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Serum Nutritional Profiles of Free-Ranging *Alouatta Caraya* in Northern Argentina: Lipoproteins; Amino Acids; Vitamins A, D, and E; Carotenoids; and Minerals

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Abstract Quantifying circulating nutrient concentrations in sera of free-ranging subjects will help to establish a basis from which we can evaluate the nutritional status and needs of the captive population. We collected serum samples from 26 free-ranging black-and-gold howlers (*Alouatta caraya*) in San Cayetano forest in northern Argentina. We analyzed them for concentrations of lipoproteins; amino

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acids; vitamins A, D, and E; carotenoids; and minerals. There are a few significant differences between sexes in concentrations of high-density lipoprotein cholesterol, certain amino acids, vitamin E, lutein + zeaxanthin, and copper. Most nutritional parameters are similar to the ones measured in free-ranging Mexican mantled howlers (*Alouatta palliata mexicana*) and in captive New World primates (NWPs). Carotenoid, vitamin D, and phosphorus concentrations are the exceptions. Carotenoid concentrations are higher in free-ranging *Alouatta caraya* than reported for other free-ranging and captive species. Vitamin D concentrations are 14 times greater in the free-ranging black-and-gold howlers than in captive NWPs. Phosphorus concentrations are also higher than expected and higher than typically occur in captive primates, leading to a 1:1.6 calcium:phosphorus ratio. Because we based our study on a small number of free-ranging howlers, additional samples from different regions and throughout the year would better define desirable nutritional parameters for captive howlers.

Keywords *Alouatta caraya* · cholesterol · howlers · mineral · protein · vitamin

Introduction

Alouatta caraya (black-and-gold howlers) inhabit regions in Northern Argentina, Paraguay, Southern Brazil, and Eastern Bolivia (Brown and Zunino 1994; DeLuycker 1995). They are herbivorous, eating leaves, fruits, and flowers (Bravo and Sallenave 2003; Crockett 1998; Crockett and Eisenberg 1987; Milton 1998). *Alouatta caraya* are classified as a Convention on International Trade in Endangered Species (CITES) Appendix II species (2006).

Alouatta caraya are the most common species of howlers in zoos (ISIS 2007); however, very little is known about their nutritional requirements. Diets for zoological garden primates are often formulated using information from laboratory primates and reports of what the animals eat in the wild. Though the information is useful, laboratory studies are often limited to more common primate research species, including rhesus and squirrel monkeys and callitrichid species. The wild dietary data do not and cannot relay the pertinent information concerning the quantity of nutrients, e.g., fats, vitamins, minerals, and proteins, a primate needs to remain healthy. Though researchers can report the concentrations of fats, vitamins, minerals, and proteins in the foods free-ranging howlers eat, the amounts of each food type consumed can only be calculated, e.g., bites per min, and not directly measured; the estimates can lead to large errors in quantifying nutrient intake of food items. Nor can one predict if there is a nutritional composition difference between the leaf or fruit parts howlers consumed and the ones discarded or not selected even though other leaves or fruits from the same tree were eaten. Accordingly, it is difficult to predict accurately nutrient requirements of free-ranging inividuals by studying the foods they consume in the wild.

Quantifying the circulating nutrient concentrations in the serum of free-ranging *Alouatta caraya* will help establish a basis from which to evaluate the nutritional health status of the captive population. If values in the captive population are

significantly different from the ones in the wild population, it may indicate that captive *Alouatta caraya* are being fed inappropriately. Our aim is to begin establishing nutrient normal concentrations of lipoproteins; amino acids; vitamins A, D, and E; carotenoids; and minerals in free-ranging black-and-gold howlers.

Methods

Study Site

The subjects inhabited several narrow strips of gallery forest in San Cayetano, Province of Corrientes, in northern Argentina (27° 30′ S, 58° 41′ W) that are located in the basin of the Rio Riachuelo, a tributary of the Rio Paraná. The area is 50–60 m above sea level and the climate is subtropical. The annual average temperature is 21.6±5.8°C and the annual average precipitation is 1467±341 mm (provided by the Argentinean Air Force and National Meteorological Service from 2000 to 2004). Rains are frequent all year, but they decrease considerably in July and August. The vegetation forms a mosaic of tall and low forests, savannas with palms, grasslands, and lower zones with lagoons and esteros (marsh habitats). The primary forest has been and is presently being logged intensively. The remnant patches of remaining forests are 5–15 ha (Kowalewski and Zunino 1999).

