

Intermittent ultraviolet B exposure increases plasma 25-hydroxyvitamin D₃ in blue-tongued skinks (*Tiliqua scincoides*)

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Objective

Ultraviolet B (UVB) guidelines for husbandry and therapeutic exposure remain limited for reptiles. We aimed to determine whether intermittent UVB exposure can elevate and maintain plasma 25-hydroxyvitamin D₃ (25-OHD₃) concentrations in blue-tongued skinks (*Tiliqua scincoides*).

Methods

A prospective, randomized experimental study was conducted using 11 subadult skinks. Six skinks received 2 hours of UVB exposure every 3 days for 30 days, while 5 controls received none. Subsequently, the control skinks received 2 hours of UVB every 7 days for 28 days. Plasma 25-OHD₃ concentrations were measured at baseline and post-UVB using chemiluminescent immunoassay.

Results

Both regimens significantly increased 25-OHD₃ in a dose-dependent manner. Two-hour exposures every 3 days elevated median concentrations from 17 [13.8 to 25] to 587.5 [303.3 to 750] nmol/L, while 7-day exposures increased concentrations from 23 [17.5 to 27] to 51 [42 to 222.5] nmol/L, even with a change to new bulbs.

Conclusions

Intermittent 2-hour UVB at 3- or 7-day intervals significantly elevated 25-OHD₃ concentrations, demonstrating that UVB can be administered as a therapeutic dose tailored to species-specific requirements. More research is needed to determine species-specific 25-OHD₃ reference intervals.

Clinical Relevance

Periodic UVB dosing may offer a sufficient alternative to continuous daily exposure for maintaining vitamin D₃ concentrations in captive blue-tongued skinks; this practical approach supports routine management while reducing the risk of prolonged artificial UVB-related concerns.

Keywords: skink, UVB, 25-hydroxyvitamin D, reptile, phototherapy

Despite the growing popularity of the pet reptile industry, species-specific husbandry information is lacking, particularly regarding vitamin D requirements and ultraviolet B (UVB) exposure. In the wild, animals experience variable UVB exposure depending on season and weather conditions; however, captive environments typically provide a daily, single, fixed artificial UVB source. It remains unclear whether such constant exposure is necessary to

maintain adequate vitamin D status. Inappropriate UVB provision can result in either adverse effects associated with excessive UVB exposure or, conversely, nutritional secondary hyperparathyroidism from insufficient UVB. Blue-tongued skinks (*Tiliqua scincoides*), diurnal omnivores native to Australia and Indonesia, exemplify this challenge. Classified within Ferguson Zones 2 to 3, corresponding to a UV index (UVI) ranging from 1.0 to 2.6, these lizards require moderate UVB exposure, yet ideal captive protocols remain poorly defined.¹⁻³

Ultraviolet B radiation (290 to 315 nm) plays a critical role in endogenous vitamin D₃ synthesis in many reptiles. Although reptiles have 2 sources of

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vitamin D, dietary intake and UVB-mediated cutaneous synthesis, previous studies⁴⁻¹¹ have shown that most reptiles, regardless of their diet, experience an increase in serum 25-hydroxyvitamin D₃ (25-OHD₃) concentrations following UVB exposure. Our previous study¹¹ revealed that blue-tongued skinks primarily use UVB-mediated cutaneous synthesis rather than dietary intake for vitamin D production. In that study, 25-OHD₃ concentrations observed in captive skinks exposed to UVB were notably high: 820 (730 to 1,251.3) nmol/L in the 12-hour group and 635 (401 to 892.5) nmol/L in the 2-hour group, even with a shelter to hide. The 12-hour daily exposure induced greater average increases in plasma 25-OHD₃; however, substantial overlap existed in concentrations achieved from both 2- and 12-hour daily exposures over 4 weeks. Although optimal vitamin D concentrations have not been established for captive reptiles, concentrations of 150 to 250 nmol/L and 409 ± 56 nmol/L have been reported for wild populations of Komodo dragons (*Varanus komodoensis*) and bearded dragons (*Pogona vitticeps*), respectively.^{4,12} While some species have demonstrated the ability to behaviorally regulate UVB exposure according to physiological needs,⁵ excessive UVB provision in the limited space of captive enclosures has been reported to result in adverse cutaneous and ocular damage in reptiles, including photokeratitis, photodermatitis, and potential skin cancer.^{13,14}

