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Circulating Levels of α-Tocopherol and Retinol in Free-Ranging African Elephants (*Loxodonta africana*)

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To date, there are no detailed reports of circulating levels of plasma α-tocopherol and retinol for large samples of free-ranging African elephants (*Loxodonta africana*). This survey study measured natural circulating levels of α-tocopherol as a measure of vitamin E activity and retinol as an indicator of vitamin A activity, in 70 free-ranging African elephants captured at Kruger National Park as part of a translocation program. Mean levels of α-tocopherol and retinol were found to be 0.613 ± 0.271 μg/mL and 0.039 ± 0.007 μg/mL, respectively, and did not vary significantly across sex or age class. Elephants appear to normally have low circulating levels of both these nutrients compared with domestic herbivore species; values from healthy, free-ranging elephant populations may provide useful data for assessing nutrient status of captive animals. Zoo Biol 18:319–323, 1999.

Key words: fat-soluble vitamins; African elephant (*Loxodonta africana*); physiological status; nutrition

INTRODUCTION

Developing appropriate diets for captive animals can be a challenging task. Captive elephant nutrition has received intense investigation in recent years in an attempt to alleviate many of the diseases associated with vitamin deficiencies [Papas et al., 1991; Dierenfeld, 1994; Sadler et al., 1994]. Vitamin E deficiency is known to

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cause disorders of the reproductive, muscular, circulatory, and nervous systems in many species and has been implicated in captive elephant deaths [Dierenfeld and Dolensek, 1988; Dierenfeld, 1994], although more recent data suggest it may be a contributory rather than the primary factor [R. Montali, pers. comm.]. It has also been suggested that vitamin E deficiency is common among captive elephants [Papas et al., 1991]. Vitamin A deficiency has been less well studied and has not been reported as common in captive elephants. However, normal levels of vitamin A intake required by healthy populations of African elephants have yet to be well established.

Recommended concentrations of vitamin E in diets of captive African elephants (*Loxodonta africana*) have been largely based on studies of captive populations with few comparative values from free-ranging animals [Dierenfeld, 1994; Dierenfeld et al., 1998; Papas et al., 1991]. Earlier studies examining circulating levels of α-tocopherol conducted on small samples of both African (*Loxodonta africana*) and Asian (*Elephas maximus*) elephants in captivity at a number of institutions found that captive elephants, in general, exhibited lower levels of α-tocopherol than their free-ranging counterparts [Papas et al., 1991; Dierenfeld and Traber, 1992; Dierenfeld, 1994; Sadler et al., 1994]. Changes to the captive diet ensued and dietary supplementation with vitamin E was recommended. Various forms of dietary vitamin E have been found effective for increasing circulating levels of this nutrient in elephants [Papas et al., 1991; Sadler et al., 1994; Wallace et al., 1992].

Recommended levels of pre-formed vitamin A in captive African elephant diets are based on fewer studies, making vitamin A status more difficult to assess. Retinol is presumed to be the principal form of the vitamin A–active substances present in blood and has generally been used as an indicator of vitamin A deficiency [Vuilleumier et al., 1983; Shrestha et al., 1998]. Plasma retinol levels have been shown to differ for captive Asian and African elephants, but comparative levels from free-ranging populations have not been adequately documented [Dierenfeld, 1994].

Although a few small sample summaries have been published [Dierenfeld and Traber, 1992; Dierenfeld et al., 1998], to date there are no detailed reports of circulating levels of plasma α-tocopherol and retinol for large samples of free-ranging African elephants (*Loxodonta africana*). This survey study examined natural circulating levels of α-tocopherol as a measure of vitamin E activity, and retinol as an indicator of vitamin A activity, in healthy free-ranging African elephants in Kruger National Park in South Africa. These data may be useful for examining efficacy of dietary supplementation and assessing nutrient status of captive African elephants.

