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VITAMIN A CONCENTRATIONS IN SERUM AND LIVER FROM FLORIDA PANTHERS

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ABSTRACT: Many of the anomalies and clinical signs afflicting the Florida panther (*Felis concolor coryi*) are suggestive of vitamin A deficiency. Our objectives in this study were to determine if a vitamin A deficiency exists in the free-ranging panther population and to determine if there are differences in vitamin A levels among various subgroups of free-ranging panthers. Retinol concentrations were used as an index to Vitamin A concentrations and were determined in serum and liver from free-ranging (serum, $n = 45$; liver, $n = 22$) and captive (serum, $n = 9$; liver, $n = 2$) juvenile and adult Florida panthers from southern peninsular Florida (USA), and in liver from free-ranging cougars (*F. concolor* subspp.) from Washington (USA) and Texas (USA) between November 1984 and March 1994. Combined juvenile (6- to 24-mo-old) and adult (>24-mo-old) free-ranging Florida panthers had mean ±SD serum retinol concentrations of 772.5 ± 229 pmol/ml. Adult free-ranging Florida panthers had mean liver retinol concentrations of 4794.5 ± 3747 nmol/g. Free-ranging nursing Florida panther kittens (age <1 mo) had mean serum retinol concentrations of 397.9 ± 69 pmol/ml. Among subgroups of free-ranging Florida panthers, females had higher corrected mean serum retinol concentrations than males and adult free-ranging Florida panthers had higher mean liver retinol concentrations than juveniles. Retinol concentrations in free-ranging Florida panthers did not differ significantly from those in captive panthers (liver and serum) or other free-ranging cougars (liver). Based on limited published values and our controls, a vitamin A deficiency could not be demonstrated in the Florida panther population nor were any subgroups or individuals considered deficient.

Key words: Cougar, endangered species, *Felis concolor coryi,* Florida panther, nutrition, retinol, vitamin A.

INTRODUCTION

The Florida panther (*Felis concolor coryi*) is an endangered subspecies of cougar with only 30 to 50 adults inhabiting the Big Cypress Swamp and Everglades ecosystems of southern Florida (USA) (Belden, 1986). This isolated population exhibits several congenital anomalies that were speculated to be the result of inbreeding (Roelke et al., 1993). Many of the clinical signs and congenital anomalies found in the Florida panther, including cardiac defects, impaired immune system, low sperm volume, cryptorchidism, poor hair coat, and low reproductive success (Roelke et al., 1993; Barone et al., 1994), have been reported in vitamin A deficient felids and laboratory animals (Wilson et al., 1953; Gershoff et al., 1957; Heywood, 1967; Krishman et al., 1974; Scott, 1975; Wallach and Hoff, 1982). Wallach and Hoff (1982: 140) state, "avitaminosis A produced defects are often attributed to inbreeding." Other researchers have suggested that an estrogenic compound toxicosis may be responsible for these anomalies (Facemire et al., 1995). The Florida panther may have elevated serum levels of estrogenic compounds (Dunbar, 1994; Facemire et al., 1995). Some estrogenic compounds have been shown to disrupt vitamin A storage and metabolism in laboratory animals (Innami et al., 1974; Chen et al., 1992).

The Florida panther is an obligate carnivore depending almost entirely on its prey, primarily white-tailed deer (*Odocoileus virginianus*) and feral swine (*Sus scrofa*) (Maehr et al., 1990), for its nutrition. Deer in some areas of the panthers range have low to negligible liver vitamin A concentrations (Cunningham, 1996). Deficiencies in prey may be reflected in the panther.

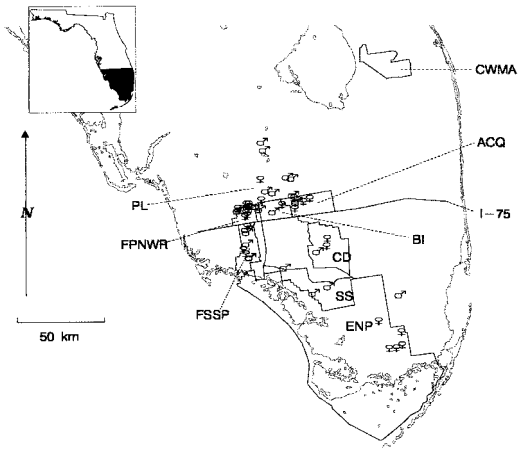

FIGURE 1. Locations of Florida panther captures for serum collection in southern Florida, 1984–94.

