Plasma 25-hydroxyvitamin D_3 concentrations in Hermann's tortoises (*Testudo hermanni*) exposed to natural sunlight and two artificial ultraviolet radiation sources

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Objective—To determine the effect of various UVB radiation sources on plasma 25-hydroxyvitamin D_3 concentrations in Hermann's tortoises (*Testudo hermanni*).

Animals—18 healthy Hermann's tortoises.

Procedures—Tortoises were exposed to sunlight in an outdoor enclosure located in the natural geographic range of Hermann's tortoises (n = 6 tortoises) or a self-ballasted mercury-vapor lamp (6) or fluorescent UVB-emitting lamp (6) in an indoor enclosure for 35 days. Plasma samples were obtained from each tortoise on the first (day 0) and last (day 35) days of the study, and concentrations of 25-hydroxyvitamin D_3 were determined. Amount of UVB radiation in enclosures was measured.

Results—Mean ± SD plasma 25-hydroxyvitamin D_3 concentrations for tortoises exposed to the mercury-vapor and fluorescent lamps were significantly lower on day 35 (155.69 ± 80.71 nmol/L and 134.42 ± 51.42 nmol/L, respectively) than they were on day 0 (368.02 ± 119.34 nmol/L and 313.69 ± 109.54 nmol/L, respectively). Mean ± SD plasma 25-hydroxyvitamin D_3 concentration for tortoises exposed to sunlight did not differ significantly between days 0 (387.74 ± 114.56 nmol/L) and 35 (411.51 ± 189.75 nmol/L). Mean day 35 plasma 25-hydroxyvitamin D_3 concentration was significantly higher for tortoises exposed to sunlight versus those exposed to mercury-vapor or fluorescent lamps. Sunlight provided significantly more UVB radiation than did the mercury-vapor or fluorescent lamps.

Conclusions and Clinical Relevance—Plasma 25-hydroxyvitamin D_3 concentrations differed between tortoises exposed to sunlight and those exposed to artificial UVB sources. Exposure to sunlight at a latitude similar to that of the natural geographic range is recommended for healthy and calcium-deficient tortoises. (*Am J Vet Res* 2012;73:1781–1786)

The primary function of vitamin D_3 in vertebrates is maintenance and regulation of calcium homeostasis.[1] Vitamin D_3 aids bone mineralization via increasing uptake of calcium from the intestinal tract.[2,3] This function of vitamin D_3 is especially important in captive reptiles because of the high incidence of calcium deficiency–related pathological changes (ie, metabolic bone disease) in these animals.[4,5] In addition, chameleons with adequate circulating concentrations of vitamin D have better reproductive success than those without adequate circulating concentrations of vitamin D.[6] The importance of vitamin D_3 in reptiles is indicated by results of studies[7–9] in which captive and wild reptiles voluntarily exposed themselves to UVB radiation.

Animals can obtain vitamin D_3 from food or via synthesis in the skin.[10–13] Photolysis of 7-dehydrocholesterol to previtamin D_3 in skin is dependent on UV radiation with a wavelength between 280 and 320 nm.[1,14] Previtamin D_3 undergoes successive temperature-dependent isomerization steps to form vitamin D_3.[1,15] Vitamin D obtained from food or via synthesis is converted by sterol 25-hydroxylase to 25-hydroxyvitamin D_3 in the liver.[16–18] The active form of vitamin D (1,25-dihydroxyvitamin D_3) is synthesized via 1-hydroxylation of 25-hydroxyvitamin D_3 in the kidneys.[19] Among assays for detection of vitamin D and its metabolites, assays for determination of serum 25-hydroxyvitamin D_3 concentrations are the most useful for determination of the vitamin D status of an animal because that metabolite has a longer half-life than other metabolites.[20]

Reptiles bask in UV light for thermoregulatory purposes and to increase vitamin D production.[8,21] In captivity, plasma concentrations of 25-hydroxyvitamin D_3 increase in basking reptiles of several species when they are exposed to UVB radiation.[7,13,22–26] To the au-

