Effects of ultraviolet radiation on plasma 25-hydroxyvitamin D_3 concentrations in corn snakes (*Elaphe guttata*)

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Objective—To determine whether corn snakes exposed to UVB radiation have increased plasma 25-hydroxyvitamin D_3 concentrations, compared with control snakes.

Animals—12 corn snakes (*Elaphe guttata*).

Procedures—After an acclimation period in individual enclosures, a blood sample was collected from each snake for assessment of plasma 25-hydroxyvitamin D_3 concentration. Six snakes were provided with no supplemental lighting, and 6 snakes were exposed to light from 2 full-spectrum coil bulbs. By use of a radiometer-photometer, the UVA and UVB radiation generated by the bulbs were measured in each light-treated enclosure at 3 positions at the basking surface and at 2.54 cm (1 inch) below each bulb surface; the arithmetic mean values for the 3 positions at the basking surface and each individual bulb surface were calculated immediately after the start of the study and at weekly intervals thereafter. At the end of the study (day 28), another blood sample was collected from each snake to determine plasma 25-hydroxyvitamin D_3 concentration.

Results—Mean ± SD plasma concentration of 25-hydroxyvitamin D_3 in snakes that were provided with supplemental lighting (196 ± 16.73 nmol/L) differed significantly from the value in control snakes (57.17 ± 15.28 nmol/L). Mean exposure to UVA or UVB did not alter during the 4-week study period, although the amount of UVA recorded near the bulb surfaces did change significantly.

Clinical Relevance—These findings have provided important insight into the appropriate UV radiation requirements for corn snakes. Further investigation will be needed before exact husbandry requirements can be determined. (*Am J Vet Res* 2008;69:294–297)

Vitamin D_3 is an important hormone that has numerous physiologic properties.[1,2] Its most widely recognized function is the regulation of calcium metabolism, which is needed for the development and maintenance of healthy bones; however, the reproductive success of panther chameleons has also been associated with optimized serum vitamin D_3 concentrations.[3,4] This hormone can be synthesized through the exposure of the skin to UVB (290 to 320 nm) radiation or via consumption of animals that have previously made this biochemical conversion.[5,6] Among vertebrate species, there is wide variation between the need for dietary intake of vitamin D_3 and the ability to synthesize the hormone.[7,8]

To date, most studies to examine the source and function of vitamin D_3 have involved mammals and birds.[9] Historically, studies[4,8,10,11] performed in reptiles have focused on dietary and basking requirements in various lizard species. Recently, an investigation[12] of the effects of UVB radiation in red-eared slider turtles (*Trachemys scripta elegans*) revealed that plasma concentration of 25-hydroxyvitamin D_3 increases significantly after exposure to UVB radiation. The authors are not aware of studies to investigate whether snakes synthesize vitamin D_3 via basking or rely on consumption of it in their diet. This is unfortunate because many of these species are raised in captivity as pets; given that these reptiles have the potential to be long-lived, it is important that the specific husbandry requirements for these animals are elucidated.

The purpose of the study reported here was to determine whether corn snakes (*Elaphe guttata*) exposed to UVB radiation have increased plasma 25-hydroxyvitamin D_3 concentrations, compared with control snakes that were not exposed to UVB radiation. The specific hypotheses were that corn snakes exposed to UVB radiation have higher plasma concentrations of 25-hydroxyvitamin D_3, compared with control snakes, and that the amount of UVB radiation emitted by commercially available fluorescent coil bulbs decreases over time.

Materials and Methods

The project was performed in accordance with the regulations established by the Institutional Animal Care and Use Committee at Louisiana State University.

Received May 23, 2007.
Accepted July 23, 2007.
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Snakes—Twelve adult male corn snakes were used in the study. The snakes were obtained from a commercial herpetoculturist. The snakes, which were housed individually in 47.3-L plastic containers[a] (26.7 × 15.8 cm × 10.8 cm), were acclimated to the laboratory for 7 days prior to the start of the study. The environmental temperature was maintained at 30.0° to 31.1°C (86° to 88°F). The snakes were not fed during the study period.

Procedures—After the initial 7-day acclimation period, a blood sample (0.5 mL) was collected from ventral tail vein or heart of each snake. This time point was designated as day 0. Blood samples were collected into tubes containing lithium heparin.[b] The samples were centrifuged within 60 minutes of collection. Plasma samples were placed on frozen gel packs and submitted to a veterinary diagnostic laboratory[c] for measurement of plasma 25-hydroxyvitamin D_3 concentration by use of a radioimmunoassay.

After the blood collection, snakes were allocated to 1 of 2 groups by use of random number generator. During the 4-week study period, corn snakes allocated to group 1 (n = 6) were not provided supplemental lighting, whereas those in group 2 (6) were provided supplemental lighting.[d] This lighting was generated from 2 bulbs placed 25.4 cm (10 inches) apart at a distance of 15.8 cm (6.2 inches) above the basking area. Light was provided for 12 continuous hours each day.

