

Summer and Winter Seasonal Changes in Vitamin D Status of Captive Rhinoceros Iguanas (*Cyclura cornuta*)

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ABSTRACT: Four long-term captive adult rhinoceros iguanas (*Cyclura cornuta*) on display at the Dallas Zoo (Dallas, TX) were maintained for several years in an outdoor enclosure in the summer and in a smaller indoor enclosure in the winter. While fully exposed to sunlight and given the opportunity to supplement their diet with live vegetation each summer, they were deprived of the benefits of full sun exposure, including ultraviolet B (UVB) exposure, and they received no dietary vitamin D for several months each winter. Maintained with this protocol, the animals seemed healthy and they reproduced. However, increasing awareness of the important role of UVB in vitamin D-mediated calcium metabolism and the potential health risk of UVB and vitamin D deprivation stimulated us to focus more on seasonal changes in vitamin D status and possible subtle changes in health status throughout winter. From September 2008 to October 2010, an 85% decline in the serum 25-hydroxyvitamin D₃ (calcidiol) concentrations was measured over each of two winters, and a half-life of 69 days was estimated for the second winter. A higher vitamin D status (calcidiol) was restored and maintained when the iguanas were outdoors and exposed themselves to 1–2 J of UVB per day, although they may have enhanced their dietary calcium and vitamin D activity by eating cactus fruit, too. Serum calcium and phosphorus concentrations remained higher than published reference values for *Cyclura* spp. A negative relationship between vitamin D status and voluntary UVB exposure during the summers was discovered, suggesting that the iguanas were regulating, rather than continually maximizing, their vitamin D status at levels lower than those recorded for *Cyclura* in the tropics. Although the iguanas seemed robust physically, had acceptable calcium and phosphorus blood concentrations, and successfully reproduced throughout the 2 yr monitoring period, we do not recommend extended periods of deprivation of UVB and vitamin D supplementation in winter for rock iguanas until minimally sufficient vitamin D status is more rigorously established.

KEY WORDS: rhinoceros iguana, vitamin D status, ultraviolet B exposure, half-life, *Cyclura cornuta*.

INTRODUCTION

For many terrestrial vertebrates, vitamin D₃ is an essential nutrient that regulates calcium uptake and other important metabolic functions (Holick, 1999, 2004, 2007). It can be obtained from the diet or endogenously by exposure of the skin to ultraviolet B radiation (UVB). UVB converts provitamin D₃ (7-dehydrocholesterol) to previtamin D₃, the latter of which is thermally isomerized to vitamin D₃ and enters the circulation. Vitamin D₃ is then converted to 25-hydroxyvitamin D₃ [25(OH)D₃, calcidiol] in the liver, re-enters the circulation, and is converted to the hormone 1, 25-dihydroxyvitamin D₃ (calcitriol) in the kidneys (Holick, 2007). Vitamin D₃ deficiency can lead to bone diseases such as rickets and osteomalacia (Holick, 2007), reproductive failure, and death (Ferguson *et al.*, 1996; Mader, 2006;

Hoby *et al.*, 2010). Calcidiol concentration is considered a measure of vitamin D status (Haddad, 1999; Holick, 2007).

Whether endogenous or dietary sources of vitamin D₃ are most important to reptiles in nature and whether the relative importance of the source differs among species remains poorly understood (Donoghue, 2006; Ferguson *et al.*, 2009; Oonincx *et al.*, 2013). However, both sources can affect vitamin D status in captivity in a variety of reptilian species (Ferguson *et al.*, 1996, 2009; Acierio *et al.*, 2006, 2008; Oonincx *et al.*, 2010; Selleri and Di Girolamo, 2012). Although UVB seems to be more effective than dietary sources in establishing and maintaining a high vitamin D status in some species, such as the bearded dragon (*Pogona vitticeps*) (Oonincx *et al.*, 2010, 2013), successful indoor maintenance and propagation of heliophilic lizards, such as the

chuckwalla (*Sauromalus obesus*), is possible relying only on dietary vitamin D (Montanucci, 1997).

In the past, adequate UVB exposure has proven difficult to provide during the winter for active tropical lizards maintained on display in large indoor enclosures. This is, in part, due to the expense of artificial light sources coupled with their limited effective distance from the light source to the animal, and sometimes unpredictable duration of effective UVB production. Also, the effectiveness of dietary vitamin D supplementation to iguanas has been questioned (Allen *et al.*, 1999; Ward and Dempsey, 2006). With neither effective dietary supplementation of vitamin D nor reliable artificial UVB exposure, a decline of vitamin D status is to be expected. However, providing UVB to large lizards in outdoor enclosures by natural sun exposure during warmer months has proven effective for restoring vitamin D status in Komodo dragons (*Varanus komodoensis*) (Gyimesi and Burns, 2002; Nijboer *et al.*, 2003).