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thors' knowledge, this response has not been identified for any terrestrial chelonian species, despite the natural basking habits of tortoises[27] and the large number of tortoises kept as pets worldwide. Moreover, metabolic bone disease may be the most common medical disorder of captive chelonians.[28] Exposure of veiled chameleons (*Chameleo calyptratus*) to adequate UVB radiation is important for prevention of metabolic bone disease[13]; exposure to adequate UVB radiation and provision of adequate dietary calcium are likely important for prevention of this disease in other reptiles.

Most reptile curators and experienced herpetoculturists believe that natural lighting is the best type of light for reptiles.[29] Various sources of UVB radiation are available for captive reptiles. Some of these UVB sources (eg, self-ballasted mercury-vapor lamps) also produce substantial amounts of thermal energy. Many anecdotal accounts suggest use of this type of lamp can reverse nutritional metabolic bone disease in reptiles.[30] Mercury-vapor lamps produce different quantities of UVB radiation than do UVB-emitting fluorescent lamps.[31] Unfortunately, equal irradiance values of different types of light sources do not indicate those light sources have the same potential for increasing vitamin D synthesis in reptiles.[32] Results of a recent study[31] indicate bearded dragons (*Pogona vitticeps*) exposed to a compact UVB fluorescent bulb producing low amounts of UVB radiation had higher circulating concentrations of 25-hydroxyvitamin D_3 than did bearded dragons exposed to light sources producing high amounts of UVB radiation. That finding suggests the biological effects of lamps regarding vitamin D production in reptiles cannot be predicted on the basis of values of radiometric variables. Determination of plasma 25-hydroxyvitamin D_3 concentration may be a more appropriate method for evaluation of the biological effects of UVB radiation sources; such biological effects could be influenced by variables other than irradiance of lamps (eg, production of a pattern and area of thermal radiation similar to that of natural sunlight).

The objective of the study reported here was to determine whether circulating concentrations of plasma 25-hydroxyvitamin D_3 in terrestrial chelonians are influenced by the type of UVB radiation source to which those animals are exposed. The hypotheses were that plasma 25-hydroxyvitamin D_3 concentrations in Hermann's tortoises (*Testudo hermanni)* would be influenced by exposure to UVB radiation and that Hermann's tortoises exposed to natural sunlight or a mercury-vapor lamp would have higher plasma concentrations of 25-hydroxyvitamin D_3 than would Hermann's tortoises exposed to a fluorescent UVB-emitting lamp.

Materials and Methods

Animals—Eighteen client-owned healthy subadult (age range, 3 to 8 years) Hermann's tortoises were used in the study. All of the tortoises had hibernated outdoors during the winter of 2010 to 2011. At the end of the hibernation period, the tortoises were placed in an outdoor enclosure for 20 days prior to the start of the study (acclimatization period). For all tortoises, the study began on April 25, 2011 (day 0). The study was performed in compliance with the directive 2010/63/EU of the European Parliament and the Council of the European Union. The owners gave written informed consent for participation of tortoises in the study and for collection of plasma samples.