In the skink study, 25-OHD₃ concentrations gradually decreased to baseline over several months following UVB withdrawal. This finding suggests that rather than maintaining constant UVB exposure, intermittent exposure protocols could be used as a targeted clinical approach in reptiles, with UVB administered as a “dose” tailored to species-specific requirements. This concept parallels findings in human medicine, where intermittent UVB phototherapy has proven more effective than daily oral vitamin D₃ supplementation for treating vitamin D deficiency.¹⁵ This approach has been successfully demonstrated in Komodo dragons, where a total of 5 months of periodic sun exposure sustained elevated 25-OHD₃ concentrations during nonexposure periods for a year.¹⁶ However, a knowledge gap remains regarding systematic dose-response relationships in reptiles needed to optimize UVB dosing protocols. Specifically, the effects of UVB exposure duration and frequency on sustained vitamin D concentrations have not been experimentally quantified.

Therefore, we aimed to investigate whether intermittent UVB exposure can both elevate and maintain 25-OHD₃ concentrations in blue-tongued skinks. Our hypotheses were (1) intermittent UVB exposure, 2 hours every 3 or 7 days, will increase skink 25-OHD₃ concentrations; and (2) 2-hour UVB exposure every 7 days will provide adequate UVB to increase and maintain 25-OHD₃ concentrations.

Methods

A prospective, randomized experimental trial was conducted in accordance with the regulations set forth by the Louisiana State University IACUC

(25-046). Eleven nonsexed, clinically healthy adult (2 years old; 798 ± 164 g) northern blue-tongued skinks were used in the study. Husbandry and feeding protocols followed a previously described method.¹¹ Briefly, each skink was housed individually in an indoor plastic enclosure (91 X 45 X 33 cm; 36 X 18 X 13 inches) equipped with a non-light-penetrating black shelter (31 X 20 X 8 cm; 12 X 8 X 3 inches) placed on one side of the enclosure. A specific brand of wet cat food (Wellness), previously used by the breeder, was offered 3 times a week (approx 60 g weekly), along with daily access to fresh tap water. This diet contained 2.5 IU/g of cholecalciferol as dry matter basis according to the company. This consistent, single-source diet was maintained to reduce any potential variability in vitamin D concentrations due to their dietary preferences. All skinks were exposed to a 12-hour photoperiod using non-UVB-producing fluorescent room lighting. The skinks had not received UVB exposure for 10 months before the study, and baseline 25-OHD₃ concentrations of trials in this study were not statistically different from previous baseline concentrations recorded before any UVB exposure.¹¹

Ultraviolet B lighting was provided using a 26-watt coil bulb (Sun Glow 5.0 UVB; Fluker Farms) mounted in a reflective dome (Sun Dome; Fluker Farms). This was placed on top of each lid of their enclosures over a large rectangular mesh window that allowed for proper ventilation and light penetration. The distance between bulbs and skinks ranged from 25 to 33 cm, depending on whether skinks were resting on the floor or rarely elevated on shelter structures. Irradiance levels at the floor of each enclosure center were set to 1.5 to 2.0 UVI, as recommended for blue-tongued skinks,³ and were measured using 2 UVB meters (Solarmeter Models 6.2R and 6.5R). Ultraviolet B output was measured weekly at the center and edges of each enclosure to ensure consistency throughout the experiments. Their irradiances at the center were 49.73 ± 6.7 (41 to 66) μW/cm² in phase I and 69.32 ± 8.5 (57 to 79) μW/cm² in phase II. All UVB exposures were conducted within the animals' enclosures, and skinks were not moved or handled during exposure sessions. Every cage was separated with thick cardboard barriers to prevent indirect exposure between groups and individuals.