For several years the Dallas Zoo (Dallas, TX) successfully maintained and successfully bred a group of adult rhinoceros iguanas (*Cyclura cornuta*). The group was rotated seasonally between an indoor winter and an outdoor summer facility; artificial UVB and dietary vitamin D were not provided in the winter. From September 2008 to October 2010, we conducted a study to monitor the variation of vitamin D status and related physiological variables (calcium and phosphorus) throughout an annual seasonal cycle to gain preliminary insight into the effects of outdoor UVB exposure and UVB deprivation on these variables. In each year of the study, the iguanas were maintained outdoors for approximately 170 days and indoors for approximately 200 days. Although not a controlled experimental study, our observational findings provide important baseline information to facilitate more controlled future studies of causes of variation of vitamin D status for this species and related health parameters.

MATERIALS AND METHODS

The four adult rhinoceros iguanas (two males, two females) were monitored from September 2008 to October 2010. At the end of the monitoring period, the largest male was approximately 13 yr old and weighed 6.7 kg. The smaller male was 13 yr old and weighed 3.8 kg. The largest female was at least 27 yr old and weighed 2.6 kg. The smallest female was 6 yr old and weighed 2.4 kg. Fertile eggs and hatchlings were produced by the group females during the summers of 2008 and 2009. All but the largest female were captive hatched. The largest female was wild caught in the Dominican Republic.

The outdoor summer enclosure consisted of an 83.6-m² open air semicircular enclosure. The enclosure surrounded a sloping substrate surface that descended into a pit below the viewing area for zoo visitors. The substrate was decorated with several large natural rock outcroppings, a sandy substrate allowing the lizards to create burrows, a shallow stream and pool at the bottom of the pit, and live desert vegetation. Perennial plants included century plants (*Agave* sp.), prickly pear cactus (*Opuntia* sp.), and yucca (*Yucca* sp.). The animals were free ranging and were able to self-regulate their temperature and UVB exposure under these seminatural conditions.

The indoor winter enclosure consisted of a small building with metal mesh partitions inside. The enclosed space for the lizards measured approximately 8.4 m², with confinement walls constructed from 2.5-cm aluminum tubing supporting 1.3-cm metal mesh. The concrete floor of the building served as the floor of the enclosure. The enclosure was furnished with cinder blocks and wood planks to create perches and refuges, along with several branches for climbing. Ambient temperature was maintained at approximately 25°C (77°F), and several 250-W incandescent lamps (medium base clear BR40, Philips, Eindhoven, The Netherlands) suspended from the ceiling provided ample basking opportunities for the lizards. While indoors, lizards received no UVB exposure from natural or artificial sources.

The diet provided to the iguanas consisted exclusively of 70% mixed greens (collards, kale, and romaine), 20% mixed vegetables (carrots, sweet potatoes, and cabbage), and 10% mixed fruits (bananas, grapes, and papayas). The lizards were offered more food than they actually consumed in 1 day. They were fed on Mondays, Wednesdays, and Saturdays. The same diet was offered at all times of the year. Vitamin D content of the *Cyclura* diet was estimated to be zero. Calcium content of the produce was estimated to be 0.73% on a dry matter basis or 0.09% as fed. Phosphorus content of the diet was 0.25 or 0.03% as fed. These values of vitamin D, calcium, and phosphorus were obtained using Zootrition™ software (version 2.6, St. Louis Zoo, St. Louis, MO) (Slifka, personal communication). No additional vitamin or mineral supplementation was provided during the monitoring period; however, the degree to which the iguanas supplemented their diet with natural and planted vegetation when outdoors is unknown. From casual observation of scat, the iguanas seemed to consume cactus pears during the summer. *Opuntia* cacti have been shown to be a source of dietary calcium and possible enhanced vitamin D activity (Ramírez-Moreno *et al.*, 2011; Guerrero-Cervantes *et al.*, 2012; Kang *et al.*, 2012).

In the two outdoor summers of the monitoring period, the natural solar UVB exposure of the iguanas in the outdoor enclosure was monitored as follows. Each iguana was observed for a 2 day period on two occasions during each summer. On the first day of a period, the investigator remained outside of the enclosure to avoid influencing the lizard's behavior or location. Using a photographic map of the enclosure, the location of the iguana was recorded from 0830 h, or from when the iguana emerged from hiding, until 1630 h, or when the iguana entered into a burrow. Observations were recorded every time the animal moved to a new location and stayed there for at least 30 sec, or every 10 min if the animal was stationary. At each observation, time and location of the lizard inside the enclosure were recorded. Visible light intensity, air temperature, and UVB irradiance were also recorded at a fixed control site outside of the enclosure. Visible light intensity (lux) was measured using a type 214 light meter (General Electric, Cleveland, OH) with the sensor positioned horizontally in full sun. Air temperature was measured using a handheld digital thermometer (Humidity Temperature Meter THWD-2, AMPROBE, Everett WA). UVB irradiance (μW/cm²) was measured in full sun with a broad band UVB meter (Solarmeter 6.2, Solartech, Harrison Township, MI) with the sensor positioned perpendicular to the direction of the sun. All measurements on the first day were taken at chest height (~1 m

above the substrate). Behaviors recorded included sun exposure (full sun, mixed sun, or full shade), and percentage of their body exposed to full sun.

