



The nocturnal leopard gecko (*Eublepharis macularius*) uses UVb radiation for vitamin D₃ synthesis

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ABSTRACT

Vitamin D is an important regulator of calcium and phosphorus homeostasis in animals. It can be acquired from the diet or synthesised de novo when skin is exposed to UVb. Vitamin D deficiency can lead to a complex of diseases collectively called metabolic bone disease (MBD). Diurnal lizards without access to UVb are prone to develop vitamin D deficiency, even when dietary vitamin D₃ is provided. A trial was conducted to determine whether juvenile nocturnal lizards require access to UVb to prevent vitamin D deficiency. All leopard geckos (*Eublepharis macularius*) were supplemented with dietary vitamin D₃. One group was exposed to low level UVb radiation (33–51 μW/cm²) from hatching until 6 months of age and a second group remained unexposed. Animals were fed ad libitum and their growth and weight gain compared with non-exposed controls. At the end of the trial, blood samples were analysed for vitamin D₃ metabolites. The concentration of the vitamin D₃ metabolite, 25(OH)D₃, was higher in UVb exposed animals (61 ± 20 vs. 38 ± 8 nmol/L), confirming cutaneous synthesis with UVb exposure. Growth and weight gain were similar in both groups, and this, together with the absence of clinical symptoms, suggests that dietary vitamin D₃ alone can meet the vitamin D requirements for growth of this nocturnal gecko, during the first six months of life. It remains to be investigated whether the higher vitamin D metabolite levels holds other health benefits for this species, such as improved bone density or immune response.

1. Introduction

Several lizard species are commonly kept as pets and are generally housed indoors, under artificial conditions. A common disorder in captive lizards is metabolic bone disease (MBD) (Oonincx & van Leeuwen, 2017). This is a complex of diseases resulting from an imbalance of calcium and/or phosphorus in the body (Laing et al., 2001; Zotti et al., 2004). Vitamin D acts as an endocrine hormone regulating the calcium and phosphorus balance via selective absorption and excretion of these minerals (Holick, 2007). Hence, vitamin D deficiency can be an underlying cause of MBD. Animals can obtain vitamin D either from the diet or via conversion of endogenous 7-dehydrocholesterol (7-DHC) to vitamin D₃ in the skin by exposure to UVb radiation (280–320 nm) and subsequent thermal isomerisation. The

latter pathway is active in the great majority of animal species studied to date, including mammals (Brot et al., 2001; Cavaleros et al., 2003; Cooper et al., 1997; Hymøller & Jensen, 2010; Jakobsen et al., 2020; Kwiecinski et al., 2001; Southworth et al., 2013; Watson et al., 2014), birds (Drake et al., 2017; Edwards, 2003; Stanford, 2006) insects (Oonincx et al., 2018), and reptiles (Acierno et al., 2006; Acierno et al., 2008; Bos et al., 2018), including diurnal lizards (Ferguson et al., 2009; Gillespie et al., 2000; Laing et al., 2001; Oonincx et al., 2010; Townsend & Cole, 1985). However, this pathway is dysfunctional in some species, including cats, dogs and seals, and has limited functionality in polar bears due to absence or low levels of 7-DHC (Bouillon & Bikle, 2019; How et al., 1994; Keiver et al., 1988; Kenny et al., 1998). These species are carnivores and fulfil their vitamin D requirements via dietary intake. Conversely, the dietary route appears ineffective as the sole source

