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Evaluating the Physiologic Effects of Short Duration Ultraviolet B Radiation Exposure in Leopard Geckos (*Eublepharis macularius)*

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Abstract

Ultraviolet B radiation (UVB) is required by many vertebrates to stimulate the photobiochemical synthesis of vitamin D. Vitamin D plays many important roles in the body, including assisting in the absorption of calcium at the level of the intestines. Deficiencies in vitamin D can lead to the development of nutritional disease. Leopard geckos (*Eublepharis macularius*) are naturally nocturnal to crepuscular; therefore, it is not known whether they benefit from UVB radiation. The purpose of this study was to measure 25-hydroxyvitamin D_3 concentrations in leopard geckos exposed to short duration UVB light. Twelve adult, male leopard geckos were used for this study. Blood samples were collected from the cranial vena cava to establish baseline 25-hydroxyvitamin D_3 concentrations. Once the baseline samples were collected, the animals were randomly divided into two groups. The animals provided UVB radiation were exposed to non-UVB producing light for 12 h and UVB for 2 h, whereas animals in the control group only received non-UVB producing light for 12 h. Exposure to the UVB light occurred for 2 h per day: 1 h at 0600 h and 1 h at 1800 h to mimic dawn and dusk, respectively. An additional blood sample was collected 30 days after the initiation of UVB exposure. There was a significant difference ($F = 9.7$, $P = 0.012$) in 25-hydroxyvitamin D_3 concentrations between the two groups, with UVB exposed geckos having significantly higher concentrations. The results of this study demonstrate that short duration exposure to UVB light can lead to increased circulating 25-hydroxyvitamin D_3 concentrations in leopard geckos.

Key Words: *Eublepharis macularius*, leopard gecko, 25-hydroxyvitamin D_3, ultraviolet B, vitamin D

Introduction

Because of their relative ease of care, longevity, and manageable size, leopard geckos (*Eublepharis macularius*) have become an attractive choice for reptile enthusiasts across different experience levels. Native to the rocky scrubland and desert regions of Pakistan, Afghanistan, Iran, and India, leopard geckos are a nocturnal and crepuscular species that spend much of the daylight hours concealed in burrows and damp crevices (Thorogood and Whimster, 1979). Leopard geckos are an insectivorous species that likely consume a varied diet in the wild. Unfortunately, in the captive setting, metabolic disturbances, such as nutritional secondary hyperparathyroidism (NSHP), are common in leopard geckos because the prey insects that are available commercially are deficient in calcium and vitamin D and disproportionately high in phosphorous. Recommendations for most insectivore diets include supplementing these prey items by offering an appropriate gut-loading diet or dusting them with a

calcium-vitamin powder. However, because of discrepancies in the nutritional content of these gut-loading diets and the variable time before prey items are ingested, this form of oral supplementation may be unreliable. Further, most of these regimens emphasize calcium supplementation but overlook the importance of vitamin D in calcium regulation.

Vitamin D is a fat-soluble hormone that can be obtained from the diet, through photobiochemical synthesis following exposure to ultraviolet B (UVB) light (280–320 nmol), or a combination of both (Webb and Holick, 1988; Webb *et al*., 1989; Allen *et al*., 1994). Vitamin D is vital to a wide array of physiologic actions within the body, most notably calcium homeostasis and metabolism (Webb and Holick, 1988; How *et al*., 1994). It has also been shown to impact reproductive success in species across taxa (Ferguson *et al*., 2002; Langerwerf, 1994; Narbaitz and Tsang, 1989). Without adequate vitamin D stores, uptake of ingested calcium from the intestines is impaired. This can lead to hypocalcemia, even in the face of calcium over supplementation. When circulating calcium concentrations drop below a certain threshold, a compensatory release of parathyroid hormone causes calcium to be released from bone. This loss of bone matrix can produce the clinical signs associated with NSHP, one of the most common nutritional diseases reported in captive reptile species (Laing and Fraser, 1999; Mader, 2005). Although it is widely accepted that many diurnal species can use ultraviolet supplementation to promote the photobiochemical conversion of precursors into vitamin D_3, much less is known about the requirements of nocturnal or crepuscular species (Bernard *et al*., 1991; Allen *et al*., 1994; Laing and Fraser, 1999; Wright, 2008). Although many of these species are successfully kept in captivity without UVB supplementation, the high prevalence of NSHP in captive insectivores, including leopard geckos, may correspond to a deficiency in vitamin D_3 and deserves further investigation.

The purpose of this study was to determine whether leopard geckos exposed to short duration UVB light would experience a rise in their 25-hydroxyvitamin D_3 concentrations. The specific hypotheses for this study were as follows: 1) leopard geckos exposed to UVB radiation for two hours per day would have higher serum 25-hydroxyvitamin D_3 concentrations than leopard geckos not exposed to UVB; and 2) that weights of these animals would not vary significantly over time between or within the UVB exposed and nonexposed groups.

