Dermato-Endocrinology 5:1, 192–198; January/February/March 2013; © 2013 Landes Bioscience

Effects of sunlight on behavior and 25-hydroxyvitamin D levels in two species of Old World fruit bats

Lizabeth O. Southworth,[1,*] Michael F. Holick,[2] Tai C. Chen[2] and Thomas H. Kunz[1]

[1]Center for Ecology and Conservation Biology; Department of Biology; Boston University, Boston, MA USA; [2]Section of Endocrinology, Diabetes, and Nutrition; Department of Medicine; Boston University School of Medicine; Boston, MA USA

Keywords: bats, calcium, Chiroptera, sunlight, UV-B radiation, vitamin D

It has long been accepted that most vertebrate animals meet their vitamin D requirements from exposure of skin to UV-B (UV-B) radiation. Many factors affect this endogenous synthesis of vitamin D, including season, latitude, time of day, age, presence of hair, and degree of skin pigmentation. Most bats roost in dark places by day and forage at night, and thus have little or no potential for sunlight exposure. Notwithstanding, some tropical species are diurnal and are known to roost in the canopy of trees where they may be exposed to sunlight for up to 12 h each day. In this study, two species of captive tropical bats (both species are active at night but one, *Rousettus aegyptiacus,* roosts in caves, tombs, and buildings, whereas the other, *Pteropus hypomelanus,* roosts in trees) were evaluated for their ability to endogenously synthesize vitamin D. Following timed periods of sunlight exposure, blood plasma was analyzed using a competitive protein binding assay (CPBA) to determine concentrations of 25-hydroxyvitamin D [25(OH)D], the major circulating vitamin D metabolite. The ability to photoconvert provitamin D (7-dehydrocholesterol, 7-DHC) in the sub-tropical winter was determined using sunlight exposed borosilicate samples of 7-DHC in hourly increments. Finally, both species were evaluated in their preference for a roost site by the release of individuals into sunlight or shade in timed trials.

Our results support the hypotheses: (1) when exposed to natural sunlight, both species exhibited an ability to endogenously synthesize vitamin D, although significant differences were found between the two, (2) photoconversion of 7-DHC to previtamin D_3 is possible during the mid-day hours of a sub-tropical winter day and (3) captive, cave roosting *R. aegyptiacus* will choose shaded roost sites while captive *P. hypomelanus* will show no preference for either shade or sun.

Introduction

For most vertebrates, vitamin D_3 is important for intestinal absorption of calcium and phosphorus and is either ingested in the diet (though few foods naturally contain vitamin D) or synthesized in the skin following exposure to solar UV-B (UV-B) radiation with wavelengths between 290–315 nm.[1-3] This synthetic process involves epidermal provitamin D, (7-dehydrocholesterol, 7-DHC), which is first converted to previtamin D_3, and subsequently isomerized to vitamin D_3 within several hours at 37°C.[4,5] Vitamin D (represented by either D_2 or D_3) synthesis from steroid precursors is an ancient evolutionary process that has been postulated to have its origin in the primordial sea, where it was used by phytoplankton as a possible "sunscreen" to prevent UV-B damage.[2,6]

With continued irradiation, vitamin D_3 can be further photoisomerized to 5,6-trans-vitamin D_3, and supersterols 1 and 2, or translocated from the skin into circulation. In the blood, it becomes bound to the vitamin D-binding protein and is transported to the liver for metabolism to 25-hydroxyvitamin D [25(OH)D], the major circulating metabolite.[7-9] Before a biological response is achieved, 25(OH)D must be hydroxylated to 1,25-dihydroxyvitamin D [$1,25(OH)_2D$] in the kidney or other tissues; however, consideration of $1,25(OH)_2D$ as a physiological index of vitamin D-status is inappropriate because vitamin D deficiency stimulates parathyroid hormone production which in turn enhances the conversion of 25(OH)D to $1,25(OH)_2D$, leading to a normal or elevated concentration of this metabolite.[3,10,11]

A number of factors affect the cutaneous synthesis of vitamin D_3 including season, latitude, time of day, age, presence of hair, and degree of skin pigmentation.[4,11,12] Ultimately, however, the single most significant factor that regulates production of cutaneous vitamin D_3 is the photosynthesis of the biologically inert photoproducts, lumisterol$_3$ and tachysterol$_3$. Production of these inert compounds limits the skin's synthesis of vitamin D_3, thus preventing hypervitaminosis that would otherwise result from prolonged exposure to sunlight.[8,9]

Most bats are nocturnally active,[13] many roost in dark places during the day,[14] and thus experience little or no exposure to solar UV-B irradiation. Consequently, they lack the opportunity for endogenous vitamin D_3 synthesis. It is possible that

*Correspondence to: Lizabeth O. Southworth; Email: lizsouthworth@mac.com
Submitted: 11/27/12; Revised: 02/11/13; Accepted: 02/15/13
http://dx.doi.org/10.4161/derm.24020

Table 1. Comparison of circulating 25(OH)D after 5 h daily sun exposure in 2 species of Old World fruit bats