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of vitamin D in certain diurnal lizards, such as Komodo dragons (*Varanus komodoensis*) and bearded dragons (*Pogona vitticeps*), which may depend on UVb radiation to obtain a suitable vitamin D status (Gillespie et al., 2000; Nijboer et al., 2001; Oonincx et al., 2010). Vitamin D₃ status is indicated by the concentration of 25(OH)D₃ in blood plasma following a primary hydroxylation in the liver (Heaney et al., 2008; Laing et al., 2001). This metabolite is subsequently hydroxylated to the biologically active form of vitamin D₃ (1,25(OH)₂D₃) in the kidney, a process tightly controlled by serum levels of calcium, phosphorus and parathyroid hormone (Holick, 2007). It is unknown whether all lizards benefit from access to UVb radiation or that this is only valid for diurnal species, or perhaps only for certain taxa or species (Adkins et al., 2003). Possibly, lizards which are primarily nocturnal require less UVb exposure to produce the same amount of vitamin D₃ as diurnal species (Carman et al., 2000). On the other hand, many lizard species kept in zoos and private collections without access to UVb survive and reproduce without clinical signs of vitamin D deficiency such as muscle weakness, tetany, or nutritional secondary hyperparathyroidism. However, vitamin D deficiency can be present without the aforementioned conditions and limit growth and weight gain (Diehl et al., 2018; Oonincx et al., 2010). The leopard gecko (*Eublepharis macularius*) is a lizard species that has been kept and bred in captivity since the early 1960s (Thorogood & Whimster, 1979). This crepuscular and nocturnal gecko has become one of the most frequently kept pet lizards and in literature associated with the pet trade, such as popular care guides, is commonly stated not to require exposure to UVb (Tremper, 2012). Hatchling leopard geckos provided with either low or high doses (319–720 IU/kg DM) of vitamin D₃, and low or high doses of calcium (0.2–0.8% DM) for 255 days had the highest serum 25(OH)D₃ concentration (103 nmol/L) when provided with high doses of both (Allen et al., 1995). Much lower 25(OH)D₃ concentrations were found when less calcium was provided (21 nmol/L) or when less vitamin D₃ was provided (12 nmol/L). Their control animals were exposed to low, but unquantified, levels of UVb for 10 h daily and had a 25(OH)D₃ concentration of 25 nmol/L (dietary Ca 1.2% DM, dietary vitamin D unknown). These authors concluded that leopard geckos provided with adequate dietary calcium have low vitamin D requirements, which are easily met by dietary provision. They suggested that vitamin D requirements might be fundamentally different between basking and nocturnal lizards. Contrarily, adult leopard geckos with daily access to UVb for 2 h had significantly increased 25(OH)D₃ concentration (from 42 to 80 nmol/L), whereas unexposed individuals had a numerical increase from 44 to 50 nmol/L after a 30 day trial (Gould et al., 2018; Mitchell, 2020). Whether the difference between the results from these two studies are due to differences in animal age, trial duration, or methodology is unclear. Furthermore, to which extent these differences in vitamin D status affect the health and welfare of this species is unknown. Therefore, the aim of this study was to determine whether UVb exposure affects the vitamin D status, weight gain or growth of juvenile leopard geckos.

2. Materials and methods

Animal housing, care and experimental procedures were filed under application number 2013083 and approved by the Committee for the Care and Use of Animals of Wageningen University (Wageningen, the Netherlands) in accordance with Dutch law.

2.1. Design and animals

This study contained two treatment groups in a parallel study design. The first group was exposed to UVb for 2 h per day, whereas the second group was not exposed to UVb. Eighteen leopard gecko (*Eublepharis macularius*) hatchlings were provided by two private breeders and transported to the university in a closed Styrofoam container within 24 h after hatching. The hatchlings were assigned and

distributed equally over the treatments with the first hatchling allocated to treatment 1, the second to treatment 2, et cetera. From the day of hatching, snout to vent length, and total length were measured, and body mass was determined with an electronic balance (type HF-2000G, A&D company ltd, Tokyo, Japan) on a weekly basis. Animals were observed during daily feeding for clinical signs of vitamin D deficiency, either manifest as signs of hypocalcemia (weakness, muscular fasciculations, neuropathy) or MBD (limb swelling, postural abnormalities, skeletal deformation, softening of bones (e.g. “rubber jaw”) or pathological fractures). Gender was determined at the end of the study by visually inspecting the cloacal region for hemipenial bulges and enlarged femoral pores.

