Do Panther Chameleons Bask to Regulate Endogenous Vitamin D₃ Production?

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ABSTRACT

Basking by ectothermic vertebrates is thought to have evolved for thermoregulation. However, another beneficial effect of sunlight exposure, specifically the ultraviolet B (UV-B) component, includes endogenous production of vitamin D₃. In the laboratory, panther chameleons exhibited a positive phototaxis to greater visible, ultraviolet A (UV-A) and UV-B light. However, with equivalent high irradiances of UV-A or UV-B, their response to UV-B was significantly greater than it was to UV-A. Exposure of in vitro skin patches of panther chameleons to high UV-B (90 μW/cm²) for 1 h significantly enhanced vitamin D₃ concentration. Voluntary exposure to higher UV-B irradiance (70 vs. 1 μW/cm²) resulted in greater circulating 25-hydroxyvitamin D₃ in female panther chameleons (604 vs. 92 ng/mL). Depending on dietary intake of vitamin D₃, chameleons adjusted their exposure time to UV-B irradiation as if regulating their endogenous production of this vital hormone. When dietary intake was low (1–3 IU/g), they exposed themselves to significantly more UV-producing light; when intake was high (9–129 IU/g), they exposed themselves to less. Vitamin D₃ photoregulation seems to be an important additional component of the function of basking.

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Introduction and Background

Many reptiles use sunlight for thermoregulation. The literature on reptilian basking behavior and thermoregulation is extensive (e.g., Cowles and Bogert 1944; Huey and Slatkin 1976; Huey 1982; Hutchinson 1989; Angilletta 2001; Blouin-Demers and Weatherhead 2001). A range of thermoregulatory precision exists. Classic thermoregulators shuttle between sun and shade to maintain a relatively constant body temperature above or below that of their immediate environment. Passive thermal conformers maintain a body temperature conforming closely to that of their immediate environment. Facultative thermoregulators (Kingsbury 1994) thermoregulate but with less precision or continuity than do classic thermoregulators. Although some chameleon species seem to tolerate more variable body temperatures than do many lizards (Burrage 1973; Hebrard et al. 1982), they definitely thermoregulate at temperature extremes (Burrage 1973; K. B. Karsten, unpublished data).

Understanding of thermoregulation has substantially increased over the years. Studies have defined in considerable detail many of the physical, physiological, and behavioral factors contributing to the balance of heat loss and heat gain (Huey 1982; Bauwens et al. 1996; Díaz 1997; Downes and Shine 1998; Pough et al. 1999 [see pp. 129–134]; Du et al. 2000; Angilletta et al. 2002). Various procedures and technologies, including the concept of the null model, have been used to quantify the precision of thermoregulatory behavior (Hertz 1992b; Hertz et al. 1993; Blouin-Demers and Weatherhead 2001). Despite the plethora of studies on thermoregulatory behavior, there have been only a few suggestions of potential functions of basking other than thermoregulation and no experiments focusing on nonthermoregulatory aspects of basking. Additional benefits of basking have often been considered to be incidental with little importance attached to their role in the evolution of basking behavior, at least until recently (Manning and Grigg 1997).

Potential benefits of basking other than thermoregulation for normal physiological maintenance include the following. Fever response to infection (Vaughn et al. 1974; Burns et al. 1996), an extended function of nonpathological thermoregulatory behavior, has been documented. Aquatic vertebrates such as turtles leave the water, dry their surface, and may benefit by removing external parasites and commensals (Pritchard and Greenhood 1968; Hutchinson 1989). Basking turtles expose the
skin of their extremities to ultraviolet (UV) light, which may stimulate vitamin D₃ synthesis (Pritchard and Greenhood 1968).

The panther chameleon, *Furcifer pardalis*, an arboreal lizard from Madagascar, requires ultraviolet B (UV-B) irradiation in captivity for proper health and reproduction (Ferguson et al. 1996, 2002a). In the course of our studies documenting the UV-B requirement, we discovered that panther chameleons are attracted to light with a strong UV-B component and voluntarily increase their exposure when deprived of dietary vitamin D₃ (Jones et al. 1996; Ferguson et al. 2002b). Here we present evidence that UV-B enhances vitamin D₃ condition in the panther chameleon, and we compile new and previous experimental evidence that behavioral vitamin D₃ photoregulation may exist for this species. An indoor experiment tested the behavioral response of juvenile panther chameleons fed either high or low vitamin D₃ diets to four different artificial UV-producing light environments set up as gradients (Table 1; Fig. 1). An additional indoor experiment compared the responses of chameleons fed a low dietary vitamin D₃ to ultraviolet A (UV-A) and UV-B light. An outdoor experiment tested the response of chameleons fed different vitamin D₃ diets to vitamin D₃-generating natural sunlight in arboreal sun/shade mosaic environments.