25(OH)D (without sunlight, ng/ml)					25(OH)D (with sunlight, ng/ml)				
Rousettus aegyptiacus	0	30 d	60 Days	90 Days	***Rousettus aegyptiacus***	0	30 d	60 Days	90 Days
1	ND[1]	ND[1]	ND[1]	ND[1]	1	7.0	24.0	48.0	36.0
2	ND[1]	ND[1]	ND[1]	ND[1]	2	5.8	40.0	68.0	94.0
3	ND[1]	ND[1]	ND[1]	ND[1]	3	6.8	17.0	ND[1]	74.0
4	8.6	ND[1]	ND[1]	ND[1]	4	5.6	11.0	35.0	54.0
5	ND[1]	ND[1]	ND[1]	ND[1]	5	6.8	50.0	100.0	105.0
Pteropus hypomelanus	0	30 d	60 Days	90 Days	***Pteropus hypomelanus***	0	30 d	60 Days	90 Days
1	ND[1]	ND[1]	ND[1]	ND[1]	1	ND[1]	7.8	ND1	7.8
2	ND[1]	ND[1]	ND[1]	ND[1]	2	ND[1]	10.5	10.0	18.5
3	23.5	ND[1]	ND[1]	ND[1]	3	7.8	13.5	10.5	12.3
4	ND[1]	ND[1]	ND[1]	ND[1]	4	9.0	5.5	9.0	13.3
5	ND[1]	ND[1]	ND[1]	ND[1]	5	11.3	15.0	15.0	17.0

[1]ND, Not Detectable (< 5.0 ng/mL).

over evolutionary time the ability to synthesize vitamin D_3 has been lost in bats, even if it existed at all. However, not all bats are nocturnal, and some tropical species spend up to 12 h each day exposed to direct sunlight.[15] For example, the island flying fox *Pteropus hypomelanus* is nocturnally active, but it typically roosts in tree crowns exposed to direct tropical sunlight during the day.[16] The Egyptian fruit bat *Rousettus aegyptiacus* is also nocturnally active but typically roosts in dark tombs, caves, and rock crevices.[17] In captivity, *P. hypomelanus* is often found roosting in full sunlight, typically with outstretched wings, whereas *R. aegyptiacus* actively avoids sunlight.[18] Skin pigmentation in these two species is also markedly different. The skin of *P. hypomelanus* is black whereas the skin of *R. aegyptiacus* is pale brown-gray with pink undertones. Both species have fully furred bodies and essentially naked wing membranes, legs, ears, and nose. Though roosting preferences and skin pigmentation are different, the diets of free-ranging *P. hypomelanus* and *R. aegyptiacus* consist of fruit and nectar that lacks vitamin D.[11,19] Thus, diet can be eliminated as a source of this nutrient for both species.

Previous studies on frugivorous captive[20,21] and wild-caught bats[22] have consistently found low levels of circulating 25(OH)D, which would indicate vitamin D deficiency when compared with a human reference range of 20–100 ng/mL.[11] However, we found extremely high levels of this metabolite in two carnivorous, nocturnally-active New World bat species that we attributed to high dietary vitamin D.[23] The work presented here is the first of its kind to evaluate endogenous vitamin D synthesis in any bat species.

Our primary goal was to assess the ability of two captive, nocturnally active plant-visiting bat species (*P. hypomelanus* and *R. aegyptiacus*) to synthesize vitamin D_3 in their skin when exposed to natural sunlight. Because melanin present in skin absorbs UV-B radiation, an *in-vitro* model was used to evaluate the maximal conversion of 7-DHC to previtamin D_3. In this model, borosilicate ampoules containing 7-DHC in ethanol were exposed to sunlight for hourly increments on a sunny winter day in Boston, MA and Gainesville, FL. Along with demonstrating maximal conversion of 7-DHC to previtamin D_3, this model examines the effects of season, latitude, and time of day on previtamin D_3 production. Assessment of vitamin D status was made by measuring 25(OH)D, the major circulating metabolite.

We tested three hypotheses as follows: (1) both species are capable of endogenous vitamin D_3 synthesis when exposed to direct sunlight; (2) both species, when presented with the opportunity to seek shady or sunny roosts will select different roosts; and, (3) photoconversion of 7-DHC to previtamin D_3 is possible during the winter in a sub-tropical environment characteristic of Gainesville, FL (29°41' N).

Results

In the timed sun exposure trials of 20 bats (10 *R. aegyptiacus* and 10 *P. hypomelanus,* divided into 2 study groups) performed in Gainesville, FL it can be seen that in September (day 0), 6 out of 10 *R. aegyptiacus* showed low levels of circulating 25(OH)D (5.6–8.6 ng/mL), whereas 4 had levels that were below the limit of detection. Also on day 0, 4 out of 10 *P. hypomelanus* showed low-moderate levels of circulating 25(OH)D (7.8–23.5 ng/mL) whereas 6 out of 10 had levels that were below the limit of detection (**Table 1**, **Fig. 1**).

In October, after 30 d without sunlight, all 5 *R. aegyptiacus* and all 5 *P. hypomelanus* had circulating 25(OH)D below the limit of detection, which remained so for the duration of the study. After 30 d of sunlight exposure, all 5 *R. aegyptiacus* and 4 out of 5 *P. hypomelanus* showed an increase in circulating 25(OH)D. By November (day 90), all 5 *R. aegyptiacus* and all 5 *P. hypomelanus* showed an increase in circulating 25(OH)D (**Table 1**, **Fig. 1**). These results show that when unexposed and exposed *R. aegyptiacus* and unexposed and exposed *P. hypomelanus* were compared, values for mean circulating 25(OH)D (< 5 ng/mL vs. 73 ng/mL and < 5 ng/mL vs. 12 ng/mL, respectively) were significantly different (**Fig. 1**, $p = 0.008$).