The second day of the observation period occurred on the next similar-weather day. On day 2, UVB measurements were taken at the recorded times by the investigator inside the enclosure at the exact locations of the lizards recorded from the previous day. If more than 50% of the lizard had been exposed to the sun, the readings were taken in full sun; if less than 50% of the lizard had been exposed to the sun, the readings were taken in full shade. In addition, at each observation time, visible light intensity, air temperature, and UVB were recorded at control sites within the enclosure. At these sites, visible light intensity and UVB were measured in full sun closest to the lizard location, whereas air temperature was measured at the nearest shaded location. For each of the four lizards there were four observation periods: two during the first summer and two during the second summer (Table 1).

To assess the similarity between the 2 days, and to adjust the readings from the lizard locations taken on the second day, if necessary, lux readings from the control sites outside of the enclosure on the first day were compared to readings at the control sites inside the enclosure on the second day of the observation period. Because the irradiances of visible and UV from the sun are generally correlated (Ferguson *et al.*, 2013), the ratio of lux readings from the control sites at each time for the first day to those from the second day was multiplied by the UVB readings at the lizard locations from the second day to adjust them to the unmeasured UVB irradiances likely to have existed at the lizard locations in the enclosure on the first day. UVB doses (irradiance $\times$ time) were calculated as average irradiance for each pair of consecutive readings multiplied by the elapsed time between readings. Dose values (J/cm) for each lizard were summed for each day. As a measure of voluntary UVB exposure or sun-seeking behavior, the percentage of maximum UVB dose available to the lizard was calculated by dividing the UVB dose at the lizard's location site by the UVB dose at the fully sun-exposed control site ($\times 100$). The percent values for each dose reading were averaged for the day.

To monitor serum concentrations of calcidiol, systematic blood samples were collected. Approximately 3.0 ml of whole blood was collected from each animal's caudal tail vein, and then the sample was centrifuged to separate serum. Samples were stored at -70°C (-94°F) at the Dallas Zoo Veterinary Hospital and then sent to the Vitamin D, Skin, and Bone Research Laboratory at Boston University School of Medicine (Boston, MA) to measure serum calcidiol concentrations (Chen *et al.*, 1990). In brief, the analysis technique involved at least two steps: 1) vitamin D and its metabolites were extracted with acetonitrile and further purified using a C18-OH cartridge to reduce the diversity of the sample compounds and 2) the sample was subjected to high-performance liquid chromatographic analyses to separate $25(\text{OH})\text{D}_3$ from 25-hydroxyvitamin D_2 . The sample content was analyzed quantitatively by comparison of the area of the sample peaks with those of a known standard solution. For three of the lizards, samples were collected on eight occasions during the monitoring period. For the other lizard, seven samples were collected (Table 1).

During the second indoor winter deprivation period, calcidiol was monitored three times, thereby enabling a more

accurate estimation of a linear regression model for the decline of each iguana. This facilitated the calculation of half-life values by using the following formula: half-life of calcidiol = natural log (ln) of 2/proportional rate of decline. The proportional rate of decline is the absolute value of the slopes (mean for the four lizards) of the regressions of calcidiol (ln transformed) versus time (Jones *et al.*, 2012). Because the data (ln transformed) passed the tests for normality and variance homogeneity, the statistical significance of the decline (α set at $P \leq 0.05$) was tested using a paired *t*-test for the first deprivation period with only beginning and ending values and a repeated measures analysis of variance (ANOVA) for the second deprivation period with beginning, intermediate, and ending values. All data processing, regression analyses, basic calculations, and figure preparations were performed using Excel (version 2007 or 2013, Microsoft, Redmond, WA), SYSTAT (version 3.5, Systat Inc., San Jose, CA), and SigmaPlot (version 11, Systat Inc.). The study was approved by the Institutional Animal Care and Use Committee of the Dallas Zoo.