Materials and Methods

This study was performed in accordance with the regulations set forth by the Institutional Animal Care and Use Committee at the University of Illinois (protocol 14-264). Twelve adult male leopard geckos obtained from a pet store (Sailfin Pets, Champaign, IL) were used for this study. Males were used to limit the potential effect of sex on the results because of sample size. The leopard geckos were housed individually in 25 cm × 34 cm × 13 cm (15 quart) Sterilite containers (Sterilite Corporation, Townsend, MA). A circular opening was cut into the lid, and galvanized wire was secured to the opening to prevent escape and ensure passage of UVB radiation into the container. Each container was outfitted with a water dish, hide box, and brown-paper lining to facilitate cleaning. The ambient temperature and humidity were kept constant at 29.4°C (85°F) and 39%, respectively. All of the geckos were offered crickets (*Acheta domestica*) on a daily basis. The number of crickets fed was equivalent to approximately 2% of the geckos' body weight. The crickets were not gut-loaded for this study to reduce the impact of any oral vitamin D supplementation. Previous unpublished research by one of the authors (MAM) has found vitamin D concentrations in these nonsupplemented crickets to be below detection limits.

The 12 geckos were allowed a 72 h acclimation period before the initial blood sample was collected. Geckos were placed, one at a time, into a gas-anesthetic induction chamber (20 L glass tank) and anesthetized using 5% isoflurane gas (IsoFlo; Abbott Laboratories, North Chicago, IL) and 2 L/min oxygen flow. Once the geckos lost their righting reflex, they were removed from the anesthetic chamber, and a blood sample was collected from the cranial vena cava (day 0). Baseline and follow-up blood samples were always collected between 1600 and 1800 h. Blood samples were stored in serum separator microtainer tubes (Becton-Dickinson, East Rutherford, NJ) and centrifuged within one hour of collection. Serum was harvested and frozen at –80°C (–112°F) until being analyzed.

After the initial blood samples were collected, the geckos were randomly assigned to two groups using a random number generator (random.org). Group one ($n = 6$) represented the treatment group, and included the geckos that would be exposed to UVB light. Group 2 ($n = 6$) represented the control group and included the geckos that would receive non-UVB producing light. Fluker Farm (Port Allen, LA) 23 watt compact fluorescent bulbs were placed over the wire tops of the geckos from group 1. A timer was connected to the lights so that the lights would turn on for one hour in the morning (0600 h) and one hour in the evening (1800 h), to mimic a crepuscular exposure pattern. All geckos were exposed to ambient non-UVB producing fluorescent lighting for 12 h per day.

Leopard geckos were monitored once daily for position within enclosure and whether they underwent an ecdysis cycle. Geckos were weighed weekly, with measurements rounded to the nearest 0.1 g. Ultraviolet radiation was also measured once weekly (1800 h) using a radiometer-photometer (Solarmeter 6.2; Solar Light Co., Inc., Glenside, PA) at a distance of 12 cm from the bulb surface from three different sites within the enclosure: directly through the mesh under the bulb and at each end of the enclosure. UV readings were taken in triplicate and the arithmetic mean calculated.

Each group was maintained under the described study conditions for 30 days. A second blood sample was

Table 1. Estimated marginal means for 25-hydroxyvitamin D_3 concentrations in leopard geckos exposed to and not exposed to UVB light.

Parameter	Group	Mean	95% CI	SE	Min–Max
25 hydroxyvitamin D_3	No UVB	49.7	33.7–65.6	7.0	12.0–81.0
	UVB	79.5	64.9–94.0	6.4	33.0–170.0

collected from each gecko on day 30. Collection, processing, and analysis of the blood samples were identical to that described for day 0. Once all samples were collected, they were transported on frozen gel packs to the Michigan State University Diagnostic Center for Population and Animal Health (Lansing, MI) for testing. A radioimmunoassay was performed to determine serum concentrations of 25-hydroxyvitamin D_3 (Holick, 1990; Laing and Fraser, 1999; Acierno *et al*., 2006, 2008).

The distribution of the data was evaluated using the Shapiro-Wilk test, skewness, kurtosis, and q-q plots. Because the data were normally distributed, a parametric test was selected. A general linear model for repeated measures was used to analyze the data, with gecko as the random variable and group and time as fixed variables. SPSS 22.0 (IBM Statistics, Armonk, NY) was used to analyze the data. A $P < 0.05$ was used to determine statistical significance.