Felids have a high vitamin A requirement compared to other species (Scott, 1960) and deficiencies frequently were encountered in captive cats (Heywood, 1967). However, little is known about vitamin A levels in free-ranging felids (Anderson, 1983). Several studies have examined vitamin A levels in free-ranging ungulates (Anderson et al., 1972; Kelley et al., 1987), however; this is the first known examination of vitamin A concentrations in a free-ranging felid. The objectives of this study were to determine if a vitamin A deficiency exists in the free-ranging panther population and to determine if there are differences in vitamin A levels among subgroups of free-ranging panthers. Without a complete understanding of all factors that may be involved in the expression of congenital anomalies in the Florida panther, management strategies designed to reduce their occurrence may be ineffective.

METHODS AND MATERIALS

The study area included public and private land in Florida south of Lake Okeechobee (25°20′ to 27°00′N, 80°20′ to 81°40′W) in Broward, Collier, Dade, Hendry, Lee, Monroe, and Palm Beach counties (Fig. 1). This region is biotically diverse with the predominant biome being tropical savannah (Hela, 1952); however, some portions of the study area are experiencing rapid human population growth and agricultural development.

The study area (Fig. 1) is divided north and south by Interstate 75 (I-75) (roughly 26°11′N). Lands north of I-75 include recently acquired public land (ACQ) and the Bear Island Unit (BI) [both part of the Big Cypress National Preserve (BCNP)], Corbett Wildlife Management Area (CWMA), the Florida Panther National Wildlife Refuge (FPNWR), and private ranches/farms (PL). Lands south of I-75 include the Corn Dance (CD) and Stair Steps (SS) Units of BCNP, the Fakahatchee Strand State Preserve (FSSP), and Everglades National Park (ENP). These lands are contiguous with other public and private lands within panther range.

Free-ranging panthers were captured using techniques described by Maehr et al. (1991) and McCown et al. (1990), and immobilized using drug combinations and dosages described by Roelke (1990). Locations for individuals were based on home range prior to collection (radio telemetry data) and not necessarily on capture/collection location. Panthers in this study frequently crossed study boundaries. Adult and juvenile free-ranging panthers were captured in ENP ($n = 6$); BCNP including ACQ ($n = 8$), BI ($n = 5$), CD ($n = 3$), and SS ($n = 3$); FPNWR ($n = 6$); FSSP ($n = 8$); and PL ($n = 10$) (Fig. 1). Some panthers were captured on more than one occasion in the same location ($n = 1$) or different locations ($n = 3$). The two free-ranging neonatal kittens were siblings captured in CD. Blood samples were collected between November 1984 and March 1994 from adult (>24-mo-old) and juvenile (6- to 24-mo-old) free-ranging [22 female (F), 23 male (M)] and captive (6F, 3M) Florida panthers. Blood was also collected from two neonates (0 to <4-wk-old; 1M, 1F) when free-ranging and again in captivity; a third neonate (F) was sampled only in captivity.

All captive panthers were wild-born and either hand-caught as nursing kittens ($n = 3$) or captured as juveniles ($n = 3$) or adults ($n = 4$). Two panthers captured as neonates were also sampled as adults. Captive panthers were held in pens at three separate locations in Florida—Jacksonville Zoological Gardens (Jacksonville), Lowry Park Zoological Garden (Tampa), and White Oak Plantation (Yulee). Captive panther kittens <4-wk-old were fed a milk replacer (KMR®, Pet Ag, Inc., Hampshire, Illinois, USA), and weaned on a mixture of milk replacer and a moist feline diet (Nebraska Feline Brand®, Central Nebraska Packing, Inc., North Platte, Nebraska, USA). Juvenile and adult panthers were fed the moist feline diet. Domestic cats require at least 6,000 IU retinol/kg diet (dry wt.) for proper growth, pregnancy, and lactation (National Research Council,

1986); Nebraska Feline Brand contains in excess of 10,230 IU/kg diet (dry wt.). Mean ± SD duration of captivity for juveniles and adults prior to sample collection was 19.8 ± 23 mo for serum samples, and 15 ± 3 mo for the two liver samples. Serum from kittens was collected after 2 wk in captivity. Some adult and juvenile panthers were sampled (serum) as both captive and free-ranging in one or more locations including FSSP (n = 1), ENP (n = 2), CD (n = 1), SS (n = 1), FPNWR (n = 1), and ACQ (n = 2); one captive panther was also sampled twice while free-ranging, 2 yr apart in CD and ENP.