METHODS

Circulating levels of plasma α-tocopherol and retinol were measured from 70 free-ranging individuals captured as part of a translocation program at Kruger National Park in South Africa. A detailed list of subjects by age and sex class is found in Table 1. Age classes (adults >12 years, young adults 6–12 years, and calves) were determined by size, weight, and physical maturity. Normally, all elephant cows are lactating; only one adult female was noted to be dry.

Sample Collection

During routine capture operations, venous blood samples were collected from elephants into two 10-mL heparinized Vacutainer (Becton Dickinson VACUTAINER

TABLE 1. Age and sex classes of wild African elephants sampled at Kruger National Park (April–June 1997)

Sex	Adult	Young adult	Calf
Female	44	9	2
Male	2	7	3
Unknown			3

Systems, Franklin Lakes, NJ) tubes using 20-gauge needles. The specimens were kept cool and were centrifuged within 4 h after collection; plasma was separated into 1.8-mL cryotubes, and stored at –20°C. Specimens were kept frozen while in transit to Roche Laboratories, Basel, Switzerland.

Analyses

Circulating levels of plasma α-tocopherol and retinol were determined using high-performance liquid chromatography (HPLC) methods developed by Vuilleumier et al. [1983]. Briefly, plasma proteins were precipitated with ethanol, and α-tocopherol and retinol were extracted from the aqueous suspension with n-hexane. After centrifugation, an aliquot of the organic phase was chromatographed isocratically (n-hexane:isopropanol 97:3) on a silicon oxide column. Separate HPLC lines are used for each of the analysis parameters; α-tocopherol was quantified fluorimetrically (excitation at 298 nm, emission at 318 nm) using internal standard preparations of crystalline purity from Hoffmann-LaRoche & Co. Ltd. (Basel, Switzerland), whereas retinol was detected by ultraviolet (UV)-absorption at a 325-nm wavelength. To maintain quality of analytical results, the laboratory performance was monitored by participation in both internal and external quality assurance programs (QAP) (NIST/NCI Micronutrient Measurement QAP, Gaithersburg, MD, and St. Heliers, Surrey, UK).

Statistical differences between sexes were evaluated using unpaired Student's *t*-tests, whereas analysis of variance was used to examine differences among age classes.

RESULTS

Mean plasma α-tocopherol and retinol values for each group are shown in Table 2. No significant differences in α-tocopherol concentrations among age categories

TABLE 2. Circulating α-tocopherol (vitamin E) and retinol (vitamin A) concentrations (μg/mL) for various age and sex classes of free-ranging African elephants sampled at Kruger National Park (April–June 1997)

		α-Tocopherol		Retinol	
	n	Mean ± standard deviation	Range	Mean ± standard deviation	Range
Age					
Adult	46	0.63 ± 0.30	0.3–1.7	0.040 ± 0.006	0.03–0.05
Young adult	16	0.55 ± 0.13	0.4–0.8	0.039 ± 0.008	0.02–0.05
Calf	8	0.64 ± 0.34	0.3–1.4	0.036 ± 0.007	0.03–0.05
Total group	70	0.61 ± 0.27	0.3–1.7	0.039 ± 0.007	0.02–0.05
Sex					
Young female	9	0.58 ± 0.15	0.4–0.8	0.041 ± 0.007	0.03–0.05
Young male	7	0.51 ± 0.09	0.4–0.7	0.036 ± 0.010	0.02–0.05

were observed [$F(2,67) = 0.552$, $P \geq 0.05$], nor were there any significant differences between α-tocopherol levels in young females and young males [$t(14) = 0.996$, $P \geq 0.05$]. There were also no significant differences among retinol concentrations in adults, young adults, and calves [$F(2,67) = 1.29$, $P \geq 0.05$], or in retinol levels between young females and young males [$t(14) = 1.23$, $P \geq 0.05$].

