Nutrient Requirements

Serum Concentrations of Lipids, Vitamin D Metabolites, Retinol, Retinyl Esters, Tocopherols and Selected Carotenoids in Twelve Captive Wild Felid Species at Four Zoos[1]

Susan D. Crissey, Kimberly D. Ange,*[2] Krista L. Jacobsen, Kerri A. Slifka, Phyllis E. Bowen,[†] Maria Stacewicz-Sapuntzakis,[†] Craig B. Langman,** William Sadler,[‡] Stephen Kahn[††] and Ann Ward[‡‡]

*Daniel F. and Ada L. Rice Conservation Biology and Research Center, Chicago Zoological Society, Brookfield, IL; *Department of Animal Science, North Carolina State University, Raleigh, NC 27695; [†]Department of Human Nutrition and Dietetics, University of Illinois at Chicago, Chicago, IL 60612; **Northwestern University, Evanston, IL and Children's Memorial Hospital, Chicago, IL 60614; [‡]Purina Mills Incorporated, St. Louis, MO 63144; [††]Loyola University Medical Center, Maywood, IL 60153; and [‡‡]Fort Worth Zoo, Fort Worth, TX 76110*

ABSTRACT Serum concentrations of several nutrients were measured in 12 captive wild felid species including caracal (*Felis caracal*), cheetah (*Acinonyx jubatus*), cougar (*Felis concolor*), fishing cat (*Felis viverrinus*), leopard (*Panthera pardus*), lion (*Panthera leo*), ocelot (*Felis pardalis*), pallas cat (*Felis manul*), sand cat (*Felis margarita*), serval (*Felis serval*), snow leopard (*Panthera uncia*) and tiger (*Panthera tigris*). Diet information was collected for these animals from each participating zoo (Brookfield Zoo, Fort Worth Zoo, Lincoln Park Zoological Gardens and North Carolina Zoological Park). The nutritional composition of the diets at each institution met the probable dietary requirements for each species except for the pallas cat. Blood samples were collected from each animal ($n = 69$) and analyzed for lipids (total cholesterol, triacylglycerides, HDL cholesterol and LDL cholesterol), vitamin D metabolites [25-hydroxycholecalciferol (25(OH)D) and 1,25-dihydroxycholecalciferol (1,25(OH)$_2$D)], vitamin A (retinol, retinyl stearate and retinyl palmitate), vitamin E (α- and γ-tocopherol) and selected carotenoids. Species differences were found for all except triacylglycerides and 1,25(OH)$_2$D. Genus differences were found for retinol, retinyl palmitate, retinyl stearate, γ-tocopherol and β-carotene. Circulating nutrient concentrations for many of the species in this study have not been reported previously and most have not been compared with the animals' dietary intakes. The large number of animals analyzed provides a substantial base for comparing the serum nutrient concentrations of healthy animals, for both wild and captive exotic species. J. Nutr. 133: 160–166, 2003.

KEY WORDS: • *Felis* • *lipids* • *vitamin A* • *vitamin D* • *vitamin E*

JN THE JOURNAL OF NUTRITION

Assessment of nutrient concentrations is an important component in the successful conservation and propagation of captive and free-ranging felid populations. Animals require lipids and vitamins D, A and E for normal growth, reproduction and health (1). In addition, carotenoids, such as β-carotene and lutein, are important in maintaining various animal immune functions (2,3). Circulating levels of β-carotene have been used to assess malabsorption and nutritional status in humans (4,5).

Attempts have been made to assess the adequacy of an animal's dietary supply of lipids and vitamins by measuring serum or plasma concentrations (6–8). However, limited information is available on concentrations of circulating lipids, vitamins and carotenoids in captive wild felids (6,8,9). These circulating nutrients can provide a basis for examining the nutritional status of such species (10). The purpose of this study was to measure and compare circulating lipids, vitamin D metabolites, retinol, retinyl esters, tocopherols and selected carotenoids in various felid species representing multiple institutions, and to define a relationship, if any, between this information and diet.

MATERIALS AND METHODS

Diet evaluation. Diet information was gathered by written survey and analyzed for nutrient content using the Animal Nutritionist program (version 2.5, 1987, N-squared computing, Silverton, OR). Dietary items were categorized into food groups, based primarily on the typical items consumed by these species in captivity (**Tables 1** and **2**). The food groups included: bones with raw meat, fish, miscellaneous items, nutritionally complete canned diets, nutritionally complete dry diets, nutritionally complete raw meat diets, organs,

[1] Gratitude is expressed to Purina Mills, which provided the funding for this study.

[2] To whom correspondence should be addressed. E-mail: kim_ange@ncsu.edu.

Manuscript received 22 May 2002. Initial review completed 19 September 2002. Revision accepted 21 October 2002.

TABLE 1

Food groups based on diet items offered to all species

Diet item	Description
Nutritionally complete raw meat diets	Canine Diet, Feline Diet.[1]
Nutritionally complete canned diets (other)	Zu/Preem canned feline diet;[2] Science Diet Feline Maintenance[3]
Nutritionally complete dry diets	Exotic Feline-Small 5M54[4]
Bones with meat/organs	Horsemeat, liver, shank bones, knuckle bones, and oxtail bones.[1]
Prey Items	Pinkies, mice, rats, chicks, crickets
Fish	Herring, trout
Miscellaneous	Hard-boiled egg, Pet-form chewable vitamin/mineral tablets[5]
Vegetables	Carrots, mixed greens

1 Nebraska Brand, Central Nebraska Packing, Inc., North Platte, NB.
2 ZuPreem, Shawnee Mission, KS.
3 Hill's Pet Nutrition, Topeka, KS.
4 Mazuri®, PMI® Nutrition International, Brentwood, MO.
5 Vet-a-Mix, Shenandoah, IA.

prey and vegetables. Computer analyses of dietary nutrients, on a dry matter basis, were compared among species and domestic felid nutrient guidelines and requirements as established by AAFCO[3] (11) and NRC (12,13). On the basis of estimates of nutrient composition and comparisons to domestic cat requirements (**Table 3**), the diets offered met or exceeded the probable requirements for lipids (crude fat), crude protein, and vitamins D, A, and E (11,12,13). The one exception was noted for the pallas cat diets in which the minimum level of dietary vitamins D and E were below the probable requirements. This value reflects the entirely whole-prey diet fed to the male pallas cat because these items were all that it would consume at the time of data collection. Among all species, there were variations in diet nutrient contents. Ranges were large, especially for fat and vitamins A and E.

