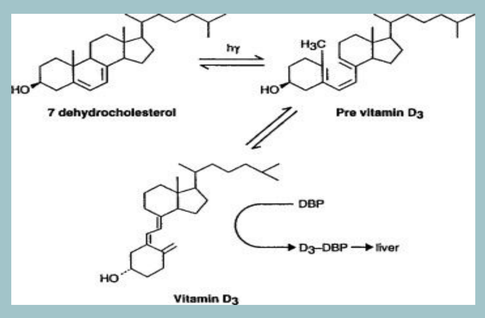
Identification of the Presence of Vitamin D in Wild Caught Mosquitoes

Baiyue Zhao[1,2], Kelly Persons[2], Michael F. Holick[2]

Ridgewood High School, 627 E Ridgewood Ave, Ridgewood, NJ 07450[1], Boston University School of Medicine, 85 Newton St, Boston, MA 02118[2]

Introduction



Sciencedirect.com

- Provitamin D (7-dehydrocholesterol; 7-DHC) in human skins is converted into vitamin D when exposed to solar ultraviolet B (UVB; 290-320 nm) radiation.
- Vitamin D in humans provide many biological functions including maintaining bone health and calcium absorption.
- Mosquitoes cannot make cholesterol, therefore have to obtain them through diet. Mosquitoes convert cholesterol into 7-DHC, the precursor for ecdysone, a necessary hormone for arthropods. 7-DHC is also the precursor for vitamin D3.

Objective:

- To determine if mosquitoes, along with other insects, contain provitamin D and/or vitamin D when caught in nature.
- Provide insights into whether mosquitoes can serve as a dietary source of vitamin D for insect-eating vertebrates including bats.

Methods

Main investigation:

1. Collect wild mosquitoes in Sudbury, MA on 7/21/19 using a CDC Light Mosquito Trap at night. Use light and dry ice to facilitate the yield of mosquitoes.
2. Methanol extraction of vitamin D related compounds followed by centrifuge. We used 3 samples of 10 mosquitoes each and 3 samples of 50 mosquitoes each.
3. Recover the supernatant liquid and dry it down under nitrogen gas
4. Dissolve the extracts in 130 microlitres of 0.8 % isopropanol alcohol in hexane
5. Run through straight-phase High Performance Liquid Chromatography (HPLC) to separate vitamin D from provitamin D.
6. Run through reverse-phase HPLC to separate vitamin D2 from vitamin D3 and provitamin D2 from provitamin D3.

Side investigation:

1. Repeat above procedures for moths that were collected.

Fig 1. The mosquito trap that was being used.

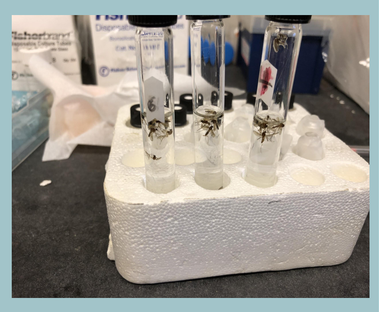


Fig 2. Extraction of mosquitoes using methanol.

Fig 3. The High Performance Liquid Chromatography (HPLC) machine.

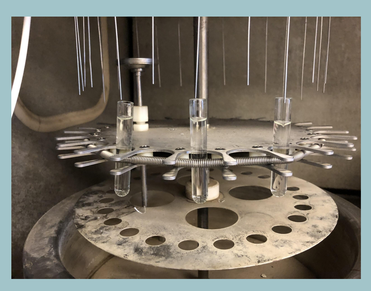


Fig 4. Samples drying under nitrogen gas.

Results

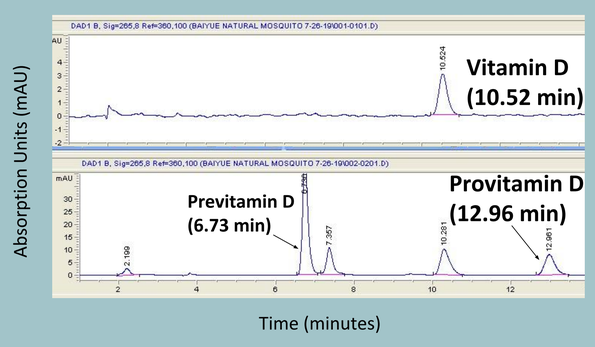


Fig 5. Chromatogram of an ampoule containing a vitamin D3 solution.

Fig 6. Chromatogram of an ampoule containing a UV irradiated provitamin D solution.

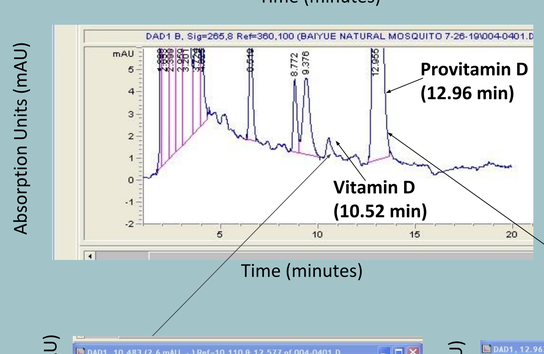


Fig 7. Chromatogram of one of the six samples of mosquitoes that contained **both provitamin D and vitamin D.**

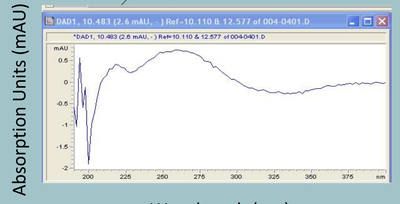


Fig 8. UV absorption spectrum of the **vitamin D** peak shown in fig 7.