### Material and Methods

In one indoor study, 26 juvenile female panther chameleons, hatched in our laboratory at Texas Christian University (TCU), were maintained in isolation in terraria in the laboratory and exposed to manipulations of dietary vitamin D₃, visible light, and UV radiation (Jones et al. 1996; Ferguson et al. 2002b). Light was provided using various combinations of GE cool white, Vita-Lite (Duro-Test), blacklight (GE BL-40), and sunlamp (Philips FS-40) fluorescent tubes (Table 1). UV-A and UV-B irradiances were monitored using Spectronics UV meters (models DM-365N and DM-300N, respectively), and visible illuminance was monitored with a GE light meter (model 214). The daily photophase was provided by 12 h illumination of either Vita-Lites (environments 1 and 2; Table 1) or cool white tubes (environments 3 and 4; Table 1). During UV treatments, a full-exposure/full-shade gradient was established by placing a board on top of the terrarium, which was illuminated from above (Fig. 1). UV treatments were 1 h/d 5 d/wk for light environments 1 and 2 (Table 1) and 3 d/wk for light environments 3 and 4. All UV treatments and observations occurred at midday, approximately 4 h into the photophase. The four light environments during the UV treatments were as follows: environment 1: higher UV-A, lower UV-B, higher visible; environment 2: lower UV-A, higher UV-B, higher visible; environment 3: lower UV-A, higher UV-B, lower visible; and environment 4: lower UV-A, lower UV-B, lower visible (Table 1).
Results for the first 6 mo of the experiment (juvenile phase of the life cycle) are reported here.

For each lizard in each of the four treatments, we calculated the percent of the time that the full (maximum possible) exposure zone was inhabited. The mean percent for a given lizard was calculated for the entire 6-mo period. Terrarium temperature gradients, provided with heat tape underneath the floor of the cage, did not correspond with light gradients (Fig. 1), so during UV treatments, equivalent temperatures were available in both the light and shaded regions of the cage.

Vitamin D$_3$ was manipulated by feeding crickets experimental grain diets differing only in vitamin D$_3$ content (Zeigler, Gardners, Pa.; Jones et al. 1996; Ferguson et al. 2002#; Table 1). Crickets were then fed to the chameleons. Dietary mass was sufficient to ensure rapid growth and maturity of the chameleons within 6–8 mo.

An additional indoor experiment used 14 hatchling lizards. The setup and procedure were the same as those previously described, but the light environments differed (environments 5–8; Table 2). Effort was made to balance the irradiance of the higher UV-A and UV-B levels and the lower UV-A and UV-B levels better than in the first experiment in order to evaluate the relative attracting value of UV-A and UV-B. Visible light and dietary vitamin D was low for all treatments in this experiment. The experiment was conducted for 8 wk.