2.2. Housing

Animals were housed at the animal facilities of Wageningen University in individual terrariums measuring 40 × 30 × 28 cm (L × W × H). Three terrariums were placed next to each other on a shelf, and per treatment three shelves above each other were used. Heat mats (29 W, 120 × 14 cm, Heatel BV, De Lier, the Netherlands) were placed at the rear end under each terrarium to provide a thermal gradient and were regulated by a thermostat set to 32 °C (600 W, HabiStat, Hayes, United Kingdom). Each week, terrariums were relocated one position (left to right, top to bottom) to account for possible location effects. Each terrarium contained a layer of masonry sand (Gamma Nederland BV, Leusden, the Netherlands), a hide box with sand that was kept moist (WB 020, EuroZoo BV, Barneveld, the Netherlands), a black polypropylene hide box with dry sand, (17 × 12.5 × 5 cm), a mealworm feeding tray (WB 015, EuroZoo BV, Barneveld, the Netherlands), a cup with calcium carbonate (M2MD, EuroZoo BV, Barneveld, the Netherlands, and Ankerpoort NV, Maastricht, the Netherlands), and a water dish (WB 120, EuroZoo BV, Barneveld, the Netherlands). UVb fluorescent tubes (Reptisun 10.0 UVb, 32 W, 120 cm, Zoo Med Laboratories Inc., San Luis Obispo, CA, USA) were mounted 45 cm above the terrarium floor and 17 cm from the rear of each terrarium. These tubes were used for 100 h before the study to stabilize UVb output and were turned on daily from 12:00 till 14:00 h during the study. Glass panes were suspended between the tubes and the terrariums in the unexposed group to filter out all UVb radiation. The UV emission of each tube was measured weekly at three locations in the terrarium: perpendicular to the tube (34 cm distance), in the centre of the terrarium (37.5 cm distance), and at the front of the terrarium (45 cm distance). Emissions were quantified with three UV meters (Solartech Inc., Harrison Township, MI, USA); Solarmeter model 5.7 for total UV intensity, model 6.2 for UVb intensity and model 6.5 for the UV-index. These devices were 11 cm high. Therefore, the distance between the light source and the sensor was approximately 8–9 cm less than the distance between the back of the animal and the light source. Surface temperatures in the rear (perpendicular to the tube), in the centre and in the front of the terrariums were measured weekly with an infrared thermometer (RayTemp 3, Electronic Temperature Instruments Ltd., West Sussex, UK).

2.3. Diet and feeding

The feeder insects were purchased from a commercial supplier (Starfood BV, Barneveld, the Netherlands) and consisted of house crickets (*Acheta domestica*), migratory locusts (*Locusta migratoria*) desert locusts (*Schistocerca gregaria*), and yellow mealworms (*Tenebrio molitor*). These insects were provided ad libitum throughout the study. Weights of the provided insects and of the feed refusals of the previous day were recorded daily per animal. This allowed calculation of feed intake and evaluation of dietary preferences between treatments. Feed conversion rate was calculated by dividing the total fresh matter intake from day 0 until day 175 by the body weight increase during that period. The provided insects were dusted with a mineral supplement

(Miner-All Indoor, Sticky tongue farms, Sun City, CA, USA) labelled to contain 4400 IU/kg vitamin D₃, ~35% calcium and no phosphorus (see results section). A sample of this supplement was taken at the start and end of the study, sealed in a plastic bag and stored at -20 °C until analysis.

The quantity of supplement adhering to the insects was determined as follows:

1–2 g of house crickets, migratory locusts or desert locusts were placed in a plastic container and weighed. Then supplement was added, and the box was carefully shaken so that the insects were dusted; that is the supplement adhered to their integument. The insects were then transferred by hand to a new, clean plastic container and weighed again. After 15 min insects were transferred again and weighed and after an additional 15 mins this was repeated so that the weight increase due to the dusting with the supplement could be calculated after 0, 15 and 30 min. This was done for two instars for the house crickets and migratory locusts and one instar for the desert locusts. The procedure was replicated 12 times per instar. The mass increase was converted to a percentage of insect body mass. To estimate total supplement provision, the average of the three time points was used, based on the observation that most insects were consumed within that time frame.