Each tortoise was allocated to 1 of 3 groups (6 tortoises/group) via a restricted randomization procedure by use of a table of random numbers. The UVB radiation sources for the tortoises were a self-ballasted mercury-vapor lamp (n = 6 tortoises), a fluorescent UVB-emitting lamp (6), or natural sunlight (6). Tortoises housed indoors (self-ballasted mercury-vapor lamp and fluorescent UVB-emitting lamp groups) were placed in open-top plastic boxes[a] (vivariums; 120 × 60 × 18.5 cm). A commercially available self-ballasted mercury-vapor lamp[b] was the source of light and heat for tortoises in that vivarium. That lamp was oriented vertically and placed 30 cm above the floor of 1 side of the box, in accordance with the manufacturer's instructions. For the vivarium-housed tortoises exposed to the fluorescent UVB-emitting lamp, the UVB fluorescent lamp[c] was the light source and an infrared lamp[d] was the heat source. In that vivarium, the fluorescent bulb was oriented diagonally with the end of the longitudinal axis 21 cm above the floor of the vivarium. The infrared lamp was oriented so that it heated the same portion of the vivarium that was exposed to UVB radiation via the fluorescent lamp. For each vivarium, light and heat were provided for 12 continuous hours each day (8:00 AM to 8:00 PM). Shelters were available for tortoises in each vivarium so that the animals could easily regulate their exposure to UVB radiation and heat. The authors observed the tortoises at 8:00 AM, 12:00 PM, 4:00 PM, and 8 PM during 5 randomly selected days of the study to verify that they had physiologic bimodal basking pattern behavior.[33] Environmental temperature in the indoor area mercury-vapor and fluorescent lamp vivariums were kept was regulated so that it was similar to the temperature in the outdoor area where the tortoises exposed to natural sunlight were housed. In the indoor area, environmental temperature was maintained at 22 ± 1°C during the day (8:00 AM to 8:00 PM) and at 19 ± 1°C during the night (8:00 PM to 8:00 AM). Environmental temperatures in basking zones (UVB radiation coverage areas) were recorded at 12:00 PM each day with an infrared thermometer[e]; temperatures in the basking zones never exceeded 37°C during the study.

The tortoises exposed to natural sunlight were housed in an outdoor, sunlight-exposed enclosure located in the natural geographic range of Hermann's tortoises.[27] To simulate natural conditions, these tortoises foraged on vegetation naturally growing in the enclosure. The tortoises kept indoors were fed that same vegetation; these plants were collected each day in the vicinity of the outdoor enclosure to minimize differences in diet among the 3 groups of tortoises. The diet consisted of Asteraceae (dandelions [*Taraxacum officinale*]), Fabaceae (clover [*Trifolium* spp]), Malvaceae (mallow [*Malva* spp]), Plantaceae (ribwort plantain [*Plantago lanceolata*]), Oxalidaceae (wood sorrels [*Oxalis* spp]), and Rosaceae (creeping cinquefoil [*Potentilla reptans*]). Water was provided to the tortoises ad libitum.

Table 1—Mean ± SD (range) 25-hydroxyvitamin D_3 concentrations in plasma samples obtained on study days 0 and 35 from Hermann's tortoises (*Testudo hermanni*) exposed to a self-ballasted mercury-vapor lamp (n = 6 tortoises), UVB-emitting fluorescent and infrared heat lamps (6), or natural, unfiltered sunlight (6) as light and heat sources and amounts of UVB radiation emitted by each of those sources on days 0 through 35.

		Plasma 25-hydroxyvitamin D_3 concentration	
Light and heat source	**UVB (μW/cm²)***	**Day 0 (nmol/L)**	**Day 35 (nmol/L)**
Self-ballasted mercury-vapor lamp	11.8 ± 1.2 (10–14)†	368.02 ± 119.34 (205.12–523.06)	155.69 ± 80.71 (77.23–304.88)‡
Fluorescent UVB and infrared heat lamps	24.7 ± 2.4 (18–29)†	313.69 ± 109.54 (145.98–423.98)	134.42 ± 51.42 (50.14–179.6)‡
Natural sunlight	205.3 ± 53.6 (87–278)	387.74 ± 114.56 (200.76–541.28)	411.51 ± 189.7 (119.36–667.04)§

*Amount of UVB radiation was determined at the basking site in each enclosure at 9:00 AM (1 hour after lamp activation; mercury-vapor and fluorescent lamps) or 1:30 PM (natural sunlight) on days 0 through 35, and mean ± SD values were calculated. †Amount of UVB radiation is significantly ($P < 0.01$) different from the value for natural sunlight. ‡Within a light and heat source group, the plasma concentration of 25-hydroxyvitamin D_3 on day 35 is significantly ($P < 0.05$) lower than the value on day 0. §Value on day 35 is significantly ($P < 0.01$) higher than values for other groups of tortoises on day 35.