RESULTS

The vitamin D status of each of the four rhinoceros iguanas was averaged at the end of the two summer outdoor periods. These averages ranged from 62.8 to 125.7 ng/ml serum calcidiol (mean of the four averages was 95.6). Status was measured following more than 5 months of living in an outdoor enclosure exposed to natural sunlight for several hours a day each year. Levels of calcidiol declined throughout the indoor winter. As with the end of the summer outdoor periods, values of each of the four individuals at the end of the two winters were averaged. These averages ranged from 6.5 to 19.8 ng/ml (mean of the four averages was 12.9). Ranges, means, and standard errors for serum calcidiol, calcium, and phosphorus, along with reference values from the literature, are presented for each blood draw in Table 1. Data for air temperatures for focal and retrace days are also presented in Table 1.

Although data were taken for only three periods throughout the second indoor winter period (October, February, and April), the decline of circulating calcidiol seemed to be linear over the 219 day period (Fig. 1). The slopes of the regressions of ln calcidiol concentrations as a function of time (absolute value) for the four iguanas averaged 0.01. The estimated half-life was 69.3 days. A repeated measures ANOVA of ln-transformed data showed a significant decline over time ($F = 23.5$, $P = 0.001$). Holm-Šidák post hoc testing revealed that the successively lower values were significantly different from each other ($P < 0.05$). The ending values for the first indoor period were also significantly lower than those at the beginning ($t = 6.9$, $P = 0.006$).

During the early part of the outdoor period, from April to June in both 2009 and 2010, vitamin D status increased as the lizards exposed themselves to solar UVB (Fig. 1). Throughout the outdoor periods, there was variance among the four individuals in both vitamin D status and UVB exposure, with a strong negative relationship between the daily dose of UVB and the percentage of voluntary exposure of maximum possible UVB exposure as a function of the vitamin D status (Figs. 2–3). There was also a strong negative relationship between the strength of sun seeking (measured by the slope of the exposure dose versus vitamin

Table 1. Air temperature during observation periods and blood chemistry for Ca, P, and serum 25-hydroxyvitamin D₃ [25(OH)D₃] at draw dates taken during indoor and outdoor monitoring of four adult rhinoceros iguanas (*Cyclura cornuta*) at the Dallas Zoo (Dallas, TX). Air temperature means are $n = 4$. Blood 25(OH)D₃ values are $n = 4$ for draws 1–6 and $n = 3$ for draws 7 and 8. Blood Ca and P concentrations are $n = 4$ for draws 1–6 and draw 8 and $n = 3$ for draw 7. Reference values for *Cyclura(1)* are from Lung (2012) and are maximum and minimum means for eight taxa of *Cyclura* (not including rhinoceros iguanas). Those for *C. cornuta(2)* are from Ramer et al. (2005) and are maximum and minimum values for seven wild-caught individuals.^a

	Begin indoors		Outdoors		Begin indoors			Outdoors		
	Draw 1	Draw 2	Draw 3		Draw 4	Draw 5	Draw 6	Draw 7		Draw 8
	19-20 Sep 2008	4-24 Apr 2009	Observe 1	Observe 2	1-3 Oct 2009	17 Feb 2010	16 Apr 2010	2-17 Jun 2010	30 Jun-21 Jul 2010	6-20 Jun 2010
Air temp on observe day			16-26 Jun 2009	13-28 Aug 2009						
			33.79 ± 0.8 [92.8]	31.04 ± 1.7 [87.9]				33.74 ± 0.4 [92.7]		34.69 ± 0.6 [94.4]
Range			(27.7-39.3)	(22.2-36.1)				(27.8-36.7)		(28.3-37.8)
Air temp on retrace day			34.93 ± 0.9 [94.9]	32.73 ± 1.1 [90.9]				33.25 ± 0.7 [91.9]		34.43 ± 0.6 [94.0]
Range			(27.8-43.3)	(27.2-40.0)				(26.7-37.8)		(27.8-38.9)
Blood Ca (mg/dl)	17.3 ± 4.1	19.6 ± 4.9	31.57 ± 10.6		24.67 ± 12.4	14.57 ± 1.3	21.35 ± 5.8		134.3 ± 120	14.1 ± 0.8
Range	(11.5-29.5)	(12.7-33.8)	(12.1-51.6)		(11.5-62.0)	(12.1-17.4)	(12.2-37.4)		(12.4-374.8)	(12.8-16.2)
Reference <i>Cyclura(1)</i>										
Min.	11.60									
Max	17.00									
(>150 gravid)										
Blood P (mg/dl)	5.35 ± 1.35	4.95 ± 1.6	7.7 ± 2.2		14.9 ± 10.7	6.13 ± 0.9	7.13 ± 1.7		29.24 ± 23.3	6.54 ± 1.9
Range	(2.7-9.3)	(3.2-9.8)	(3.4-12.5)		(3.6-47.2)	(4.7-9.0)	(4.6-12.1)		(5.3-75.8)	(3.9-12.2)
Reference <i>Cyclura(1)</i>										
Min.	4.79									
Max	8.10									
(>16 gravid)										
Blood 25(OH)D ₃ (ng/ml)	95 ± 24.7	12.7 ± 2.3	87.1 ± 3.2		93.7 ± 10.6	26.4 ± 5.6	13.0 ± 4.3		88.9 ± 13.3	98.2 ± 24.2
Range	(87-167)	(6.1-16.8)	(78.1-92.8)		(69.5-111.7)	(13.6-39.7)	(6.9-25.3)		(63.7-108.7)	(56.0-140.0)
Reference <i>C. cornuta(2)</i>										
Min.	104.00									
Max	148.00									

^a Air temperatures are mean ± SE in degrees Celsius [degrees Fahrenheit], and blood chemistry concentrations are mean ± SE. Reference values are given as minimum (Min.) and maximum (Max).