2.4. Blood sampling and processing

Leopard geckos can voluntarily self-amputate their tail (caudal autotomy). When such trauma occurs, a combination of arterial sphincters and venous valves limits blood loss (Gilbert et al., 2013). Therefore, at an age of 180 ± 5 d, the ventral skin of the tail was anointed with lidocain and prilocain ointment (both 25 mg/g; EmlaR, AstraZeneca BV, Zoetermeer, the Netherlands) aiming to prevent pain induced blood flow reduction. This should be used with caution to prevent toxicity or other unwanted effects. Approximately 1 h later, the leopard geckos were anaesthetised by mask with sevoflurane (SevoraneR, AbbVie B.V., Hoofddorp, the Netherlands) until the righting reflex was absent. Then, a blood sample was taken from the caudal vein, near the base of the tail, with a syringe (1-mL, Becton Dickinson and Company, Franklin Lakes, NJ, USA) fitted with a fine needle (25G 0.5 × 0.16 mm microlance 3, Becton Dickinson and Company, Franklin Lakes, NJ, USA). These were previously flushed with heparin (Heparine 5000 IU/mL, LEO pharma A/S, Ballerup, Denmark). The obtained blood was centrifuged at 10,000 rpm for 10 min at 10 °C and the plasma was then stored at -80 °C until analysis. After successful blood sampling, animals were given 14 days to recover and were then returned to the breeder. If blood sampling was unsuccessful on the first attempt, a second attempt was made 3 weeks later.

2.5. Laboratory analyses

Concentrations of 25(OH)D₃ and 1,25(OH)₂D₃ in plasma were determined at the Department of Clinical Chemistry of the VU University Medical Centre Amsterdam (Amsterdam, the Netherlands). 25(OH)D₃ was determined by ID-XLC-MS/MS as described in Heijboer et al. (Heijboer et al., 2012), with modifications for lizards as described in Oonincx et al. (Oonincx et al., 2013). 1,25(OH)₂D₃ was also determined using 2D-ID-UPLC-MS/MS, as described in Dirks et al. (Dirks et al., 2016); intra-assay variation was < 12%. The vitamin D₃ content of the vitamin-mineral supplement was determined following the methodology for feed materials reported in Oonincx et al. (Oonincx et al., 2018).

The spectral power distribution of the fluorescent tubes utilised in the experiment was determined. Tubes were run on UK line voltage (nominally 230-240v) and warmed up for 30 min. Spectral analysis was performed with an Ocean Optics Inc. USB2000+ miniature spectrometer with a UVb compatible fibre optic sensor with cosine adaptor, calibrated for absolute irradiance. Scans were made with and without a

Table 1

Temperature and UV emissions (UV index, UVA and UVb intensity) during a 175 day trial measured perpendicular to the tube, and in the centre and front of terrariums housing UVb exposed (*n* = 261) and UVb unexposed (*n* = 253) leopard geckos. Data presented as average ± standard deviation.

		UVb exposed	UVb unexposed
Temperature (°C)	Perpendicular	32.9 ± 1.1	33.0 ± 1.1
	Centre	29.9 ± 1.1	29.9 ± 1.1
	Front	29.8 ± 1.1	29.8 ± 1.1
UV Index	Perpendicular	1.6 ± 0.12	0
	Centre	1.3 ± 0.12	0
	Front	1.0 ± 0.08	0
UVA (μW/cm ²)	Perpendicular	64.8 ± 6.65	35.5 ± 1.78
	Centre	69.2 ± 8.08	31.4 ± 2.32
	Front	37.5 ± 4.90	21.4 ± 2.26
UVb (μW/cm ²)	Perpendicular	51.4 ± 3.98	0
	Centre	44.5 ± 2.98	0
	Front	33.3 ± 2.62	0

sample of the glass plates placed between the spectrometer probe and the tubes as used in the control treatment to block UVb.