For group 2, UVA and UVB radiations generated by the coil bulbs were measured by use of a radiometer-photometer.[e] Levels of UVA and UVB radiation were measured on days 0, 7, 14, and 21. With the exception of day 0, when the reading was recorded immediately after the lights were activated, the measurements were made at the same time of the day in each successive week. Measurements were made from 5 points in each enclosure: at 2.54 cm (1 inch) from each bulb surface, at 15.8 cm (6.2 inches) directly beneath each bulb at the basking surface, and at the basking surface at a location below the midpoint between the 2 bulbs. The UVA and UVB radiation levels were measured in triplicate at each location by the same author (MKR). The arithmetic mean value of the 9 measurements obtained at the basking surface was used to determine the mean UV exposure in each enclosure. The arithmetic mean value of the 3 measurements obtained at each individual bulb surface was used for the statistical analysis.

The snakes were also weighed at the start and end of the study. Weight measurements were rounded to the nearest 0.1 g.

At the end of the 4-week study (day 28), another blood sample was collected for measurement of plasma 25-hydroxyvitamin D_3 concentrations. Sample collection, processing, and analysis were similar to the techniques used for the samples collected at the start of the study.

Statistical analysis—The data were evaluated by use of a Kolmogorov-Smirnov test and were normally distributed. The mean, SD, and range (minimum and maximum) are reported. A paired sample *t* test was used to determine whether plasma 25-hydroxyvitamin D_3 concentration and body weight changed in individual snakes during the study period. An unpaired *t* test was used to compare the mean differences for 25-hydroxyvitamin D_3 concentration and weight between treatment groups. A repeated-measures ANOVA was used to assess the quantity of UVA and UVB radiation generated at the bulb surfaces and the mean amount of radiation reaching the basking surface over the 4-week study. Commercially available software[f] was used for all analyses, and a value of $P \leq 0.05$ was considered significant.

Results

Between the start and end of the study period, the plasma 25-hydroxyvitamin D_3 concentration in group 2 increased significantly (63.0 ± 36.96 nmol/L and 196 ± 16.73 nmol/L, respectively; $P = 0.003$; **Table 1**). Group 1 snakes had no significant ($P = 0.987$) increase in 25-hydroxyvitamin D_3 concentration. At the end of the study (day 28), plasma concentration of 25-hydroxyvitamin D_3 in groups 1 and 2 differed significantly ($P < 0.001$).

Body weight in groups 1 and 2 did not differ significantly at the start ($P = 0.991$) or end of the study ($P = 0.944$). Therefore, weights were pooled and evaluated over time. At day 0, mean ± SD weight was 340.8 ± 19.90 g; at day 28, mean weight was 323.3 ± 18.68 g. This reduction in body weight was not significant ($P = 0.528$).

Although the amount of UVB at the bulb surface appeared to decrease over the course of the study, this

Table 1—Mean ± SD (range) plasma 25-hydroxyvitamin D_3 concentration in corn snakes that did not (group 1; n = 6) or did (group 2; 6) receive UV radiation via supplemental lighting at the start (day 0) and end (day 28) of a 4-week period.

Time point	Group	25-hydroxyvitamin D_3 (nmol/L)
Day 0	1	57.33 ± 45.59 (0–132)
	2	63.0 ± 36.96 (0–108)
Day 28	1	57.17 ± 15.28 (21–128)
	2	196.0 ± 16.73[a] (121–232)

[a]Value significantly ($P < 0.001$) different from value for group 1 at this time point.

Table 2—Amounts of UVB radiation (mean ± SD [range]) provided by 2 coil fluorescent bulbs in each of 6 enclosures housing a corn snake as measured on days 0, 7, 14, and 21. Assessments were made near the bulb surface* and at the basking surface.†

Location	Day	UVB‡ (μW/cm²)
Bulb	0	199.9 ± 41.73 (133.6–266.0)
	7	267.1 ± 169.3 (152.0–790.0)
	14	164.4 ± 48.47 (92.0–248.0)
	21	213.1 ± 58.93 (141.7–356.3)
Basking surface	0	10.19 ± 1.81 (8.03–12.87)
	7	9.66 ± 2.09 (7.67–12.43)
	14	9.72 ± 1.54 (7.833–12.43)
	21	10.37 ± 2.22 (8.467–14.17)

*Measured in triplicate at 2.54 cm (1 inch) from each bulb surface. †Measured 15.8 cm (6.2 inches) directly beneath each bulb at the basking surface and at the basking surface below the midpoint between the 2 bulbs; arithmetic mean value was calculated for each enclosure. ‡Data were obtained immediately after lights were activated on day 0 and 4 hours after activation at other time points (12 hours of continuous lighting/d).

Table 3—Amounts of UVA radiation (mean ± SD [range]) provided by 2 coil fluorescent bulbs in each of 6 enclosures housing a corn snake as measured on days 0, 7, 14, and 21. Assessments were made near the bulb surface* and at the basking surface.†

Location	Day	UVA‡ (μW/cm²)
Bulb§	0	1,274.0 ± 154.7 (1,003.0–1,497.0)
	7	1,382 ± 103.1 (1,257.0–1,581.0)
	14	1,032 ± 353.6 (336.0–1,356.0)
	21	1,239 ± 128.2 (903.3–1,381.0)
Basking surface	0	55.20 ± 3.59 (51.30–61.73)
	7	56.05 ± 9.38 (44.77–68.53)
	14	57.84 ± 8.40 (48.20–68.07)
	21	57.02 ± 6.98 (50.00–66.17)

§The amount of UVA measured at the bulb surface was significantly ($P = 0.006$) different throughout the study.