Experimental procedures—On day 0, a blood sample (0.3 mL) was collected from a jugular vein of each tortoise. Blood samples were obtained from the subcarapacial sinus when blood could not be obtained from the jugular vein within 2 attempts. Blood samples were collected in tubes containing lithium heparin[f] and were centrifuged (1,650 × *g* for 5 minutes) within 30 minutes after collection. Plasma was harvested and stored immediately at –25°C. Another plasma sample was obtained from each tortoise on day 35 of the study by use of that same technique. Plasma 25-hydroxyvitamin D_3 concentrations were estimated by use of an enzyme immunoassay.[g]

During the study, the amount of UV radiation with a wavelength between 280 and 320 nm to which tortoises were exposed was measured by use of a digital portable radiometer.[h] The amount of UV radiation was measured at the basking site in each enclosure.[9] To determine variations in amount of UVB radiation at the basking areas in the enclosures during the daytime, measurements were obtained every 30 minutes on days 0 and 35 and mean ± range values were calculated. To determine variations in amount of UVB radiation at the basking areas in the enclosures during the 35-day study period, measurements were obtained once every day in the outdoor enclosure at the time of peak UVB radiation (1:30 PM) and once every day in the indoor vivaria 1 hour after lamp activation (9:00 AM).

Statistical analysis—Statistical analysis was performed with commercially available software.[i] Plasma concentrations of 25-hydroxyvitamin D_3 were evaluated for normality via the Shapiro-Wilk test. Plasma concentrations of 25-hydroxyvitamin D_3 on days 0 and 35 and daily UVB measurements among groups of tortoises were analyzed via a 1-way ANOVA and a Tukey honestly significant difference post hoc comparison test. A *t* test for correlated samples was used to determine differences between day 0 and day 35 plasma 25-hydroxyvitamin D_3 concentrations for tortoises in each group. Values of $P < 0.05$ were considered significant.

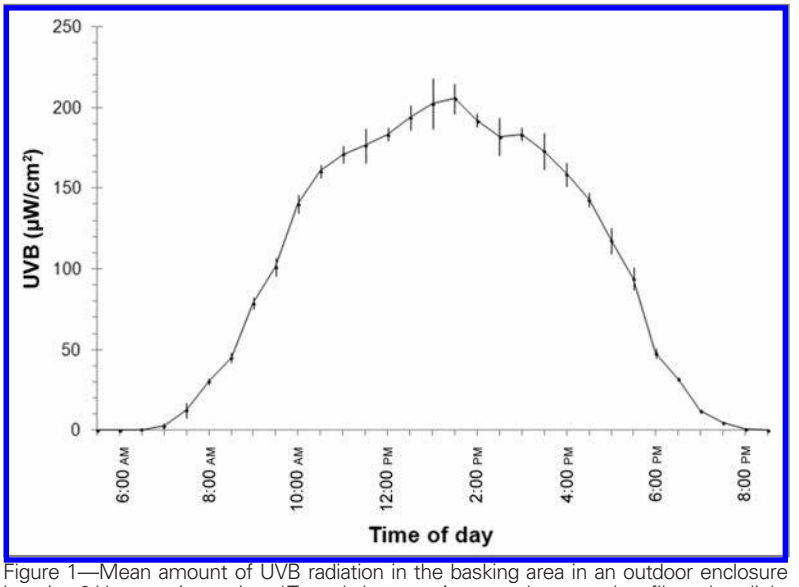

Figure 1—Mean amount of UVB radiation in the basking area in an outdoor enclosure housing 6 Hermann's tortoises (*Testudo hermanni*) exposed to natural, unfiltered sunlight from 6:00 AM through 8:30 PM on days 0 and 35 of the study. Error bars represent range.