Blood was collected into serum separator tubes from free-ranging and captive panthers by venipuncture of the cephalic, medial saphenous, or jugular veins and allowed to clot. Blood tubes were kept on ice (1–6 hr) then centrifuged at 2000 rpm for approximately 10 min. The serum was stored in plastic vials at ≤ −20 C for between 1 mo and 10 yr before analysis. Blood from free-ranging panthers was collected predominantly between January and May of each year.

Liver was collected at necropsy from 22 juvenile and adult free-ranging (17M, 5F) and two juvenile captive female Florida panthers during this study period and frozen at ≤ −20 C for between 3 mo and 10 yr until analyzed. There were no liver samples from kittens for comparison. Necropsied free-ranging panthers were recovered from ENP (n = 3), FPNWR (n = 1), FSSP (n = 4), PL (n = 13), and CWMA (n = 1). Samples were collected from parenchyma of different lobes and condition ranged from fresh to moderately autolyzed. Necropsied panthers include those dying from vehicular collision (n = 9), intraspecific aggression (n = 3), cardiac defects (n = 2), acute viral disease (n = 2), mercury toxicosis (n = 1), or unknown causes (n = 5). All serum and liver samples collected were obtained from animals believed to be free of any chronic disease or condition that could have affected vitamin A levels. Necropsied female panthers were neither pregnant nor nursing. Deaths occurred during all seasons of the year. Some panthers were sampled while living (serum) and again at necropsy (liver) (12 free-ranging, 2 captive).

Liver samples were also collected from one juvenile and two adult cougars (*F. concolor stanlyana*) from Texas (USA) and three hunter-killed adult female cougars (*F. concolor oregonensis*) from Washington (USA). Exact ages for these animals were unknown. These cougars were considered healthy prior to injury/death. One cougar from Texas died from unknown trauma and two from illegal gunshot while free-ranging in north Florida (30°10′ to 31°10′N, 82°10′ to 82°40′W). Cougars from Texas were part of a Florida panther reintroduction feasibility study (Belden and Hagedorn, 1993). Liver samples were stored similarly to those from Florida panthers and storage time ranged from 2 mo to 6 yr.

Serum and liver samples were analyzed for vitamin A (retinol and retinyl esters) at the Animal Health Diagnostic Laboratory (Michigan State University, East Lansing, Michigan, USA) by normal phase isochromatic high speed liquid chromatography (HPLC) as described by Dennison and Kirk (1979) and Stowe (1982). Values for retinyl esters are not included in this report due to inaccuracies in the data. Therefore, concentrations of retinol were used as an indicator of vitamin A concentrations. Liver values are reported as dry weight. Comstock et al. (1993) reported retinol to be stable for >15 yr when stored at ≤ −20 C.

Panther age was based on known birth dates or estimated by tooth wear and facial, body, and pelage characteristics (Shaw, 1983). Ages ranged from 2 wk to >12 yr and were grouped into three classes of (1) nursing kitten (<1 mo.), (2) juvenile (6 to 24 mos.), and (3) adult (>24 mos.). Age class 1 was not included in statistical analysis. Genotype was based on analysis of mitochondrial DNA and nuclear markers. Two genotypes were delineated and used for comparison: authentic Florida panthers and panther intergrades (O'Brien et al., 1990).

Retinol concentrations in panthers from areas with deer having low liver retinol concentrations (PL), were compared to values from the remaining study area. Private lands (PL) included Collier Enterprise Land (approximately 26°21′N to 26°24′N, 81°16′W to 81°20′W location) where retinol depleted deer were collected. Comparisons also were made between panthers in the Big Cypress Swamp (PL, BI, FPNWR, FSSP, ACQ, CD, and SS) and Everglades (ENP) ecosystems.

Concentration values were transformed to logarithms prior to statistical analysis. All computations were performed using the SAS System (SAS Institute, Inc., 1990). Retinol concentrations in serum and liver from free-ranging Florida panthers were examined using analysis of variance (ANOVA) for effects of sex (male, female), age (juvenile, adult), genotype (authentic, intergrades), deer vitamin A status (normal, low), and ecosystem (Everglades, Big Cypress Swamp). Analysis was problematic because of the sparseness of the data, so an iterative model-fitting procedure was followed. In the first iteration, an initial model was fitted containing all main effect terms and as many two-way interaction terms as could be supported by the data. In each subsequent iteration, the term with the highest P-value according to

TABLE 1. Mean, standard deviation, and range of serum retinol (pmol/ml) in free-ranging and captive Florida panthers collected from southern Florida in 1984–94.