2.6. Statistical analyses

Data on body mass, snout to vent length and total length at the start of the study and after 175 days were analysed for the effects of UVb exposure. The same was done for total feed intake, feed preference, feed conversion efficiency and supplement provision during the study and vitamin D metabolite (25(OH)D₃ and 1,25(OH)₂D₃) concentrations in blood plasma at the end of the study. All analyses were performed via the ANOVA procedure in SPSS Statistics 22 (IBM Corporation, Armonk, NY, USA). Differences were considered significant at *P* < 0.05.

3. Results

3.1. Treatment and housing

Temperatures were similar over treatments, averaging 33.0 °C in the rear, 29.9 °C in the centre and 29.8 °C in the front of the terrariums (Table 1). The UV emissions remained stable over time and differed between treatments regarding UVI, UVA and UVb (Table 1). In the UVb exposed group, the average UVb intensity at animal level was 51.4 μW/cm² directly underneath the tube, and this gradually decreased to 33.3 μW/cm² towards the front of the terrarium. Similarly, UVI and UVA intensity decreased away from the tube, towards the front of the terrariums. In the unexposed group, the glass plate between the terrariums and the tube effectively filtered out all UVb and part of the UVA (320–365 nm), whereas the glass did not filter longer wavelengths (Fig. 1).

3.2. Animals and diet

The 18 animals in this trial consisted of 4 males and 14 females. One male was in the UVb exposed group and the three others were part of the unexposed group. One female from the UVb exposed group stopped feeding at day 133 due to sand impaction. After five days of treatment with 0.3 mL olive oil daily, the impaction was cleared out. Data from this animal were included in further analysis. The other 17 leopard geckos remained visibly healthy and no clinical signs of MBD were observed for any of the animals throughout the trial. At the start and at the end of the trial, average weight, snout vent length and total length were similar between treatments (Table 2). Also feed intake, feed preference and feed conversion ratio were similar for the UVb exposed and unexposed animals (Table 3).

Regarding the feeder insects, the percentage of weight increase due to dusting were similar between instars, hence the data were pooled per

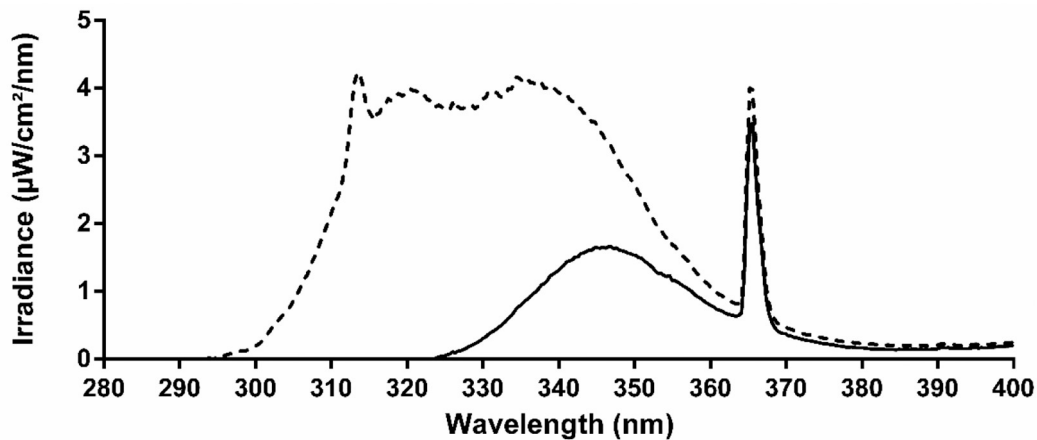


Fig. 1. Average spectral power distribution of a UV emitting lamp (Reptisun 10.0 UVB 32 W 120 cm, Zoo med laboratories Inc., San Luis, USA) with ($n = 3$) and without ($n = 2$) a glass plate filter.

Table 2

Start and end (day 175) data on weight, snout vent length and total length of UVB exposed and UVB unexposed leopard geckos. Data presented as average \pm standard deviation; $n = 9$, and analysed by ANOVA.