Results

Mean ± SD day 0 plasma 25-hydroxyvitamin D_3 concentration for all tortoises was 356.49 ± 112.32 nmol/L; the distribution of values was normal ($P < 0.01$), and no significant ($P = 0.52$) differences in day 0 plasma 25-hydroxyvitamin D_3 concentration were detected among the 3 groups of tortoises. Mean ± SD plasma 25-hydroxyvitamin D_3 concentrations were significantly lower on day 35 than they were on day 0 for tortoises exposed to the self-ballasted mercury-vapor lamp (day 0 value, 368.02 ± 119.34 nmol/L; day 35 value, 155.69 ± 80.71 nmol/L; $P = 0.017$) and tortoises exposed to the fluorescent UVB-emitting lamp (day 0 value, 313.69 ± 109.53 nmol/L; day 35 value, 134.42 ± 51.42 nmol/L; $P = 0.007$; **Table 1**). Concentrations of 25-hydroxyvitamin D_3 in plasma samples obtained on days 0 (387.74 ± 114.56 nmol/L) and 35 (411.51 ± 189.75 nmol/L) from tortoises exposed to natural sunlight were not significantly ($P = 0.64$) different. The ANOVA results indicated day 35 plasma 25-hydroxyvitamin D_3 concentrations differed significantly ($P = 0.002$) among the 3 groups of tortoises; results of post hoc analysis indicated values for tortoises exposed

to natural sunlight were significantly ($P < 0.01$) higher than they were for tortoises exposed to mercury-vapor or fluorescent lamps.

Mean ± SD UVB radiation outputs of the mercury-vapor lamp (at 9:00 AM on days 0 through 35), the fluorescent lamp (at 9:00 AM on days 0 through 35), and natural sunlight (at 1:30 PM on days 0 through 35) were 11.8 ± 1.2 μW/cm^2, 24.7 ± 2.4 μW/cm^2, and 205.3 ± 53.6 μW/cm^2, respectively (Table 1). The ANOVA results indicated the amount of UVB radiation emitted by the 3 UVB sources differed significantly ($P < 0.001$); results of post hoc analysis indicated natural sunlight provided significantly ($P < 0.01$) more UVB radiation than did mercury-vapor or fluorescent lamps. Amounts of UVB radiation emitted by the mercury-vapor and fluorescent lamps were not significantly different. The amount of UVB radiation in the basking area of the outdoor enclosure exposed to natural sunlight on days 0 and 35 from 6:00 AM through 8:30 PM was determined (**Figure 1**). The mercury-vapor and fluorescent lamps provided constant amounts of UVB radiation (ie, substantial variations during the day were not detected). The 3 groups of tortoises had similar basking patterns; none of the tortoises avoided the basking zone.