Some of the main plant species free-ranging black-and-gold howlers consume throughout the year include *Celtis* spp., *Forsteronia glabrescens*, *Gleditsia amorphoides chlorophora tinctoria*, *Ficus monckii*, *Eugenia uniflora*, *Eugenia pungens*, and *Acrocomia* spp. (Delgado 2006). Researchers have completed limited nutrient analyses on them (Zunino 1989).

Subjects

In November 2004 we collected samples opportunistically by adding serum sampling to the protocol of another study that was anesthetizing subjects for tagging and identification. At the time of sampling, we recorded information on approximate age, sex, body mass, body condition, and health status of each individual. We collected samples from 39 adult individuals, all of which were ≥4 yr of age according to physical characteristics of sexual maturation (Rumiz 1990). Twenty samples are from females and 19 are from males. The females ranged in age from 4 to ≥12 yr, with an average age of 7.4±0.6 yr. Female masses ranged from 3.8 to 6.8 kg and averaged 5.9±0.2 kg. Four of the females were pregnant and 5 were lactating. The males ranged in age from 4 to ≥16 yr, with an average age of 8.4± 0.8 yr. Their masses ranged from 5.0 to 10.3 kg and average 8.3±0.3 kg. We evaluated all individuals with regard to body condition and health status; most received a rating of good or very good for both assessments. One ≥16-yr-old male was not good in body condition, but good with regard to his health status. One male and 1 female received questionable ratings on health status due to missing hair on some parts of their bodies, but we classified both as good in relation to body condition assessment.

Sample Collection and Processing

With ≥2 veterinarians assisting and monitoring vital signs, we darted monkeys via a Pneu Dart Model 178B Air Pump Rifle loaded with 1cc Pneu-dart type P darts (Pneu-Dart, Williamsport, PA). We prepared each dart with 0.9 ml of ketamine (50 mg/ml) and 0.1 ml of xylazine. The preferred injection site was the hindquarters. We avoided the individual's, thorax, lumbar region, abdomen, shoulder, neck, head, and face and darted the animal when it was facing away from us. A howler that fell was caught in a nylon mesh net held by 2–3 people. If the subject did not fall, we manually retrieved it by climbing the tree. Once the subjects were captured, and with the wounds from the darts treated, we measured, weighed, and marked them with colored ear-tags and anklets made of tubular webbing; we then collected blood samples. After we sampled each individual, we placed it in an open cloth bag for recovery from anesthesia. When the individual fully recovered, we released it at the point of capture and monitored it for several hours to make certain it had recovered fully.

We collected blood samples (*ca.* 8 ml) from the femoral vein of each subject via a syringe (BD, Franklin Lakes, NJ). We then injected the sample into a vacutainer tube (BD), which we packed in ice and stored in a cooler without exposure to light until the lab processed the sample; collection to processing time was <1 h. We centrifuged each sample to separate serum from red blood cells. We then transferred the serum via plastic pipettes (Fisher Scientific, Hampton, NH) and aliquoted it into 4 cryovial tubes (Fisher Scientific) with 0.5 ml in each and froze them at −18°C. We shipped samples frozen via a Thermosafe Deep Chill Insulated Shipper (Thermosafe, Arlington Heights, IL) and U-tek® gel packs (Thermosafe) to Lincoln Park Zoo, where laboratory personnel immediately placed them in an ultralow freezer (−80°C) for temporary storage.

Statistical Design and Analyses

We collected a total of 2 ml of serum from each individual, but it was not enough serum to run a full panel of analyses. To compensate, we separated the serum samples by sex and arranged them sequentially by age within sex. We assigned each of the 5 analyses, including lipoproteins, amino acids, vitamins, carotenoids, and minerals, a number and used a random number generator to assign the numbers into groups of 4. We then allotted the numbered analysis groups to each sample.