		UVb exposed	UVb unexposed	P-value
Weight (g)	Start	3.2 \pm 0.54	3.3 \pm 0.48	0.668
	End	52.0 \pm 6.81	59.6 \pm 12.36	0.126
Snout vent length (mm)	Start	51.2 \pm 2.22	52.1 \pm 1.83	0.369
	End	120.0 \pm 2.45	123.1 \pm 5.35	0.132
Total length (mm)	Start	86.6 \pm 5.66	87.3 \pm 5.98	0.780
	End	186.8 \pm 9.97	192.1 \pm 10.87	0.308

Table 3

Feed intake (g), subdivided by insect species, and feed conversion ratio of UVB exposed and UVB unexposed leopard geckos during a 175 trial. Data presented as average \pm standard deviation; $n = 9$ and analysed by ANOVA.

		UVb exposed	UVb unexposed	P-value
Total feed intake		208.4 \pm 26.02	234.8 \pm 38.53	0.108
House crickets (<i>Acheta domestica</i>)		46.9 \pm 16.05	59.6 \pm 22.97	0.193
Migratory locusts (<i>Locusta migratoria</i>)		68.4 \pm 16.88	84.8 \pm 18.78	0.069
Desert locusts (<i>Schistocerca gregaria</i>)		65.5 \pm 20.35	72.3 \pm 15.44	0.436
Yellow mealworms (<i>Tenebrio molitor</i>)		27.6 \pm 24.90	18.1 \pm 18.94	0.373
Feed conversion ratio		4.3 \pm 0.46	4.3 \pm 0.67	0.877

Table 4

Percentage of mass increase directly after dusting and after 15 and 30 min, for house crickets, migratory locusts and desert locusts. Data presented as average \pm standard deviation.

Sample	Time (min)	Percentage of mass increase		
		0	15	30
House crickets (<i>Acheta domestica</i>)	24	13.4 \pm 1.37	8.7 \pm 2.30	4.5 \pm 1.88
	24	5.6 \pm 1.13	2.6 \pm 0.78	1.4 \pm 0.41
Desert locusts (<i>Schistocerca gregaria</i>)	12	5.5 \pm 0.83	2.6 \pm 0.48	1.6 \pm 0.69

species. House crickets with supplement were 13.4% heavier directly after dusting and this percentage decreased to 4.5% after 30 min (Table 4). Values for the two locust species were lower than for house crickets, but similar between the locust species. The vitamin D₃ content of the supplement was 15,500 IU/kg, which was \sim 3.5 times higher than

declared on the label (4400 IU/kg).

3.3. Blood sampling and vitamin D metabolite levels

In 13 out of 18 animals sufficient blood was obtained to determine 25(OH)D₃ concentrations ($n = 6$ UVB exposed, $n = 7$ unexposed), for 10 animals ($n = 5$ in both treatment groups) 1,25(OH)₂D₃ concentrations were also determined. For three animals a second attempt was required to obtain a blood sample. Their vitamin D metabolite concentrations fell within the ranges of the first group. Two animals self-amputated their tails several hours after blood sampling was conducted. In the UVB exposed animals, 25(OH)D₃ plasma concentrations were higher ($P = 0.017$) than in the unexposed group (61 \pm 20 vs. 38 \pm 8 nmol/L; Fig. 2A). Plasma concentrations of 1,25(OH)₂D₃ were similar between the UVB exposed and unexposed group (39 \pm 13.5 pmol/L vs. 33 \pm 12.6 pmol/L; Fig. 2B).

4. Discussion

Leopard geckos exposed to UVB had higher plasma 25(OH)D₃ concentrations than unexposed specimens, indicating that this species can synthesize vitamin D₃ de novo. The concentration of the biologically active metabolite of vitamin D₃, 1,25(OH)₂D₃, was not influenced by UVB exposure. Concentrations of the latter metabolite are tightly regulated and generally are less strongly affected by factors such as age, season or gender than 25(OH)D₃ (Moan et al., 2009). However, in a trial with bearded dragons in which UVB unexposed individuals had extremely low 25(OH)D₃ concentrations (9.9 nmol/L), there was a fivefold difference in 1,25(OH)₂D₃ concentrations between UVB exposed and unexposed individuals (Oonincx et al., 2010).