Comparing the ability of sunlight exposed *R. aegyptiacus* and *P. hypomelanus* to endogenously synthesize vitamin D_3, the results show that circulating levels of 25(OH)D were significantly higher in the former than the latter (73 ng/mL vs. 12 ng/mL). This is despite the fact that *P. hypomelanus* received slightly more daily sunlight exposure (5.2 vs. 4.5 h) than *R. aegyptiacus*.

7-DHC ampoules exposed to direct sunlight on a clear, sunny day in January, showed a percent conversion to previtamin D_3 which peaked at 6.1% in Gainesville, FL (29°41' N), whereas it scarcely reached 0.5% in Boston, Massachusetts (42°33' N, **Fig. 2**). The total percent conversion to previtamin D_3, indicated by the area under each curve, is 18 times greater in Gainesville than in Boston. The peak conversion in both locations was between the mid-day hours of 1100–1500 h.

Provided with an opportunity to select a sunny vs. shaded roost site, *R. aegyptiacus* preferred shade in all trials, whereas *P. hypomelanus* was equally likely to choose either shade or sun (**Table 2**). Furthermore, with the exception of one individual, all *R. aegyptiacus*, when released into the sun flew to a shaded location in less than 3 sec, demonstrating a strong preference for shade. By contrast, only 4 out of 10 *P. hypomelanus* when released into the sun flew to a shaded location. Latency to alternate condition for this species was 11.58 sec–4 min 23 sec. Hourly observations of unmanipulated individuals indicate that at no time did *R. aegyptiacus* roost in the sun, whereas *P. hypomelanus* was most often found roosting in direct sunlight (**Table 3**).

Discussion

For most animals, vitamin D is vital to the maintenance of appropriate extracellular concentrations of calcium and phosphorus.[12] Given the importance of calcium to both metabolic function and skeletal health in vertebrates, it is not surprising that most can synthesize their own vitamin D when they are exposed to adequate UV-B irradiation. However concentrations of 7-DHC are extremely low in the skin of some vertebrates and it is thought that these animals do not have the ability to endogenously synthesize vitamin D_3 following exposure to UV-B irradiation.[6,24-27] For example, How et al.[24] compared both the initial concentration of the prohormone (7-DHC) in skin and the ability of the Norway rat (*Rattus norvegicus*), dog (*Canis familiarus*), and cat (*Felis catus*) skin to synthesize vitamin D_3 following UV-B irradiation. They found that 7-DHC concentrations were ten times higher in rats as compared with dogs and cats and concluded that dogs and cats had ineffective endogenous vitamin D_3 synthesis, and that this nutrient was most likely obtained from their diet.

Prior to this time, no studies had been conducted to test the effect of sunlight on production of circulating vitamin D, especially in a vertebrate known to be nocturnally active. A number of factors known to critically influence this synthetic process include latitude, time of year, and time of day.[6,26,27] In temperate regions of the world (north and south of 40° latitude), the increased zenith angle of the sun in winter means that solar radiation must travel farther to reach the Earth's surface.[28] This ultimately decreases the intensity of UV-B radiation through scattering and absorption by the ozone layer, and thus reduces the endogenous synthesis of vitamin D_3 in animals and humans.[29,30] We show, in our 7-DHC ampoule irradiation experiment, that on a clear, cloudless day in January, previtamin D_3 production is possible during the winter in sub-tropical Gainesville, FL, when compared with a northerly location (Boston, Massachusetts) where it is not. This observation also supports results of previous studies, in which seasonal previtamin D_3 production is markedly altered in locations north and south of 40° latitude.[29,30]