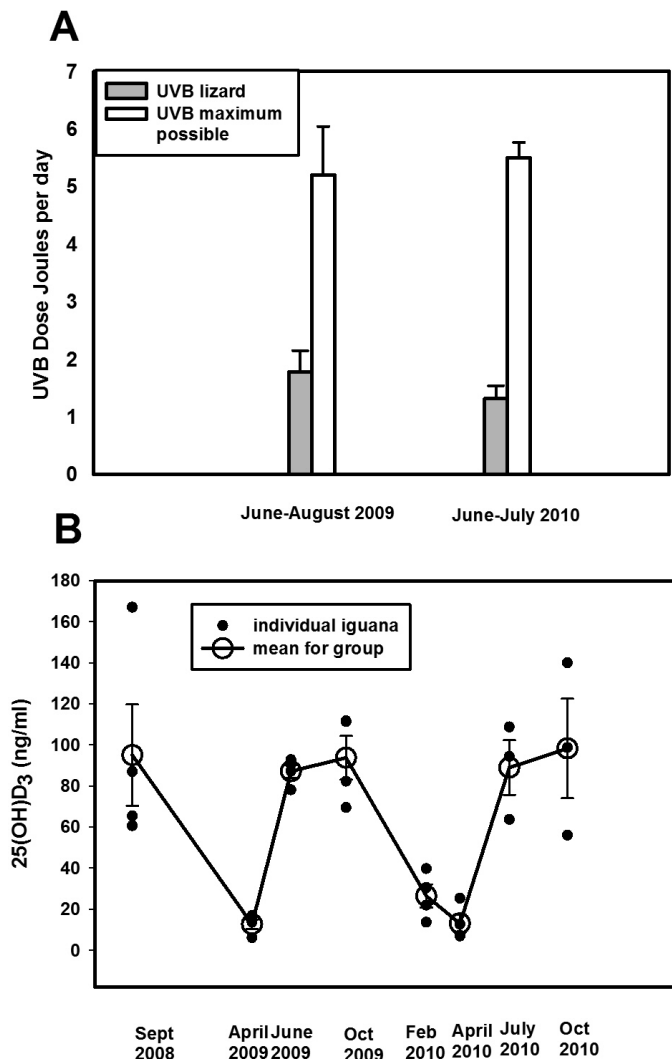


Figure 1. Time line of ultraviolet B (UVB) dose (A) and vitamin D status (serum 25-hydroxyvitamin D₃ or calcidiol) (B) of four captive rhinoceros iguanas (*Cyclura cornuta*) maintained as a display group at the Dallas Zoo (Dallas, TX). The group was maintained indoors without artificial UVB or dietary vitamin D from October to April of each year. They were maintained outdoors with access to natural sunlight exposure from April to October of each year. For (A), bars show mean dose; capped lines show 1 SE. For (B), solid symbols show individual values and open symbols show mean; capped lines show 1 SE. Line connecting means shows the seasonal trend.

D status regression for each individual) as a function of the individual's average vitamin D status at the end of the summer (Fig. 4). With the small number of observation/vitamin D status pairs for each individual, the probability for each slope exceeded that needed to show statistical significance for all but the small female (Fig. 2). However, the trend among individuals of decreasing slope of the regression with vitamin D status at the end of summer was highly significant ($P = 0.002$; Fig. 4).

Throughout the monitoring period, serum calcium (total calcium [TCA]) and phosphorus concentrations varied, with peak concentrations occurring in midsummer and lower concentrations maintained during the winter (Table 1).

