultaneously on the same gel and were stained with Coomassie Blue after development. Proteins migrated left from the origin. The heights of the deflections reflect intensity of stain. Arrows indicate relative migration distances.

in the $d > 1.21$ g/ml bottom was included the total lipid recovery in all plasma fractions was $98.5 \pm 3.2\%$ of that extracted from whole plasma, which confirmed the absence of HDL₃. The lipid compositions of the major LP classes are shown in Table 2. Howler VLDL and LDL lipid compositions were quite similar to human VLDL and LDL. HDL lipid composition corresponded more closely to human HDL₂ than HDL₃, in agreement with its flotation properties.

The FA profiles of the major lipid classes of VLDL, LDL and HDL pooled within social groups are shown in Table 3. About half of the FA in all three LPs were saturated. In TG and CE, approx. 90% of the total saturated FA was palmitate (16:0) whereas in lecithins (PC) 16:0 and 18:0 (stearate) were about equal. Of the total FA in TG, 16:0 and 18:1 (oleate) together made up 60–70%, whereas the major FA of CE (30–50% of total FA) was linoleate (18:2). Overall, PC FAs were quite similar among all four groups of monkeys in all LP fractions. However, there were some striking differences between the groups in HDL TG and CE FA profiles. For example, the 16:0 content of HDL TG varied between 30–52% while 18:2 and 18:3 each were only about

1% of total FA in group 3 but reached 14 and 18% in group 4. Also, HDL CE of group 3 were strikingly enriched with 16:0 (38%) and depleted in 18:2 (5%) compared with the other three groups (respectively 18–20% and 37–50%).

The phospholipid class compositions of howler VLDL, LDL and HDL are shown in Table 4. Similarly to most mammalian species, the major PL class of all three LP fractions was PC. However phosphatidylethanolamine (PE) was unusually elevated in LDL and HDL, especially when compared with VLDL. LDL appeared deficient in sphingomyelin compared with the other LP fractions.

Polyacrylamide gel electrophoresis of the LDL and HDL apoproteins revealed prominent bands with mol. wts similar to human apoB-100 (513,000) and to apoA-I (28,000) and apoC (6500–9000), respectively (Fig. 2). A small amount of apoE (mol. wt = 34,000) was also observed in howler HDL, but there was no apoA-II dimer and no apoprotein with a mass similar to that of human apoA-IV (46,000).

DISCUSSION

The present report is the first description of plasma lipids and lipoproteins in the New World monkey genus *Alouatta* and, to our knowledge, represents the first report of lipoproteins in free-ranging primates of any genus. The following comparisons between howling monkeys and other primates may thus be confounded by the defined diets and restricted confinement of the captive primates reported previously. With this caveat, howlers show many similarities to and some differences from, both New and Old World primates and humans. Also, individual foraging groups showed marked variations in plasma FA profiles (see Table 3). These variations may be the result of dietary differences, or may possibly be due to differences in genetic makeup.

Overall, howler plasma total lipid levels were similar to the original normal human plasma lipid concentration range cited by Skipski *et al.* (1967) but were somewhat lower than currently accepted human values of 550–600 mg/dl, due mainly to a lower PL concentration in the howlers (human PL 200–250 mg/dl). Howler total lipids were distinctly higher than those of squirrel monkeys, *Saimiri sciureus* (284 mg/dl, Illingworth, 1975).

The three LP fractions found in howler plasma (VLDL, LDL and HDL) contained roughly similar

Table 2. Plasma lipoprotein lipid composition

	Density (g/ml)		TG	PL	UC	CE
VLDL	<1.01	wt%*	36.0 ± 2.1	27.9 ± 0.5	9.5 ± 0.3	26.5 ± 1.9
		mg/dl	50 ± 7	40 ± 5	13 ± 2	40 ± 5
		% of plasma†	42.3 ± 4.3	21.5 ± 2.6	36.0 ± 4.1	28.1 ± 3.7
LDL	1.01–1.063	wt%	19.0 ± 1.7	31.8 ± 0.7	11.0 ± 0.3	38.2 ± 1.5
		mg/dl	24 ± 3	41 ± 4	14 ± 1	49 ± 6
		% of plasma	23.1 ± 2.6	22.9 ± 1.9	38.1 ± 2.3	37.7 ± 3.0
HDL	1.09–1.15	wt%	13.0 ± 1.1	43.2 ± 0.9	7.9 ± 0.4	35.8 ± 1.6
		mg/dl	9 ± 2	30 ± 5	6 ± 1	25 ± 5
		% of plasma	8.5 ± 1.6	16.7 ± 2.8	16.2 ± 3.4	17.7 ± 4.0

Data are means ± SE of nine animals. All animals contributed equal lipid masses to the LP pools. The aberrant male (251) and the lactating female (351) have been omitted.