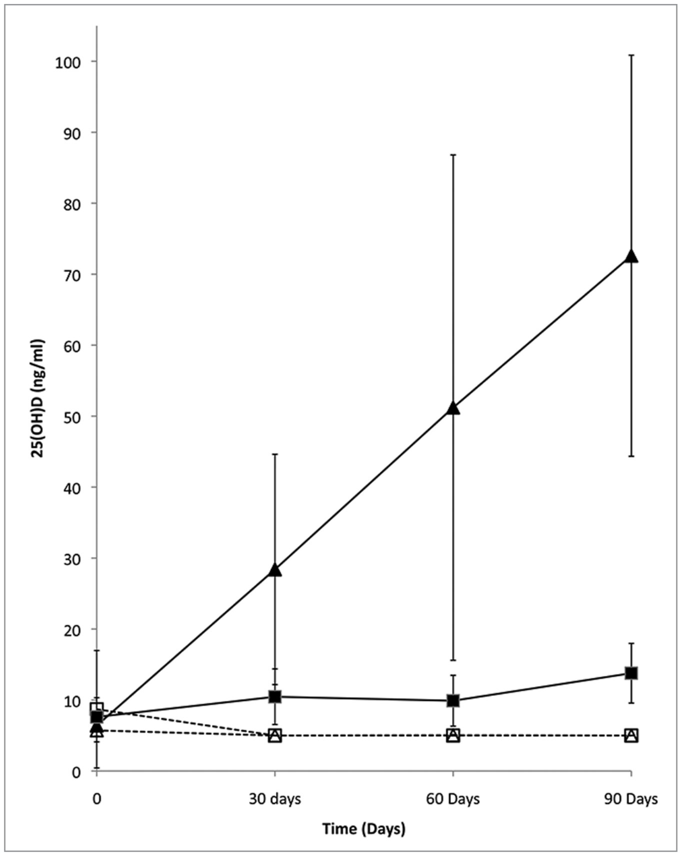

Figure 1. Comparison of circulating 25(OH)D after 5 h daily sun exposure in 2 species of old world fruit bats. ▲ *R. aegyptiacus* (with sunlight), □ *R. aegyptiacus* (without sunlight), ■ *P. hypomelanus* (with sunlight), □ *P. hypomelanus* (without sunlight). The data shown is the mean +/- the standard deviation of 5 test animals. At 90 d the difference is significant at $p = 0.008$ for bats exposed to sunlight vs. those not exposed to sunlight.

A similar decrease in the intensity of UV-B radiation occurs as the Earth rotates on a daily basis. This is readily observed in the results of our 7-DHC ampoule irradiation experiment which showed that previtamin D_3 production is limited to the hours between 0900 and 1600 h in the subtropics. These results support previous findings on the influence of time of day on previtamin D_3 synthesis[11] and refute the hypothesis of Cavaleros et al.[21] that nocturnal bats may venture out during crepuscular periods to gain access to sunlight and thereby synthesize vitamin D. Ampoule conversion of 7-DHC to previtamin D_3 represents the maximal quantity possible, without the effects of melanin. Prior to 0900 and after 1600 h essentially no previtamin D_3 is produced, thus it is highly improbable that any animal with pigmented skin would have the ability to synthesize previtamin D_3 outside this range.

The confirmation that sunlight in winter in Gainesville, FL, is sufficient for in vitro previtamin D_3 production was supported

Table 2. Preference for sun or shade in two species of Old World fruit bats when released into the sun or shade

Released into the Sun					
Pteropus hypomelanus	LAC[1]	5 min	*Rousettus aegyptiacus*	LAC[1]	5 min
1	11.58 s	Shade	1	2.95 s	Shade
2	none	Sun	2	1.67 s	Shade
3	none	Sun	3	1.75 s	Shade
4	4 min 23.00 s	Shade	4	1.82 s[2]	Shade
5	22.54 s	Shade	5	2.17 s[2]	Shade
6	28.22 s	Shade	6	0.96 s	Shade
7	none	Sun	7	2.40 s	Shade
8	none	Sun	8	9.94 s	Shade
9	none	Sun	9	0.98 s[2]	Shade
10	none	Sun	10	1.47 s	Shade
			11	0.74 s[2]	Shade
Released into the Shade					
Pteropus hypomelanus	LAC[1]	5 min	*Rousettus aegyptiacus*	LAC[1]	5 min
1	none	Shade	1	none	Shade
2	none	Shade	2	none	Shade
3	none	Shade	3	none	Shade
4	none	Shade	4	none	Shade
5	none	Shade	5	none	Shade
6	none	Shade	6	none[2]	Shade
7	4 min 23.00 s	Sun	7	none[2]	Shade
8	none	Shade	8	none	Shade
9	none	Shade	9	none[2]	Shade
10	none	Shade	10	none	Shade
			11	none[2]	Shade

[1]LAC, Latency to Alternate Condition. [2]Indicates brief, usually 2–3s flight into and out of alternate condition, sometimes more than once and in one case for 17s. However, the bat always returned to the shade.

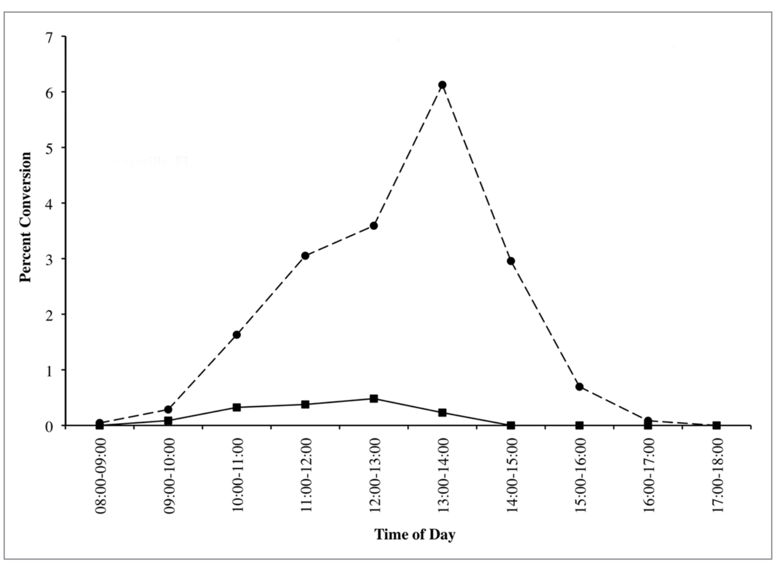

Figure 2. Percent conversion of provitamin D to previtamin D and its photoproducts on a clear sunny day in January in ● Gainesville, FL. and ■ Boston, MA.

