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Fatty acid profiles of major food sources of howler monkeys (*Alouatta palliata*) in the neotropics

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Abstract. Wild howler monkeys (*Alouatta palliata*) get most of their calories from carbohydrates (65%) and fats (18%) of native tropical plants, but little is known about their intake of individual fatty acids. The fatty acid composition of several natural food sources of howler monkeys collected in Panama was determined by gas-liquid chromatography. The predominant fatty acids were palmitic (30%), linoleic (23%), α-linolenic (16%) and oleic (15%). Fatty acids with less than 16, and more than 18, carbon chains were uncommon (0–7%). Although total saturated fatty acids were high in some specific food sources (22–54% of total fatty acids and 8 energy %), most of the calories from fat in the animals' diets are derived from mono- and polyunsaturated fatty acids (9.75 energy %.) All food sources had significant amounts of the ω-3 fatty acid, α-linolenic acid (2.9 energy %). In terms of human diets, the howler monkey's fat consumption would not be considered atherogenic. Unless these animals show a particular adverse susceptibility to dietary fat, it is unlikely that their fat intake is the primary cause of the low, but significant, incidence of atherosclerosis that develops in these animals in the wild state.

Key words. Fatty acids; primates; ω-3 fatty acids; saturated fats; howler monkeys; wild plant parts; neotropics.

Little is known about the fatty acid (FA) intake of wild primates. They are primarily herbivorous, and some species, as for example, mantled howler monkeys (*Alouatta palliata*), are exclusively so. In Panama, the howler monkey diet is composed entirely of fruits, leaves, and occasional flowers of tropical forest trees and vines[1–4]. Howler monkeys especially consume the fruits (figs) and protein-rich young leaves of wild *Ficus* species[5]. Previous analyses of these food sources of wild howler monkeys have shown unexpectedly high yields of ascorbic acid (88 mg/kg/day) and dietary fiber (44% of dry weight of food consumed daily, 88 g/day)[2–5]. Information on the fat intake of wild primates would help to fill a notable gap in our knowledge of their nutritional intake in the natural environment. Since optimal fat intake and fatty acid ratios are currently under investigation in humans[6–8], such knowledge might also provide some insight into the FA intake of prehistoric humans[9–12]. This knowledge should also be of interest if howler monkeys are to be preserved, since the continuing destruction of the tropical forests that provide their natural food sources threaten their survival in the wild[13–17]. The present study reports the fatty acid content of several key plant sources from Panama that are important in the diet of wild howler monkeys.

Materials and methods

Plant samples were collected from trees growing in the moist, lowland tropical forest on Barro Colorado Island, Republic of Panama[2]. All samples were sealed immediately in plastic bags, transported to a freezer within 30 min., and kept frozen until analyzed. They were packed in dry ice for transport from Panama to Berkeley, CA, and again for transport from Berkeley to the Western Human Nutrition Research Center, USDA, at the Presidio, San Francisco, CA. Species identification of plant samples analyzed are given in the caption to table 1.

Lipid extraction and fatty acid analysis. Tissues were extracted by the procedures described by Nelson and co-workers[18–20] using chloroform/methanol (2:1, v/v). As the leaf samples contained considerable fibrous material, the samples were frozen in liquid N_2 and then ground to a powder before extraction with solvent. Fruit samples were placed in a blender with the extracting solvent and homogenized briefly until no visible fragments over a few millimeters in size were present. The solid, non-lipid material was removed by filtration, and then the total lipid material recovered by removing the solvents in a rotary evaporator under N_2[20]. The total lipid extracts were further purified by redissolving them in dry chloroform/methanol (19:1, v/v) and filtering the solution through a medium grade, fritted-glass funnel. The purified total lipid extracts were then transmethylated with methanolic-HCl (7%, w/w) by the procedures described elsewhere[20,21]. After the samples were transmethylated, the impurities, extracted into the hexane phase after stopping the reaction, were removed by

Table 1. Fatty acid profiles of major food sources of howler monkeys (*Alouatta palliata*) in the neotropics (Panama)

