Effects of ultraviolet radiation on 25-hydroxyvitamin D_3 synthesis in red-eared slider turtles (*Trachemys scripta elegans*)

Mark J. Acierno, DVM; Mark A. Mitchell, DVM, PhD; Marlana K. Roundtree; Trevor T. Zachariah, DVM

Objective—To determine whether there are increased concentrations of 25-hydroxyvitaminn D_3 in red-eared slider turtles (*Trachemys scripta elegans*) after exposure to UV radiation.

Animals—12 yearling turtles recently removed from aestivation.

Procedures—Turtles were randomly allocated to 2 groups (6 turtles/group). An initial blood sample was collected from all turtles for measurement of 25-hydroxyvitamin D_3 concentrations. Turtles of 1 group were then provided no supplemental lighting, whereas turtles of the other group were exposed to full-spectrum coil bulbs at a distance of 22.86 cm. The UV-A and UV-B radiation generated by the supplemental lighting was measured by use of a radiometer-photometer at weekly intervals. Measurements were collected 2.54 and 22.86 cm from the bulb surface. The study was continued for a 4-week period. At the end of the study, a second blood sample was collected from all turtles for measurement of 25-hydroxyvitamin D_3.

Results—Mean ± SD 25-hydroxyvitamin D_3 concentrations differed significantly between turtles provided supplemental UV radiation (71.7 ± 46.9 nmol/L) and those not provided UV radiation (31.4 ± 13.2 nmol/L).

Conclusions and Clinical Relevance—Appropriate husbandry recommendations for raising and maintaining red-eared slider turtles should include use of sunlight that is unobstructed by UV-B filtering material or provision of an artificial source of UV-B radiation. (*Am J Vet Res* 2006;67:2046–2049)

Vitamin D_3 is an important hormone that is involved in numerous physiologic processes.[1,2] Although the most widely recognized function of this hormone is the regulation of calcium metabolism, which is needed for the development and maintenance of healthy bones, the reproductive success of some reptilian species has also been associated with optimized amounts of vitamin D_3.[3,4] Vitamin D_3 can be obtained through the diet or synthesized through exposure of the skin to UV-B (290 to 320 nm) radiation.[5,6] There is wide variation among vertebrate species between the need for dietary vitamin D_3 and the ability to synthesize the hormone.[7,8]

The source and function of vitamin D_3 have been examined in mammals and birds.[9] Studies[4,8,10,11] that have been performed in reptiles have focused on dietary requirements and synthesis during basking in various lizards. The authors are not aware of any studies to determine whether chelonians synthesize vitamin D_3 during basking or obtain vitamin D_3 from the diet. This is unfortunate because many of these species are raised in captivity as pets. Because these animals potentially have long lives, it is important that specific husbandry requirements be elucidated for them.

In addition to maintaining these animals as pets, there is an increased interest in the conservation of these reptiles. Currently, programs are underway to prepare juvenile chelonians in captivity for release to the wild. These programs typically release larger animals that are less likely to be preyed on. If exposure to UV-B radiation is required for chelonians to maximize serum 25-hydroxyvitamin D_3 concentrations, then full-spectrum lights capable of inducing production of this hormone should be used.

The purpose of the study reported here was to determine whether red-eared slider turtles (*Trachemys scripta elegans*) exposed to UV-B radiation under controlled conditions would have increased concentrations of 25-hydroxyvitamin D_3 concentrations, compared with concentrations for control turtles. We proposed to test 3 specific hypotheses. First, turtles exposed to UV-B radiation would have higher concentrations of 25-hydroxyvitamin D_3, compared with concentrations for control turtles. Second, turtles fed a diet that included amounts of vitamin D_3 would have an increase in 25-hydroxyvitamin D_3 concentrations over time. Finally, the amount of UV-B radiation generated by commercially available coil fluorescent bulbs would decrease over time.

Materials and Methods

Animals—Twelve yearling red-eared slider turtles were used in the study. The turtles were being housed outdoors for the winter of 2005 to 2006 and were removed from aestivation for the study. Turtles were allowed to acclimate in the laboratory environment for 7 days prior to the start of the study. This project was performed in accordance with the regulations established by the Institutional Animal Care and Use Committee at Louisiana State University (protocol No. 05-112).