Source	n[a]	Serum retinol		
		Mean	SD[b]	Range
Free-ranging Florida panther				
adult and juvenile[c]	45	772.5	229	342.1–1,256.5
nursing kitten[d]	2[e]	397.9	69	349.0–446.8
Captive Florida panther				
adult and juvenile	9	914.1	215	666.7–1,308.9
nursing kitten[f]	3	636.4	449	324.6–1,151.8

[a] Sample size.
[b] Standard deviation.
[c] >6 mo to >12 yr.
[d] <4 wk.
[e] 1 male, 1 female (siblings).
[f] Same 2 kittens as listed for free-ranging and 1 other female after 2 wk in captivity.

a Type IV hypothesis test was deleted, consistent with maintaining a hierarchical model, and the reduced model refitted. Iteration continued until all terms in the model were significant at the $\alpha = 0.05$ level (SAS Institute, Inc., 1990).

ANOVA was used to make comparisons of serum retinol concentrations between free-ranging and captive Florida panthers. The ANOVA model contained terms for status (captive or free-ranging), and as many factors found to significantly affect serum retinol concentrations in free-ranging panthers and interactions of status with those factors, as could be supported by the data. Comparisons of liver retinol concentrations between free-ranging and captive Florida panthers, and between free-ranging cougars and Florida panthers, were similarly performed using ANOVA, with the exception that if the model contained only terms for status, then a t-test was performed.

Because only age class was found to affect liver retinol concentrations in the ANOVA (see Results), a nonlinear regression approach was adopted in order to further explore liver retinol accumulation. Inspection of scatterplots of log (concentration) versus age suggested that liver retinol accumulation with age could be approximated by a two-phase linear model. In the first phase, log (concentration) increased approximately linearly until a critical age was reached, and in the second phase log (concentration) was either constant or changed linearly with age at a different rate. Therefore, the following parametrization of the two-phase regression model was fitted to the data:

$$\log(\text{concentration}) = \begin{cases} \beta_0 + \beta_1 m + \epsilon & \text{for } m \le t \\ \beta_0 + \beta_1 t + \beta_2 (m - t) + \epsilon & \text{for } m > t, \end{cases} \quad (1)$$

where m = age in months, t = age at which the phase change occurred, β_0 = intercept term, β_1 = slope in the first phase, β_2 = slope in the second phase, and ϵ = experimental error. If the slope in the second phase was not significantly different from 0, then the model was reduced to the linear-plateau model, and refitted. The linear-plateau model was parametrized as (1) with $\beta_2 = 0$, i.e.

$$\log(\text{concentration}) = \begin{cases} \beta_0 + \beta_1 m + \epsilon & \text{for } m \le t \\ \beta_0 + \beta_1 t + \epsilon & \text{for } m > t. \end{cases} \quad (2)$$

Using this parametrization, the log (concentration) value at plateau was $k = \beta_0 + \beta_1 t$. Models (1) and (2) were fitted using ordinary least squares non-linear regression, as implemented in PROC NLIN in the SAS System (SAS Institute, Inc., 1990). For model (2), the plateau was estimated as $\hat{k} = \beta_0 + \beta_1 \hat{t}$, with variance estimated by the delta method (Agresti, 1984). R^2 values were computed as the proportional reduction in the residual sum of squares relative to the mean model.

RESULTS

Mean ± standard deviation (SD) and ranges for serum and liver retinol concentrations for free-ranging Florida panthers are shown in Table 1 and 2, respectively. In free-ranging Florida panthers, differences in serum vitamin A concentrations depended on gender only. Females had higher corrected mean ± SE serum retinol concentrations (856.0 ± 51 pmol/ml; $P = 0.0285$) than males (692.5 ± 39.7 pmol/ml respec-

TABLE 2. Mean, standard deviation, and range of dry weight liver retinol (nmol/g) in free-ranging and captive Florida panthers and free-ranging western cougars collected 1984–94.