*% of total lipid mass extracted from the isolated LP fractions (Folch *et al.*, 1957). Calculations as in Table 1.

†Lipid content in LP expressed as % of total extracted from whole plasma.

Table 3. Fatty acid mol % composition of lipoprotein triglycerides, cholesterol esters and lecithins

Group	N	FA	TG			CE			PC				
			VLDL	LDL	HDL	VLDL	LDL	HDL	VLDL	LDL	HDL		
18	2	14:0	2.1	2.0	1.6	1.1	1.7	0.4	0.1	5.2	0.4		
		16:0	40.7	38.0	35.3	20.4	18.8	18.1	20.7	27.1	23.3		
		16:1	3.2	3.9	3.3	4.0	2.2	3.4	0.9	1.4	1.2		
		(17:0)*	0	3.7	3.7	0	0.7	0	1.7	1.6	1.6		
		18:0	5.5	5.8	7.5	2.2	1.9	0	21.4	12.4	21.3		
		18:1	24.7	22.2	22.8	17.9	17.6	19.2	14.7	10.6	12.6		
		18:2	10.7	10.6	10.9	46.8	47.7	49.8	25.7	26.6	29.6		
		18:3	10.8	9.8	10.4	6.6	6.5	6.7	4.2	5.4	3.9		
		20:1 + 20:2 + 20:3	0	0.3	0.7	0	0	0	4.3	2.3	0.7		
		20:4	0	0.9	1.0	0	0	0	5.0	2.5	4.2		
		unknown†	2.4	2.8	1.9	1.1	3.0	3.0	2.2	5.9	2.5		
		11	4	14:0	2.7	2.1	0	1.2	1.1	1.3	0.2	0.2	0.3
				16:0	39.8	39.1	29.9	19.6	17.4	19.5	26.9	26.5	23.7
16:1	3.5			3.6	2.1	4.2	4.0	4.9	1.1	0.9	1.4		
(17:0)	2.2			3.1	3.1	0.9	0.6	0.9	2.1	1.9	1.9		
18:0	4.2			5.0	6.7	2.4	1.9	2.0	18.4	18.7	16.6		
18:1	18.8			20.2	24.5	20.5	21.4	20.1	13.7	13.0	12.7		
18:2	11.1			10.6	13.6	36.2	39.0	37.2	26.7	28.1	29.3		
18:3	14.8			13.5	17.9	10.7	10.1	9.5	5.2	5.4	6.7		
20:1 + 20:2 + 20:3	0			0	0	0.7	0	0	0.9	1.0	2.1		
20:4	0.7			0.6	1.0	0.7	0.8	0.9	3.3	2.8	3.8		
unknown	2.3			2.2	1.2	3.6	3.9	1.1	3.6	1.5	3.6		
3	2			14:0	1.7	3.1	9.3	1.7	1.6	7.4	0.3	0.3	0.6
				16:0	38.7	40.9	52.2	20.3	19.2	38.4	24.3	23.7	21.4
		16:1	4.1	4.4	4.6	4.7	6.1	11.0	1.0	0.9	5.3		
		(17:0)	3.2	3.6	6.7	1.2	1.1	8.1	2.0	1.9	2.2		
		18:0	4.5	4.6	6.3	2.2	2.0	4.8	20.1	20.1	16.7		
		18:1	23.6	21.5	14.8	19.5	19.3	19.3	13.1	12.8	14.6		
		18:2	7.3	6.8	1.2	39.0	38.7	5.2	26.1	26.4	24.4		
		18:3	12.8	10.6	1.3	7.4	7.5	0	5.9	5.8	6.4		
		20:1 + 20:2 + 20:3	0	0	0	0	0	0	1.7	2.1	2.3		
		20:4	0.6	0.5	0	0	1.0	0	4.0	3.5	3.5		
		unknown	3.4	4.0	3.7	3.4	3.6	5.7	3.7	4.5	5.0		
		19	1	14:0	2.1	1.6	4.7	1.2	0.8	1.1			0.2
				16:0	32.0	31.0	36.2	18.9	17.5	19.6			28.9
16:1	2.6			3.9	4.4	4.0	3.3	3.2			2.1		
(17:0)	2.9			3.9	4.7	1.0	0.7	0			2.2		
18:0	4.2			5.4	6.1	1.6	1.6	1.1			25.6		
18:1	29.5			27.4	21.3	19.2	19.2	19.1			15.0		
18:2	12.5			11.8	9.6	45.3	46.5	44.4			21.1		
18:3	14.1			12.4	10.1	5.7	5.7	10.3			1.9		
20:1 + 20:2 + 20:3	0			0	0	0	0	0			0.9		
20:4	0			0.6	0.5	1.2	1.0	0			1.0		
unknown	0			2.1	2.7	1.9	3.8	1.3			1.3		

