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VITAMIN D_3 IN THE HEMOLYMPH OF GOLIATH BIRDEATER SPIDERS (*THERAPHOSA BLONDI*)

Trevor T. Zachariah, D.V.M., M.S., and Mark A. Mitchell, D.V.M., Ph.D.

Abstract: Vitamin D_3 is an important vitamin in vertebrates. This fat-soluble vitamin is associated with the regulation of many physiologic processes, most importantly calcium metabolism. The presence or importance of vitamin D_3 has been determined in only a handful of invertebrate species. In this study, hemolymph was collected from six wild-caught, subadult goliath birdeater spiders (*Theraphosa blondi*) and analyzed for the presence of 25(OH)-vitamin D_3, the precursor to the active form of vitamin D_3. The metabolite 25(OH)-vitamin D_3 was detected in all of the spiders (mean: 5.7 nmol/L, SD: 1.5 nmol/L, range: 3–7 nmol/L). The method by which spiders acquire vitamin D_3 is unknown. It is possible, though unlikely, that they synthesize it via exposure to ultraviolet radiation. Many of the invertebrate species upon which theraphosid spiders prey are not known to have high circulating levels of vitamin D_3 or its precursors. However, dietary intake is a possible means of vitamin D_3 acquisition in this study.

Key words: Hemolymph, spider, Theraphosidae, *Theraphosa blondi*, vitamin D_3, 25(OH)-vitamin D_3.

BRIEF COMMUNICATION

The physiology of spiders has been studied extensively. Much of the research has been focused on elucidating the metabolic processes of these animals as a comparative method to further explain vertebrate physiology. However, a literature search reveals a conspicuous absence in this body of work in the area of calcium and vitamin D_3 metabolism. This pilot study was conducted to confirm the presence of vitamin D_3 in the goliath birdeater spider (*Theraphosa blondi*).

Six (four male and two female) wild-caught, subadult *T. blondi* were obtained from an invertebrate importer in Florida (LASCO, Naples, Florida 34119, USA). The spiders were housed in rectangular, 5.7-L plastic storage containers with screen tops. A 50:50 mixture of potting soil and vermiculite was used for the substrate. The spiders had ad lib access to chlorinated tap water and were fed five adult crickets weekly. The crickets had access to a high-calcium cricket food and water source (High Calcium Cricket Diet and Cricket Quencher Calcium, Fluker Farms, Port Allen, Louisiana 70767, USA) until being offered to the spider. The cricket food also contained cholecalciferol, a precursor molecule to 25(OH)-vitamin D_3. The temperature and humidity in the enclosures were maintained at approximately 23.9°C (75°F) and 80%, respectively. The spiders were held under these conditions for 12 wk before diagnostic samples were obtained.

An intracardiac hemolymph sample was collected from each of the *T. blondi* according to the following procedure: each spider was placed into a square, 3-L plastic storage container that was modified into a gas anesthetic chamber. The container was customized by drilling a hole on one side and inserting an endotracheal tube adapter. Each spider was anesthetized with 5% isoflurane (Isoflo, Abbott Laboratories, North Chicago, Illinois 60064, USA) at a flow rate of 1 L/min oxygen. Once the spider had lost its ability to right itself, it was removed from the anesthetic chamber and weighed. A 26-gauge, 1.9-cm (3/4-inch) needle fastened to a 3-ml syringe was used to collect an intracardiac hemolymph sample. Collection of each sample was accomplished by inserting the needle at approximately a 45° angle through the exoskeleton at the midpoint of the dorsal midline of the opisthosoma. A total of 0.5 ml hemolymph was collected from each individual. Hemolymphstasis was accomplished by applying a small amount of Nexaband glue (Veterinary Products Laboratories, Phoenix, Arizona 85067, USA) to the collection site. The spiders were recovered in 100% oxygen, and recovery from anesthesia was uneventful.

From the Department of Veterinary Clinical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, Louisiana 70803, USA. Present addresses (Mitchell): Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois, Urbana, Illinois 61802, USA; (Zachariah): Chicago Zoological and Aquatic Animal Residency, Brookfield, Illinois 60513, USA. Correspondence should be directed to Dr. Zachariah (zachariahdvm@yahoo.com).

Each sample was placed in a lithium heparin Microtainer tube (Becton Dickinson, Franklin Lakes, New Jersey 07417, USA) and centrifuged for 10 min at 1,411 *g*. Once the sample was centrifuged, the supernatant was removed and frozen in a Cryovial (Nalge Nunc International, Rochester, New York 14625, USA) at −62.2°C (−80°F). The samples were then transported on frozen gel packs to the Endocrine Service of the Diagnostic Center for Population and Animal Health at Michigan State University for detection of 25(OH)-vitamin D_3 by quantitative radioimmunoassay analysis.