Results

There was a significant difference in 25-hydroxyvitamin D_3 concentrations over time ($F = 12.99$, $P = 0.006$) and between groups ($F = 9.72$, $P = 0.012$). Leopard geckos exposed to UVB had significantly higher 25-hydroxyvitamin D_3 concentrations than those not provided UVB light (Table 1). There was no significant difference in body weight within or between groups ($F = 2.7$, $P = 0.103$). All geckos, irrespective of light exposure, showed a strong preference for staying within a hide box whenever observed; however, this was not unexpected as they were only evaluated during the daytime. It is important to note that both groups of geckos were found to be outside of their shelters during the study; therefore, we know direct exposure did occur in the UVB group. None of the geckos produced a shed during the 30 day trial. The UVB radiation measurements for the treatment group were 12–52 μwatts/cm^2; the higher end of the range was under the bulb and the lower end of the range at the sides of the enclosure.

Discussion

The results of this study confirm that leopard geckos exposed to short-duration (2 h) UVB radiation are capable of significantly increasing their 25-hydroxyvitamin D_3 concentrations over time compared with control geckos not exposed to UVB but fed a similar diet. This is an important finding because it further affirms that leopard geckos can use UVB radiation to photobiochemically generate 25-hydroxyvitamin D_3 and that it can be done using limited UVB exposure versus a more common 12 h exposure (Wangen *et al*., 2013).

To date, the majority of the research evaluating the effects of UVB radiation on reptiles has focused on diurnal species (Allen *et al.,* 1999; Laing and Fraser, 1999; Carman *et al*., 2000; Laing *et al*., 2001; Ferguson *et al*., 2003, 2005; Acierno *et al*., 2006, 2008; Oonincx *et al*., 2010; Selleri and Di Girolamo, 2012). Although originally focused on lizards (Allen *et al.,* 1999; Laing and Fraser, 1999; Carman *et al*., 2000; Laing *et al*., 2001; Ferguson *et al*., 2003, 2005; Oonincx *et al*., 2010), more recent examples in chelonians (Acierno *et al*., 2006; Selleri and Di Girolamo, 2012) and a snake species (Acierno *et al*., 2008) also suggest that UVB can play an important role in the circulating concentrations of 25-hydroxyvitamin D_3 in these reptiles too. However, it should not be assumed that UVB light is a requirement for vitamin D regulation in all diurnal vertebrates, as dogs and cats do not use this method for acquiring vitamin D and a recent study in captive ball pythons (*Python regius*) found that UVB had no impact on circulating 25-hydroxyvitamin D_3 concentrations in that species (How *et al*., 1994; Hedley and Eatwell, 2013). These findings re-affirm that we must evaluate each species separately to determine how they acquire vitamin D such that, when in captivity, we can provide appropriate husbandry and care to minimize the likelihood for iatrogenic disease (e.g., NSHP).

Evolutionary adaptations appear to be in place for some species to maximize their ability to synthesize vitamin D following limited exposure to sunlight. Several studies have shown that crepuscular and nocturnal reptiles may have a more sensitive and efficient mechanism for converting UVB to vitamin D (Carman *et al*., 2000; Ferguson *et al*., 2005). When comparing two species of Jamaican anoles, the shade-dwelling species had a higher rate of photoconversion than the basking species, while simultaneously consuming less dietary vitamin D (Ferguson *et al*., 2005). Upon exposure to ultraviolet radiation, the nocturnal/crepuscular house gecko (*Hemidactylus turcicus*) was also shown to similarly convert ultraviolet radiation more effectively than the diurnal Texas spiny lizard (*Sceloporus olivaceus*) (Carman *et al*., 2000). Further, the latter study also found evidence suggesting behavioral regulation by the house gecko to maximize sun exposure by emerging earlier (if west-facing) or remaining active longer (if east-facing). Behavioral regulation has been proposed as a means of self-regulating circulating vitamin D concentrations, evidenced by the panther chameleon (*Furcifer pardalis*) that will alter its basking time depending on dietary intake of vitamin D (Karsten *et al*., 2009). When given less access to dietary vitamin D, the frequency and duration of basking behavior significantly increases, showing particular preference for UVB-generating light (Ferguson *et al*., 2003). Our findings in leopard geckos similarly suggest that this crepuscular species can increase circulating 25-hydroxyvitamin D_3 concentrations when exposed to artificial lighting that produces UVB radiation, even when maintained on a

vitamin D_3 deficient diet. It was interesting to note that the 25-hydroxyvitamin D concentrations in the control group did not decrease; however, this was not unexpected as decay (half-life) of this hormone over time has been found to be 69.3 days and 83 days in rhinoceros iguanas (*Cyclura cornuta*) and bearded dragons (*Pogona vitticeps*), respectively (Ferguson *et al.,* 2015; Oonincx *et al*., 2013).