Discussion

Results of this study supported the hypothesis that circulating 25-hydroxyvitamin D_3 concentrations in Hermann's tortoises are influenced by exposure to UVB radiation. However, the hypothesis that tortoises exposed to natural sunlight or a mercury-vapor lamp would have higher circulating concentrations of 25-hydroxyvitamin D_3 than tortoises exposed to a fluorescent UVB-emitting lamp was not completely supported. To the authors' knowledge, this is the first study in which plasma 25-hydroxyvitamin D_3 concentrations were determined for terrestrial chelonians exposed to natural sunlight in an environment within their natural geographic range. Mean circulating 25-hydroxyvitamin D_3 concentrations in *Testudo* spp tortoises maintained outdoors in the United Kingdom without an additional source of UVB radiation,[34] adult desert tortoises (*Gopherus agassizii*) housed in outdoor pens in the Mojave desert, and African spurred tortoises (*Geochelone sulcata*) and juvenile desert tortoises kept in indoor enclosures are 28.41, 20.5, and < 12.5 nmol/L, respectively.[35] Compared with chelonians in those other studies, the tortoises in this study had a higher mean concentration of 25-hydroxyvitamin D_3 (356.49 nmol/L) at the start of the study. Although this difference may be attributable to species differences, the tortoises in the present study may have been exposed to more UVB radiation (from sunlight) prior to the start of the study versus tortoises in those other studies. In addition, the diet for tortoises in the present study (naturally growing vegetation) may have contributed to the high circulating 25-hydroxyvitamin D_3 concentrations in these animals. Plants of several types, such as alfalfa and perennial ryegrass, contain ergosterol (provitamin D_2).[12,36] Theoretically, ingested vitamin D and vitamin D produced in animals via exposure to UV radiation can be differentiated via chromatographic separation of 25-hydroxyvitamin D_2 and 25-hydroxyvitamin D_3.[37,38] To the author's knowledge, no studies have been conducted in which this technique was used for determination of vitamin D concentrations in reptiles. Results of other studies[13,24] indicate certain reptiles (eg, red-eared sliders [*Trachemys scripta elegans*] and veiled chameleons) meet their need for vitamin D via both dietary sources and production in skin. Although the ability of reptiles to regulate dietary vitamin D intake is not known,[9] results of a recent study[26] indicate bearded dragons with low circulating 25-hydroxyvitamin D_3 concentrations preferentially eat food that is rich in vitamin D. Despite this finding, excessive dietary vitamin D intake has toxic effects in reptiles,[39,40] birds,[41] mammals other than humans,[42–44] and humans.[45,46] Plasma 25-hydroxyvitamin D_3 concentrations in tortoises in the present study that were kept outdoors were higher than those previously reported for other chelonians.[24,34,47] The high plasma 25-hydroxyvitamin D_3 concentration in tortoises in the present study might have had toxic effects or have led to development of hypervitaminosis D, but this seemed unlikely because healthy tortoises were included, tortoises were exposed to sunlight in their natural geographic range, tortoises were provided protective shelters against UVB radiation, and endogenous production theoretically does not cause an excess of vitamin D_3. Previtamin D_3 can undergo thermal isomerization to form vitamin D_3 or can undergo photochemical isomerization to form biologically inert products (ie, lumisterol and tachysterol), which limits synthesis of previtamin D_3 in skin during prolonged exposure to UVB radiation.[48] Because exposure to UV radiation is a more efficient method for increasing circulating 25-hydroxyvitamin D_3 concentrations in reptiles versus supplementation of the diet with vitamin D[26,49] and because reptiles seem to regulate exposure to UV light better than they regulate dietary intake of vitamin D, stimulation of endogenous vitamin D_3 production via exposure to UV light may be preferable to addition of supplemental vitamin D to food for reptiles. However, exposure to UVB radiation can cause adverse effects in reptiles[50]; therefore, use of appropriate UVB lamps and careful adherence to the manufacturer's instructions are suggested.

Results of the present study indicated significant differences in circulating vitamin D concentrations between tortoises exposed to natural sunlight and those exposed to artificial UVB sources. This finding was attributed to differences in UVB emission of natural sunlight versus that of artificial lamps; it was considered unlikely that other factors caused this result because the conditions in which the groups of tortoises were kept were similar, other than the UVB radiation sources for the 3 enclosures. The amount of UVB radiation in the outdoor enclosure exposed to sunlight was approximately 8 times as high as it was in the vivarium with fluorescent UVB-emitting and infrared lamps and 17 times as high as it was in the vivarium with the self-ballasted mercury-vapor lamp. Differences were detected between the 2 types of artificial lamps regarding amount of UVB radiation emitted, but values were not significantly different. Plasma 25-hydroxyvitamin D_3 concentrations were not significantly different between tortoises exposed to each type of artificial lamp. Results

of this study suggested a fluorescent UVB-emitting lamp and a thermal source (eg, infrared lamp or ceramic heater) stimulated production of 25-hydroxyvitamin D_3 in tortoises as well as a mercury-vapor lamp did.