Animals and blood collection. Blood samples from 69 felids from Brookfield Zoo, Fort Worth Zoo, Lincoln Park Zoological Gardens, and North Carolina Zoological Park were collected opportunistically during routine or diagnostic physical examinations between April 1996 and March 2000 and analyzed chemically. All animals were housed in their usual exhibit areas and were fed their usual diets. Because of the quantity of blood required, not all analyses were performed on serum from all animals. Species included: caracal (*Felis caracal*; 1 male and 1 female, each 10 y old); cheetah (*Acinonyx jubatus*; 3 males and 1 female,5–10 y old); cougar (*Felis concolor*; 1 male and 3 females, 7–9 y old); fishing cat (*Felis viverrinus*; 3 males, each 1 y old); leopard (*Panthera pardus*; 2 males and 1 female, 5–8 y old), lion (*Panthera leo*; 8 males and 6 females, 1–17 y old); ocelot (*Felis pardalis*; 2 males, 3–4 y old); pallas cat (*Felis manul*; 1 male, 3 y old and 1 female, 6 y old); sand cat (*Felis margarita*; 5 males and 4 females, 3–7 y old); serval (*Felis serval*; 4 females, 8–11 y old); snow leopard (*Panthera uncia*; 4 males and 8 females, 2–17 y old); and tiger (*Panthera tigris*; 4 males and 6 females, 2–18 y old). Blood from four geriatric lions known to have feline immunodeficiency virus, but with no clinical signs, was included; otherwise, animals known to be of abnormal health status were not included in these data. Animals were deprived of food overnight before immobilization, and veterinary staff drew blood into tubes with no added anticoagulants. The protocol was performed according to the guidelines of the Brookfield Zoo Animal Care and Use Committee. Serum was separated by centrifugation (2300 × *g*, 10 min), wrapped in foil as appropriate to protect light sensitive substances, labeled and frozen for a mean of 6 mo at −80°C until thawed for analysis.

Blood analyses. Total cholesterol (TC) concentrations were analyzed at the Brookfield Zoo Veterinary Services Laboratory using the Eastman Kodak Ortho Clinical Diagnostics 250 Analyzer (Rochester, New York). Triacylglyceride (TG) and HDL-cholesterol concentrations were measured at Loyola University Medical Center (Maywood, IL) using the Synchron Delta CX7 Analyzer (Beckman-Coulter, Brea, CA) (14–16). A direct HDL-cholesterol method (Sigma Diagnostics, St. Louis, MO) was adapted to the Synchron Delta CX7 analyzer. LDL-cholesterol was calculated based on a modification of the Friedewald formula using TC minus HDL cholesterol and TG/6.25 (representing VLDL) (17). This calculation is valid for TG levels up to 4.56 mmol/L.

Samples were assayed for the vitamin D metabolites, 25(OH)D and $1,25(OH)_2D$, at the Mineral Metabolism Laboratory, located at the Children's Memorial Hospital (Chicago, IL), following methods outlined by Reed et al. (18). Aliquots of thawed serum were analyzed for vitamin A (retinol, retinyl stearate, and retinyl palmitate), vitamin E (α- and γ-tocopherol) and carotenoids (α- and β-carotene, lutein/zeaxanthin, lycopene, and β-cryptoxanthin) by HPLC at the University of Illinois, Chicago, following methods outlined by Stacewicz-Sapuntzakis et al. (19). *Cis/trans* isomers of the carotenoids were not separated.

Statistical analyses were performed using SPSS for Windows (SPSS 10.0 1999, SPSS, Chicago, IL). Only main effects were analyzed because of the limited number available within independent variables (sex and age). Differences among genera and species were compared by a multivariate General Linear Model (GLM) procedure. Limited values for γ-tocopherol and carotenoids were not included in the multivariate GLM procedure, and were instead evaluated by univariate ANOVA using species, and genus as independent variables. The Bonferroni test was used for post-hoc multiple comparisons; significance for all analyses was set at $P \leq 0.05$. Means for species were calculated from single values for each individual. Most animals were sampled only once, but for those with multiple samples, a mean value was calculated before performing statistics. Values are presented as means ± SEM.

RESULTS AND DISCUSSION

Lipids. Lipid levels are listed by genus and species in **Table 4** and comparisons to published values are shown in **Table 5**. Lipid levels did not differ among genera, although levels of cholesterol, HDL cholesterol and LDL cholesterol differed among species ($P < 0.001$). Hyperlipidemia is the general term for the presence of any or all elevated lipids in the bloodstream (20). In contrast to humans, healthy cats do not exhibit hyperlipidemia in the fasting state (20,21).

Total cholesterol levels of tigers, cougars, cheetahs, leopards, lions and snow leopards were similar to those previously published (Tables 4 and 5). We contend that the single previously published value for TC in servals may not be representative of normal serval serum. This published value was very low (0.1 mmol/L) and the concentrations reported in the present study are likely to be more representative of the captive serval TC levels (Table 4). The lions, pallas cats, sand cats, servals and snow leopards all had TC levels within the domestic cat range of 3.1–4.3 mmol/L. In contrast, cheetahs, cougars, fishing cats, leopards, ocelots and tigers had TC concentrations greater than those for the domestic cat. Caracals were the only species to have lower TC (2.1 mmol/L) than domestic cats. They also had lower levels compared with the published TC levels of all other exotic cats (with the exception of the published serval level already discussed).