Phase I (2-hour UVB exposure every 3 days)

Six skinks were randomly selected using a random number generator (<https://www.random.org>) to receive UVB exposure for 2 hours (8 AM to 10 AM) every 3 days for 30 days (10 total exposures). The remaining 5 skinks served as a control group and received no UVB during this period.

Blood samples were collected on day 0 and day 31 using a 25-gauge needle and a 1-mL syringe from the ventral tail vein. Due to a shipping issue, samples from 2 individuals in the treatment group were recollected on day 38; these values were within the range of the remaining 4 skinks that were measured on day 31 and used as the day 31 endpoint for those individuals. Samples were transferred in lithium

heparin tubes (BD Microtainer; Becton, Dickinson and Company), centrifuged at 1,800 *g* for 5 minutes, and stored in cryovials (VWR) at -80°C until being analyzed. All blood samples were collected at 8 AM, before feeding, to minimize potential circadian influences on 25-OHD₃ concentrations. Plasma samples were transported on frozen gel packs to the Veterinary Diagnostic Laboratory at Michigan State University for analysis. Plasma 25-OHD₃ concentrations were quantified using a chemiluminescent immunoassay.¹⁷ The assay detects both 25-OHD₂ and 25-OHD₃ with equal sensitivity; however, given that UVB exposure exclusively stimulates cutaneous synthesis of vitamin D₃,¹⁸ any changes in measured values were attributed to 25-OHD₃.

Phase II (2-hour UVB exposure every 7 days)

After completing phase I, the 5 skinks that served as controls were assigned to a new treatment group and received UVB exposure for 2 hours (8 AM to 10 AM) every 7 days for 28 days (4 total exposures). Blood was collected on day 29, and the day 31 values from phase I were adopted as the baseline for the current phase. All samples were processed using the methods described for phase I.

Statistical analysis

The sample size for phase I was determined a priori using the following parameters: α of 0.05, power of 0.8, an expected mean difference in 25-OHD₃ concentrations between the control and treatment groups of 25 nmol/L, and an SD of 12 nmol/L in each group. For phase II, a paired sample design was used to calculate the required sample size: α of 0.05, power of 0.9, an expected mean difference of 25 nmol/L between baseline and day 29, and an SD of 12 nmol/L. A higher statistical power was selected for phase II due to its within-subject design. The distribution of the 25-OHD₃ concentrations was assessed using the Shapiro-Wilk test, Q-Q plots, skewness, kurtosis, and histograms. Normally distributed data are presented as mean $\pm$ SD, while non-normally distributed data are described as median [IQR], with minimum and maximum values in parentheses.

Weekly UVB irradiance output was evaluated using a repeated-measures ANOVA, with week as the within-subject factor and individual lamp as the subject to assess temporal consistency of lamp output. Changes in 25-OHD₃ concentrations during the 30-day UVB exposure period in phase I were analyzed using a linear mixed model, with time (day 0 and day 31) as the within-subject factor and group (control vs treatment) as the between-subject factor. Individual skinks were included as a random effect to account for individual variability. Post hoc comparisons were conducted using paired samples *t* tests within each group. In phase II, a Wilcoxon signed-rank test was used to assess differences in 25-OHD₃ concentrations between day 0 and day 29, as no between-subject factor was present. Post UVB 25-OHD₃ concentrations from phase I and phase II and the magnitude of increase in post UVB 25-OHD₃

concentrations between 2 phases were compared using the Mann-Whitney test. Although parametric tests were applied based on the normality of residuals, all descriptive statistics are reported as median [IQR] because post-UVB data were not normally distributed. All analyses were performed using SPSS V26.0 (IBM Statistics). Although we set directional hypotheses, two-tailed *P* values were reported, and $P < .05$ was considered statistically significant.