Determining how a reptile acquires vitamin D, either through the diet, UVB exposure, or a combination of both, is important for making "best practice" recommendations for that species in captivity. Historically, supplementation of vitamin D for captive vertebrates has been through the diet. However, it is possible to over supplement vitamin D in captive vertebrates, causing hypervitaminosis D. Animals that develop hypervitaminosis D can develop soft-tissue mineralization, particularly renal and aortic calcification, leading to organ dysfunction and organ failure (Wallach, 1996; Mader, 2005; Watson and Mitchell, 2014). Ferguson *et al*. (1996) found that reproductively active female panther chameleons had an increased mortality when fed a high vitamin D diet (9.1 IU/g cholecalciferol). In humans, over supplementation via intramuscular injections (600,000 U of vitamin D) can result in nausea, vomiting, anorexia, increased thirst and urination, weakness, and altered sensorium (Pandita *et al*., 2012). Comorbidities included chronic kidney disease, urinary tract infection, diabetes mellitus, osteoarthritis, and hypertension (Pandita *et al*., 2012). In comparison, the cutaneous biosynthesis of vitamin D, which occurs through exposure to natural sunlight or artificial ultraviolet radiation, seems to provide a protected means of acquiring vitamin D (Webb and Holick, 1988). Vitamin D is produced through a series of interactions whereby 7-dehydrocholesterol (7-DHC, provitamin D_3) is photoconverted to previtamin D. From here, there are several possible biochemical options forward. When metabolic stores of circulating vitamin D are diminished, previtamin D undergoes a thermochemical change into vitamin D_3, which, after passing through both the liver and kidneys, becomes the metabolically active 1,25-dihydroxyvitamin D (1,25(OH)2D, calcitriol). When the body has adequate circulating levels of vitamin D, previtamin D_3 is shunted down alternative pathways to become biologically inert photoproducts such as tachysterol or lumisterol (Webb and Holick, 1988; Holick, 1990, 2007). These inactive byproducts can either be degraded or recycled back to previtamin D_3 for conversion to active vitamin D_3 based on need (Webb and Holick, 1988). To date, there are no known studies describing hypervitaminosis D associated with photobiosynthesis.

Based on our current understanding of hypervitaminosis D, it appears that the provision of UVB radiation, for those species that can use it, is preferred over the sole provision of dietary vitamin D until we perform the necessary studies to determine appropriate dietary levels. However, there are also negative side effects associated with UVB exposure that should be considered to minimize the likelihood of them occurring. In a preliminary study evaluating the ability of leopard geckos to synthesize vitamin D_3 following UVB exposure, providing an unprotected (no shelter) 12 h photoperiod with high concentrations of UVB resulted in weekly episodes of ecdysis (Wangen *et al*., 2013). This frequent skin shedding was compared to the erythema (sunburn) noted in other species of vertebrates that receive high doses of ultraviolet radiation. Excess unprotected exposure has also been shown to negatively affect hatching rates and survivorship in some species of amphibian (Blaustein *et al*., 1994, 1998) and lead to ocular dysfunction, lethargy, and weight loss in a variety of vertebrate taxa (Gerhman, 1994; Flamarique *et al.,* 2000; Fris *et al*., 2006; Gardiner *et al*., 2009). There is also some concern that reports of squamous cell carcinoma in bearded dragons, especially around the head, may be associated with excessive ultraviolet radiation exposure (Hannon *et al*., 2011). These findings, however, are likely compounded by other negative extraneous environmental factors and a particularly high output of ultraviolet radiation. This highlights the need for careful selection of ultraviolet lamp sources, appropriate light distance from the light source to the basking location, the provision of shelter and a UVB gradient, the amount of time ultraviolet exposure is provided, and close monitoring of the animal to ensure deleterious effects of irradiation do not occur (Ferguson *et al*., 2002, 2010; Adkins *et al*., 2003; Baines *et al*., 2016).

Conclusions

Leopard geckos are a popular pet reptile, and nutritional disease, especially NSHP, is a common finding in these animals. The results of this study demonstrate that short-term exposure to UVB radiation is sufficient to increase circulating 25-hydroxyvitamin D_3 concentrations in leopard geckos. Because the prey species offered to leopard geckos in captivity are naturally low in vitamin D (e.g., crickets, mealworms), exposure to UVB may help offset dietary deficiencies, or concerns about hypervitaminosis associated with supplements. However, there remains much we still need to learn about this subject. Prospective, longitudinal studies are required to determine reference intervals for 25-hydroxyvitamin D_3 in leopard geckos, as well as how much UVB exposure and dietary vitamin D are required to achieve these concentrations. In addition, it is important for us to determine the role vitamin D plays in the pathophysiology of NSHP in this species. Until further research is done, the authors believe that leopard geckos should be provided protected UVB exposure to ensure that they can behaviorally control their vitamin D concentrations.

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