In the current study, 25(OH)D₃ concentrations of the unexposed specimens were similar to reported values for adult male leopard geckos prior to UVB exposure (38 vs. 43 nmol/L serum) (Mitchell, 2020). However, those adult males had a higher concentration after 1 month of UVB exposure than the specimens in the current study after 6 months of UVB exposure (80 vs. 61 nmol/L). This could be because growing leopard geckos require more vitamin D than adults or because the timing of UVB availability differed. In the current study, 2 h of illumination were provided mid-day, whereas Gould et al. (Gould et al., 2018) provided 1 h in the early morning and 1 h in the early evening.

Also, methodological differences can result in different 25(OH)D₃ values. For instance, in the current study this metabolite was quantified via ID-XLC-MS/MS and Gould et al. (Gould et al., 2018) used a radioimmunoassay. While both methods are suitable to determine relative differences within studies, they can result in different values, even for the same sample (Heijboer et al., 2012). Another relevant source of

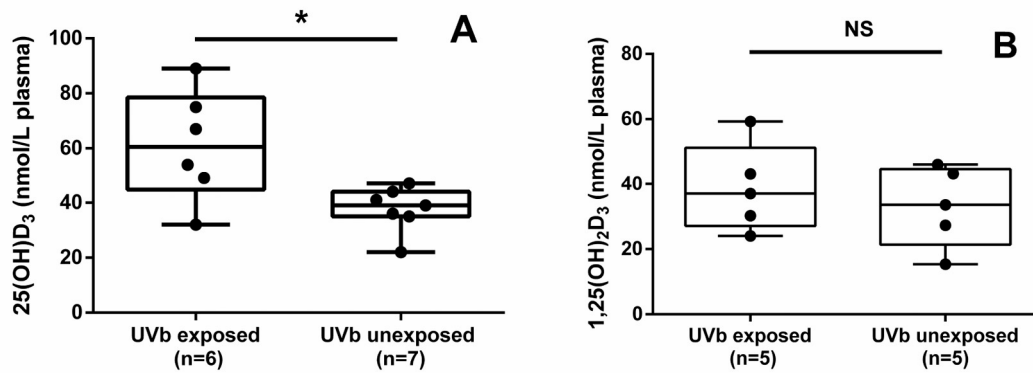


Fig. 2. Boxplot and individual datapoints for 25(OH)D₃ (Panel A) and 1,25(OH)₂D₃ (Panel B) plasma concentrations of UVb exposed and UVb unexposed leopard geckos.

NS, Not significant ($P > 0.05$); *, $P < 0.05$.

difference can be UVb lamp emissions. Even though the total UVb irradiances were similar between studies (up to 51 and 52 $\mu\text{W}/\text{cm}^2$), differences in their spectral power distribution can significantly influence vitamin D synthesis and, thereby, 25(OH)D₃ concentrations (Diehl et al., 2018). A low potency of the UVb lamp in the study of Allen et al. (Allen et al., 1995) might explain the low 25(OH)D₃ concentration (25 nmol/L) in those juvenile leopard geckos. However, the higher 25(OH)D₃ concentration (103 nmol/L) for animals provided with a high calcium diet (0.8% DM) and a high concentration of dietary vitamin D (720 IU/kg DM) compared to the current study, is less easily explained. Throughout the current study animals had access to a cup filled with calcium carbonate and crickets and locusts were dusted with a supplement consisting of ~35% calcium (according to the manufacturer; not analysed). This supplement alone would increase the absolute calcium concentration of the house crickets by ~2.8% (DM) and that of the locust species by ~1.1%. If calcium has a sparing effect on vitamin D utilisation, these levels should suffice. Furthermore, this supplement increased vitamin D₃ concentrations of house crickets by 1261 IU/kg and by 487 IU/kg for the locusts. It is unclear why in our study 25(OH)D₃ plasma concentrations were lower than those on a high calcium, high vitamin D diet in the study of Allen et al. (Allen et al., 1995), while provided with more calcium and similar concentrations of vitamin D₃.