Although TG did not differ among genera or species, some of the feline species had TG levels much different than previously published information (Tables 4 and 5). Most notably, the current value for snow leopards (0.38 mmol/L) deviated

[3] Abbreviations used: AAFCO, American Association of Feed Control Officials; GLM, General Linear Model; 25(OH)D, 25-hydroxycholecalciferol; $1,25(OH)_2D$, 1,25-dihydroxycholecalciferol; TC, total cholesterol; TG, triacylglycerides.

TABLE 2

Food group composition of the felid diets at Brookfield Zoo, Fort Worth Zoo, Lincoln Park Zoological Gardens and North Carolina Zoological Park

Species	*n*	Raw meat diets[1]	Diets (other, canned)[1]	Dry diets[1]	Bones with meat	Prey items	Fish	Miscellaneous	Vegetables
					g/100 g[2]				
Caracal	2	54–72	0	0	21–39	6–7	0	0	0
Cheetah	4	67–93	0	0	8–25	0–5	0	0–1	0–3
Cougar	4	0–93	0–90	0–9	1–25	0–5	0–2	0–1	0–3
Fishing cat	3	64–71	0	0	20–27	0–1	7–8	0	0
Leopard	3	67–100	0	0	0–25	0–5	0	0–1	0–3
Lion	14	86–97	0	0	3–11	0–3	0	<1	0–2
Ocelot	2	87	0	0	6	7	0	0	0
Pallas cat	2	0–82	0	0	0	18–100	0	0	0
Sand cat	9	64	0	0	30	6	0	0	0
Serval	4	0–34	0–97	0	3–49	0	0	0–1	0–7
Snow leopard	12	67–83	0	0	12–25	0–5	0	0–1	0–3
Tiger	10	85–95	0	0	5–15	0–2	0	<1	0–2

[1] Nutritionally complete.
[2] As fed to the animals.

substantially from the published literature concentration for snow leopards (1.21 mmol/L). From Tables 4 and 5, it can be seen that all of the animals except for cougars had TG levels less than those of domestic cats.

Among published data, only values for domestic cats were available for both LDL and HDL cholesterol. Although measuring the lipid profile of the study animals may not correspond directly to the dietary requirement for fat, examining an array of circulating lipids provides a more complete lipid profile, and normal parameters can be established. All species, except the caracal, had considerably greater levels of HDL cholesterol than domestic cats. Compared with the LDL cholesterol levels of domestic cats (0.9 mmol/L), fishing cats had a much greater concentration (2.4 mmol/L). In contrast, caracals (0.3 mmol/L) and snow leopards (0.4 mmol/L) had considerably lower levels.

Previous studies reported that cats, unlike humans, appear to have 5 to 6 times more HDL than LDL cholesterol (22,23), although other researchers reported HDL cholesterol levels in cats ~2 times greater than LDL cholesterol (24). Among felids in this study, most had HDL cholesterol levels ~2–3 times greater than LDL cholesterol. With relatively high levels of HDL cholesterol, cats are not known to suffer from coronary heart disease caused by hyperlipidemia (23).

Caracals had very little fat in their diet compared with the other species and this could be associated with the caracal's low serum lipid levels. However, the fishing cats also had very little fat in their diet and they had some of the highest serum lipid concentrations. Therefore, it is clear that the lipid concentrations of the diets must be further examined before any relationships between serum lipid concentrations and dietary fat can be considered.

TABLE 3

Calculated felid diet nutrient composition at Brookfield Zoo, Fort Worth Zoo, Lincoln Park Zoological Gardens and North Carolina Zoological Park[1]

Species	*n*	Dry matter	Fat	Crude protein	Vitamin D[2]	Vitamin A[3]	Vitamin E[4]
		g/100 g			*mg/kg*		
Caracal	2	30	19–21	65–68	600–800	15,000–18,000	30–39
Cheetah	4	30–36	22–39	51–63	550–1000	24,000–126,000	47–144
Cougar	4	36–38	37–39	44–51	1900	126,000	144
Fishing cat	3	29	21	66	700	21,000	36
Leopard	3	36	35–39	51–53	2000	90,000–129,000	123–144
Lion	14	31–37	22–41	49–62	1500–2000	24,000	50
Ocelot	2	37	40	50	2000	117,000	146
Pallas cat	2	32–35	24–27	58–60	225–975	21,000–150,000	16–42
Sand cat	9	29	20	66	1750	18,000	35
Serval	4	29–39	27–44	43–62	600–750	132,000–180,000	73–222
Snow leopard	12	33–36	29–39	51–59	750–1000	69,000–126,000	94–144
Tiger	10	31–37	22–41	49–62	250–1250	24,000–135,000	50–155
Probable requirements[5]		—	5–11	18–33	550	5500–30,000	27–34

[1] Calculated on a dry matter basis (with the exception of dry matter).
[2] Vitamin D in the calculated diets was primarily cholecalciferol, with some diets containing small amounts of ergocalciferol.
[3] Vitamin A in the calculated diets was retinol, carotenoids or both.
[4] Vitamin E in the calculated diets was either α-tocopherol or total tocopherol, depending on the source of the data.
[5] Probable requirements are based on those established for the cat (11–13).

TABLE 4

Serum lipid concentrations in felids at Brookfield Zoo, Fort Worth Zoo, Lincoln Park Zoological Gardens, and North Carolina Zoological Park[1,2]