Turtles were housed in 78 × 53 × 41.9-cm plastic containers.[a] Each container was filled with 35 L of chlorinated tap water. Two concrete stones were placed in each container to provide a basking area. The laboratory environment was maintained at a temperature of 30.0° to 31.1°C. Water temperature in each container was maintained at 25.5° to 26.6°C. Water in the containers was replaced every 48 hours with fresh chlorinated tap water; a water filtration system was not used. Each day, commercially available chow formulated for turtles[b] was provided in each container.

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From the Department of Veterinary Clinical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70810.
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Address correspondence to Dr. Acierno.

Table 1—Plasma concentrations of 25-hydroxyvitamin D_3 for 2 groups of red-eared slider turtles (6 turtles/group) at the beginning (day 0) and end (day 30) of the study in which 1 group of turtles received supplemental UV radiation and the other group was exposed to only ambient light.

Sample	Group	Mean ± SD (nmol/L)	Minimum (nmol/L)	Maximum (nmol/L)
Day 0	No UV radiation	11.2 ± 4.3	6.0	16.0
	Supplemental UV radiation	10.7 ± 3.4	5.0	14.0
Day 30	No UV radiation	31.4 ± 13.2*	15.0	44.0
	Supplemental UV radiation	71.7 ± 46.9*†	34.0	155.0

*Value differs significantly ($P = 0.001$) from value for the same group on day 0. †Value differs significantly ($P < 0.05$) from the value for the other group on day 30.

Experimental procedures—After the initial 7-day acclimation period, a blood sample (0.5 mL) was collected from the subcarapacial sinus of each turtle (day 0). Blood samples were stored in lithium heparin microtainers.[c] Blood samples were centrifuged within 60 minutes after collection. Plasma was harvested and frozen. Plasma samples were submitted on frozen gel packs to a university laboratory[d] for measurement of 25-hydroxyvitamin D_3 concentrations.

After collection of the initial blood sample, turtles were allocated into 2 groups (6 turtles/group) by use of a random number generator. Turtles of 1 group were not provided supplemental lighting, whereas turtles of the other group were provided with supplemental lighting. Lights[e] used to provide supplemental lighting were positioned at a height of 22.86 cm directly over the basking stones. Lighting was provided for 12 continuous hours each day.

Radiation (UV-A and UV-B) generated by the coil bulbs was measured by use of a radiometer-photometer.[f] Measurements were collected at a distance of 2.54 and 22.86 cm from the bulb surface. Amounts of UV-A and UV-B radiation were measured in triplicate at each distance, and the arithmetic mean value was calculated and used for statistical analysis. Measurements of UV-A and UV-B were also collected at the surface of the basking stone for the turtles that did not receive supplemental lighting. Amounts of UV-A and UV-B were measured on a weekly basis at the same time of day during each successive week, with the exception of the first measurement, which was recorded immediately after the lights were turned on.

The turtles were weighed weekly. Weight measurements were rounded to the nearest 0.1 g. Weight measurements were collected after the bulbs had been on for 4 hours.

The study was continued for 4 weeks. At the end of that period, a second blood sample was collected (day 30) from each turtle for use in measuring 25-hydroxyvitamin D_3 concentrations. Collection of blood samples, processing, and shipment to a university laboratory for testing were similar to the techniques described for the blood samples obtained on day 0.

Statistical analysis—Distribution of the data was evaluated by use of the Shapiro-Wilk test. Mean, SD, minimum, and maximum values were reported for data that had a normal distribution, whereas the median, 10th to 90th percentiles, minimum, and maximum values were reported for data that did not have a normal distribution. Data that were not normally distributed were logarithmically transformed for parametric analysis.

A paired-sample *t* test was used to determine within-subject differences throughout the study for 25-hydroxyvitamin D_3 concentrations and body weight. An unpaired *t* test was used to assess differences in 25-hydroxyvitamin D_3 concentrations and body weight between turtles provided UV radiation and those that did not receive UV radiation. A repeated-measures ANOVA was used to assess the quantity of UV-A and UV-B radiation generated at the bulb surface and the surface of the basking stone during the 4-week study. When differences were found, post hoc comparisons were made by estimating the marginal means. A value of $P \leq 0.05$ was used to determine significance. A commercially available statistical program[g] was used to analyze the data.

Table 2—Amount of UV-B radiation measured at the bulb surface and surface of the basking stone during the course of the study.