See Table 2 for remainder of key.

reduction was not significant ($P = 0.06$; **Table 2**). There was a significant ($P = 0.006$) difference in the amount of UVA measured at the bulb surface throughout the study **(Table 3)**. The amounts of UVA and UVB radiation reaching the basking surface were measured in triplicate directly under each of the 2 bulbs and also at the location below the midpoint between the 2 bulbs. These 9 values were averaged to calculate the mean UVA and UVB exposure for each snake. There was no change ($P = 0.240$) in UVB exposure during the course of the study. The mean UVA exposure did not differ ($P = 0.686$) throughout the study.

Discussion

The synthesis of vitamin D is the result of the photosynthetic conversion of 7-dehydrocholesterol to previtamin D_3 in the skin of vertebrates after exposure to UVB.[1,13] Previtamin D_3 is an unstable molecule that undergoes temperature-dependent isomerization to vitamin D_3.[13] The newly formed vitamin is transported to the liver where it is hydroxylated to 25-hydroxyvitamin D_3.[3,11] This represents the storage form of the hormone, which is bound to protein and circulated systemically.[11] The kidneys are responsible for the final conversion of 25-hydroxyvitamin D_3 to 1,25-dihydroxyvitamin D_3, which is the active form of the hormone.[1,3,11]

Vitamin D_3 can be obtained directly through the exposure of the skin to UVB radiation (wavelength, 290 to 320 nm) or via consumption of prey that has already performed the biosynthesis. The need for appropriate plasma concentrations of vitamin D_3 is so important that the skin of some nocturnal species of lizards, such as the Mediterranean house gecko (*Hemidactylus turcicus*), has developed the ability to synthesize the hormone under minimal light conditions, whereas other species modify their basking behaviors to compensate for variations in dietary amounts of vitamin D_3.[14,15] Not all animals have the same capacity to biosynthesize this hormone or extract it from their diet. Some carnivorous mammals, such as cats, are unable to biosynthesize any vitamin D and must rely totally on dietary intake[7]; by contrast, some lizards are reliant primarily on biosynthesis of the hormone.[16] Because snakes are carnivores, the general assumption among herpetoculturists and veterinarians was that these animals also derived vitamin D from their diet and that UVB radiation–induced synthesis was likely of no importance. The findings of the present study suggest otherwise.

In the present study, plasma 25-hydroxyvitamin D_3 concentration significantly increased in snakes that were exposed to supplemental lighting during a 4-week period, whereas control snakes that were not exposed failed to develop any change in plasma 25-hydroxyvitamin D_3 concentration. There was no significant increase in the weight of snakes exposed to UV radiation or the weight of snakes in the control group.

The coil fluorescent bulbs selected for use in our study were recently included in the selection of reptile full-spectrum lights available through the pet trade. Historically, fluorescent tubes have been the most popular lights for reptile husbandry.[17,18] Results of previous research[18] indicated that these bulbs provide adequate UVB radiation in the 290- to 320-nm wavelength range. In the experience of one of our group, the coil bulbs that we used provide a greater quantity of UVB radiation at the bulb surface and at distances of 6 and 12 inches from the surface than that provided by the more commonly used types of full-spectrum fluorescent tubes. It was for this reason that these bulbs were selected.

The UVB radiation generated by the bulbs used in our study did not decease over time. This is most likely because of the short duration of the study. There was a difference in the amount of UVA radiation released, although most of the difference was attributable to readings obtained from 2 bulbs during the third week of the study. It is possible that the measurements at those bulb surfaces were measured incorrectly. However, the same author (MKR) measured the UVA and UVB radiation levels throughout the study. This was done to minimize the likelihood of error. Interestingly, the amount of UVA radiation at the basking surface did not decrease during the study period.

Corn snakes have the potential for living for long periods in captivity and are popular as pets. It is therefore important to consider the effect of UV radiation on a snake's human caregivers. As newer, more powerful bulbs are developed, they may begin to pose health risks. To the authors' knowledge, this risk has yet to be evaluated.

The findings of the present study have provided important new information regarding the biosynthesis of vitamin D in corn snakes. For appropriate husbandry recommendations to be developed, further studies are required to determine the health and developmental consequences of provision or withholding of supplemental UVB radiation (wavelength, 290 to 320 nm) to these animals.

a. Rubbermaid Home Products, Fairlawn, Ohio.
b. Becton-Dickinson, Franklin Lakes, NJ.
c. Diagnostic Center for Population and Animal Health, East Lansing, Mich.
d. Sun-glow coil bulbs, Fluker Farms, Port Allen, La.
e. Model #1400, International Light Inc, Newburyport, Mass.
f. Prism, version 4.0 for Macintosh, GraphPad Software Inc, San Diego, Calif.

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