We analyzed equality of variances between sexes by an F-test with a significance level of $p<0.05$. We tested means with unequal variances via Cochran t-tests ($p<0.05$). We tested means with equal variances via a 2-group t-test ($p<0.05$). We determined differences between measured and calculated LDL concentrations via a paired t-test (SAS 2004).

Serum Analyses

Lipoproteins Total cholesterol, triglyceride, high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol concentrations were quantified via Synchron LX Systems® (Beckman Coulter, Fullerton, CA) Cholesterol Reagent, Triglycerides GPP Reagent, HDL Cholesterol Reagent, and LDL Cholesterol

Reagent, respectively, on a Beckman Coulter Synchron® LX20 (Beckman Coulter) at Loyola University Medical Center (Maywood, IL; Allain *et al.* 1974; Beckman Coulter 2000, 2002; Bucolo and David 1973; Pinter *et al.* 1965; Roeschlau *et al.* 1974; Trinder 1969). Both HDL cholesterol and LDL cholesterol concentrations were obtained via direct methods. The laboratory also calculated LDL cholesterol concentrations via Friedewald's equation (Friedewald *et al.* 1972). The subjects did not fast before serum collection. Friedewald's equation to calculate LDL cholesterol concentrations requires sampling from fasted individuals. Using Friedewald's equation on nonfasted individuals may result in higher LDL cholesterol concentrations, which do not accurately reflect the status of the subject.

Amino Acids A panel of free, physiological amino acids was quantified using a Beckman 121MB amino acid analyzer (Beckman, Fullerton, CA, Beckman 1975, 1980), and the analyses were completed at Experiment Station Chemical Laboratories, University of Missouri, Columbia, MO. The amino acid panel includes 1-methyl-histidine, 3-methyl-histidine, α-amino-adipic acid, α-amino-*n*-butyric acid, β-alanine, β-amino-isobutyric acid γ-amino-butyric acid, alanine, anserine, arginine, asparagine, aspartic acid, carnosine, citrulline, cystathionine/allocystathionine, cystine, ethanolamine, glutamic acid, glutamine, glycine, histidine, homocystine, hydroxylysine, hydroxyproline, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, phosphoethanolamine, phosphoserine, proline, sarcosine, serine, taurine, threonine, tryptophan, tyrosine, urea, and valine.

Vitamins and Carotenoids We quantified vitamin A (retinol, retinyl palmitate, retinyl stearate), vitamin E (α-tocopherol, γ-tocopherol), and carotenoid (lutein/zeaxanthin, lycopene, α- and β-cryptoxanthin, α- and β-carotene) concentrations in the Department of Human Nutrition, University of Illinois (Chicago, IL). We completed the analyses via a Waters NovaPak C18 column on a Waters 490 Programmable Multiwavelength Detector (Waters Corporation, Milford, MA; Stacewicz-Sapuntzakis *et al.* 1987).

We measured vitamin D concentrations —$25(OH)D_3$— at Vitamin D Research Laboratory, Boston University Medical Center (Boston, MA). We determined concentrations via a protein-binding assay with rat serum vitamin D-binding protein (Chen *et al.* 1990).

Minerals We quantified serum mineral concentrations of calcium, copper, iron, magnesium, phosphorus, potassium, sodium, and zinc via a serum gravimetric method on a Axial ICP-AES (Vista AX CCD Simultaneous ICP-AES; Varian, Palo Alto, CA) (Braselton *et al.* 1981; Stowe *et al.* 1985, 1986). The samples were analyzed at Diagnostic Center for Population and Animal Health (Lansing, MI).

Results

There are few significant differences between sexes, but the HDL cholesterol concentrations were significantly different between males and females (Table I). There is a significant difference ($p<0.001$) between measured LDL cholesterol

Table I Total cholesterol, triglyceride, HDL cholesterol, and LDL cholesterol concentrations (mmol/L) in sera of free-ranging *Alouatta caraya* compared to other New World primates