Besides the supplement, also vitamin D in the feeder insects themselves contributed to total dietary intake. These insects are expected to contain less than 200 IU/kg based on their own dietary sources (Ooninx et al., 2010), however this could be increased in the UVb exposed treatment due to de novo synthesis (Ooninx et al., 2018). Quantitatively, this contribution would be limited for house crickets and migratory locusts (124–226 IU/kg of fresh matter). However, yellow mealworms can reach 1700 IU of vitamin D₃/kg of fresh matter with 2 h of UVb exposure (Ooninx et al., 2018). This raises the question whether the higher 25(OH)D₃ levels in the UVb exposed group are due to direct de novo synthesis, or indirectly via the feeder insects. If the latter were the case, individuals in the UVb exposed group consuming more yellow mealworms would have higher 25(OH)D₃ plasma concentrations. One individual in that group strongly preferred yellow mealworms; they composed 46% of its diet, whereas this was 2–20% for the other geckos. It also had the lowest 25(OH)D₃ plasma concentration (32 nmol/L) of the UVb exposed group. Hence, this suggests that the higher 25(OH)D₃ concentrations in UVb exposed leopard geckos are due to their UVb exposure and not that of the feeder insects.

The overall diet composition of the UVb exposed and the unexposed group were similar; migratory locusts and desert locusts made up the main part of the diet of the exposed animals (31 and 34% of the fresh weight, respectively), followed by house crickets (24%) and lastly

mealworms (11%). Besides the diet, also total feed intake and feed conversion efficiency were similar between groups. The feed conversion ratio of these ad libitum fed leopard geckos was somewhat higher than that of bearded dragons (4.3 vs. 3.7) provided with a comparable diet (Ooninx et al., 2010). Growth and weight gain of the leopard geckos was not affected by UVb exposure. Numerically higher values were found in the unexposed group (Table 2). Conversely, female bearded dragons deprived of UVb during the first 6 months of life were smaller and weighed less than those with access to UVb (Ooninx et al., 2010).

Several studies on diurnal lizards point towards the benefit of UVb exposure as indicated by improved growth (Ooninx et al., 2010), disappearance of clinical signs of MBD (Diehl et al., 2018; Gillespie et al., 2000), higher hatchability of eggs (Townsend & Cole, 1985) and higher survival of juveniles (Laing et al., 2001). The data from the current study does not indicate a benefit of UVb exposure for 2 h per day for growing leopard geckos if provided with adequate dietary intake of vitamin D₃. Bone density was not evaluated in the current study, but would be a parameter of interest to allow evaluation of calcium deposition in further studies.

Future studies could determine 25(OH)D₃ concentrations in free-roaming leopard geckos to establish reference values. Furthermore, benefits of UVb exposure for reproducing females, which are likely to have a higher vitamin D requirement could be explored. Such a study should include egg hatchability, and the growth and development of resulting juveniles. Beneficial effects could be present as vitamin D has an important role in embryonic development and positively affects hatchability (Packard & Packard, 1984). Lastly, it would also be of interest to investigate whether the elevated levels of 25(OH)D₃ is of biological relevance for the functioning of the lizard immune system. Such a relationship is well-known in humans but in animals, let alone lizards, has received little attention (Holick, 2007).

In conclusion, this study shows that juveniles of the nocturnal leopard gecko attain a higher 25(OH)D₃ plasma concentration when given access to UVb. However, under the investigated circumstances, concentrations of the biologically active metabolite 1,25(OH)₂D₃, growth, and weight gain remained unaffected. Hence, unlike most diurnal lizards, a lack of UVb exposure does not seem to hinder normal development of juvenile leopard geckos, when provided with sufficient dietary calcium and vitamin D.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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