The metabolite 25(OH)-vitamin D_3 was detected in all six spiders. The Shapiro-Wilk test was used to assess the normality of the data, and the data were found to follow a Gaussian distribution ($P = 0.212$). Measures of central tendency and dispersion were then calculated: mean, 5.7 nmol/L; SD, 1.5 nmol/L; and range, 3–7 nmol/L. The significance level was set at $\alpha = 0.05$. The statistical analysis was performed using a commercial software package (SPSS 15.0, SPSS Inc., Chicago, Illinois 60606, USA).

To the authors' knowledge, this is the first time that vitamin D_3 has been measured in any species of spider. Limited studies in other taxa reveal that there may be some variability in vitamin D levels in invertebrates. Vitamin D_3 and a vitamin D-dependent calcium binding protein have been found in two species of terrestrial snails.[12] Seven species of insect, both adults and larvae, were found to have no detectable levels of vitamin D_3.[4] It has been stated that there is no evidence that insects require vitamin D_3.[7] Also, it has been shown that vitamin D_2 may have a role in the nutrition of copepods.[5]

Vitamin D_3 plays an important role in calcium metabolism of vertebrate animals. Whether it is absorbed from the diet or synthesized in the skin due to ultraviolet-B (UVB) radiation exposure, the liver hydroxylates the compound to 25(OH)-vitamin D_3 via the enzyme 25-hydroxylase. This compound is either stored in adipose tissue when calcium levels are adequate, or hydroxylated by the kidneys to form 1,25$(OH)_2$-vitamin D_3 when increased calcium absorption is required. The 1,25$(OH)_2$-vitamin D_3, also known as calcitriol, is the active form of vitamin D_3 and is responsible for promoting calcium uptake and transport in the body. In addition to this function, vitamin D_3 promotes various other metabolic functions in tissues throughout the body.[3]

Many invertebrates possess tissues containing calcium, though the mechanisms of calcium metabolism in these species are not well studied. Spiders probably require calcium to ensure striated muscle function and various other physiological cell processes. In a study involving *T. blondi* and Chilean rose spiders (*Grammostola rosea*), it was found that hemolymph calcium levels were greater (11.9 ± 1.7 mg/dL and 16.9 ± 1.8 mg/dL, respectively)[13] than those of typical mammals (e.g., dogs and cats, 9.0–11.5 mg/dL).[8] For the theraphosid spider *Eurypelma californicum* (nomen dubium, unknown species probably belonging to the genus *Aphonopelma*[9]), hemolymph calcium levels have also been found to be greater than those in mammals (15.76 ± 0.52 mg/dL).[10] These findings suggest that theraphosid spiders require relatively high levels of calcium, although the method by which they achieve them is unknown.

The 25(OH)-vitamin D_3 levels measured in these theraphosid spiders was low in comparison to mammalian (dog: 60–215 nmol/L, cat: 65–170 nmol/L)[6] and reptilian (red-eared slider turtle: 31.4 ± 13.2 nmol/L)[1] vertebrates. Vertebrates have the opportunity to store calcium in their skeletons. The absence of such a storage depot in spiders may be associated with the higher circulating levels of calcium in the body. This physiologic finding may also play a role in the intertaxa vitamin D_3 level differences detected. These facts also suggest that vitamin D_3 may play a minimal or no role in calcium metabolism in spiders.

Most spiders prey on arthropods, although theraphosid spiders will occasionally capture and eat vertebrates. Due to the previously stated lack of vitamin D_3 in insects, it is unlikely that wild spiders ingest the compound from their prey. In captivity, however, the insect prey may serve as a source of vitamin D_3 if they are offered commercial diets with cholecalciferol. From this study, it is not possible to determine whether the crickets or the spiders possess 25-hydroxylase, but one of the species may convert cholecalciferol to 25(OH)-vitamin D_3. Where the hydroxylation of cholecalciferol occurs, and whether it is further processed to 1,25$(OH)_2$-vitamin D_3, remains to be investigated.

Spiders may synthesize vitamin D_3; however, the process may not be similar to that previously described for vertebrate species. Anecdotally, theraphosid spiders in captivity are usually kept without the presence of a source of UVB, and these animals appear to be clinically normal. The spiders in this study were kept without UVB radiation and still maintained the reported

calcium levels. Many species of theraphosid spider are considered nocturnal or hide out of the sunlight in the wild;[11] however, exposure to low-intensity light during crepuscular activity is feasible. Although it is possible that the spiders in this study obtained 25(OH)-vitamin D_3 before their capture, it is difficult to determine if the metabolite measured in this study was present from that time. The half-life of 25(OH)-vitamin D_3 in humans is approximately 1 mo.[2] This value is unknown for theraphosid spiders; however, it seems unlikely that any vitamin D_3 would have remained after the 3 mo of captivity prior to the hemolymph sample collection for this study.

The finding of vitamin D_3 in *T. blondi* is intriguing. Further study into the metabolism of vitamin D_3 and calcium in spiders, including organs involved and possible storage sites, is needed. Research to determine what role, if any, UVB radiation or dietary intake has on these metabolites is recommended. Investigation into vitamin D_3 and calcium levels in free-ranging theraphosid spiders would also enhance the understanding of these metabolic processes.

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