Genus and species name	Status	*N*	Total cholesterol	Triglyceride	HDL cholesterol	Calculated LDL cholesterol	Measured LDL cholesterol
Alouatta caraya	Free-ranging						
	Males	12	4.9±0.4	—	1.5±0.1[a]	—	—
	Males	13	—	1.0±0.1	—	3.2±0.2	3.0±0.2
	Females	13	4.0±0.3	1.1±0.1	1.0±0.1[b]	2.5±0.3	2.4±0.3
A. palliata mexicana[c]	Free-ranging	6	3.9±0.3	1.4±0.3	1.4±0.1	2.0±0.1	Not reported
Alouatta caraya[d]	Captive	7	3.6±0.3	1.2±0.1	0.9±0.1	2.2±0.3	2.2±0.2
Ateles geoffroyi[e]	Captive	7	5.6±0.5	1.7±0.3	1.2±0.1	4.0±0.4	Not reported
Cebidae[f]	Captive	Not reported	3.4–7.5	0.6–2.8	0.3–2.1	Not reported	Not reported
Callimico goeldii[d]	Captive	4	2.9±0.3	0.7±0.1	2.0±0.2	0.7±0.2	1.3±0.2
Leontopithecus chrysomelas[d]	Captive	5	1.4±0.2	1.2±0.2	Not reported	Not reported	0.8±0.2
Callitrichidae[g]	Captive	Not reported	1.8±0.1	1.1±0.1	0.5±0.2	1.3±0.1	Not reported

Results are means ± SEM.

[a,b] Means in the same column for the current study with different superscripts are significantly different ($p<0.05$).

[c] Crissey *et al.* (2003).

[d] Crissey *et al.* (unpublished).

[e] Crissey *et al.* (1999).

[f] Carroll and Feldman (1989).

[g] Loeb and Quimby (1989).

Table II Amino acid concentrations (μmol/L) in sera of free-ranging *Alouatta caraya* compared to captive howlers and captive golden-headed lion tamarins (*Leontopithecus chrysomelas*)

Amino acid	*Alouatta caraya*[a]		*Alouatta caraya*[b]	*Leontopithecus chrysomelas*[b]
	Males (n=12)	Females (n=14)	(n=2)	(n=5)
3-Methyl-histidine	23.5±2.5	23.9±2.1	10.8±0.1	Not detected
α-amino-adipic acid	42.0±7.7	38.7±6.7	Not detected	1.7±1.7[c]
α-amino-*n*-butyric acid	14.9±0.9	15.9±1.9	4.4±1.0	10.5±1.4
β-alanine	2.2±0.6[d]	0.1±0.1[e]	Not detected	Not detected
γ-aminobutyric acid	1.7±0.2	1.4±0.2	Not detected	Not detected
Alanine	494.7±72.7	615.1±72.6	614.4±172.4	141.1±21.1
Anserine	0.03±0.0	0.15±0.1	Not detected	Not detected
Arginine	153.4±8.3	119.1±14.3	156.3±39.1	37.3±4.5
Asparagine	0.3±0.2	0.7±0.4	Not detected	Not detected
Aspartic acid	63.5±4.3	67.0±9.6	34.7±0.7	16.0±3.4
Carnosine	0.6±0.6	1.0±0.7	Not detected	Not detected
Citrulline	17.9±1.1	16.4±1.2	31.6±4.0	46.5±2.5
Cystathionine/allocystathionine	4.5±0.6	4.8±0.6	5.5±0.7	3.3±0.4
Cystine	8.8±1.8[d]	3.7±1.4[e]	36.8±1.2	47.0±8.5
Ethanolamine	9.8±1.3	11.3±1.6	22.6±1.5	6.3±0.5
Glutamic acid	144.4±10.9	159.5±10.6	182.4±28.0	61.4±12.0
Glutamine	435.9±33.7	449.6±24.6	440.9±93.4	529.3±17.7
Glycine	678.7±70.0	641.3±46.8	744.8±10.1	503.6±60.8
Histidine	70.7±4.7	75.9±4.8	89.9±22.8	74.4±3.1
Homocystine	0.5±0.1[d]	0.8±0.1[e]	Not analyzed	Not analyzed
Hydroxylysine	0.6±0.1	0.6±0.1	Not analyzed	Not analyzed
Hydroxyproline	23.4±1.7	22.0±3.5	Not analyzed	Not analyzed
Isoleucine	45.0±2.4	50.0±4.9	44.5±5.1	44.5±5.6
Leucine	99.2±4.1	111.0±10.5	93.4±14.1	88.6±11.7
Lysine	114.4±10.3	134.3±15.5	88.7±18.1	73.5±6.5
Methionine	24.2±2.5	30.1±2.9	46.7±8.8	34.3±4.0
Ornithine	77.5±6.4	97.9±16.7	64.4±14.0	39.2±5.4
Phenylalanine	52.7±2.7	55.7±4.8	54.5±6.2	56.2±4.2
Phosphoserine	13.8±1.3	11.3±0.6	6.9±2.4	11.0±0.3
Proline	217.4±25.0	200.7±17.0	214.6±13.9	105.8±14.4
Sarcosine	171.7±25.7	156.9±21.4	Not analyzed	Not analyzed
Serine	197.0±12.8	248.5±21.3	235.6±43.6	40.0±6.1
Taurine	104.0±9.4	93.1±17.2	223.7±16.6	144.0±46.5
Threonine	195.1±13.5	238.4±19.9	140.2±29.4	120.0±13.7
Tryptophan	45.0±4.4[d]	68.3±3.9[e]	Not analyzed	Not analyzed
Tyrosine	48.4±3.7	42.2±3.7	68±4.2	49.8±6.2
Urea	6867.1±674.6	6031.9±489.9	Not analyzed	Not analyzed
Valine	211.9±8.7	236.8±16.4	159±16.7	154.4±16.4