Fatty acid	% of total fatty acids in each plant part[a, b]					
	#1	#2	#3	#4	#5	#6
12:0	0.00	0.00	0.00	1.10	0.00	0.00
14:0	0.00	0.30	0.30	1.20	0.70	0.30
16:0	25.10	26.20	40.80	10.40	25.90	30.00
16:1n7	0.00	0.90	0.40	0.00	0.00	0.50
17:0	0.30	0.40	0.30	0.00	0.30	0.50
18:0	14.60	5.90	5.40	8.30	4.50	9.30
18:1[c]	4.20	3.50	12.10	20.00	35.30	14.30
18:2n6	30.90	35.70	26.80	8.50	3.20	27.10
18:3n3	9.00	13.80	7.00	48.00	20.70	10.80
20:0	7.10	1.10	0.00	0.90	1.90	1.50
20:1n9	0.40	0.00	0.40	1.50	1.00	0.60
20:2n6	0.30	0.40	0.00	0.00	0.00	0.00
22:0	6.00	1.50	5.60	0.00	2.60	2.70
24:0	1.00	4.70	1.00	0.00	1.80	2.50
24:1n9	0.00	0.00	0.00	0.00	0.70	0.00
Unknown & trace	1.10	5.50	0.00	0.00	1.10	0.00
% Saturated	54.1	40.1	53.4	21.9	37.7	46.8
% Monounsaturated	4.6	4.4	12.9	21.5	37.0	15.4
% Polyunsaturated	40.2	49.9	33.8	56.5	23.9	37.9
Total unsaturated	44.8	54.3	46.7	78	60.9	53.3
P/S Ratio	0.74	1.24	0.63	2.58	0.63	0.81
n3/n6 Ratio	0.29	0.38	0.26	5.65	6.47	0.40

[a] #1, rolled flush (leaf) of *Ficus insipida*; #2, young leaves of *Cecropia insignis*; #3, fruits of *Ficus insipida*; #4, fruits of *Hyeronima laxiflora*; #5, fruits of *Spondias mombin*; #6, flower of *Pseudobombax septenatum*. [b] Do not always equal 100% due to rounding. [c] Combined n7 and n9 isomers of 18:1.

thin-layer chromatography as described by Nelson[19]. The conditions of the capillary gas-liquid chromatography (GLC) have been described previously[18, 19, 22]. The column was a 30 m × 0.025 mm fused silica, open tubular column coated with SP-2340 purchased from Supelco Inc. (Bellefonte, PA). The GLC data were processed with a Perkin-Elmer Corp. (Norwalk, CT) Model 7500 data station using Perkin-Elmer Chrom 3 software. Individual fatty acids were identified by their retention times as compared to pure reference standards purchased from Nu-Chek-Prep, Elysium, MN. Quantitative accuracy was estimated to be better than 5% for fatty acids present in more than 5% of the mixture and 15% for most minor components.

Results

The results are presented in tables 1 and 2. The food sources varied considerably in fat content. Overall the animals are estimated to receive 18% of their total caloric intake from fat, 65% from carbohydrate, and 17% from protein. The predominant fatty acids were palmitic (30% of total fatty acids), linoleic (23%), α-linolenic (16%) and oleic (15%). Fatty acids with less than 16 and more than 18 carbon chains were uncommon (0–7%). Although total saturated fatty acids were high in some specific food sources (22–54% of total fatty acids and 8 energy %), most of the calories from fat in the animal's diets are derived from mono- and polyunsaturated fatty acids (9.75 energy %.) All food sources had significant amounts of the ω-3 fatty acid, α-linolenic acid (18:3n3) (2.9 energy %). Palmitic acid (16:0) (energy % = 5.5) was the dominant saturated fatty acid, with fruits of *Ficus insipida*, one of the most important fruit sources of these monkeys' diet, showing the highest value (41%). The rolled young leaves of this species showed a high content of linoleic acid (18:2n6; 31%) (energy % = 4.1). Total saturated FA in all sources varied from 22 to 54% (energy % = 8.1), yet unsaturated FA provide most of the fat calories (energy % = 9.75). The highest P/S ratios were seen in fruits of *Hyeronima laxiflora* (2.6) and the young leaves of *Cecropia insignis* (1.2), with all sources averaging 0.85. All food sources showed significant amounts of ω-3 FA (18.3n3; 7–48%; energy % = 2.85), with fruits of *Spondias mombin* and *Hyeronima laxiflora* showing ω-3/ω-6 ratios of 6.5 and 5.7, and the fruits of *Ficus insipida* 0.3. One of the six samples showed small amounts (0.7%) of nervonic acid (24:1n9). The total amount of monounsaturated FA varied from 3.5 to 35.3% of the total FA. Monounsaturated FA contributed on the average 16% (energy % = 2.81) of the animals' fatty acid intake, and all plant parts had other monounsaturated FA besides oleic acid, usually in small amounts.

