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

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Introduction

For most vertebrates, vitamin D₃ 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. 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 D₂ 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.

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 D₃ 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 D₃ is the photosynthesis of the biologically inert photoproducts, lumisterol3 and tachysterol3. Production of these inert compounds limits the skin’s synthesis of vitamin D₃, thus preventing hypervitaminosis that would otherwise result from prolonged exposure to sunlight.5,9

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 D₃ synthesis. It is possible that...
over evolutionary time the ability to synthesize vitamin D₃ 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.¹³ For example, the island flying fox Pteropus hypomelanus is nocturnally active, but it typically roosts in dark tombs, caves, and rock crevices.¹⁴ 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 captive²⁰,²¹ and wild-caught bats²² 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.²³ 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.²⁴ 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-visitng bat species (P. hypomelanus and R. aegyptiacus) to synthesize vitamin D₃ 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 D₃. 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 D₃, this model examines the effects of season, latitude, and time of day on previtamin D₃ 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 D₃ 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 D₃ 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 D₃, 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

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) |
|------------------|----------------------------------|---------------------------------|
|                  | 0 | 30 d | 60 Days | 90 Days | 0 | 30 d | 60 Days | 90 Days |
| Rousettus aegyptiacus | 1 | ND¹ | ND¹ | ND¹ | ND¹ | 1 | 7.0 | 24.0 | 48.0 | 36.0 |
|                  | 2 | ND¹ | ND¹ | ND¹ | ND¹ | 2 | 5.8 | 40.0 | 68.0 | 94.0 |
|                  | 3 | ND¹ | ND¹ | ND¹ | ND¹ | 3 | 6.8 | 17.0 | ND¹ | 74.0 |
|                  | 4 | 8.6 | ND¹ | ND¹ | ND¹ | 4 | 5.6 | 11.0 | 35.0 | 54.0 |
|                  | 5 | ND¹ | ND¹ | ND¹ | ND¹ | 5 | 6.8 | 50.0 | 100.0 | 105.0 |
| Pteropus hypomelanus | 0 | 30 d | 60 Days | 90 Days | 0 | 30 d | 60 Days | 90 Days |
|                  | 1 | ND¹ | ND¹ | ND¹ | ND¹ | 1 | ND¹ | 7.8 | ND¹ | 7.8 |
|                  | 2 | ND¹ | ND¹ | ND¹ | ND¹ | 2 | ND¹ | 10.5 | ND¹ | 18.5 |
|                  | 3 | 23.5 | ND¹ | ND¹ | ND¹ | 3 | 7.8 | 13.5 | 10.5 | 12.3 |
|                  | 4 | ND¹ | ND¹ | ND¹ | ND¹ | 4 | 9.0 | 5.5 | 9.0 | 13.3 |
|                  | 5 | ND¹ | ND¹ | ND¹ | ND¹ | 5 | 11.3 | 15.0 | 15.0 | 17.0 |

¹ND, Not Detectable (< 5.0 ng/mL).
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. 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. For example, How et al. compared both the initial concentration of the prohormone (7-DHC) in skin and the ability of the Norway rat (*Rattus norvegicus*), dog (*Canis familiaris*), 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. 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. 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.

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.

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 and refute the hypothesis of Cavaleros et al. 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 by the study of How et al., who found that 7-DHC concentrations were ten times higher in rats as compared with dogs and cats. The data shown in the figure is the mean +/- the standard deviation of 5 test animals. At 90 days the difference is significant at p = 0.008 for bats exposed to sunlight vs. those not exposed to sunlight.
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)

| 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.
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 D₃ 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. 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. found that *C. damerensis* had a minimal response to supplementary vitamin D₃ and that calcium absorption was nonsaturable and vitamin D-independent. Similarly, Buffenstein and Yahav found that vitamin D₃ 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. 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.

In summary, our results support the hypotheses: (1) photoconversion of 7-DHC to previtamin D₃ 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 D₃ 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 D₃, 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.

Finally, our results have important implications for bats maintained in captivity for long periods at temperate latitudes, which may depend on endogenously synthesized vitamin D₃ 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 × 0.60 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 × 0.60 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

| 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     |       |       |       |
| **Rousettus aegyptiacus** | 11 | |       |       |       |       |       |       |
| Sun         | 0              | 0  | 0     | 0     | 0     |       |       |       |
| Shade       | 11             | 11 | 11    | 11    | 11    | 11    |       |       |

### Table 3. Preference for sun or shade in two species of Old World fruit bats at different times of the day
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. This assay does not distinguish between 25(OH)D2 and 25(OH)D3 without the effects of skin pigmentation, we used triplicate, and to measure the maximal conversion of 7-DHC to previtamin D3 in the skin. In: Goldsmith LA, ed. Biochemistry and Biomedical Implications. Orlando, Academic Press, 1998:123-63.

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