	n	TC	TG	HDL cholesterol	LDL cholesterol
			mmol/L		
Genus[3]					
Acinonyx	3	4.9 ± 0.39	0.32 ± 0.054	3.6 ± 0.17	1.2 ± 0.24
Felis	21	4.5 ± 0.31	0.35 ± 0.042	3.2 ± 0.23	1.2 ± 0.13
Panthera[4]	38	4.3 ± 0.22	0.43 ± 0.032	3.3 ± 0.16	0.9 ± 0.09
Species[5]					
Caracal	2	2.1 ± 0.01[w]	0.24 ± 0.110[w]	1.7 ± 0.10[w]	0.3 ± 0.05[w]
Cheetah	3	4.9 ± 0.39[x,y,z]	0.33 ± 0.054[w]	3.6 ± 0.17[w,x,y]	1.2 ± 0.24[w,x,y]
Cougar	4	5.4 ± 0.58[y]	0.54 ± 0.170[w]	4.1 ± 0.53[x,y]	1.0 ± 0.10[w,x,y]
Fishing cat	3	6.3 ± 0.31[z]	0.41 ± 0.060[w]	3.8 ± 0.25[w,x,y]	2.4 ± 0.20[y]
Leopard	3	4.4 ± 0.82[w,x,y,z]	0.40 ± 0.111[w]	3.4 ± 0.73[w,x,y]	0.8 ± 0.24[w,x]
Lion[6]	13	4.1 ± 0.32[w,x,y]	0.44 ± 0.055[w]	2.8 ± 0.25[w,x]	1.0 ± 0.12[w,x]
Ocelot	2	5.4 ± 0.72[y,z]	0.25 ± 0.020[w]	4.1 ± 3.36[x,y]	1.1 ± 0.05[w,x,y]
Pallas cat	2	4.2 ± 0.22[w,x,z]	0.34 ± 0.015[w]	3.0 ± 0.21[w,x,y]	1.1 ± 0.01[w,x,y]
Sand cat	4	3.5 ± 0.27[w,y]	0.33 ± 0.060[w]	2.4 ± 0.35[w,x]	0.9 ± 0.13[w,x]
Serval	4	3.9 ± 0.40[w,y]	0.25 ± 0.045[w]	2.7 ± 0.35[w,x]	1.1 ± 0.23[w,x]
Snow leopard[7]	12	3.3 ± 0.13[w,x]	0.38 ± 0.052[w]	2.8 ± 0.11[w,x]	0.4 ± 0.07[w]
Tiger[8]	10	5.7 ± 0.39[z]	0.50 ± 0.069[w]	4.2 ± 0.24[y]	1.3 ± 0.19[x,y]

1 Values are means ± SEM.
2 TC, total cholesterol and TG, triacylglycerides.
3 Genera did not differ, $P \geq 0.05$.
4 $n = 32$ for LDL cholesterol.
5 Species means within a column with different superscripts differ, $P < 0.05$.
6 $n = 9$ for LDL cholesterol.
7 $n = 11$ for LDL cholesterol.
8 $n = 9$ for LDL cholesterol.

Vitamin D. Serum concentrations of vitamin D metabolites are compared among genera and species in **Table 6**, and species data are compared with published data in Table 5. Levels of both 25(OH)D and $1,25(OH)_2D$ did not differ among genera. Significant species differences were shown for 25(OH)D; snow leopards, cheetahs and lions had the greatest levels, whereas tigers and cougars had the lowest. Although not significantly different, cheetahs, lions and snow leopards

TABLE 5

Published serum total cholesterol, triacylglycerides, vitamin D metabolites and vitamin A, E, and carotenoid levels in various species of felids[1–4]

Species	TC	TG	Retinol	Retinyl palmitate	Retinyl stearate	β-Carotene	α-Tocopherol
	mmol/L				*nmol/L*		*μmol/L*
Caracal	UA	UA	664 ± 73.4 (2)[45]	172 ± 59.2 (2)[45]	UA	298 (1)[9]	16.6 ± 11.98 (2)[45]
Cheetah	4.6 ± 0.28 (4)[6]	0.40 ± 0.079 (4)[6]	1608 ± 195.8 (4)[6]	420 ± 303.4 (4)[6]	561 ± 352.6 (4)[6]	UA	21.2 ± 0.62 (4)[6]
			1818 ± 108.4 (88)[44]				18.1 ± 0.53 (88)[44]
Cougar	5.3 (68)[39]	UA	804 (1)[6]	UA	UA	UA	6.4 (1)[6]
	3.8 ± 0.07 (94)[40]						
Fishing cat	UA	UA	1014 ± 115.4 (2)[45]	496 ± 74.4 (2)[45]	325 ± 325.5 (2)[45]	93 ± 1.86 (2)[9]	16.6 (2)[45]
Leopard	4.6 ± 0.25 (2)[6]	0.88 ± 0.113 (2)[6]	1259 ± 293.7 (2)[6]	267 (1)[6]	trace (2)[6]	UA	24.2 ± 6.32 (2)[6]
			1434 ± 17.5 (2)[42]				15.3 ± 0.02 (2)[42]
Lion	4.0 ± 0.62 (8)[6]	0.58 ± 0.124 (8)[6]	455 ± 115.4 (8)[6]	trace (8)[6]	trace (8)[6]	503 ± 40.1 (3)[9]	19.5 ± 4.81 (8)[6]
	3.5 (mean)[38]		559 (6)[42]				12.1 (6)[42]
Ocelot	UA	UA	UA	UA	UA	652 (1)[9]	UA
Pallas cat	UA	UA	2413 ± 433.6 (9)[45]	7061 ± 1185.1 (9)[45]	14358 ± 56.1 (9)[45]	186 ± 33.5 (9)[9]	28.8 ± 3.39 (9)[45]
Sand cat	UA	UA	1154 (1)[45]	4580 (1)[45]	8128 (1)[45]	652 (1)[9]	46.9 (1)[45]
			1224 ± 202.8 (8)[43]	2786 ± 290.1 (8)[43]		410 ± 65.2 (8)[43]	
Serval	0.1 (1)[6]	0.25 (1)[6]	105 (1)[6]	UA	UA	UA	139.3 (1)[6]
Snow leopard	2.7 ± 0.34 (7)[6]	1.21 ± 0.429 (7)[6]	2203 ± 104.9 (7)[6]	76 ± 19.1 (7)[6]	72.3 ± 25.3 (7)[6]	615 ± 63.3 (5)[9]	13.0 ± 2.5 (7)[6]
Tiger	5.9 ± 0.55 (25)[38]	0.76 ± 0.271 (10)[6]	664 ± 115.4 (10)[6]	trace (10)[6]	trace (10)[6]	987 ± 160.2 (3)[9]	33.9 ± 5.90 (10)[6]
	5.4 ± 0.62 (10)[6]		804 (3)[42]				20.4 (3)[42]
Domestic cat	4.3 ± 0.46 (61)[41]	0.63 ± 0.056 (61)[41]	734 ± 55.9 (7)[6]	324 ± 78.2 (7)[6]	579 ± 115.7 (7)[6]	37 ± 3.7 (29)[44]	22.9 ± 3.85 (7)[6]
	3.1 ± 0.16 (10)[24]	0.53 ± 0.068 (10)[24]	3357 ± 2692.3 (29)[44]				16.2 ± 2.44 (29)[44]

1 Values are means ± SEM (*n*).
2 TC, total cholesterol and TG, triacylglycerides.
3 Superscript numbers are those of the references cited.
4 UA, unavailable.