Results

Weekly UVB irradiance was evaluated for each phase before analysis of biological outcomes to verify the stability and consistency of UVB exposure across the experiment. The higher irradiance in phase II resulted from replacing degraded bulbs before this phase; however, both phases maintained irradiance within a 1.4- to 2.1-UVI range. There were no significant differences in weekly UVB output in phase I ($F_{1,4,7} = 1.5$; $P = .28$) or phase II ($F_{1,1,4,5} = 3.468$; $P = .13$). A significant between-lamps effect was observed in both phase I ($F_{5,20} = 3.1$; $P = .03$) and phase II ($F_{4,16} = 6.1$; $P = .003$), indicating differences in average UVB output across individual lamps; however, all 95% CIs of each bulb were overlapping, and this finding was thus considered part of the accepted variance. No changes in behavior, appetite, or clinical signs were observed during the trials.

Phase I (2-hour UVB exposure every 3 days)

Plasma 25-OHD₃ concentrations from both phases are presented in **Table 1**, **Supplementary Table S1**, and **Supplementary Figure S1**. Baseline concentrations across all skinks were similar and ranged from 12 to 36 (20.5 ± 7.5) nmol/L.

Significant main effects were detected for group ($F_{1,18} = 18.6$; $P < .001$), time ($F_{1,18} = 19.1$; $P < .001$), and group $\times$ time interaction ($F_{1,18} = 19.0$; $P < .001$). While the control group showed no significant

Table 1—Plasma 25-hydroxyvitamin D₃ (25-OHD₃) concentrations of blue-tongued skinks before and after being exposed to 2 hours of ultraviolet B light for every 3 or 7 days over 30 or 28 days, respectively.

Group/time	25-OHD ₃ (nmol/L)	Minimum– maximum	<i>P</i> values
2 hours every 3 days			
Control			
Day 0	21 [15.5–29.5]	12–36	—
Day 31	23 [17.5–27]	13–30	.96
Treatment			
Day 0	17 [13.8–25]	13–31	—
Day 31	587.5 [303.3–750]	130–885	.005 ^a
2 hours every 7 days			
Treatment			
Day 0	23 [17.5–27]	13–30	—
Day 29	51 [42–222.5]	34–321	.043 ^b

Median [IQR] and minimum–maximum values are reported. *P* values were obtained from paired *t* tests within each group.

— = Not applicable.

^{a,b}Values with different superscripts are significantly ($P < .05$) different from their baselines.

change over time ($t_4 = -0.05$; $P = .96$), the treatment group exhibited a marked increase in 25-OHD₃ concentration after 30 days ($t_5 = -4.84$; $P = .005$), rising from 17 [13.8 to 25] to 587.5 [303.3 to 750] nmol/L.

Phase II (2-hour UVB exposure every 7 days)

Skinks exposed to UVB weekly also showed a significant increase in 25-OHD₃ concentrations ($Z = -2.02$; $P = .043$), with values increasing from 23 [17.5 to 27] to 51 [42 to 222.5] nmol/L. However, the postexposure 25-OHD₃ concentration was significantly lower than that of phase I ($P = .008$). Also, the magnitude of increase was significantly smaller in phase II compared to phase I ($P = .008$).

Discussion

In this study, both intermittent UVB regimens significantly increased plasma 25-OHD₃ concentrations in blue-tongued skinks, demonstrating that daily UVB exposure may not be essential for increasing vitamin D concentrations in this species. This finding suggests that UVB exposure delivered at defined intermittent intervals may represent an alternative approach to elevate and maintain 25-OHD₃ concentrations, particularly for species that exhibit selective basking rather than full-day sun exposure in the wild. From a practical standpoint, intermittent UVB exposure can help limit cumulative artificial UVB exposure and, consequently, reduce the risk of UVB-related complications reported in reptiles,^{13,14} while also offering benefits such as lower energy consumption and reduced lamp degradation.