Source	n[a]	Retinol		
		Mean	SD[b]	Range
Free-ranging Florida panther				
adult[c]	16	4,794.5	3,747	589.9–12,041.9
juvenile[d]	6	1,289.1	1,140	101.2–2,991.3
Captive Florida panther				
juvenile	2	3,595.1	49	3,560.2–3,630.0
Western cougar[e]				
adult	5	2,399.3	1,027	1,225.1–3,298.4
juvenile	1	2,855.1		

[a] Sample size.
[b] Standard deviation.
[c] >2 yr.
[d] >6 mo, ≤2 yr.
[e] Cougars from Texas (n = 2 adults, 1 juvenile), cougars from Washington (n = 3 adults).

tively). Adult free-ranging Florida panthers had higher liver retinol than juveniles (P = 0.009) (Table 2). Levels plateaued at about 30-mo-old (Fig. 2). Free-ranging nursing Florida panther kittens, both from CD, had mean serum retinol values (Table 1) similar to those of the same kittens and 1 other sampled after 2 wk in captivity. Retinol concentrations in liver and serum in free-ranging Florida panthers did not differ significantly from those in captive panthers or liver retinol concentrations in other free-ranging cougars. No differences in vitamin A concentrations were found between genotypes, between areas with low, and normal vitamin A concentrations in deer, or between ecosystems.

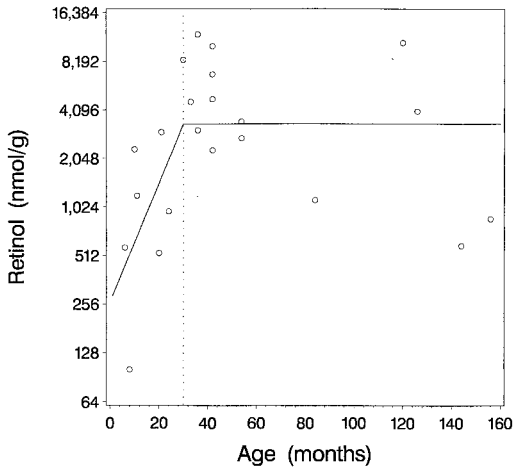

FIGURE 2. Fitted linear plateau model of log (liver retinol concentration) versus age for free-ranging Florida panthers collected in southern Florida during 1984–94.

DISCUSSION

Liver vitamin A concentrations are considered the most reliable indicator of vitamin A status (Schweigert et al., 1990), and concentrations in adult free-ranging Florida panthers were comparable to those in other free-ranging *F. concolor* subspps. However, adequate vitamin A stores do not guarantee proper vitamin A transport or utilization. Protein-calorie malnutrition (Smith et al., 1973) and estrogenic compound toxicity can disrupt vitamin A metabolism. Nevertheless, a vitamin A deficiency was not apparent for any segment of the Florida panther population including any individual.

Captive panthers were on a nutritionally balanced commercial diet and, thus, are assumed to have received adequate dietary vitamin A. However, it should be noted that differences between free-ranging and captive animals are not restricted to diet. Disparity in activity level, stress, disease, and other factors may affect vitamin A

concentrations. Liver retinol in captive Florida panthers is represented solely by two juveniles. In the only known publication of serum vitamin A levels in cougars, Schweigert et al. (1990) reported a retinol value from a captive cougar that was comparable to our findings. Other factors possibly affecting results include location of sample collection within the liver and degree of autolysis.

The increase in liver retinol with age in panthers is similar to published reports for domestic cats (Moore et al., 1963), although we found no mention of an age plateau for any species.

Mean serum retinol for free-ranging panthers resembled published values for domestic house cats (Schweigert et al., 1990; Fox et al., 1993). The physiological significance of higher serum retinol in female panthers, if any, is unknown. Pregnancy in free-ranging panthers ($n = 5$) apparently had no effect on circulating retinol levels (sampled between days 10 and 45 of a 92 day gestation period). Free-ranging nursing Florida panther kittens from CD had lower serum retinol concentrations than juvenile and adult panthers. This is probably normal although we found no published serum vitamin A values for felidae kittens for comparison. These same kittens had similar serum vitamin A values after 2 wk in captivity nursed on KMR®. Vitamin A status of panther kittens from other regions is unknown.

The establishment of baseline values of physiological parameters, including vitamin A concentrations, is requisite to the understanding of the chronic effects of toxicity and deficiency in free-ranging wildlife, and especially true in endangered species. The establishment of baseline vitamin A concentrations in the Florida panther should assist researchers and managers in understanding the complex of environmental variables affecting the endangered Florida panther.

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