Discussion

Howler monkeys eat approximately 1 kg/day (wet wt) of a plant diet composed of 60% fruits and 40% leaves when their total food intake is averaged over a year[3–5].

Table 2. The average fatty acid composition of the howler monkey diet and the potential energy derived from individual fatty acids in the diet

Fatty acid	Percent	gm/day	Calories/fatty acid	Energy %/total fat	Energy %/total calories*
12:0	0.15	0.01	0.13	0.15	0.03
14:0	0.44	0.04	0.40	0.44	0.08
16:0	30.24	3.02	27.22	30.24	5.46
16:1n7	0.30	0.03	0.27	0.30	0.05
17:0	0.28	0.03	0.25	0.28	0.05
18:0	7.04	0.70	6.34	7.04	1.27
18:1	14.56	1.46	13.10	14.56	2.63
18:2n6	22.53	2.25	20.28	22.53	4.07
18:3n3	15.78	1.58	14.20	15.78	2.85
20:0	1.52	0.15	1.37	1.52	0.27
20:1n9	0.59	0.06	0.53	0.59	0.11
20:2n6	0.09	0.01	0.08	0.09	0.02
22:0	3.83	0.38	3.45	3.83	0.69
24:0	1.52	0.15	1.37	1.52	0.27
24:1n9	0.10	0.01	0.09	0.10	0.02
Unknown & trace	1.02	0.10	0.92	1.02	0.18
Totals	100.00	10.00	90.00	100.00	18.07
% Saturated	45.01	4.50	40.51	45.01	8.14
% Monounsaturated	15.56	1.56	14.00	15.56	2.81
% Polyunsaturarated	38.40	3.84	34.56	38.40	6.94
Total unsaturated	53.96	5.40	48.57	53.96	9.75
P/S Ratio	0.85	0.85	0.85	0.85	0.85
n3/n6 Ratio	0.70	0.70	0.70	0.70	0.70

*Based on an average energy intake of 498 Kcalories per day (ref. 3).

On the average, 80% of this diet is water; they drink little, if any, standing water[4]. For two months of the year they may consume predominantly leaves. Their daily dry mass consumption (200–300 g) is made up of approximately 44% dietary fiber (88 g/day), 40% carbohydrates (80 g/day; 65 energy %), 11% protein (20–25 g/day; 17 energy %) and 5% fat (10 g/day; 18 energy %). Howler monkeys, like other primates, are known to extract metabolic energy via gut microbes (31% of calories)[3,4]. Thus, they have reliable and varied sources of energy throughout the year, especially when fruits are not available. They must have a diverse diet to meet their nutritional fatty acid and other requirements[4].

Howler monkeys on Barro Colorado Island consume no cholesterol other than fortuitous consumption of trace amounts found in insects and larvae attached to fruits. Their serum cholesterol levels are low to moderate (100–200 mg/dl)[19,20]. Their P/S ratio on the natural diet is 0.85 whereas humans eating a typical Western diet have a P/S ratio of 0.4 currently[23]. The present study shows that, although howler monkeys consume 9.8% of their calories from unsaturated FA, 8% of their total calories come from saturated FA, amounting to 41% of their fat calories. Nevertheless, there is no reason to suspect that their diet is atherogenic, unless these animals have a genetic predisposition for this disease[24,27]. The reported atherosclerosis found in the howler monkey, though its incidence is low in the wild, may be initiated by several other means such as blood rheology, viruses, aging phenomena, and/or fungi contamination of their diet[28–30]. It is not likely, therefore, that the assorted fatty acids eaten by them in the wild are the primary cause of the observed, but low, incidence of atherosclerosis in this species. As postulated for early hominids[11,31], howler monkeys get their calories primarily from plant carbohydrates and plant fats. Such wild foods also may contain high levels of vitamin C and no doubt other antioxidants[32–34] which have been speculated to be protective for atherosclerosis, at least in humans[35]. Also they may be protected from vascular disorders by their high intake of plant sterols and/or saponins[36].