After reaching maturity, several chameleons exposed daily to environments 3 and 4 (Table 1), which differed substantially in UV-B, were bled to measure UV-B effects on circulating 25-hydroxyvitamin D$_3$ (calcifediol). Plasma calcifediol was measured using a competitive-binding assay (Chen et al. 1990). Cricket and grain samples were analyzed for vitamin D$_3$ content using high-performance liquid chromatography (HPLC; Chen et al. 1992). Patches of skin (0.25 cm$^2$) were excised from two panther chameleons, which were killed by decapitation followed immediately by brain destruction, and placed on a piece of filter paper soaked in physiological saline. The skin patches were immediately exposed to a Philips sunlamp (90 W/cm$^2$) and fed crickets maintained on the low vitamin D$_3$ grain diet. Beginning 2 mo before being taken to outdoor enclosures, crickets for three of the lizards were dusted by agitation at each feeding with a high vitamin D$_3$ mineral-based powder (Rep-Cal [Rep-Cal Research Labs, Los Gatos, Calif.] or Miner-all I [Sticky Tongue Farms, Menifee, Calif.]). Crickets dusted with Miner-all I provided 129 IU/g of vitamin D$_3$ to the lizards. Crickets dusted with Rep-Cal containing vitamin D$_3$ provided 66 IU/g of vitamin D$_3$. Crickets for the other three lizards were dusted with pure calcium carbonate or Miner-all 0, neither of which resulted in a concentration in the crickets of more than 3 IU/g of vitamin D$_3$. The six chameleons were taken to cylindrical outdoor enclosures measuring approximately 2 × 2 m. Each enclosure was constructed from 1.25 × 2.5-cm welded-wire mesh and surrounded one or two chickasaw plum trees, *Prunus angustifolia*, trimmed to prevent the chameleons from reaching and climbing onto the wire. Metal flashing was placed along the inside base of the enclosure to prevent chameleons from climbing the wire from the ground. Black vinyl curtains were placed on top of each enclosure to shade about one-third of the enclosure throughout the day. Each enclosure housed a single chameleon, which was visually isolated from chameleons in the other enclosures. After a week of acclimation in the enclosures, observations began. On the first day, lizard location in the tree was accurately monitored throughout the 12-h day using a coordinate system (K. B. Karsten and G. W. Ferguson, unpublished data). Locations were recorded every 20 min for all lizards. On the following day, a series of four in vitro models (ampoules containing a solution of provitamin D$_3$; Lu et al. 1992) were used to retrace the previous-day path of the lizard (Carman et al. 2000). A model was attached to the perch site, relocated as necessary, and replaced every 3 h throughout the day. Exposure of the model to UV-B irradiation causes conversion to previtamin D$_3$ and other photoproducts at a rate

### Table 2: Voluntary exposure of juvenile female panther chameleons fed low vitamin D$_3$ diets to available light in four additional light environments (5–8)

<table>
<thead>
<tr>
<th>Light Environment (No. of Lizards)</th>
<th>Maximum UV-A (μW/cm$^2$)</th>
<th>Maximum UV-B (μW/cm$^2$)</th>
<th>Maximum Illuminance (lux)</th>
<th>% Full Exposure with Low Vitamin D Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 (3)</td>
<td>4</td>
<td>67</td>
<td>344</td>
<td>49.6 ± 9.0</td>
</tr>
<tr>
<td>6 (4)</td>
<td>63</td>
<td>72</td>
<td>387</td>
<td>43.4 ± 7.8</td>
</tr>
<tr>
<td>7 (4)</td>
<td>69</td>
<td>5</td>
<td>344</td>
<td>15.6 ± 7.8</td>
</tr>
<tr>
<td>8 (3)</td>
<td>1.4</td>
<td>0</td>
<td>409</td>
<td>17.5 ± 9.0</td>
</tr>
</tbody>
</table>

Note. Percent full exposure values are mean ± 1 SE and represent the mean percent of the observations during a UV treatment period that a lizard located itself in the full exposure region of the light gradient (see Fig. 1). Low vitamin D$_3$ diet included crickets containing 1 IU/g of vitamin D$_3$ (Jones et al. 1996). The high UV-B effect was significant.
Discussion

For more than half a century, studies have focused on thermoregulation of ectothermic vertebrates. However, many findings are difficult to explain. For example, some thermoregulators shift preferred temperature seasonally for unknown reasons (Christian and Bedford 1995; Seebacher and Grigg 1997). Although a shift can be explained as a shift in cost-benefit balance involving energy efficiency or predator avoidance, the phenomenon is widespread and can also be explained as compromises for competing physiological benefits of basking. Thus, seeking a higher body temperature during the breeding season can maximize energy utilization and allow a female lizard to produce more young, reproduce faster, or run faster to escape a predator. But it can also allow an animal to bask longer to attain higher vitamin D₃ levels at a time of maximum vitamin D₃ requirements.