by our results that both captive *P. hypomelanus* and *R. aegyptiacus* are able to endogenously synthesize vitamin D_3 when exposed to daily natural sunlight over a period of 90 d. Both species showed significant increases in circulating 25(OH)D, although the 25(OH)D values for black-skinned *P. hypomelanus* at 90 d were significantly lower (8–18 ng/mL) than the 90 d values for pale brown-skinned *R. aegyptiacus* (36–105 ng/mL) despite the fact that *P. hypomelanus* received greater daily exposure to the sun. The lower values for black-skinned *P. hypomelanus* support published reports for humans and other mammals that darkly pigmented skin, when compared with lightly pigmented skin, requires a longer period of sun exposure to synthesize the same amount of vitamin D_3 (because melanin absorbs UV-B photons in the same wavelengths as does 7-DHC, and thus acts as an effective sunscreen).[6,19] In contrast, the much higher values for pale brown-skinned *R. aegyptiacus* demonstrates their ability to efficiently produce vitamin D with minimal sun exposure. And while these values are very high, they are not excessive. In a study by Haddad and Chyu,[31] similarly high values (64.4 ng/mL) were obtained when lifeguards were exposed to approximately 53.0 h of weekly sunlight. Although to our knowledge this has not been studied in bats, the high values obtained in this study are unlikely to cause vitamin D toxicity, for as in humans, it is expected that bats are able to photoisomerize previtamin D_3 into the biologically inert photoproducts lumisterol and tachysterol following extended exposure to sunlight.

Although both species are nocturnally active, they exhibit very different roosting behavior. *Pteropus hypomelanus* typically roosts in tree crowns exposed to tropical sunlight, whereas *R. aegyptiacus* typically roosts in dark places under natural conditions and thus is not exposed to UV-B radiation.[16,17] Similar observations were made in our behavioral experiment with captive bats, where we showed *P. hypomelanus* was commonly observed roosting in direct sunlight whereas *R. aegyptiacus* actively avoided sun exposure. This may help explain the unexpected decrease in circulating 25(OH)D in one individual of *R. aegyptiacus* at 90 d. This species exhibited tight clustering behavior when exposed to natural sunlight which may have led to a decrease in UV-B irradiation to the skin of that individual. The undetectable 25(OH)D in another individual at 60 d may represent assay error or mislabeling as the value rapidly increased at 90 d (**Table 1**). It is clear that specific and distinct roosting differences exist in the two species reported in this study, not only in free-ranging animals but also when individuals are housed in captivity where alternative conditions are available.

The importance of these differences to vitamin D status may, however, depend on the overall importance of vitamin D to calcium metabolism in *R. aegyptiacus* and *P. hypomelanus*. Interestingly, the Damara mole rat (*Cryptomys damerensis*) and the naked mole rat (*Heterocephalus glaber*) are thought to passively absorb intestinal calcium independent of any source of vitamin D. Skinner et al.[32] found that *C. damerensis* had a minimal response to supplementary vitamin D_3 and that calcium absorption was nonsaturable and vitamin D-independent. Similarly, Buffenstein and Yahav[33] found that vitamin D_3 supplementation had no effect on circulating calcium or phosphorous in *H. glaber* concluding that the animals employed a vitamin D-independent method of mineral homeostasis.

It is quite possible that nocturnal, free-ranging bats that have no known dietary source of vitamin D have evolved to also absorb intestinal calcium independent of vitamin D. Indeed, Keegan et al.[34] found that the small intestine of *R. aegyptiacus* was freely permeable to calcium in both directions indicating a vitamin D-independent process. It is also possible that a currently unknown source of dietary vitamin D is available to free-ranging animals as has been suggested by others.[35,36]

In summary, our results support the hypotheses: (1) photoconversion of 7-DHC to previtamin D_3 is indeed possible during the winter in sub-tropical Gainesville, FL; (2) both species of bats studied will select different roosting sites when presented with the opportunity to seek shade or sun; and most importantly (3) vitamin D is synthesized endogenously by both species of captive fruit bats following exposure to sunlight; however, the cave-roosting species with paler skin pigmentation (*R. aegyptiacus*), synthesized greater amounts of vitamin D_3, despite slightly less daily sunlight exposure, than did the tree-roosting species with darker skin pigmentation (*P. hypomelanus*). In these two species at least, our data strongly suggest that the ability to endogenously synthesize vitamin D following exposure to sunlight has been conserved in bats even though it may prove unnecessary to intestinal calcium absorption and mineral homeostasis.

Table 3. Preference for sun or shade in two species of Old World fruit bats at different times of the day

		Time of day					
Species	**n**	**10:00**	**11:00**	**12:00**	**13:00**	**14:00**	**15:00**
Pteropus hypomelanus	9						
Sun		9	9	9	7	7	7
Shade		0	0	0	2	2	2
Rousettus aegyptiacus	11						
Sun		0	0	0	0	0	0
Shade		11	11	11	11	11	11

Finally, our results have important implications for bats maintained in captivity for long periods at temperate latitudes, which may depend on endogenously synthesized vitamin D_3 to meet their physiological needs, especially during lactation in females, when demands for calcium are high.