TABLE 6

Serum vitamin D metabolite and vitamin A and E concentrations in felids at Brookfield Zoo, Fort Worth Zoo, Lincoln Park Zoological Gardens and North Carolina Zoological Park[1,2,3]

	25(OH)D	$1,25(OH)_2D$	Retinol	Retinyl palmitate	Retinyl stearate	α-Tocopherol	γ-Tocopherol	β-Carotene
	nmol/L	*pmol/L*	*nmol/L*	*nmol/L*	*nmol/L*	*μmol/L*	*nmol/L*	*nmol/L*
Genus[4]								
Acinonyx	95 ± 25.2^{a} (4)	132 ± 21.8^{a} (4)	3217 ± 919.6^{a} (2)	76 ± 38.2^{b} (2)	181 ± 130.2^{b} (2)	44.9 ± 4.19^{a} (2)	ND (2)	$448 \pm 449.8^{a,b}$ (2)
Felis	69.9 ± 9.0^{a} (6)	53 ± 11.8^{a} (6)	1189 ± 101.4^{b} (16)	1660 ± 561.1^{a} (16)	2532 ± 880.6^{a} (16)	38.9 ± 5.79^{a} (16)	288 ± 62.4^{a} (16)	280 ± 50.4^{b} (16)
Panthera	92 ± 7.0^{a} (17)	69.6 ± 7.2^{a} (17)	1224 ± 171.3^{b} (21)	57 ± 11.5^{b} (21)	36 ± 9.0^{b} (21)	41.0 ± 3.49^{a} (21)	144 ± 43.2^{b} (21)	653 ± 72.8^{a} (21)
Species[5]								
Cheetah	95 ± 25.2^{y} (4)	132 ± 21.8^{x} (4)	3217 ± 919.6^{w} (2)	76 ± 38.2^{y} (2)	181 ± 130.2^{x} (2)	$44.9 \pm 4.19^{y,z}$ (2)	ND (2)	$448 \pm 449.8^{x,y,z}$ (2)
Cougar	47 ± 0.5^{w} (2)	86 ± 0.0^{x} (2)	$1888 \pm 35.0^{x,z}$ (2)	76 ± 22.9^{y} (2)	72 ± 23.5^{x} (2)	23.5 ± 1.50^{z} (2)	ND (2)	$579 \pm 18.7^{x,y,z}$ (2)
Fishing cat	NA	NA	$839 \pm 83.9^{y,z}$ (3)	344 ± 40.1^{y} (3)	416 ± 66.9^{x} (3)	82.0 ± 3.53^{x} (3)	672 ± 48.0^{x} (3)	$355 \pm 42.9^{y,z}$ (3)
Lion	92 ± 12.7^{y} (3)	70 ± 15.8^{x} (3)	$804 \pm 62.9^{x,y}$ (9)	38 ± 5.7^{y} (9)	54 ± 7.2^{x} (9)	34.4 ± 4.25^{z} (9)	48 ± 55.2^{y} (9)	560 ± 106.4^{z} (9)
Ocelot	$80 \pm 5.5^{x,y}$ (2)	45.6 ± 8.9^{x} (2)	NA	NA	NA	NA	NA	NA
Pallas cat	$80 \pm 21.5^{x,y}$ (2)	24 ± 2.4^{x} (2)	NA	NA	NA	NA	NA	NA
Sand cat	NA	NA	$1154 \pm 115.3^{x,y}$ (8)	3149 ± 835.9^{z} (8)	4882 ± 1318.3^{y} (8)	27.9 ± 2.96^{z} (8)	312 ± 33.6^{y} (8)	131 ± 37.3^{y} (8)
Serval	NA	NA	$1049 \pm 230.8^{x,y}$ (3)	38 ± 5.7^{y} (3)	9 ± 4.9^{x} (3)	$35.69 \pm 8.29^{y,z}$ (3)	$ND^{y,z}$ (3)	$411 \pm 106.4^{y,z}$ (3)
Snow leopard	107 ± 7.7^{y} (9)	84 ± 9.1^{x} (9)	1818 ± 349.7^{x} (8)	605 ± 70.6^{y} (8)	ND (8)	$38.9 \pm 3.78^{y,z}$ (5)	144 ± 76.8^{y} (8)	$560 \pm 74.6^{x,z}$ (8)
Tiger	62 ± 8.5^{x} (5)	45.6 ± 9.4^{x} (5)	$1049 \pm 223.8^{x,y}$ (4)	134 ± 26.7^{y} (4)	90 ± 30.7^{x} (4)	$60.2 \pm 9.66^{x,y}$ (4)	$288 \pm 45.6^{x,z}$ (4)	1082 ± 132.5^{x} (4)

[1] Values are means ± SEM (*n*).
[2] NA, not analyzed; ND, levels were below detectable limits.
[3] Neither lycopene or α-carotene was detected in any species. Lutein + zeaxanthin and β-cryptoxanthin were detected only in sandcats and their values were 9 ± 2.1 and 18 ± 3.6 nmol/L, respectively. α-Cryptoxanthin was detected only in cheetahs (215 ± 215.2 nmol/L), lions (78 ± 77.8 nmol/L), and sandcats (15 ± 2.9 nmol/L).
[4] Genus means in columns with different superscripts differ, $P < 0.05$.
[5] Species means in columns with different superscripts differ, $P < 0.05$.

had the greatest levels of $1,25(OH)_2D$, whereas pallas cats and ocelots had the lowest.