Dietary saturated FA do not act alike in their effect on plasma cholesterol in non-human primates. Palmitic acid (16:0) is perhaps less hypercholesterolemic than lauric acid (12:0), while myristic acid (14:0) may be the most hypercholesterolemic of the dietary fats[37]. Both latter fatty acids were very low in the analyzed dietary sources in this study (0.03–0.08 energy %).

It is interesting to compare the composition of the wild howler monkey's diet with the diet of the Tarahumara Indians of northern Mexico as reported by Conner et al.[38]. These people represent a group of rural Amerindians who have subsisted almost exclusively on corn, beans, and squash for over 8,000 years[39]. Their mean serum cholesterol is 125 mg/dl, with 12 energy % from fat, 75 energy % from carbohydrate, and 13 energy % from protein. Their diets are adequate in all known nutrients and, presumably, antiatherogenic. Perhaps a high fat diet is atherogenic in human populations eating

a Westernized diet and in caged non-human primates because these species did not evolve eating high fat, cholesterol-laden diets[11,15].

The diet of howler monkeys in the wild also lacks highly unsaturated ω-3 fatty acids with more than 18 carbon atoms in the fatty acid chain. The diets do contain relatively high amounts of α-linolenic acid (18:3n3). Thus, these animals must have adequate metabolic pathways to desaturate and elongate α-linolenic acid to EPA (20:5n3) and DHA (22:6n3), the latter of which may be an essential component of nervous and reproductive tissues. As the nervous system develops in utero and before weaning, the fetal and infant howler monkeys must get their supply of DHA entirely from de novo synthesis or exogenously from their mothers' milk. The high relative proportion of ω-3 FA found in the food sources analyzed here is quite similar to that reported in flax (*Linum usitatissimum*)[8] and purslane (*Portulaca oleracea*)[34,42], the latter a known source of ω-3 fatty acids for 'prehistoric' (770 BC–1300 AD) Chaco peoples of the American Southwest[43]. The average ω-3/ω-6 ratio in howler monkeys is 0.7, over three times that observed in large human population samples (0.21) eating typical Western diets[8,9].

Clark et al.[44] analyzed the lipoproteins, plasma lipids, and plasma lipoprotein fatty acid composition of wild howler monkeys living in Costa Rica. They found that these animals had lipid levels in their plasma not unlike various human populations[31,38], particularly vegetarian populations. The striking difference between human plasma fatty acid composition and that observed in the wild howler monkeys was the large amount α-linolenic acid in these animals. Human plasma usually contains less than 1 percent of this fatty acid[22] while the plasma from the animals studied by Clark et al. contained average levels several times that found in human plasma (4 to 15%). As reported here, the howler monkey consumes on the average about 16% of its total fatty acid as α-linolenic acid. Thus, the plasma fatty acid composition reflects the composition of the dietary fat. In short term feeding studies with human volunteers consuming high levels of α-linolenic acid, the plasma α-linolenic acid level (3%) did not approximate that (21%) in the experimental diet (cf. ref. 45). The difference between the α-linolenic acid content of human and howler monkey plasma may be caused by an adaptation in howler monkeys to the large quantity of α-linolenic acid in their diet, but its biological significance, if any, is unknown.

Thus, although a low incidence of atherosclerosis is found in howler monkeys in the wild, the animals eat a diet that is, by human standard, antiatherogenic. It is low in fat, high in carbohydrate. Its P/S ratio is 0.85, and the major saturated fatty acid is palmitic acid. Fatty acids containing more than 18 carbons contribute little to the diet. Metabolically important fatty acids with 20 or more carbon atoms, such as arachidonic acid (20:4n6), EPA (20:5n3), and DHA (22:6n3), must be entirely synthesized endogenously. The data presented here may provide clues to the fatty acid composition of the diets postulated by some authorities to have been consumed by early hominids[11,31], and in turn yield information about the putative adverse health consequences of the current 'Western' human diet.

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