In addition to changes in thermal preference, some thermal conformers bask for unknown reasons. Thus, the Australian freshwater turtle *Emydura signata* is frequently observed to bask, but temperature is not elevated significantly above water
Our current understanding of the role, acquisition, and regulation of vitamin D₃ comes from studies of terrestrial vertebrates, mostly endotherms. Vitamin D₃ is an important nutrient complexly involved in calcium and phosphorus metabolism (for summaries, see Holick 1996, 1999a, 1999b, 1999c). Vitamin D₃ may also play critical roles in a number of other poorly understood physiological processes (Bidmon and Stumpf 1996; Holick and Jung 1996, 1999). It can be obtained either in the diet or by endogenous synthesis in the skin. Exposure of the skin to UV-B irradiation converts provitamin D₃ (7-dehydrocholesterol), a steroid widely available in the skin of vertebrates, to previtamin D₃, which is then thermally isomerized to vitamin D₃ or cholecalciferol. Cholecalciferol is transported from the skin by vitamin D–binding protein through the bloodstream first to the liver, where it becomes 25-hydroxyvitamin D₃, or calcifiediol (Holick and Clark 1978). Then, it is carried to the kidney, where it is hydroxylated into its most biologically active form, 1,25 dihydroxyvitamin D₃, or calcitriol (Holick et al. 1971). Calcitriol is a hormone whose best-known role is to maintain circulating calcium levels (Holick 1999b, 1999c). This occurs primarily by promoting absorption of calcium from the gut and resorption from the kidney filtrate. Calcitriol deficiency results in depletion of body calcium and serious bone demineralization. This is mediated principally by a rise in parathyroid hormone, the primary stimulator of calcium resorption from bone (Hurwitz 1989).

The regulation of vitamin D₃ is complex and not fully understood. In endotherms, there is evidence for physiological regulation at several levels (Holick 1999b, 1999c). First, excess previtamin D₃ production with prolonged UV-B exposure is diverted with the conversion of previtamin D₃ to the biologically inert photoproducts lumisterol and tachysterol (Holick et al. 1981). These compounds can serve as reservoirs of previtamin D₃ since the reaction is reversible. Excess vitamin D₃ remaining in the skin is unstable after exposure to UV radiation, which converts it to relatively inert compounds such as suprasterol I, suprasterol II, and 5,6, transvitamin D (Webb et al. 1989). There is some feedback at the first hydroxylation conversion in the liver (Bell 1985), but this is relatively weak in mammals, and levels of the liver-produced metabolite calcifediol fluctuate substantially with input of vitamin D₃ from the skin and gut. Thus, circulating level of calcifediol is considered a good measure of “vitamin D condition” of an animal (Haddad and Stamp 1974; Haddad and Walgate 1976). Regulation of the hormonally active calcitriol is more precise in the kidney, and circulating levels are relatively constant. Decreased circulating calcium triggers the release of parathyroid hormone, which stimulates the kidney to produce calcitriol. As calcium increases from enhanced absorption from the gut or bone, parathyroid hormone and calcitriol decrease possibly under the direct influence of elevated phosphorus (Holick 1999b). Although incompletely studied, much of this scenario seems to apply to lizards (Holick et al. 1995; Liang and Fraser 1999).

Although the complexity of physiological and biochemical regulation of vitamin D₃ is under active investigation, the potential role of behavior remains virtually ignored. Can some vertebrates detect UV-B radiation and adjust their exposure on the basis of internal vitamin D₃ condition? In ectotherms, might this be an important step in vitamin D₃ regulation?

Our data suggest that panther chameleons are able to assess temperature (Manning and Grigg 1997). Manning and Grigg (1997) suggested that there are functions for basking other than thermoregulation in this species. Vitamin D₃ synthesis has been suggested to be an important factor stimulating turtles to bask (Pritchard and Greenhood 1968; Hutchinson 1989), but the specific role of behaviorally basking for UV-stimulated vitamin D synthesis has not been addressed experimentally before our studies.

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<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient</th>
<th>SE</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y-intercept of model</td>
<td>.34</td>
<td>.039</td>
<td>8.6</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Vitamin D₃</td>
<td>-.01</td>
<td>.003</td>
<td>-3.5</td>
<td>.002</td>
</tr>
<tr>
<td>UV-B</td>
<td>.001</td>
<td>.001</td>
<td>2.3</td>
<td>.034</td>
</tr>
<tr>
<td>UV-A</td>
<td>.001</td>
<td>&lt;.001</td>
<td>3.0</td>
<td>.007</td>
</tr>
<tr>
<td>Visible</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>3.5</td>
<td>.002</td>
</tr>
</tbody>
</table>

Note. Each of the light variables had a significant (P < .05) positive effect on exposure; dietary vitamin D₃ had a significant negative effect. The regression model explained most of the variance of voluntary exposure (r² = 0.84, P < .001).