In phase I, 2-hour UVB exposure every 3 days resulted in a 35-fold increase in 25-OHD₃ concentrations from baseline, whereas 2-hour exposure every 7 days also produced a smaller but significant 2-fold increase. This dose-dependent response is consistent with our previous study,¹¹ in which blue-tongued skinks exposed to daily 12- or 2-hour UVB resulted in overlapping but discernible concentration ranges in vitamin D synthesis. Notably, the median concentration achieved in the 2-hour every 3-day group (587.5 [303.3 to 750] nmol/L) was nearly equivalent to that observed under the 2-hour daily regimen (635 [401 to 892.5] nmol/L),¹¹ further indicating that comparable vitamin D concentrations can be achieved without continuous daily exposure. In the 2-hour every 7-day group, the overall increase was smaller; however, some individuals achieved concentrations as high as 321 nmol/L, 2.5 times higher than the lowest observed value in the every 3-day regimen. Moreover, 80% (4 of 5) of individuals in the 7-day interval group exceeded 50 nmol/L, confirming a clear positive response even at low exposure frequency.

The physiological interpretation of these values remains challenging because a 25-OHD₃ reference range for wild blue-tongued skinks is currently unknown. Consequently, it is not yet possible to determine whether the 2-hour every 7-day protocol represents a suboptimal provision or an adequate maintenance dose. However, given that both regimens produced concentrations spanning 34 to 321 and 130 to 885 nmol/L, encompassing the reported

normal ranges for other reptiles (200 to 400 nmol/L for iguanids and bearded dragons),^{12,19} these findings indicate that weekly exposure may be insufficient, whereas a more frequent interval is appropriate to maintain 25-OHD₃ concentrations under captive conditions. Notably, an interval longer than 3 days may be more adequate, as 2 post-UVB values from phase I (every 3 days), 361 and 615 nmol/L, were obtained 1 week after the scheduled blood collection (day 31). These 2 skinks would be expected to have exhibited even higher concentrations on day 31. Additionally, all blood samples were collected several days after the final UVB exposure (ie, at intervals matching the exposure schedule) to assess maintenance rather than peak concentrations. Therefore, higher 25-OHD₃ concentrations would be expected if examined immediately after the last exposure.

Even with 2-hour every 3-day exposure, 25-OHD₃ concentrations exceeded 500 nmol/L in 4 of 6 skinks, and 3 individuals reached or were expected to exceed 700 nmol/L. In humans, plasma 25-OHD₃ concentrations above 750 nmol/L have been suggested as the threshold for toxic symptoms, considerably higher than a previously accepted upper limit (250 nmol/L).²⁰ Given the intrinsic photoregulatory mechanisms and renal feedback control, as well as the high tolerance of plasma 25-OHD₃ concentrations observed in wild Ricord iguanas (*Cyclura ricordii*) and captive outdoor green iguanas (*Iguana iguana*), vitamin D intoxication, typically associated with rapid oral overdosing, may not be accompanied with this protocol.²¹⁻²⁴ Supporting this, a 16-year retrospective study²⁵ in humans with elevated 25-OHD concentrations found only a weak correlation between 25-OHD concentration, clinical vitamin D toxicity, and serum calcium concentrations.

Nonetheless, considering the limitations of using artificial UVB lamps that do not reproduce the full spectrum of natural sunlight and our short (1-month) observation period, even the 2-hour every 3-day protocol may provide higher UVB exposure than required for some individuals under these captive conditions. Longer term studies in green iguanas have demonstrated that 25-OHD₃ continues to accumulate over 200 days under continuous UVB.²⁶ Therefore, higher post-UVB 25-OHD₃ concentrations are likely to be achieved if UVB is provided for extended durations (eg, every 3 days over several months) because we did not measure the cumulative effect of repeated UVB exposure. This conservative interpretation is further supported by recent concerns that narrow-spectrum UVB-LED lamps (290 to 315 nm dominant) may limit natural photoregulatory processes active at longer UVB wavelengths (315 to 335 nm).²⁷ Although compact fluorescent UVB lamps used in this study likely provide broader UVB spectra than UVB-LEDs, the unnatural distribution and its adequacy for normal photoproduct balance remain uncharacterized, warranting cautious interpretation of the elevated 25-OHD₃ concentrations observed in this study.