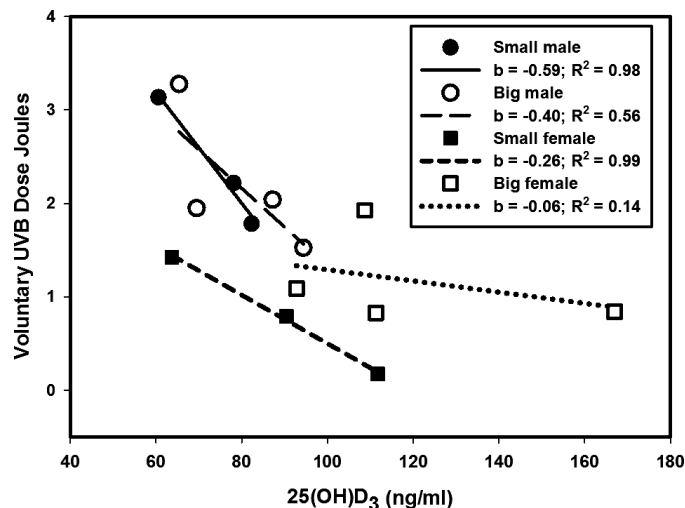


Figure 2. Voluntary ultraviolet B (UVB) dose as a function of vitamin D status (serum 25-hydroxyvitamin D₃ or calcidiol) for each of four rhinoceros iguanas (*Cyclura cornuta*) maintained as a display group in captivity at the Dallas Zoo (Dallas, TX). The slope of the regression for each individual (b) is negative, indicating that iguanas sought more UVB exposure when their vitamin D status was lower. Slope value (absolute) indicates strength of vitamin D status restoration due to greater voluntary UVB exposure when vitamin D status was lower. For each individual the dose was paired with the vitamin D status value taken close to the time of the dose (e.g., June dose with July draw value or August dose with October draw value).

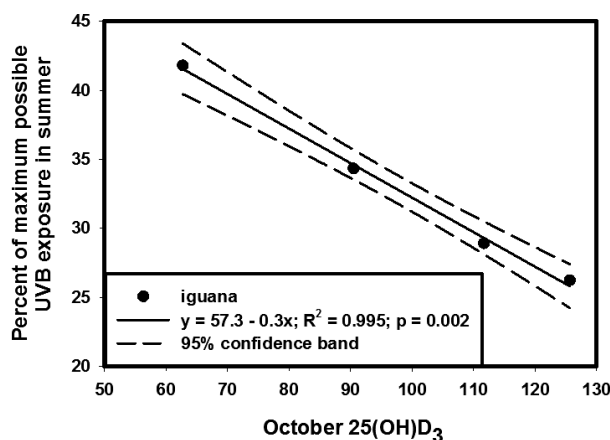


Figure 3. Percentage of maximum possible ultraviolet B exposure (sun-seeking behavior) as a function of vitamin D status (serum 25-hydroxyvitamin D₃ or calcidiol) of four rhinoceros iguanas (*Cyclura cornuta*) maintained as a display group in captivity at the Dallas Zoo (Dallas, TX). For each individual, the percent values for the four summer observation periods (two each in 2009 and 2010, respectively) were averaged, as were the vitamin D status values for the two subsequent October blood draws (2009 and 2010).

DISCUSSION

Storage of vitamin D in the body and depletion when deprived of external sources has been studied in humans and other vertebrates. In humans and other mammals, circulating vitamin D and calcidiol can be stored in body tissue,

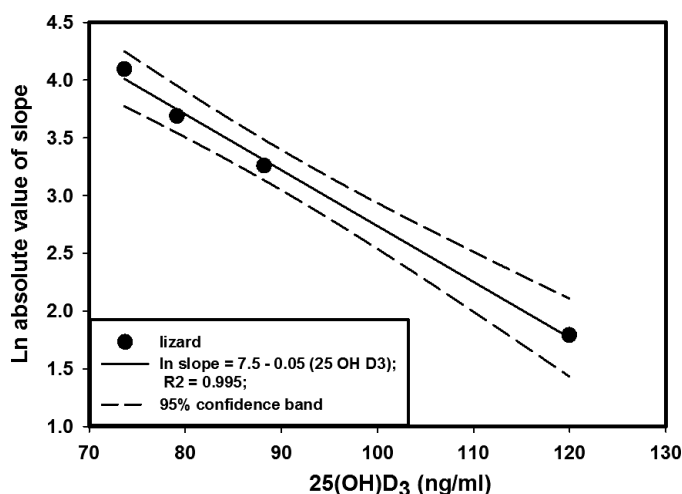


Figure 4. Natural log of the absolute slope value (strength of vitamin D status restoration from Fig. 2) as a function of average October vitamin D status for four rhinoceros iguanas (*Cyclura cornuta*) maintained as a group in captivity at the Dallas Zoo (Dallas, TX).

mostly in adipose tissue, for slow release (Rosenstreich and Volwiler, 1971; Holick, 2007), whereas the water-soluble vitamin D metabolite calcitric acid is rapidly excreted in the bile. Measurement of calcidiol concentration in the blood is considered to be the best index of vitamin D status (Haddad, 1999; Holick, 2007). In humans, during deprivation or after a large single dose, circulating calcidiol has a half-life ranging from 10 to 40 days (mean = 20; Jones *et al.*, 2012). The half-life of calcidiol during deprivation of UVB and dietary vitamin D remains largely unstudied in reptiles, but it has been estimated for captive-raised juvenile black-throated monitors (*Varanus albigularis*) to be 128–139 days (Ferguson *et al.*, 2009).