Table 4: Multiple regression analysis of the effects of dietary vitamin D₃ and light variables on voluntary exposure of juvenile female panther chameleons to artificial light in laboratory environments 1–4

![Figure 2](image)

Figure 2. Effect of dietary vitamin D₃ of chameleons in outdoor enclosures on percent conversion of 7-dehydrocholesterol (provitamin D₃) to photoproducts in ampoules retracing their previous-day locations from 10:00 A.M. to 1:00 P.M. Capped lines are standard errors. The diet effect was significant.
their internal vitamin D₃ status, perceive their UV environment, and behaviorally adjust their exposure in a regulatory manner. Existing information suggests that the prerequisites for this ability may exist in lizards such as the panther chameleon. Thus, vitamin D receptors, identified in the brain of Anolis lizards (Bidmon and Stumpf 1996), suggest a mechanism for perceiving the level of circulating vitamin D₃. Lizards, including the panther chameleon, have UV-sensitive retinas (Fleishman et al. 1993; E. R. Loew, personal communication). Hence, they should be able to detect UV radiation, provided it is not filtered out completely by the lens of the eye. The greater attraction to UV-B than to UV-A demonstrated in this study does not prove that panther chameleons can directly perceive UV-B. Data such as optic nerve activation in response to UV-B stimulation through an intact eye are needed to verify perceptual acuity. However, greater attraction to UV-B does show that panther chameleons can discriminate a light environment that provides a beneficial physiological effect, that is, enhanced vitamin D₃ condition. Ours is perhaps the first experimental support for the hypothesis that regulation of vitamin D₃ in ectotherms may extend beyond the physiological mechanisms previously described and involve phototactic behavior.

An interesting question is, How would such an ectotherm balance the functions of behavioral thermoregulation and vitamin D₃ photoregulation? Might it involve temporal shifts in thermal preference (Christian and Bedford 1995; Seebacher and Grigg 1997), that is, allowing the body to tolerate more sun exposure and the resulting higher body temperature during periods of high vitamin D₃ requirements? Alternatively, might an ectotherm be able to uncouple these functions? A chameleon may bask to elevate its temperature during the early morning hours when UV irradiation is low (Lu et al. 1992). Then, it might retreat to shady environments to prevent overheating during late morning. If the animal needed to replenish its pre-vitamin D₃, it could expose itself to full or partial sun during midday, when UV irradiance is highest (Lu et al. 1992). However, at midday, bouts of exposure would have to be brief and terminated when body temperature began to elevate. Whether short midday bouts would be sufficient to relieve low vitamin D₃ condition is unknown. One might think that desert-dwelling lizards would be in such a light-rich environment that UV irradiation might never be limited. However, the heat loads in such environments may curtail daytime activity to the extent that it is dangerous to be exposed at times when UV is most readily available.

UV-B limitation suggests another issue. How do lizards adapted to low UV-B light environments obtain vitamin D₃? Must they seek vitamin D-rich foods, or are they still able to generate vitamin D₃ endogenously with low levels of UV-B? We have begun to address this issue (Carman et al. 2000). Nocturnal/crepuscular house geckos Hemidactylus turcicus are active in low UV-B light environments, yet they have skins highly sensitive regarding UV-B-induced vitamin D₃ synthesis. Conversely, diurnal Texas spiny lizards are active in higher UV-B light environments but have skins less sensitive regarding UV-B-induced vitamin D₃ synthesis. The natural dietary levels of vitamin D₃ seem to be lower for the geckos than they are for Texas spiny lizards. We currently do not know whether these species actively show phototactic responsiveness to UV-B, which would suggest that they possess vitamin D–regulating behavior. However, low opportunity may be compensated by enhanced ability. An interesting comparison would be relative opportunity and compensatory ability for UV-B-induced vitamin D₃ production for species or populations within a clade, such as sun-dwelling and shade-dwelling Anolis (Hertz 1992a, 1992b).

In conclusion, the potential role of basking behavior for UV-stimulated vitamin D photoregulation has been virtually unstudied. However, our results clearly show a link between basking behavior and internal vitamin D condition in the panther chameleon. Because vitamin D photosynthetic ability and opportunity may vary among squamate species (Carman et al. 2000), ectotherms may vary in their vitamin D–regulating systems, and these may differ from those of mammals. This previously underemphasized complexity in basking behavior may help explain some of the puzzling findings (e.g., shifts in thermal preference) in previous studies of thermoregulation.

Acknowledgments

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