Materials and Methods

Animals/blood collection. Captive groups of the island flying fox, *P. hypomelanus* (n = 10; body mass 484–796 g) and the Egyptian fruit bat, *R. aegyptiacus* (n = 10; body mass 102–168 g), born, raised, and housed at the Lubee Bat Conservancy in Gainesville, FL, were randomly assigned to either an "exposed" or "unexposed" group. Thus, four groups containing five animals each were formed and were housed together. All "exposed" animals were provided with approximately 5 h of natural sunlight daily by accessing an outside holding cage of 1.83 min × 1.83 min × 1.37 min for *P. hypomelanus* and 0.60 min × 60 min× 0.91 min for *R. aegyptiacus*. "Unexposed" animals were maintained indoors without access to natural sunlight for the duration of the 90 day study. On day 14, we observed that *R. aegyptiacus* began to develop moderate erythema. Thus, we decreased their exposure time to 2–4 h/day on sunny days over 78°F, while exposure time of *P. hypomelanus* was maintained at approximately 5 h/day. Indoor illumination was provided by 60 Watt incandescent bulbs. *Pteropus hypomelanus* was housed in 1.83 min × 1.83 min × 1.37 min wire holding cages whereas *R. aegyptiacus* was housed in 0.60 min × 60 min × 0.91 min wire holding cages. All bats prior to, and during the study, were maintained on a vitamin D deficient, mixed fruit and vegetable diet, supplemented with a commercially prepared, vitamin D deficient, powdered vitamin and mineral supplement (Lubee fruit bat supplement). All animals were fed daily in the late afternoon, consistent with the feeding practice at the Lubee Bat Conservancy prior to the study. Water was available to the bats *ad libitum*.

To facilitate handling during blood collection, each bat was anesthetized with isoflurane (5% decreased to 2.5%) in oxygen supplied from a mask. Blood samples were collected within 5 min of capture and on the following schedule: baseline, 30 d, 60 d, and 90 d. The collected blood was transferred to either a 1.1 mL or 3 mL plasma separator tube and centrifuged at 3,000 rpm for 10 min. The plasma fraction was transferred by pipette to a 1 mL cryotube and held at -20°C (or lower) until analyzed, whereas the red cell fraction was discarded.

Sample analysis. Plasma 25(OH)D was obtained by absolute ethanol extraction followed by a competitive protein-binding assay (CPBA) using the plasma vitamin D-binding protein from laboratory rats, which has a high affinity for 25(OH)D.[37] This assay does not distinguish between 25(OH)D_2 and 25(OH)D_3 and thus represents the total circulating 25(OH)D concentration. It has 100% cross-reactivity with 24,25$(OH)_2$D but levels of this metabolite in humans circulates in the blood at only 10–15% of the concentration of 25(OH)D. A CPBA alone, without the addition of chromatographic separation, is sufficient for detecting circulating 25(OH)D[37,38] and is comparable to liquid chromatography tandem mass spectroscopy.[38] The lower limit of detection for this assay is 5 ng/mL and the intra- and interassay coefficients of variance are 8% and 15%, respectively.

7-DHC ampoule exposure and analysis. To investigate the effects of latitude and time of day in Massachusetts and Florida, and to measure the maximal conversion of 7-DHC to previtamin D_3 without the effects of skin pigmentation, we used triplicate, unlabeled, borosilicate ampoules (Sigma-Aldrich) containing 7-DHC in 700 μL ethanol (50 μg/mL). The ampoules were placed on a black cloth over ice and exposed to natural sunlight on a clear, cloudless day. These triplicate samples were exposed for hourly increments from 0800–1600 h, three ampoules were exposed for the entire duration (0800–1600 h), and three were retained unexposed, wrapped in foil and placed on ice. When exposure periods were completed, the ampoules were labeled and covered with foil, protected from light and stored in a freezer at -20°C until analyzed. At the time of analysis, 100 μL aliquots were removed from each ampoule and transferred to clean, labeled vials, dried under a stream of nitrogen, and redissolved in 0.5% isopropanol alcohol (IPA) in hexane. Control and exposed samples were applied to a high-performance liquid chromatograph (HPLC) with an UV detector under following conditions: column, Zorbax silica 5 μm, 250 × 4.5 mm (Altech Assoc. Inc.); mobile phase, 0.5% IPA in hexane at a flow rate of 1.6 mL/minute.[39] Vitamin D_3, 7-DHC and all of the photoproducts were quantified by UV spectrophotometry using a Hitachi spectrophotometer. A standard curve for each of the compounds was determined by the peak height and area under the curve using a 254 nm UV detector by HPLC chromatography. The lower limit of detection for each of the compounds was less than 5 ng/mL.

Sun vs. shade behavioral experiment. To determine if captive *P. hypomelanus* and *R. aegyptiacus* had a strong preference for sun vs. shaded roosts, *P. hypomelanus* (n = 10) and *R. aegyptiacus* (n = 11) were released individually in both sun and shaded conditions in a 9.1 min × 7.0 min × 2.44 min holding cage. For trial one, each animal was randomly assigned to a condition (sun vs. shade) and released on the cage ceiling at which time the "releaser" immediately departed from the cage. From outside the cage, the "observer" timed the animal's latency to alternate roost conditions (LAC) with a stopwatch while the total 5 min observation period was recorded with another stopwatch. The amount of time elapsed between a change in location (sun to shade or shade to sun) was recorded. Trial two was run in the same cage two days later, using the opposite condition for each animal.

In addition to the above experimental protocols, hourly observations on the selection of roost site with unmanipulated *R. aegyptiacus* (n = 11) and *P. hypomelanus* (n = 9) were made by recording their specific roost site at hourly intervals throughout the day (1000–1500 h) while they occupied their normal roosting cages.