*An unknown FA with the same retention time as 17:0 was not identified further.

†Combined amounts of up to 4 small, unidentified FAs.

amounts of lipid although LDL carried more than HDL (33 vs 24% of total lipid). This contrasts with squirrel monkeys which carried <1% of plasma lipid in VLDL and 70% in HDL (Illingworth, 1975). The difference in plasma VLDL levels between the two species probably results at least in part from differences between fasting and non-fasting states; the howlers were post-prandial when sampled and the VLDL fraction would have contained chylomicrons and chylomicron remnants, possibly in considerable amounts.

Table 4. Phospholipid class compositions of plasma lipoproteins

	PI + PS	PE (mol % of total PL)	PC	Sph	LPC
VLDL	1.3	1.2	90.2	6.3	0.4
LDL	2.8	14.8	78.7	1.0	1.1
HDL	1.2	17.1	74.0	6.4	0.7

Lipoprotein fractions were pooled among the nine animals of Table 2.

PI, phosphatidylinositol; PS, phosphatidylserine; PC, phosphatidylcholine (lecithin); Sph, sphingomyelin; LPC, lysolecithin; PL, phospholipid.

HDL of most species is heterogeneous, with two major subclasses, HDL₂ (d 1.063–1.125 g/dl) and HDL₃ (d 1.125–1.21 g/dl). Howler HDL appeared devoid of HDL₃. In humans, HDL₃ is the predominant form of HDL but in chimpanzees (genus *Pan*), orangutans (genus *Pongo*) and gorillas (genus *Gorilla*; Nelson *et al.*, 1984), squirrel monkeys (*Saimiri sciureus*; Illingworth, 1975), marmosets (*Callithrix jacchus*; Chapman *et al.*, 1979), baboons (genus *Papio*; Kruski, 1983), rhesus (genus *Macaca*; Scanu *et al.*, 1973) and other Old World monkeys (Chapman, 1980) HDL₃ is minor or absent. The lipid composition of howler HDL conformed generally to that of human HDL₂, which has a lipid composition of 4.6 ± 0.6% TG, 53.0 ± 2.9% PL, 6.9 ± 0.7% UC and 35.4 ± 2.6% CE (Kuksis *et al.*, 1979).

Of the total cholesterol present in plasma, only about 16% of UC and 18% of CE was found in HDL (see Table 2). This is considerably less than in any of the 12 New and Old World primate species studied by Srinivasan *et al.* (1974). In that series, the lowest proportion of HDL (α lipoprotein) cholesterol was