Results are means±SEM. Essential amino acids are in **bold**.

[a] 1-Methyl-histidine, β-amino-isobutyric acid, and phosphoethanolamine not detected in these free-ranging animals.

[b] Crissey *et al.* (unpublished).

[c] Concentration detected in only 1 of 5 individuals; 4 individuals had concentrations lower than the detection limit and were considered to be 0. This is the average of all 5 individuals.

[d,e] Means in the same row within the current study with different superscripts are significantly different ($p<0.05$).

Table III Vitamin A, E, and D concentrations in sera of free-ranging *Alouatta caraya* compared to other New World primates

Genus and species name	Status	*n*	Vitamin A (μmol/L)			Vitamin D (nmol/L)	Vitamin E (μmol/L)	
			Retinol	retinyl palmitate	Retinyl stearate	25(OH)D	γ-Tocopherol	α-Tocopherol
Alouatta caraya	Free-ranging							
	Males	12	0.9±0.1	0.1±0.0	0.07±0.01	704.8±58.1	0.2±0.1	15.5±1.4[a]
	Females	14	1.0±0.1	0.1±0.0	0.05±0.01	771.1±61.8	0.1±0.0	11.9±1.0[b]
A. palliata mexicana[c]	Free-ranging	6	0.6±0.1	0.1±0.03	0.03±0.02	207.2±40.7	1.4±0.4	23.1±2.3
Saguinus oedipus[d]	Free-ranging	18	Not analyzed	Not analyzed	Not analyzed	190.7	Not analyzed	Not analyzed
Ateles geoffroyi[e]	Captive	7–9	0.6±0.05	0.02±0.02	Not analyzed	48.7±7.5	0.84±0.07	31.3±2.3
Alouatta caraya[f]	Captive	3–6	0.7±0.1	0.1±0.02	0.07±0.02	49.4±10.0	0.7±0.1	10.1±1.0
Aotus trivirgatus[f]	Captive	2	0.4±0.1	0.4±0.2	Not analyzed	Not analyzed	2.5±0.1	53.0±25.0
Callimico goeldii[f]	Captive	5	0.4±0.2	0.01±0.006	Not analyzed	Not analyzed	1.8±0.1	16.8±1.4
Leontopithecus chrysomelas[f]	Captive	3	0.3±0.1	0.1±0.02	Not analyzed	Not analyzed	0.8±0.01	15.5±0.4
Callithrix jacchus[g]	Captive	15	0.4±0.3	Not analyzed	Not analyzed	Not analyzed	Not analyzed	13.9±24.0
Saguinus fusciollis[g]	Captive	17	0.3±0.2	Not analyzed	Not analyzed	Not analyzed	Not analyzed	7.7±5.7
Cebidae/Callitrichidae[h]	Captive	Not reported	Not analyzed	Not analyzed	Not analyzed	334.5±59.4	Not analyzed	Not analyzed

Results are means ± SEM.