Published vitamin D concentrations in exotic cat serum were not available. Recent data (25) for domestic kittens show that a 25(OH)D concentration > 50 nmol/L reflects an adequate level of this metabolite. The normal 25(OH)D level in domestic dogs is 70–95 nmol/L (26). All felids in this study likely possessed adequate levels of this metabolite with the possible exception of cougars who had 25(OH)D levels of 47 nmol/L. No published values for $1,25(OH)_2D$ for felids were available. Compared with normal levels in domestic dogs of 36–127 pmol/L (25), only the pallas cats had slightly less $1,25(OH)_2D$ at 24 pmol/L. However, due to the lack of data for domestic cats, deficiencies cannot necessarily be suspected. It is interesting to note that one of the pallas cats was the only feline in the study receiving a diet that was slightly deficient in vitamin D. Although cheetahs and lions had the highest levels of both metabolites, their diets did not contain the highest level of the vitamin.

Circulating serum 25(OH)D is indicative of liver stores, and $1,25(OH)_2D$ reflects the active form and possibly recent intake (4,27). Thus, the precursor form may be the best indicator of status (1). Levels of 25(OH)D differed among species, whereas the more active hormone $1,25(OH)_2D$ did not. This may be interpreted in one of two ways: *1)* there is tight regulation of $1,25(OH)_2D$ compared with 25(OH)D, and the increase of 25(OH)D does not increase the $1,25(OH)_2D$ levels, or *2)* there may be mild substrate depletion relative to need. Lack of the UV-light produced 7-dehydrocholesterol in domestic cats raises the possibility that more efficient absorption from the diet occurs because this backup system to poor diet intake is absent (28,29).

Vitamin A. Differences were found among genera for circulating retinol, retinyl palmitate, and retinyl stearate ($P < 0.05$) (Table 6). *Acinonyx* had greater retinol concentrations than both *Felis* and *Panthera* ($P < 0.001$). However, *Felis* had greater levels of retinyl palmitate and retinyl stearate, due primarily to the high levels in sand cats within that genus. Cheetahs had greater retinol (3217 nmol/L) concentrations than all other species. All of the feline species measured had retinol concentrations within the domesticated cat range of published values (734–3357 nmol/L). Sand cats had substantially greater retinyl palmitate (3149 nmol/L) and retinyl stearate (4882 nmol/L) concentrations than all other species including domestic cats. Previously reported sand cat retinyl palmitate and retinyl stearate concentrations were also very high and therefore consistent with the current results. Unfortunately, we were unable to have pallas cat blood tested for retinyl palmitate and retinyl stearate. It appears from published data that pallas cats also have very high naturally occurring levels of these two metabolites (Table 5). Retinyl stearate was not detected in snow leopards.

Hepatic concentrations may more accurately reflect the status of vitamins A and E (30–32) than circulating levels, although given its invasive nature, tissue analysis is impractical for routine application to zoo animals. Circulating retinol concentrations for cheetahs, cougars and snow leopards appeared to reflect the dietary levels of vitamin A. However, sand cats, with one of the lowest levels of dietary vitamin A, had markedly greater levels of retinyl esters. Vitamin A transport by esters in carnivores can be influenced directly by dietary vitamin A intake (6). Circulating retinyl palmitate has been reported to indicate excessive dietary vitamin A in some animals (7). Vitamin A has been examined in select carnivores and it has been shown that carnivores can secrete vitamin A in the urine in a unique manner and therefore may have high levels of circulating vitamin A metabolites without concern for toxicity (7).

Vitamin E. α-Tocopherol levels were greatest among fishing cats, followed by tigers and cheetahs; cougars and sand cats had the lowest levels (Table 6). There were no differences among genera. All α-tocopherol concentrations, except in sand cats, were greater than previously published data for the species examined. It has been shown that serum levels of α-tocopherol can be correlated with dietary intake (33,34). Dierenfeld (35) suggested that exotic animals have a 5- to 10-fold greater vitamin E requirement than current livestock recommendations. It is not known whether this applies to all exotic species because not all have been studied. The high α-tocopherol concentrations in this study seemed to reflect the relatively high dietary levels reported in Table 3 with one exception. The cougar had one of the greatest dietary vitamin E levels (144 mg/kg) but the least circulating α-tocopherol (23.5 μmol/L).

There were genus and species differences in serum γ-tocopherol ($P < 0.05$). Fishing cats had the greatest serum γ-tocopherol but it was not different than tigers. γ-Tocopherol was not detected in cheetahs, cougars or servals. The importance of circulating γ-tocopherol is not known. Current opinion in human studies is that γ-tocopherol may have beneficial physiologic effects such as lowering cholesterol in patients with high cholesterol (36). Although α-tocopherol is the most active form of vitamin E in the diet, small amounts of γ-tocopherol also are present in most nutritionally complete feeds (37). γ-Tocopherol is present in plant products such as corn, barley, oats, rye, wheat and various vegetable oils (37). Thus, the animals in this study may have obtained γ-tocopherol via vegetable products in their diets or plant material in their exhibits. Literature describing γ-tocopherol in felids is not available and its relevance to their nutritional health is unknown.

Carotenoids. Nearly all felids had measurable serum β-carotene, and the levels differed among genera (Table 6). Tigers, snow leopards and lions had the greatest level of β-carotene, whereas sand cats had the least. One cheetah had an undetectable level, whereas the other had 896 nmol/L; hence, the species and genus (*Acinonyx*) mean was 448 ± 449.8 nmol/L. Within *Felis*, only sand cats had detectable levels of α-cryptoxanthin, lutein + zeaxanthin and β-cryptoxanthin. Some cheetahs, lions and sandcats had detectable levels of α-cryptoxanthin. However, again, one cheetah had undetectable levels of α-cryptoxanthin, whereas the other had 430.5 nmol/L, giving a mean of 215 ± 215.0 nmol/L for the species and the genus. Lycopene and α-carotene were not detected in any species.