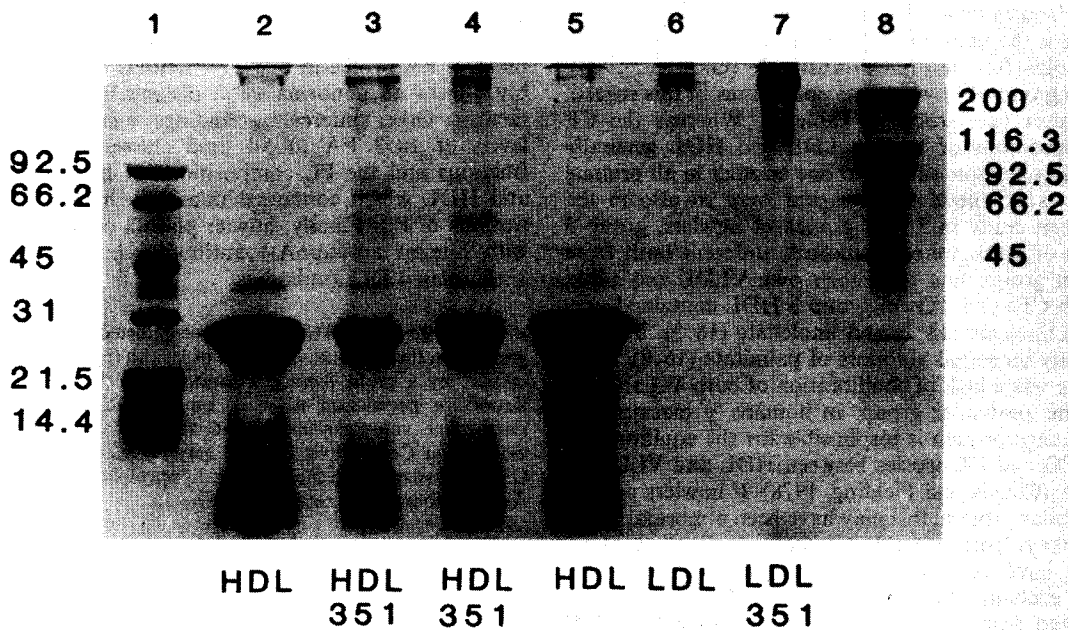


Fig. 2. Apolipoproteins of human and howling monkey lipoproteins. Low and high molecular weight standards were loaded in lanes 1 and 8 respectively. Lanes 2 and 5: human HDL (d 1.08–1.21 g/ml). Lanes 3 and 4: howler HDL. Lane 6: human LDL (d 1.025–1.05 g/ml). Lane 7: howler LDL. Lanes 4 and 5: without 2-mercaptoethanol. Lanes 2 and 3: with 2-mercaptoethanol.

also found in a New World primate (*Ateles geoffroyi*, spider monkey); however in another New World species (*Cebus albifrons*, capuchin) HDL carried more than two-thirds of the total plasma cholesterol. Differences in dietary habits probably contribute to these inter-species variations, although no detailed studies have yet been performed. Spider monkeys, which are fruit-eaters and howlers, which are leaf-eaters, both ingest little fat. Capuchins, which eat seeds, ingest large amounts of fat. Clearly, further work is needed to identify fully the causes of the enormous species differences which exist among primates with regard to the major cholesterol-carrying LP class.

Treatment of howler plasma with antibodies to human apoA-I, the major apoprotein of HDL, caused precipitation of howler A-I-containing LPs. A similar observation was made by Chapman *et al.* (1979) for marmoset plasma. The high degree of cross-reactivity of howler and human apoA-I implies significant structural and conformational homology between the apoproteins associated with lipoproteins in these two species. Anti-human LDL antibodies, on the other hand, were not antigenic for howler LDL, possibly because of differences in the conformation of the sole LDL apoprotein, apoB-100, in the two species. This contrasts with the considerable cross-reactivity between marmoset and human LDL observed by Chapman *et al.* (1979) and between baboon and human LDL by Blaton *et al.* (1977) and baboon and rhesus by Goldstein *et al.* (1977). That apoB-100 was nevertheless present in considerable amounts in howler LDL was revealed by PAG electrophoresis (see Fig. 2). Howler LDL and HDL differed from their human counterparts further in displaying

noticeably faster electrophoretic mobilities in agarose gels, which implies a higher charge density for the howler LPs (see Fig. 1). A similar finding was observed but not commented upon in marmoset LPs by Chapman (1979), but appears not to have been noted in other primates to date.

The lipoprotein lipid compositions of the howling monkeys displayed some unusual features. As shown in Table 4, LDL and HDL contained very high proportions of PE (15–17% of total PL). In our experience human VLDL, LDL and HDL total PL all typically contain only 2–4% PE, while rhesus HDL PL contains about 3–6% PE. Rosseneu *et al.* (1979) and Blaton *et al.* (1974) reported similarly low PE levels in chimpanzee LDL and HDL. However, baboon LDL and HDL have been reported to contain as much as 10–11% of PL as PE (Peeters *et al.*, 1970; Blaton *et al.*, 1977). To date, no structural or metabolic significance has been attached to this PL class distribution.