[a,b] Means in the same column within the current study with different superscripts are significantly different ($p<0.05$).

[c] Crissey *et al.* (2003).

[d] Power *et al.* (1997).

[e] Crissey *et al.* (1999).

[f] Crissey *et al.* (unpublished).

[g] Schweigert *et al.* (1991).

[h] Adams *et al.* (1985).

Table IV Carotenoid concentrations (μmol/L) in sera of free-ranging *Alouatta caraya* compared to other New World primates

Genus and species name	Status	*n*	Lutein + zeaxanthin	Lycopene	α-Carotene	β-Carotene	α-Cryptoxanthin	β-Cryptoxanthin
Alouatta caraya	Free-ranging							
	Males	12	2.7±0.2[a]	0.1±0.03	0.8±0.05	2.6±0.2	0.6±0.10	0.5±0.09
	Females	14	1.9±0.2[b]	0.1±0.03	0.7±0.06	2.4±0.3	0.5±0.09	0.5±0.06
A. palliata mexicana[c]	Free-ranging	6	0.5±0.2	Not detected	0.06±0.01	0.09±0.02	0.03±0.002	0.3±0.01
Alouatta caraya[d]	Captive	6	0.4±0.08	0.006±0.006[e]	0.2±0.06	1.0±0.1	0.15±0.02	0.1±0.01
Ateles geoffroyi[f]	Captive	9	0.8±0.1	0.01±0.004	1.4±0.3	1.0±0.2	0.2±0.03	0.1±0.01
Callimico goeldii[d]	Captive	5	0.08±0.02	Not detected	0.008±0.004	0.03±0.02	0.17±0.06	0.005±0.003

Results are the means ± SEM.

[a,b] Means in the same column within the current study with different superscripts are significantly different ($p<0.05$).

[c] Crissey *et al.* (2003).

[d] Crissey *et al.* (unpublished).

[e] Concentration detected in only 1 of 6 individuals; 5 individuals had concentrations lower than the detection limit and were considered to be zero. This is the average of all 6 individuals.

[f] Crissey *et al.* (1999).

Table V Mineral concentrations in free-ranging *Alouatta caraya* compared to other New World primates

Animal	Status	*n*	Ca (mmol/L)	Cu (μmol/L)	Fe (μmol/L)	K (mmol/L)	Mg (mmol/L)	Na (mmol/L)	P (mmol/L)	Zn (μmol/L)
Alouatta caraya	Free-ranging									
	Males	13	2.3±0.05	14.2±1.6[a]	50.1±7.2	4.2±0.2	0.8±0.02	134.5±0.6	3.8±0.2	16.8±1.5
	Females	13	2.3±0.05	22.0±3.1[b]	50.1±5.4	4.5±0.2	0.8±0.04	136.8±2.1	3.7±0.2	16.8±1.5
A. palliata mexicana[c]	Free-ranging	6	1.9±0.03	14.2±3.1	30.4±1.8	5.0±0.3	0.7±0.04	147.3±3.0	1.6±0.1	7.7±1.5
Alouatta caraya[d]	Captive	5	2.3±0.2	6.3±1.6	40.8±7.2	5.1±1.0	0.8±0.05	144.1±4.9	1.8±0.1	16.4±1.5

Results are means ± SEM.

[a,b] Means in the same column within the current study with different superscripts are significantly different ($p<0.05$).

[c] Crissey *et al.* (2003).

[d] Crissey *et al.* (unpublished).

concentrations and calculated LDL cholesterol concentrations, which we determined via Friedewald's equation (Friedewald *et al.* 1972).

There are significant sex differences in β-alanine, cystine, homocystine, and tryptophan concentrations for amino acids (Table II). The only essential amino acid concentration that proved significantly different between sexes was tryptophan.

Levels of vitamins A and D are not significantly different between males and females (Table III). Vitamin E as α-tocopherol is the only vitamin with a significant difference in concentration between the sexes.