Although median 25-OHD₃ concentrations differed approximately 10-fold between the 2 exposure

regimens, substantial individual variation was observed within each protocol. For instance, in the phase I group, the lowest and highest post-UVB concentrations differed by 6.8-fold, and a similar 9.4-fold difference occurred within the phase II group. Potential explanations for this variability include behavioral differences (eg, shade-seeking tendencies with a shelter or substrate or feeding time preferences), skin pigmentation or thickness, metabolic variations, differences in vitamin D-binding protein levels, storage capacity of vitamin D stored in adipose tissue, and sex-associated differences.^{18,26,28–30} Sex was not considered a factor as all skinks were subadults before sexual maturation and were maintained under uniform husbandry conditions without seasonal variation, thereby minimizing potential sex-related differences. Nevertheless, sex-related differences in circulating vitamin D metabolites have been reported in the Hermann tortoises (*Testudo hermanni*), with males exhibiting higher concentrations than females throughout the year.³⁰ Although 95% CIs of all UVB lamp outputs largely overlapped, variation in lamp irradiance may have also contributed, as the 2 skinks showing the lowest post-UVB concentrations in each phase were exposed to the weakest UVB intensity. These wide interindividual differences highlight the limitations of a “one-size-fits-all” UVB protocol and suggest that individualized monitoring and a target range approach may be more clinically appropriate. Moreover, because of these differences, the authors recommend 25-OHD₃ concentrations be measured in reptiles as a component of their annual examination to establish patient reference values. The magnitude of the UVB response also varies markedly among reptile species. For example, juvenile bearded dragons exposed to a lamp with similar output to that used in this study for 2 hours daily achieved 25-OHD₃ concentrations of only 41.00 ± 12.85 nmol/L, approximately one-tenth of those measured in wild conspecifics.¹² This species-specific variability in UVB-mediated vitamin D synthesis underscores the importance of developing tailored, evidence-based UVB exposure protocols rather than relying on generalized recommendations.

Beyond regular husbandry, intermittent “dosed” UVB exposure represents a viable phototherapeutic option for vitamin D-deficient reptiles. Our findings demonstrated that 2-hour exposures at 3- or 7-day intervals significantly elevated 25-OHD₃ concentrations within 1 month. Moreover, previous work¹¹ in this species showed that elevated concentrations persisted for more than 4 months following cessation of daily exposure, which is consistent with prolonged maintenance after UVB withdrawal observed in other species.^{16,31,32} In contrast, high-dose oral supplementation in green iguanas and black-throated monitors (*Varanus albigularis*), while rapidly increasing 25-OHD₃ concentrations, poses a greater risk of intoxication and shows faster decline postcessation.^{26,32,33} Collectively, these findings support that intermittent UVB is capable of increasing and maintaining circulating plasma 25-OHD₃ concentrations

as a sustainable approach for both maintenance and therapeutic management of vitamin D status in captive blue-tongued skinks. This periodic UVB provision mimics natural photobiological processes and aligns with the negligible contribution of dietary vitamin D in most wild herbivorous and omnivorous squamates, even in species capable of absorbing dietary vitamin D.³⁴ However, not all intermittent UVB protocols are equally effective; weekly 10- to 20-minute exposures failed to restore 25-OHD₃ concentrations in black-throated monitors despite higher irradiance (144 μW/cm²),³² underscoring the need to identify appropriate dose-duration combinations on a species-specific basis.

Additional limitations of this study include the small sample size and considerable interindividual variability, which limit the generalizability of these findings. Additionally, 25-OHD₃ concentrations were only measured twice (before and after exposure) in this study. More frequent and shorter sampling during the exposure phase would allow for characterization of the rate and dynamics of vitamin D synthesis, providing a more comprehensive understanding of dose-response kinetics and the photoregulatory process. Overall, this study provides foundational evidence for establishing phototherapy protocols in blue-tongued skinks, but further research incorporating larger populations, extended observation periods, and wild population comparisons will be essential to refine and validate safe, species-specific UVB dosing guidelines.

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Supplementary Materials

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