Several factors may affect the rate of decline of vitamin D status during deprivation of both UVB and dietary vitamin D. These factors include age, body condition, which may be largely influenced by fat deposition, and metabolic rate. Also, the rate of depletion may vary with vitamin D status, so it may be more accurately estimated if studied over a longer period of depletion. Thus, comparison of the half-life of juvenile *Varanus* of 128–139 days (Ferguson *et al.*, 2009) with a high initial vitamin D status (150–200 ng/ml) measured over a short period of 71 days with only ~35% depletion may not reflect accurately a real species difference from the half-life of adult *Cyclura* with a lower initial status (62–165 ng/ml) measured through a longer period of 219 days with 85% depletion. More research is needed to understand variation in storage and depletion of vitamin D and its metabolites within and among animal taxa.

Given that unregulated exposure of lizard skin to UVB increases vitamin D production and vitamin D status (Holick *et al.*, 1995; Aucone *et al.*, 2003; Ferguson *et al.*, 2003, 2005; Oonincx *et al.*, 2010), the strong negative relationship between UVB exposure and vitamin D status during the summer suggests that the iguanas in this report were attempting to regulate their vitamin D status at set points at or below 100 ng/ml. Two lizard species, the panther chameleon (*Furcifer pardalis*) and the Cuban brown anole (*Anolis sagrei*), have been shown to regulate their exposure to UVB

and to reduce their exposure when dietary vitamin D is increased (Ferguson *et al.*, 2003, 2013; Karsten *et al.*, 2009). A positive relationship between UVB exposure and vitamin D status would have indicated that the iguanas in this report were trying to achieve the higher levels observed for this and related species in tropical environments (Ramer *et al.*, 2005), but perhaps they were constrained by the more limited seasonal availability of UVB.

Interestingly, the two lizards with the highest vitamin D status at the end of both summers were the two smallest individuals, which also happened to be the two female members of the group. They were also the individuals with the lower UVB exposures during their two observation periods. Further research is needed to see whether the sexes differ in optimal levels of circulating calcidiol and whether there are sexual differences in the efficiency of vitamin D production in the skin for a given UVB exposure (see Ferguson *et al.*, 2005 for differences in skin sensitivity among lizard species).

Rock iguanas are a group of tropical lizards with serious conservation issues (Lemm and Alberts, 2012). Captive breeding is considered an important part of their recovery programs. Further research is necessary to determine minimum vitamin D concentrations required for optimum health and reproduction in captivity. The mean concentration of blood calcidiol in rhinoceros iguanas at the end of summer (95 ng/ml) was lower than that previously measured for rhinoceros iguanas (mean $\pm$ SE = 133 $\pm$ 19 ng/ml for wild-caught and 127 $\pm$ 33 ng/ml for head-started captives) maintained outdoors year-round at tropical locations (Ramer *et al.*, 2005), but was similar to values measured for free-living chuckwallas (mean 85 ng/ml, range 55–130; Aucone *et al.*, 2003), a medium-sized herbivorous desert iguanid lizard that hibernates in winter. Does higher vitamin D status necessarily signify better health? Furthermore, although levels measured at the end of winter in our study were considerably below those of outdoor-living *Cyclura* living in the tropics and monitored year-round (Ramer *et al.*, 2005; Lung, 2012), the iguanas in our study seemed robust and healthy throughout the indoor winter period and quickly adapted to their outdoor environment.

Despite the apparent good health and physiological condition of the four iguanas throughout our study, more controlled experimental investigation of the effects of deprivation using more health indicators and larger sample sizes is warranted. All concentrations of blood total calcium (TCA) and phosphorus, including those at the end of winter, exceeded reference values for *Cyclura* successfully maintained outdoors (Table 1). However, although the animals seemed robust throughout the monitoring period, with no external clinical signs of metabolic bone disease, we obtained no data on bone density or ionized calcium values, which more accurately reflect physiological availability than TCA. Assessment of decreased bone density in winter might have given a more accurate assessment of whether there was incipient metabolic bone disease during the UVB deprivation periods.