Lutein + zeaxanthin concentration in males is significantly higher than in females (Table IV). Lycopene, α-carotene, β-carotene, α-cryptoxanthin, and β-cryptoxanthin concentrations are similar in the sexes.

Copper is the only mineral significantly different between males and females (Table V). Concentrations of circulating calcium, iron, potassium, magnesium, sodium phosphorus, and zinc are similar for the free-ranging male and female black-and-gold howlers.

The randomization of samples resulted in samples from 4 lactating females in the vitamin and lipoprotein analyses, 3 samples in the amino acid and fatty acid analyses, and 2 samples in the mineral analyses. We included samples from 4 pregnant females in the amino acid analyses; 3 samples in the minerals and vitamin A and E analyses; and 2 samples in vitamin D, lipoprotein, and fatty acid analyses. We did not consider pregnancy and lactation status in the statistical analysis.

Discussion

Most dietary requirements for optimal health in exotic animals are unknown. Determining the requirements typically involves invasive studies that are not appropriate for zoo animals. Researchers often compare nutrient blood parameters in exotic subjects to values measured in domestic animals, which, though helpful, may not be the best standard for evaluating exotic animals. In the first study to encompass several primate species from captive settings, Crissey *et al.* (1999) collected and analyzed plasma/serum samples for lipid; vitamins A, D, and E; and carotenoid concentrations from 9 primate species living at 4 zoos. The ideal basis for evaluating the results from captive subjects would be serum nutrient concentrations from free-ranging species. If the values measured in captive individuals are drastically different from values in free-ranging individuals, it may indicate that captive individuals are being fed incorrectly, which may lead to health-related problems.

For reasons scientists do not fully understand, captive New World primate species are appear to be more susceptible to metabolic bone disease than captive Old World primate species are (Ullrey and Bernard 1999), possibly because of a higher vitamin D requirement for the former, linked to their inability to effectively utilize the plant form of vitamin D (Adams *et al.* 2003; Chun *et al.* 2001; Hunt *et al.* 1966). Vitamin D is instrumental in the process of calcium absorption in the small intestine. When individuals are deficient in vitamin D for prolonged periods, they cannot absorb sufficient calcium from the diet to meet their physiological needs, so they begin to use calcium from bone. The process weakens bone structure and makes the individual susceptible to fractures. Dietary calcium and phosphorus concentrations

also need to be in an appropriate ratio to avoid an imbalance. We thus believed it was especially important to quantify circulating vitamin D, calcium, and phosphorus concentrations in free-ranging howlers for assessment of the captive population.

We obtained samples from only adult individuals with an intended target of equal numbers from males and females. It is not possible to determine if this small sample size ($n=26$ for each analysis) is truly representative of the free-ranging population. A factor that may affect our results was the inability to fast the free-ranging howlers, as is typically done with captive primates. Another aspect that may lead to differences between our study and previous ones may be method of analysis. Analytical methods typically change and improve over time, a factor that may lead to different concentrations.

The variables that are significantly different between males and females include HDL cholesterol, β-alanine, cystine, homocystine, tryptophan, α-tocopherol, lutein + zeaxanthin, and copper concentrations. Reasons for the sex differences are not readily apparent, but could be because of the small sample size and may not be truly representative of the larger population.

Total cholesterol, triglyceride, HDL cholesterol, and LDL cholesterol concentrations are similar to those of free-ranging Mexican mantled howlers (*Alouatta palliata mexicana*) and other captive NWPs (Crissey *et al.* 1999, 2003, unpublished). Captive apes have significantly higher cholesterol concentrations than those of their free-ranging counterparts, which may be affecting their cardiovascular health (Schmidt *et al.* 2006), but appears not to be a problem with captive howlers. Although there is a significant difference between calculated and measured LDL cholesterol concentrations, it is not appropriate to use Friedewald's equation on samples from nonfasted individuals (Friedewald *et al.* 1972). Numerically the difference is not large and may not be biologically relevant.