DISCUSSION

Although comparative data for semi-free ranging Asian elephants were recently published [Shrestha et al., 1998], limited data on plasma α-tocopherol and retinol concentrations in free-ranging African elephants make it difficult to interpret vitamin status in captive individuals with any degree of certainty. Further, suggested differences in circulating concentrations of these nutrients among elephant species [Sadler et al., 1994], based solely on captive animal information, have not been substantiated.

Mean vitamin E levels for small samples of free-ranging African elephants were previously reported to range from 0.5 to 0.8 μg/mL [Papas et al., 1991; Dierenfeld and Traber, 1992; Dierenfeld et al., 1998]. Figures from this study fell within the low end of this range. It was suggested that seasonal variations associated with location, vegetation, and other factors could affect vitamin E levels [Dierenfeld and Traber, 1992]. Given that all samples in this study were taken during the dry season, available food choices due to seasonality could have caused this result. Future studies may investigate causes behind the reported variation in range of vitamin E levels for free-ranging populations in more detail.

Previous studies of captive populations showed that dietary supplementation produces levels of α-tocopherol similar to those found in this survey population [Sadler et al., 1994]. If further investigation shows seasonality of plasma α-tocopherol levels in free-ranging animals, additional studies will be needed to determine whether it is appropriate to supplement captive diets only to the level of dry season forage. Overall, results of this study indicate that acceptable levels of circulating α-tocopherol based on free-ranging populations need to be more firmly established.

Plasma retinol values for captive elephants (for African elephants: $X = 0.15 \pm 0.14$ μg/mL; range, 0.05–0.48 μg/mL; for Asian elephants: $X = 0.063 \pm 0.003$ μg/mL; range, 0.01–0.12 μg/mL) [Dierenfeld, 1994; Dierenfeld et al., 1998; Shrestha et al., 1998] compare favorably to values reported here, although variability appears greater in captive animals. It was suggested that plasma retinol may not accurately reflect current intake of vitamin A–active compounds due to the large stores of vitamin A in the liver, as well as a host of external factors that induce fat-soluble vitamin antagonisms, such as stress or disease [Dierenfeld et al., 1998; Gibson, 1990; Shrestha et al., 1998]. However, since alternative methods to provide a more detailed interpretation of vitamin A status have not been developed, even for domestic herbivores, establishing baseline plasma retinol levels in free-ranging animals can serve as initial indicators in determining a normal range for healthy animals [Dierenfeld et al., 1998].

Circulating α-tocopherol and retinol concentrations in both free-ranging and zoo-held elephants were reported to average approximately one fourth the value seen in domestic herbivore species, indicating that although the horse appears to be a good physiological model for exotic equids, it is not an appropriate model for assessing vitamin E or retinol status in elephants [Dierenfeld et al., 1998]. The low levels of α-tocopherol and retinol seen in this study further support this statement.

Although the gut anatomy of horses and elephants is similar, storage of vitamin E may differ due to the habitats in which each species evolved [Dierenfeld and Traber, 1992]. For a temperate-evolved species like the horse, storing nutrients in fat for later retrieval in time of need would be ecologically advantageous. For the tropically evolved elephant, body fat would be detrimental to heat exchange, making storage of nutrients in fat physiologically disadvantageous. The disparity in nutrient concentrations between the two species does not appear to be due to a difference in plasma lipids or lipoproteins [Dierenfeld and Traber, 1992].

Since circulating levels of both α-tocopherol and retinol appear lower in elephants than other domestic herbivore species, establishing baseline levels based on healthy, free-ranging elephant populations may aid in developing dietary recommendations for captive animals.

CONCLUSIONS

1. Sex and age did not affect circulating levels of α-tocopherol (vitamin E) and retinol (vitamin A) in free-ranging African elephants sampled from the same population.

2. Values for circulating levels of α- tocopherol (vitamin E) and retinol (vitamin A) from healthy, free-ranging populations may provide an important measure for examining efficacy of dietary supplementation and assessing nutrient status of captive animals.

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