Location	Day	Mean ± SD ($\mu W/cm^2$)	Minimum ($\mu W/cm^2$)	Maximum ($\mu W/cm^2$)
Bulb*	0	686 ± 109.4[a,b,c]	495	803
	7	488 ± 67.5[a]	400	564
	14	414 ± 67.3[b]	309	489
	21	426 ± 58.4[c]	348	489
Basking stone†	0	14.1 ± 1.7[e,f,g]	12.1	16.1
	7	18.6 ± 3.3[e]	14.0	24.3
	14	17.3 ± 2.3[f]	13.4	19.9
	21	18.2 ± 3.6[g]	15.0	24.9

*Radiation was measured 2.54 cm from the bulb surface. †Radiation was measured 22.86 cm from the bulb surface.
Day 0 = First day of the study.
[a-g]Values with different superscript letters differ significantly ($P < 0.05$).

Results

Concentrations of 25-hydroxyvitamin D_3 differed significantly ($P = 0.001$) between days 0 and 30 for both groups (**Table 1**). Mean ± SD concentrations of 25-hydroxyvitamin D_3 differed significantly between turtles provided supplemental UV radiation (71.7 ± 46.9 nmol/L) and turtles that were not provided supplemental UV radiation (31.4 ± 13.2 nmol/L).

Body weight did not differ significantly ($P = 0.50$) between the 2 groups of turtles. Therefore, body weights for both groups were pooled and evaluated over time. Body weight increased significantly ($P = 0.001$) during the course of the study. At the beginning of the study, mean ± SD body weight for the turtles was 115.9 ± 23.4 g (minimum, 88 g; maximum, 170 g). At the end of the study, body weight of the turtles had increased by 10% (mean, 128.6 ± 21.8 g; minimum, 102 g; and maximum, 174 g).

We detected significant differences in the amount of UV-B radiation at the bulb surface ($F = 20.9$; $P = 0.006$) and surface of the basking stone ($F = 11.9$; $P = 0.002$) during the course of the study (**Table 2**). There was also a significant ($F = 89.8$; $P < 0.001$) difference in the amount of UV-A radiation at the bulb surface during the course of the study (**Table 3**). However,

Table 3—Amount of UV-A radiation measured at the bulb surface and surface of the basking stone during the course of the study.

Location	Day	Mean ± SD ($\mu W/cm^2$)	Minimum ($\mu W/cm^2$)	Maximum ($\mu W/cm^2$)
Bulb*	0	3,463 ± 248.3[a]	3,160	3,800
	7	2,595 ± 160.6[b]	2,340	2,750
	14	2,438 ± 258.0[b]	2,160	2,910
	21	2,318 ± 104.2[b]	2,180	2,470
Basking stone†	0	73.7 ± 9.5	61.9	82.9
	7	73.5 ± 8.6	65.6	84.5
	14	76.4 ± 6.1	69.7	85.5
	21	78.1 ± 9.7	65.0	91.0

See Table 2 for key.

there was not a significant ($F = 0.4$; $P = 0.70$) difference in UV-A radiation at the surface of the basking stone during the study. The amount of UV-B (< 0.01 W/cm^2) and UV-A (< 10 W/cm^2) radiation measured at the surface of the basking stone for turtles provided only ambient light (ie, no supplemental radiation) was negligible.

Discussion

All vitamin D is ultimately the result of the photosynthetic conversion of 7-dehydrocholesterol to previtamin D_3 in the skin of vertebrates exposed to UV-B.[1,12] Previtamin D_3 is an unstable molecule that undergoes temperature-dependent isomerization to become vitamin D_3.[12] Newly formed vitamin is transported to the liver, where it is hydroxylated to form 25-hydroxyvitamin D_3.[3,11] This represents the storage form of the hormone, which is bound to protein for systemic circulation.[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 exposure of the skin to UV-B radiation (290 to 320 nm) or through 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 in minimal light conditions, whereas other species can modify their basking to compensate for variations in dietary amounts of vitamin D_3.[13-15] Interestingly, animals vary in their capacity to photosynthesize or extract this important hormone from their diet. Some carnivorous mammals, such as cats, are unable to photosynthesize vitamin D and must rely totally on dietary sources, whereas some lizards rely primarily on photosynthesis to produce vitamin D.[7,16]

In the study reported here, 25-hydroxyvitamin D_3 concentrations increased significantly in all turtles from the time they were removed from aestivation until the end of the study. Although we cannot eliminate ambient UV-B radiation as a possible cause for this increase, half of the turtles had exposure to only insubstantial amounts of UV-B radiation (< 0.01 W/cm^2). Therefore, absorption of vitamin D_3 from the diet is the most likely explanation for the observed increase. Turtles that were exposed to supplemental UV-B radiation had significantly higher 25-hydroxyvitamin D_3 concentrations than the turtles that did not receive supplemental lighting.