Carotenoids can be important for immune function, and establishing normal circulating levels may become increasingly important (2). However, with the exception of β-carotene, most carotenoids were not detected in most felid species studied. Sand cats are the primary exception. This agrees with previously published literature; however, some of these reports were from our own laboratory (Table 5) (6,9). The research data presented here confirm that felids are high to moderate carotenoid accumulators for β-carotene. Other carotenoids may be present in some species, however, at concentrations below the detection limits. Given that dietary carotenoid levels were not known in this study, the effect of diet on circulating carotenoids cannot be determined. However, we contend that carotenoids were present in the dietary items as well as in the plant material in exhibits.

In summary, we evaluated serum levels of lipids, vitamins D, A and E, and carotenoids in various healthy captive exotic felids. There was considerable variation in numbers of felids sampled within a species, which may have skewed the means used to compare genera; however, many of the circulating nutrients in these species have never before been reported. Because of small numbers in a few species, comparisons to the most closely related species such as the domestic cat are appropriate. Most circulating nutrients in captive wild felines have never been compared with dietary levels and although knowledge of the dietary levels of some nutrients such as lipids was incomplete, any knowledge gained from comparing circulating levels with diet is important. It was not expected that the circulating nutrient levels would be similar in humans and felids, but considerable data are available for humans and thus a comparison with established normal ranges for humans as well as domestic cats puts these felid data in some perspective. This information provides a substantial database for evaluating differences across these species, and could be valuable for future nutritional health assessments. Furthermore, data presented in this paper provide reference serum nutrient concentrations for captive felids that potentially can be used to assess the health status of other exotic felid species both in captivity and in the wild.

The diet of each animal studied was not measured quantitatively; although all diets met or exceeded nutritional requirements with one exception, they differed among genera and species of felines. The nutritional differences that may be caused by seasonal differences within and among zoos also contribute to the variations in the means. However, the data presented in this paper do show that species such as the serval that are from two separate zoos and fed two completely different diets do not have substantially different circulating nutrient concentrations. Similarly, species such as the fishing cat that are fed relatively the same diet and all housed at the same zoo, stand out as a species outlier in many nutritional measurements. Therefore, this study provides researchers with a basis of information upon which to begin performing more advanced species-specific research to determine why feline species differ in their circulating nutrient concentrations.

ACKNOWLEDGMENTS

We thank Robyn Barbiers and Ann Manharth at Lincoln Park Zoological Gardens; Ron Morris, Lorraine Smith, and Mike Loomis at North Carolina Zoological Park; and the Veterinary and keeper staffs at Brookfield and Fort Worth Zoos.

LITERATURE CITED

1. Rucker, R. B. & Morris, J. G. (1997) The vitamins. In: Clinical Biochemistry of Domestic Animals (Kaneko, L. A., Harvey, J. W. & Bruss, M. L., eds.), pp. 771–714. Academic Press, New York, NY.
2. Chew, B. P. (1993) Role of carotenoids in the immune response. J. Dairy Sci. 76: 2804–2811.
3. Kim, H. W., Chew, B. P., Wong, T. S., Park, J. S., Weng, B. C., Cerveny, C. G., Hayek, M. G. & Reinhart, G. E. (1999) Dietary lutein stimulates cell-mediated and humoral immunity in cats. FASEB J. 13: A552 (abs.).
4. Miller, S. M. (1985) Vitamins. In: Clinical Chemistry—Principles, Procedures, Correlation (Bishop, M. L., Duban-vonLaufan, J. L. & Fody, E. P., eds.), pp 368–371. J. B. Lippincott, Philadelphia, PA.
5. Jandacek, R. J. (2000) The canary in the cell: a sentinel role for β-carotene. J. Nutr. 130: 648–651.
6. Schweigert, F. J., Ryder, O. A., Rambeck, W. A. & Zucker, H. (1990) The majority of vitamin A is transported as retinyl esters in the blood of most carnivores. Comp. Biochem. Physiol. 4: 573–578.
7. Schweigert, F. J, Thomann, E. & Zucker, H. (1991) Vitamin A in the urine of carnivores. Int. J. Vitam. Nutr. Res. 61: 110–113.
8. Dierenfeld, E. S. (1993) Nutrition of captive cheetahs: food composition and blood parameters. Zoo Biol. 12: 143–150.
9. Slifka, K. A., Bowen, P. E., Stacewicz-Sapuntzakis, M., & Crissey, S. D. (1999) A survey of serum and dietary carotenoids in captive wild animals. J. Nutr. 129: 380–390.
10. Crissey, S. D., Swanson, J. A., Lintzenich, B. A., Brewer, B. A. & Slifka, K. A. (1997) Use of a raw meat-based diet or a dry kibble diet for sand cats (*Felis margarita*). J. Anim. Sci. 75: 2154–2160.
11. American Association of Feed Control Officials (2001) Pet Food Regulations. In: Official Publication of the American Association of Feed Control Officials. AAFCO, Atlanta, GA.
12. National Research Council (1978) Nutrient Requirements of Domestic Cats. National Academy Press, Washington, DC.
13. National Research Council (1986) Nutrient Requirements of Domestic Cats. National Academy Press, Washington, DC.
14. Bucolo, G. & David, H. (1973) Quantitative determination of serum triglycerides by the use of enzymes. Clin. Chem. 19: 476–482.