The FA profiles of individual howler LPs also deserve comment. Arachidonate (20:4) was uniformly low in all lipid classes of all LPs (see Table 3). This important FA typically comprises 5–14% of TG and CE FAs in baboon (Kruski, 1983; Blaton *et al.*, 1970, 1977) and human plasma (Schrade *et al.*, 1961). Rosseneu *et al.* (1979) found chimpanzee VLDL, LDL and HDL PL and CE to contain 5–6% 20:4 but TG contained only 1–2% arachidonate, similar to howler TG.

Linoleate (18:2) was the most prominent FA in CE of all howler LP fractions, similar to baboon (Kruski, 1983), chimpanzee (Rosseneu *et al.*, 1979) and human LPs, suggesting that the plasma cholesterol esterifying enzyme may have similar FA

specificities in all these primate species. In humans, 18:2 is the preferred FA for the enzyme responsible, lecithin-cholesterol acyltransferase (Glomset, 1968). One group of howlers was anomalous in this regard, however (see group 3; Table 3). Whereas the CE compositions of VLDL, LDL and HDL generally tend to be quite similar to one another in all primate species examined to date, and were so also in the present study in 3 of 4 groups of howlers, group 3 CEs of HDL were significantly different both from other groups and from their own VLDL and LDL. Both CEs and TGs of group 3 HDL contained very little linoleate (18:2) and linolenate (18:3), but had greatly increased amounts of palmitate (16:0). Thus there was a lack of equilibration of both TG and CE in this particular group. In humans, a plasma lipid exchange protein is responsible for the equilibration of TG and CE species between HDL and VLDL or LDL (Chajek and Fielding, 1978). If howlers possess a similar protein, this may have been abnormal in the monkeys from group 3. Alternatively, these monkeys may have elevated levels of a plasma inhibitor for this exchange reaction, similar to that described in human plasma by Morton and Zilversmit (1981). Either of these potential explanations would further suggest a possible familial factor operating within this group. A diet deficient in essential FA, eaten only by this group, may also have contributed to the findings but cannot alone explain them, because phospholipids contained normal amounts of 18:2 in all LP fractions of group 3 (see Table 3). Furthermore, linolenate (18:3), another essential FA derived solely from diet, was present in large amounts in all lipid classes from all the groups including group 3. Indeed, 18:3 levels generally were much higher than those reported for captive baboons or chimpanzees fed defined "control" diets, which therefore probably are deficient in 18:3 FA (Kruski, 1983; Rosseneu *et al.*, 1979).

Of the three lipid classes—TG, CE and PC—TG tended to vary most among the four groups, probably because of differences in the lipids of their respective diets which were obtained from different geographical home ranges.

Apoproteins of howler HDL were similar to those of other non-human primates such as baboons (Blaton *et al.*, 1977), rhesus (Edelstein *et al.*, 1973) and marmosets (Chapman *et al.*, 1979) and were different from human HDL apoproteins in having no dimeric apoA-II. ApoA-IV was missing from howler HDL, perhaps because, as in many primates, dissociation of this labile apoprotein occurred during ultracentrifugation. Other apoproteins (E and Cs) had similar electrophoretic mobilities to their human counterparts (see Fig. 2). Howler LDL was unremarkable, containing mainly one protein with a mol. wt similar to human apoB-100, the major LDL protein in all species examined so far.

To summarize, a study of plasma lipid and lipoprotein profiles in 11 howling monkeys from four different social groups has revealed considerable differences between the groups in the FA compositions of individual lipid classes. There was no apparent trend in lipid class concentrations or lipoprotein lipid compositions with increasing age. One aberrant animal was found which had extremely low

plasma lipids, possibly due to a genetic abnormality. Two animals from another group displayed an unusual FA profile in one LP fraction, HDL, possibly due to an abnormality in plasma lipid transfer factors. Other interesting findings were the high levels of 18:3 FA in all lipid classes of all LP fractions and the PL compositions of howler LDL and HDL which contained unusually high concentrations of PE. Finally, howler plasma cross-reacted with human anti-apoA-I antibodies but not with antihuman LDL antibodies.

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