Recent laboratory research on smaller insectivorous and omnivorous lizards indicates that they serve as good models for future studies of UVB, vitamin D, and calcium interactions in *Cyclura*. Studies on the insectivorous veiled chameleon (*Chamaeleo calyptratus*) show that dietary vitamin D supplementation has a positive effect on vitamin D status that is independent of UVB exposure and that the strongest

effect is seen with a combination of UVB exposure and supplemental dietary vitamin D (Hoby *et al.*, 2010; Haxhiu *et al.*, 2014). Adult female bearded dragons (*Pogona vitticeps*), which are omnivorous, were provided UVB for 6 months and maintained a high vitamin D status into adulthood (Oonincx *et al.*, 2010). When they were subsequently deprived of UVB, they maintained high levels of calcidiol and calcitriol for 83 days with no apparent decline. During this period of UVB deprivation, they received low, but not zero, dietary intake of vitamin D₃ (Oonincx *et al.*, 2013). The low vitamin D diet without UVB exposure had previously been shown to maintain a vitamin D status much lower than that seen with UVB supplementation in growing juveniles of this species. Although the dietary vitamin D₃ was insufficient to attain a high vitamin D status, it may have prevented or decelerated the decline during deprivation of UVB in adult females. The Dallas Zoo currently provides both artificial UVB and dietary vitamin D supplements to all lizards maintained indoors. Until a minimally appropriate vitamin D status is determined for *Cyclura*, we do not recommend long-term deprivation of UVB, dietary vitamin D, or both to captive lizards maintained indoors. Future studies that include UVB deprivation should closely monitor all bioindicators of health.

The four iguanas in this study were fed a prepared fruit and vegetable diet estimated to contain no vitamin D, but an adequate amount of calcium and phosphorus. When outdoors in summer, the iguanas had opportunities to supplement their diet with live plants containing unknown mineral and vitamin D concentrations. One perennial plant group present in the outdoor enclosure that is known to contain bioavailable calcium and also shown to have a positive effect on bone density when consumed by laboratory rats is *Opuntia* spp. (Ramírez-Moreno *et al.*, 2011; Guerrero-Cervantes *et al.*, 2012; Kang *et al.*, 2012). The effect on bone density was, in part, due to suppression of parathyroid hormone. Because this hormone suppresses the production of calcitriol (Holick, 2007), production of calcitriol should increase. Consequently, calcium transport from the gut to the circulation, which is stimulated by calcitriol, should have increased. There was evidence from scat that the iguanas consumed the fruits of this plant during the summers of this study. In future studies designed to test the relative importance of UVB and dietary contributions to vitamin D status, the palatable plants used for display decoration should be inaccessible to the lizards.

Rock iguanas of the genus *Cyclura*, like many heliophilic lizards, are strong thermoregulators and maintain relatively constant body temperatures ($\geq 36^{\circ}\text{C}$ [96.8°F]) throughout their daily activity (Christian *et al.*, 1986). At high midday environmental temperatures, individuals seek shade to avoid overheating. Thermoregulatory behavior can strongly influence the pattern of UVB exposure during the day in free-living lizards (Ferguson *et al.*, 2010, 2014). However, daily dose of UVB may not be markedly reduced on days when high environmental temperatures constrain midday activity (Ferguson *et al.*, 2014). Although mean and maximum air temperatures varied among the observation days in our study (Table 1), our small sample size precluded detection of an effect of temperature on daily UVB dose. More research is needed to determine the relative effects of temperature and vitamin D status on voluntary UVB dose in rhinoceros iguanas. Lung (2012) emphasized the importance of considering the opportunity for solar exposure (including the

opportunity to regulate it) when setting up outdoor enclosures to head start rock iguanas.

To fully understand calcium uptake and physiological availability and to interpret calcium and vitamin D status most accurately, future studies should assess or emphasize one or combinations of the following topics: 1) ionized calcium and TCA of the diet, blood, and organ systems (especially the gut, liver, kidney, adipose tissue, skeleton, skin, and brain); 2) as many vitamin D metabolites as feasible in the diet, blood, and organs; 3) histological structure and mineral density of bone, joints, and soft tissue; 4) other nutrients and hormones that might interact with vitamin D and calcium metabolism (e.g., vitamin A, phosphorus, and parathyroid hormone); 5) light quality and quantity, including UVB, UVA, and visible; 6) behavioral responses to light quality and quantity; and 7) use of single and multifactorial experimental designs to assess cause, effect, and interaction of morphological, physiological, and behavioral variables. Recent studies of the veiled chameleon, bearded dragon, and panther chameleon serve as model approaches that should be expanded, refined, and extended to other squamate taxa as knowledge accumulates (see Ferguson *et al.*, 2003; Karsten *et al.*, 2009; Hoby *et al.*, 2010; Oonincx *et al.*, 2010, 2013; Haxhiu *et al.*, 2014). Behavioral, ecological, and physiological monitoring studies of model organisms in their natural habitats, where feasible, should be undertaken. Field reference values are important for understanding the results of laboratory studies. Although rock iguanas are potential candidates for model organism status for these kinds of studies, their size and endangered status limits their utility for in-depth studies requiring large sample sizes and invasive procedures, particularly those requiring euthanasia.

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