Statistical analysis. Statistical analysis was performed on Graphpad Prism for MacIntosh, version 4. Changes in 25(OH)D were analyzed for each group using Friedman's tests. Post hoc analyses with Wilcoxon signed-rank test, and/or Dunnett's test for non-parametric multiple comparisons, were explored for significant effects. Comparisons between groups and between species were performed using the Mann-Whitney U-test. Significance levels were accepted at $p < 0.05$.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

This study was funded by the Center for Ecology and Conservation Biology, Boston University, and The Lubee Bat Conservancy, Gainesville, FL, and was conducted in accordance with the American Society of Mammalogists Guidelines for Capture, Handling, and Care of Mammals, and Boston University's Institutional Animal Care and Use Committee. The authors would also like to thank DeeAnn Reeder for assistance with the behavioral protocol and Maurice Southworth for critical reading of the manuscript.

References

1. Bender DA. Nutritional Biochemistry of the Vitamins. Cambridge: Cambridge University Press, 1992.
2. Holick MF. Phylogenetic and evolutionary aspects of Vitamin D from phytoplankton to humans. In: Pang PKT, Schreibman MP, eds. Vertebrate Endocrinology: Fundamentals and Biomedical Implications. Orlando, FL: Academic Press, Inc., 1989:7-43.
3. Holick MF, Vitamin D. The Underappreciated D-lightful hormone that is important for skeletal and cellular health. Curr Opin Endocrinol Diabetes 2002; 9:87-98; http://dx.doi.org/10.1097/00060793-200202000-00011.
4. Holick MF. Photobiology of Vitamin D. In: Feldman, Glorieux, Pike, eds. Vitamin D: Academic Press, Inc., 1997.
5. MacLaughlin JA, Holick MF. Photobiology of vitamin D_3 in the skin. In: Goldsmith LA, ed. Biochemistry and Physiology of the Skin. New York Oxford: Oxford University Press, 1983:734-54.
6. Holick MF, Adams JS. Vitamin D Metabolism and Biological Function. In: Avioli LV, Krane SM, eds. Metabolic Bone Disease. San Diego, California: Academic Press, 1998:123-63.
7. Holick MF, MacLaughlin JA, Clark MB, Holick SA, Potts JT Jr., Anderson RR, et al. Photosynthesis of previtamin D_3 in human skin and the physiologic consequences. Science 1980; 210:203-5; PMID:6251551; http://dx.doi.org/10.1126/science.6251551.
8. Holick MF, MacLaughlin JA, Doppelt SH. Regulation of cutaneous previtamin D_3 photosynthesis in man: skin pigment is not an essential regulator. Science 1981; 211:590-3; PMID:6256855; http://dx.doi.org/10.1126/science.6256855.