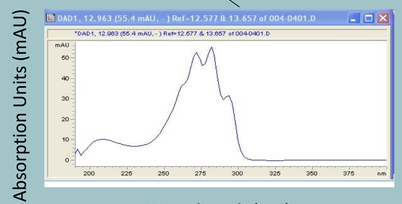


Fig 9. UV absorption spectrum of the **provitamin D** peak shown in fig 7.

- In this one sample of mosquitoes, the vitamin D level was 7 ng / 10 mosquitoes (0.28 IU) and the provitamin D level was 1098 ng / 10 mosquitoes

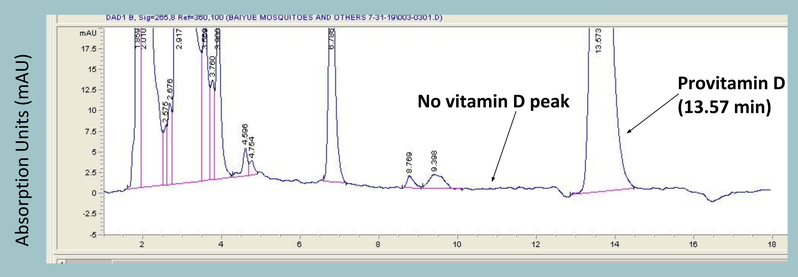


Fig 10. Representative chromatogram for the other 5 samples that **contained only provitamin D without vitamin D presence.**

- An average of 690 ng / 10 mosquitoes of provitamin D was found in the other 5 samples, however, no vitamin D presence was identified.

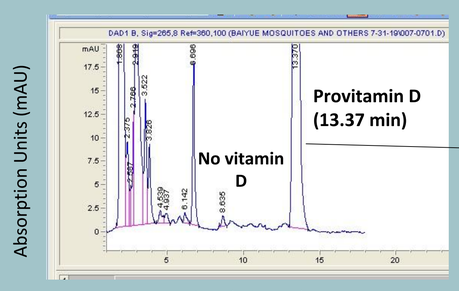


Fig 11. Chromatogram of moths that contained **provitamin D**.

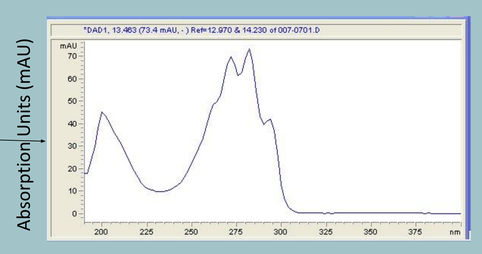


Fig 12. UV absorption spectrum of the **provitamin D peak** shown in fig 11.

- Similar to the mosquitoes, an average of 2423 ng/sample of provitamin D was found in both samples of moths. However, no vitamin D was identified.
- An average of 4900 ng of provitamin D were found in 1 g of mosquitoes.
- An average of 48500 ng of provitamin D were found in 1 g of moths.

Discussion/ Conclusions

- Consistent with previous findings, mosquitoes and moths captured had provitamin D, indicating a potential for them to produce vitamin D when exposed to sunlight.
- The low amount of vitamin D might be due to the fact that the mosquitoes were collected during the night. They are more likely to be nighttime active mosquitoes that hide away from sunlight during the day. It is unclear whether daytime active mosquitoes would be able to produce more vitamin D.
- Only one out of six samples of mosquitoes showed presence of vitamin D. It is likely that one or multiple mosquitoes in this sample were active during the daytime and exposed to more sunlight than others.
- It is unknown whether the mosquitoes are utilizing vitamin D for any biological functions.
- It is also likely that mosquitoes, especially daytime mosquitoes, along with other insects, might serve as a dietary source of vitamin D for insect eating vertebrates including bats.
- A single bat eats about 6,000 insects every night. Assuming each insect contains 0.7 ng of vitamin D as found in one of the six samples of this study. A bat would consume 4200 ng (168 IU) in a night.

Fig 13. The mosquitoes used in this experiment.

Fig 14. Other insects used in this experiment.

References

1. Holick, M. F. Vitamin D Deficiency. *New England Journal of Medicine.* **2007**, 357, 266-81.
2. Wacker, Matthias; Holick, M. F. Sunlight and Vitamin D: a global perspective for health. *Dermato-Endocrinology.* **2013,** 51-108.
3. Deluca, H. F. (n.d.). [The photolysis of 7-dehydrocholesterol to produce vitamin D3]. Retrieved August 6, 2019, from https://www.sciencedirect.com

Acknowledgements

I would like to thank the RISE program and the faculty who provided me with this six-week opportunity to study in a research lab. I would like to thank Kelly Persons who assisted me in lab techniques and data analysis. Special thanks to Dr. Holick who patiently guided me and allowed me to be a part of the lab over the summer.