In the present study, distances between the floors of the vivariums and the lamps were selected in accordance with the minimum distances indicated in the manufacturer's instructions; a decrease in those distances would likely have increased the UVB radiation received by the tortoises but could possibly have caused ocular and dermal lesions.[50] Although minimum adequate circulating concentrations of 25-hydroxyvitamin D_3 have not been determined for tortoises, the manufacturer's recommendations for safe use of lamps may not allow adequate 25-hydroxyvitamin D_3 synthesis in tortoises. Further studies would be necessary to determine whether changes in lamp location and distance from vivarium floors would increase UVB radiation received by tortoises and increase circulating 25-hydroxyvitamin D_3 concentrations in tortoises without causing harm.

To the authors' knowledge, the effects of sun exposure in tortoises at different latitudes have not been evaluated. Comparison of results of the present study with those of another study[34] in which Hermann's tortoises were exposed to sunlight in the United Kingdom suggested that circulating concentrations of 25-hydroxyvitamin D_3 in tortoises exposed to sunlight depend on latitude, as they do for humans.[51] Moreover, plasma 25-hydroxyvitamin D_3 concentrations in Hermann's tortoises exposed to artificial UVB sources in the present study were higher than those in tortoises exposed to sunlight at northern latitudes in that other study.[34] These findings suggested that tortoises kept in a location other than their natural geographic range may more effectively synthesize vitamin D when they are exposed to UVB lamps versus natural sunlight, depending on the latitude at which they are kept and other related environmental factors (eg, climate). Because tortoises were not deprived of UVB radiation or dietary vitamin D prior to the start of the present study, the high plasma 25-hydroxyvitamin D_3 concentration in the tortoises kept indoors may have been attributable to exposure to natural sunlight before the study started. The duration of this study was determined on the basis of the half-life of circulating 25-hydroxyvitamin D_3 in humans (approx 3 weeks),[20] although the half-life of circulating 25-hydroxyvitamin D_3 in black-throated monitors (*Varanus albigularis*) is 128 to 139 days.[52] However, exposure to sunlight in the natural geographic range of tortoises in the present study induced greater production of 25-hydroxyvitamin D_3 versus exposure to artificial UVB sources.

Results of the present study provided important information regarding biosynthesis of vitamin D in Hermann's tortoises and indicated biosynthesis of vitamin D in these tortoises was UVB dependent. Additional studies conducted during a longer period would be required to determine the long-term biological effects of different UVB radiation sources for tortoises. However, the finding of the present study that tortoises exposed to natural sunlight produced a greater quantity of 25-hydroxyvitamin D_3 than tortoises exposed to artificial UVB sources suggested that tortoises with metabolic bone disease should be exposed to natural, unfiltered sunlight at a latitude similar to that of their natural geographic range to maximize dietary calcium uptake.

a. Tontarelli S.p.a., Castelfidardo (AN), Italy.
b. Solar Glo, 125-W mercury-vapor bulb, Exo Terra, Rolf C. Hagen Inc, Montréal, QC, Canada.
c. Repti Glo 5.0, 24-W fluorescent bulb, Exo Terra, Rolf C. Hagen Inc, Montréal, QC, Canada.
d. Heat Glo, 75-W infrared heat lamp, Exo Terra, Rolf C. Hagen Inc, Montréal, QC, Canada.
e. Minitemp MT4, Raytek, Santa Cruz, Calif.
f. Sarstedt Ag & Co, Numbrecht, Germany.
g. 25-Hydroxy Vitamin D EIA, AC-57F1, Immunodiagnostic Systems Ltd, Boldon, England.
h. Solarmeter, model 6.2, serial No. 03787, Solartech Inc, Harrison Township, Mich.
i. SPSS, version 16.0, SPSS Inc, Chicago, Ill.

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