9. Webb AR, DeCosta BR, Holick MF. Sunlight regulates the cutaneous production of vitamin D_3 by causing its photodegradation. J Clin Endocrinol Metab 1989; 68:882-7; PMID:2541158; http://dx.doi.org/10.1210/jcem-68-5-882.
10. DeLuca HF. William C. Rose lectureship in biochemistry and nutrition. Some new concepts emanating from a study of the metabolism and function of Vitamin D. Nutr Rev 1980; 38:169-82; PMID:7010229; http://dx.doi.org/10.1111/j.1753-4887.1980.tb05887.x.
11. Holick MF. Vitamin D deficiency. N Engl J Med 2007; 357:266-81; PMID:17634462; http://dx.doi.org/10.1056/NEJMra070553.
12. Holick MF. McCollum Award Lecture, 1994: vitamin D--new horizons for the 21st century. Am J Clin Nutr 1994; 60:619-30; PMID:8092101.
13. Speakman JR. Why do insectivorous bats in Britain not fly in daylight more frequently? Funct Ecol 1991; 5:518-24; http://dx.doi.org/10.2307/2389634.
14. Kunz TH. Roosting ecology of bats. In: Kunz TH, ed. Ecology of Bats. New York: Plenum Press, 1982:1-55.
15. Kunz TH, Lumsden LF. Ecology of cavity and foliage roosting bats. In: Kunz TH, Fenton MB, eds. Bat ecology. Chicago, Illinois: University of Chicago Press, 2003:3-89.
16. Jones DP, Kunz TH. *Pteropus hypomelanus*. Mammalian Species, No 639 2000:1-6.
17. Kwiecinski GG, Griffiths TA. Rousettus aegyptiacus. Mamm Species 1999; 611:1-9; http://dx.doi.org/10.2307/3504411.
18. Ochoa H, Kunz TH. Behavioral thermoregulation in the island flying fox Pteropus hypomelanus. J Therm Biol 1999; 24:15-20; http://dx.doi.org/10.1016/S0306-4565(98)00033-3.
19. McDowell LR. Vitamin D. In: Mcdowell LR, ed. Vitamins in Animal Nutrition: Comparative Aspects to Human Nutrition. San Diego, New York, Boston, London: Academic Press, Inc., 1989:55-92.
20. Dierenfeld ES, Seyjagat J. Plasma fat-soluble vitamin and mineral concentrations in relation to diet in captive pteropodid bats. J Zoo Wildl Med 2000; 31:315-21; PMID:11237137.
21. Cavaleros M, Buffenstein R, Ross FP, Pettifor JM. Vitamin D metabolism in a frugivorous nocturnal mammal, the Egyptian fruit bat (Rousettus aegyptiacus). Gen Comp Endocrinol 2003; 133:109-17; PMID:12899852; http://dx.doi.org/10.1016/S0016-6480(03)00150-3.
22. Kwiecinski GG, Zhiren L, Chen TC, Holick MF. Observations on serum 25-hydroxyvitamin D and calcium concentrations from wild-caught and captive neotropical bats, *Artibeus jamaicensis*. Gen Comp Endocrinol 2001; 122:225-31; PMID:11316428; http://dx.doi.org/10.1006/gcen.2001.7635.
23. Southworth LO, Holick MF, Chen T, Kunz TH. Variation in Serum 25-Hydroxyvitamin D in Free-ranging New-World Tropical Bats. Acta Chiropt 2009; http://dx.doi.org/10.3161/150811009X485675.
24. How KL, Hazewinkel HA, Mol JA. Dietary vitamin D dependence of cat and dog due to inadequate cutaneous synthesis of vitamin D. Gen Comp Endocrinol 1994; 96:12-8; PMID:7843559; http://dx.doi.org/10.1006/gcen.1994.1154.
25. Kenny DE, Irlbeck NA, Chen TC, Lu Z, Holick MF. Determination of vitamins D, A, and E in sera and vitamin D in milk from captive and free-ranging polar bears (*Ursus maritimus*), and 7-dehydrocholesterol levels in skin from captive polar bears. Zoo Biol 1998; 17:285-93; http://dx.doi.org/10.1002/(SICI)1098-2361(1998)17:4<285::AID-ZOO3>3.0.CO;2-5.
26. MacLaughlin JA, Holick MF. Aging decreases the capacity of human skin to produce vitamin D_3. J Clin Invest 1985; 76:1536-8; PMID:2997282; http://dx.doi.org/10.1172/JCI112134.
27. Tian XQ, Holick MF, Allen M. Comparative studies of cutaneous vitamin D_3 photosynthesis in terrestrial vertebrates. In: Jung EG, Holick MF, eds. Biologic Effects of Light 1995: Walter de Gruyter & Co., 1995:39-48.
28. Paul ND, Gwynn-Jones D. Ecological roles of solar UV radiation: towards an integrated approach. Trends Ecol Evol 2003; 18:48-55; http://dx.doi.org/10.1016/S0169-5347(02)00014-9.
29. Webb AR, Kline L, Holick MF. Influence of season and latitude on the cutaneous synthesis of vitamin D_3: exposure to winter sunlight in Boston and Edmonton will not promote vitamin D_3 synthesis in human skin. J Clin Endocrinol Metab 1988; 67:373-8; PMID:2839537; http://dx.doi.org/10.1210/jcem-67-2-373.
30. Pettifor JM, Moodley GP, Hough FS, Koch H, Chen T, Lu Z, et al. The effect of season and latitude on in vitro vitamin D formation by sunlight in South Africa. S Afr Med J 1996; 86:1270-2; PMID:8955733.
31. Haddad JG, Chyu KJ. Competitive protein-binding radioassay for 25-hydroxycholecalciferol. J Clin Endocrinol Metab 1971; 33:992-5; PMID:4332615; http://dx.doi.org/10.1210/jcem-33-6-992.
32. Skinner DC, Moodley G, Buffenstein R. Is vitamin D_3 essential for mineral metabolism in the Damara mole-rat (*Cryptomys damarensis*)? Gen Comp Endocrinol 1991; 81:500-5; PMID:1647351; http://dx.doi.org/10.1016/0016-6480(91)90178-9.
33. Buffenstein R, Yahav S. Cholecalciferol has no effect on calcium and inorganic phosphorus balance in a naturally cholecalciferol-deplete subterranean mammal, the naked mole rat (*Heterocephalus glaber*). J Endocrinol 1991; 129:21-6; PMID:1851511; http://dx.doi.org/10.1677/joe.0.1290021.
34. Keegan JD, Levine LS, Balgobind ND. Absorption of calcium in the small intestine of the bat, *Rousettus aegyptiacus*. S Afr J Sci 1980; 76:328.
35. Boland RL. Plants as a source of vitamin D_3 metabolites. Nutr Rev 1986; 44:1-8; PMID:3005932; http://dx.doi.org/10.1111/j.1753-4887.1986.tb07543.x.
36. Gardner RM, Reinhardt TA, Horst RL. The biological assessment of vitamin D3 metabolites produced by rumen bacteria. J Steroid Biochem 1988; 29:185-9; PMID:2831435; http://dx.doi.org/10.1016/0022-4731(88)90264-6.
37. Chen TC, Turner AK, Holick MF. Methods for the determination of the circulating concentration of 25-hydroxyvitamin D. J Nutr Biochem 1990; 1:315-9; PMID:15539221; http://dx.doi.org/10.1016/0955-2863(90)90067-U.
38. Holick MF, Siris ES, Binkley N, Beard MK, Khan A, Katzer JT, et al. Prevalence of Vitamin D inadequacy among postmenopausal North American women receiving osteoporosis therapy. J Clin Endocrinol Metab 2005; 90:3215-24; PMID:15797954; http://dx.doi.org/10.1210/jc.2004-2364.
39. Lu Z, Chen TC, Kline L, Markestad T, Pettifor JM, Ladizesky M, et al. Photosynthesis of previtamin D_3 in cities around the world. In: Holick MF, Kligman AM, eds. Biologic Effects of Light. Atlanta, Georgia: Walter de Gruyter, 1992:48-52.