15. Rifai, N. & Warnick, G. R., eds. (1994) Laboratory Measurement of Lipids, Lipoproteins and apoproteins. AACC Press, Washington, DC.
16. Beckman-Coulter (1997) Synchron Delta CX7 Technical Procedure Manual. Brea, CA.
17. Friedewald, W. T., Levy, R. I. & Frederickson, D. S. (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma without the use of the centrifuge. Clin. Chem. 18: 449–502.
18. Reed, A. M., Haugen, M., Pachman, L. M. & Langman, C. B. (1993) Repair of osteopenia in children with chronic rheumatic disease. J. Pediatr. 122: 693–696.
19. Stacewicz-Sapuntzakis, M., Bowen, P., Kikendall, J &. Burgess M. (1987) Simultaneous determination of serum retinol and various carotenoids: their distribution in middle-aged men and women. J. Micronutr. Anal. 3: 27–45.
20. Jones, B. (1989) Feline hyperlipidemia. In: Textbook of Veterinary Internal Medicine. Diseases of the Dog and Cat (Ettinger, S. J., ed.), 3rd ed., pp. 198–202. W. B. Saunders Company, Philadelphia, PA.
21. Backues, K. A., Hoover, J. P., Bauer, J. E., Campbell, G. A. & Barrie, M. T. (1997) Hyperlipidemia in four related male cheetahs (*Acinonyx jubatus*). J. Zoo Wildl. Med. 28: 476–480.
22. Demacker, P.N.M., van Heijst, P. J., Hak-Lemmers, H.L.M. & Stalenhoef, A. F. H. (1987) A study of the lipid transport system in the cat, (*Felix domesticus*). Atherosclerosis 66: 113–123.
23. Bauer, J. E. (1992) Diet-induced alterations of lipoprotein metabolism. J. Am. Vet. Med. Assoc. 201: 1691–1694.
24. Dimski, D. S., Buffington, C. A., Johnson, S. E., Rosol, T. J. & Sherding, R. D. (1992) Serum lipoprotein concentrations and hepatic lesions in obese cats undergoing weight loss. Am. J. Vet. Res. 53: 1259–1262.
25. Morris J. G., Earle, K. E. & Anderson, P. A. (1999) Plasma 25-hydroxyvitamin D in growing kittens is related to dietary intake of cholecalciferol. J. Nutr. 129: 909–912.
26. Puls, R. (1994) Vitamin levels in animal health. In: Health: Diagnostic Data and Bibliographies (Puls, R., ed.), p. 87. Sherpa International, Clearbrook, British Columbia, Canada.
27. Holick, M. (1999) Vitamin D. In: Modern Nutrition in Health and Disease (Shils, M. E., Olson, J. A., Shike, M. & Ross, A. C., eds.), 9th ed. pp. 329–345. Williams and Wilkins, Baltimore, MD.
28. How, K. L., Hazewinkel, A. W. & Mol, J. A. (1994) Dietary vitamin D dependence of cat and dog due to inadequate cutaneous synthesis of vitamin D. Gen. Comp. Endocrinol. 96: 12–18.
29. Ullrey, D. E. & Bernard, J. B. (1999) Vitamin D: metabolism, sources, unique problems in zoo animals needs. In: Zoo and Wildlife Medicine: Current Therapy 4 (Fowler, M. E. & Miller, R. E., eds.), pp. 63–78. W. B. Saunders, Philadelphia, PA.
30. Gallo-Torres, H. E. (1980) Transportation and metabolism. In: Vitamin E: A Comprehensive Treatise (Machlin, L. J. ed.), pp. 193–267. Marcel Dekker, New York, NY.
31. Olson, J. A. (1996) Vitamin A. In: Present Knowledge in Nutrition (Zeigler, E. E. & Filer, L. J., eds.), 7th ed., pp. 109–119. ISLI Press, Washington, DC.
32. Sokol, R. J. (1996) Vitamin E. In: Present Knowledge in Nutrition (Zeigler, E. E. & Filer, L. J., eds.) 7th ed., pp. 130–135. ISLI Press, Washington, DC.
33. Willett, W. C., Stampfer M. J., Underwood, B. A., Taylor, J. O. & Hennekens, C. H. (1983) Vitamins A, E, and carotene: effects of supplementation on their plasma levels. Am. J. Clin. Nutr. 38: 559–566.
34. Brush, P. J. & Anderson, P. H. (1986) Levels of plasma alpha-tocopherol in zoo animals. Int. Zoo Yearb. 24/25: 316–321.
35. Dierenfeld, E. S. (1994) Vitamin E in exotics: effects, evaluation and ecology. J. Nutr. 124: 2579S–2581S.
36. Traber, M. G. (1999) Vitamin E. In: Modern Nutrition in Health and Disease (Shils, M. E., Olson, J. A., Shike, M., & Ross, A. C., eds.), 9th ed., pp. 347–362. Williams and Wilkins, Baltimore, MD.
37. McDowell, L. R. (1989) Vitamins in Animal Nutrition (Cunha, T. J., ed.), pp. 93–131. Academic Press, San Diego, CA.
38. Fowler, M. E. (1999) Felidae. In: Zoo and Wild Animal Medicine (Fowler, M. E. & Miller, R. E., eds.), pp. 831–841. W. B. Saunders, Philadelphia, PA.
39. Currier, M. & Russell, K. (1982) Hematology and blood chemistry of the mountain lion (*Felis concolor*). J. Wildl. Dis. 18: 99–104.
40. Dunbar, M. R., Nol, P. & Linda, S. B. (1997) Hematologic and serum biochemical reference intervals for Florida panthers. J. Wildl. Dis. 33: 783–789.
41. Fox, P. R., Trautwein, E. A., Hayes, K. C., Bond, B. R., Sisson, D. D. & Moise, N. S. (1993) Comparison of taurine, α-tocopherol, selenium, and total triglycerides and cholesterol concentrations in cats with cardiac disease and in healthy cats. J. Vet. Res. 54: 563–569.
42. Ghebremeskel, K., & Williams, G. (1988) Plasma retinol and alpha-tocopherol levels in captive wild animals. Comp. Biochem. Physiol. 89B: 279–283.
43. Crissey, S. D., Maslanka, M. & Ullrey, D. E. (1999) Assessment of nutritional status of captive and free-ranging animals. In: Nutrition Advisory Group Handbook Fact Sheet 8, American Zoo and Aquarium Association (Baer, D. J. & Baer, C. K., eds.). Brookfield, IL.
44. Baker, H., Schor, S. M., Murphy, B. D., DeAngelis, B., Feingold, S. & Frank, O. (1986) Blood vitamin and choline concentrations in healthy domestic cats, dogs, and horses. Am. J. Vet. Res. 47: 1468–1471.
45. Slifka, K. (1994) A Survey of Carotenoids, Retinols and Tocopherols in Captive Wild Animals. Master's thesis, University of Illinois, Chicago.