The information reported here reinforces results of a study[17] of another species of freshwater turtle (*Emydura signata*) in which it was reported that those turtles were commonly observed basking, but their body temperature was in thermoconformity with the water. Considering the thermal conductivity of water, compared with that of air, it seems logical that an aquatic species would gain little thermal benefit from basking for relatively short periods. Thus, it would appear that in at least some freshwater turtles, sun-seeking behavior is a strategy for vitamin D synthesis rather than a thermoregulatory adaptation.

Body weight of all turtles increased significantly from the time they were removed from aestivation until the end of the study. There was no difference in body weight between turtles exposed to supplemental UV-B and turtles that did not receive supplemental lighting. Vitamin D_3 is important in the development and maintenance of healthy bones. Because these turtles were all from the same colony and had been housed outdoors, it seems unlikely that measurable differences in body weight from bone demineralization or changes in general health would be detectable in such a short study (ie, 4 weeks). Even had the study period been longer, we would have needed to conduct a more sensitive measure of bone density to detect weight attributable to demineralization of bone.

The coil fluorescent bulbs used in the study were the most current addition to the selection of full-spectrum lights available for reptiles. Historically, fluorescent tubes have been the most commonly used lights.[18,19] Those bulbs provide adequate UV-B radiation in the range of 290 to 320 nm.[19] In the experience of one of the authors, the coil bulbs used in the study reported here generated greater quantities of UV-B radiation at the bulb surface and distances of 15 and 30 cm, compared with the amount generated by the more common full-spectrum fluorescent tubes. It was for this reason that the coil fluorescent bulbs were selected for use in the study.

The bulbs produced 40% and 27% less UV-B and UV-A radiation, respectively, at the bulb surface during the course of the study. Investigators in another study[h] also found that the quantities of UV-B radiation produced by fluorescent tubes significantly decreased over time. Interestingly, the amount of UV-A radiation at the surface of the basking stone did not decrease during the course of the study. One possibility is that the initial measurements for the bulbs were obtained as soon as they were energized, and they may not have had sufficient time to generate maximum output. In our experience, these bulbs do not necessarily produce a maximal quantity of radiation immediately after being turned on. It is also possible that initial measurements at the surface of the basking stone were conducted incorrectly. However, the same 2 investigators (MJA and MKR) measured the UV-A and UV-B radiation throughout the study in an effort to minimize the chance of error. If either scenario was true, then the difference at the bulb surface would have been even greater.

Currently, we are evaluating the long-term production of UV-B and UV-A radiation by these bulbs to determine their spectral characteristics and longevity. Interestingly, UV-B radiation at the surface of the basking rock generated by the coil florescent tube was consistent with UV-B radiation measured during overcast conditions in early May in Louisiana. Red-eared slider turtles have the potential for living long periods in captivity and are popular as pets. Therefore, it is important to consider the effect of UV radiation on the human caregivers. As newer, more powerful bulbs are developed, they may begin to pose health risks to humans. To the authors' knowledge, this risk has not yet been evaluated.

The study reported here provides important new information regarding the husbandry of red-eared slider turtles. It would appear that the appropriate husbandry recommendations for raising and maintaining these aquatic turtles should include sunlight that is unobstructed by UV-B filtering material or an artificial source of UV-B radiation (290 to 320 nm) that is located no more than 23 cm from the basking area.

a. Rubbermaid Home Products, Fairlawn, Ohio.
b. Aquatic turtle diet, Fluker Farms, Port Allen, La.
c. Microtainer tube with lihium heparin, Becton-Dickinson, Franklin Lakes, NJ.
d. Diagnostic Center for Population and Animal Health, College of Veterinary Medicine, Michigan State University, East Lansing, Mich.
e. Sun-glow coil bulbs, Fluker Farms, Port Allen, La.
f. Model No. 1400, International Light Inc, Newburyport, Mass.
g. SPSS, version 11.0, SPSS Inc, Chicago, Ill.
h. Bernard JB. *Spectral irradance of fluorescent lamps and their efficacy for promoting vitamin D synthesis in herbivorous reptiles.* PhD thesis, Department of Science, Michigan State University, East Lansing, Mich, 1995.

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