Although we only have amino acid data from 2 captive howlers and 5 captive golden-headed lion tamarins (*Leontopithecus rosalia chrysomelas*) for comparison, most of the amino acid concentrations appear to be similar between free-ranging and captive groups. However, cystine and taurine concentrations in the captive howlers were *ca.* 6 and 2 times higher, respectively, than the average concentrations of the free-ranging monkeys (Crissey *et al.* unpublished), most likely due to dietary supplementation of the amino acids in captive primates through nutritionally complete primate feeds. In the captive golden-headed lion tamarins, several amino acid concentrations, including α-aminoadipic acid, alanine, arginine, aspartic acid, glutamic acid, proline, and serine, were lower than those detected in captive and free-ranging black howlers. Perhaps the differences can be attributed to captive diets, genetic differences between the species, or small sample sizes.

All forms of vitamin A —retinol, retinyl palmitate, and retinyl stearate— and vitamin E (as γ-tocopherol) appear similar in concentration to the ones measured in free-ranging Mexican howlers and other captive NWP species (Crissey *et al.* 2003, unpublished). Some vitamin E concentrations were numerically higher in sera of captive primates and may be attributed to dietary supplementation. Vitamin E is added to primate feeds at higher concentrations than may be needed because vitamin E acts as an antioxidant both in the product and the subject. Vitamin E is well tolerated in doses at least twice as high as the individual's nutritional requirement (Combs 1998).

Vitamin D concentrations were the most interesting. They are not only dramatically higher at 14 times the concentrations measured in captive NWPs (Adams *et al.* 1985; Crissey *et al.* 1999, unpublished), but also *ca.* 3.5 times higher than those in free-ranging Mexican mantled howlers (Crissey *et al.* 2003) and free-ranging cotton-top tamarins (Power *et al.* 1997). Nutritionally complete feeds manufactured for NWPs can have 2–4 times the concentration of vitamin D, depending on the manufacturer, than other primate feeds. The captive primate values in Table III may have been from animals receiving feeds formulated for NWPs with higher concentrations of dietary vitamin D, though it is not evident by serum vitamin D concentrations. Although we measured only a small portion of the population at a specific time in the calendar year, we think that the vitamin D concentrations in captive black howlers should be more similar to those measured in their free-ranging counterparts. We need to find a safe and effective way to increase circulating vitamin D concentrations in captive black-and-gold howlers.

Most of the carotenoids we measured are at concentrations 1.5–28 times higher than those of free-ranging Mexican mantled howlers (Crissey *et al.* 2003) and 2–80 times higher than captive NWP concentrations (Crissey *et al.* 1999, unpublished). Some carotenoids are precursors to vitamin A, while others function as antioxidants. Unlike the study with Mexican mantled howlers, we detected low concentrations of lycopene in the serum.

Stacewicz-Sapuntzakis *et al.* detected other, unidentified carotenoids in high concentrations in the serum from the subjects in our study (Stacewicz-Sapuntzakis *pers. comm*.). University of Illinois-Chicago researchers are pursuing their identification and classification.

Copper concentrations are significantly different between males and females, though the reason is not apparent. All mineral concentrations appeared to be similar to those of free-ranging Mexican mantled howlers (Crissey *et al.* 2003) and captive black howlers (Crissey *et al.* unpublished). Calcium, potassium, magnesium, and zinc are within normal concentrations of captive primates (Kaneko 1989). However, phosphorus concentrations are much higher at 3.8 mmol/L than the 1.42–1.78 mmol/L range considered average (Kaneko 1989). The high P concentration leads to an inversed Ca:P ratio at 1:1.6, while the average Ca:P ratio in captive primates is exactly opposite at 1.6:1 (Kaneko 1989). It is difficult to tell if this is a true reflection of free-ranging howlers or if it could be due to small sample size, a reflection of seasonal diet, or some other unidentified reason.

Most of the nutritional parameters quantified in free-ranging black-and-gold howlers appeared similar to concentrations recorded for captive primates. However, free-ranging howlers had higher concentrations of vitamin D and phosphorus, 2 components necessary to build bone. Carotenoid concentrations are also higher than those of captive NWPs, but how and if their deficiency directly affects the health of captive NWPs is unknown. Expanding the study by gathering additional samples from free-ranging *Alouatta caraya* black howlers throughout the year and from different regions would give a clearer picture of the nutritional parameters for captive *A. caraya*.

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