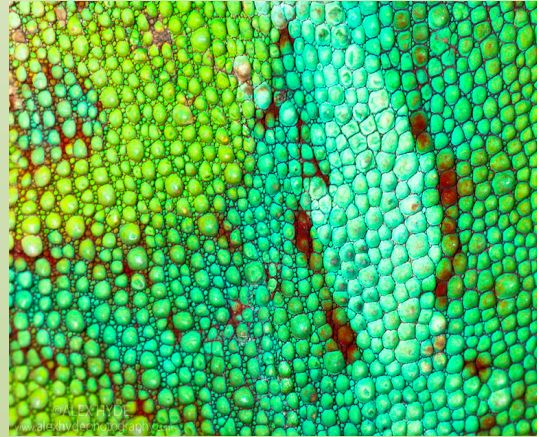


Production of cutaneous vitamin D3 after UVB radiation in reptiles and amphibians

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Abstract

The aim of this study was to investigate the differences in the ability of different reptile and amphibian species to synthesize sufficient amounts of vitamin D in the skin. Skin tissue of 32 different species were collected from dead animals from a terrarium specialist. Wistar rat skin served as a positive control. Cholesterol, 7-DHC, and vitamin D levels were determined after UVB exposure at 37°C, and compared to non-irradiated skin. Overall, there was no significant effect of UVB irradiation on 7-DHC and vitamin D concentrations of the skin in different reptile and amphibian species. The relatively low cutaneous levels of the vitamin D precursor 7-DHC observed in this study suggest that most reptiles and amphibians are unable to synthesize sufficient amounts of vitamin D. However, some reptiles and amphibians species could be capable of synthesizing sufficient amounts of vitamin D in their skin. The results have to be taken into account when appropriate husbandry and food recommendations are given for these species when held under captive conditions.

Table of Contents

Introduction	4
Materials and methods	5
Data Analysis	6
Results	11
Discussion	13
Conclusion	17
Acknowledgements	17
References	17

Introduction

Vitamin D is of great importance in a number of physiological processes, such as the regulation of calcium metabolism that ensures the development and maintenance of bones (Ganong W. 2001, Boyer 1996). Metabolic bone disease (MBD) is considered the most prevalent and complex disease in captive reptiles (Mader 2006, Laing, Fraser 1999, Laing, Trube et al. 2001, Kik, Beynen 2003). Vitamin D may be optimized by the exposure of the skin to ultraviolet B light (290 to 320 nm, UVB) radiation (Webb, Holick 1988) or by an animal's diet (How, Hazewinkel et al. 1994).

The synthesis of vitamin D is a more complex process. It starts with the vitamin D precursor 7-dehydrocholesterol (7-DHC). Through the process of photo isomerisation it forms pre-vitamin D out of 7-DHC. Pre-vitamin D can be converted into vitamin D under influence of the skin temperature. Vitamin D is bound to vitamin D binding protein (DBP) in the blood. This can be determined by measuring the 25-hydroxy vitamin D (25OHD) levels (Tian, Chen et al. 1993, Tian, Chen et al. 1994, Holick, Tian et al. 1995). If there is an over-expression of the vitamin D precursor 7-DHC, it can make a shift to a different pathway. This other metabolic pathway can lead to cholesterol synthesis. This cascade contains the making of cholesterol and desmosterol. This leads to the conclusion that cutaneous vitamin D synthesis can be less if the diet includes the consumption of animals that have already made this biochemical conversion (Morris 1999).

There is not much knowledge about the cutaneous vitamin D synthesis in reptiles and amphibians. Research that has been done is limited to a few different species. The conclusions of some of this research are described below. In panther chameleons there was a direct link between UVB exposure and internal vitamin D3 levels. An increased amount of UVB exposure, lead to higher levels of vitamin D3 in laboratory using artificial UVB (Karsten, Ferguson et al. 2009). In addition, it is also known that an optimal calcium concentration in chameleon is important for reproductive success, especially in females (Ferguson, Jones et al. 1996, Ferguson, Gehrman et al. 2002). A study with juvenile bearded dragons concluded that exposure for 2 or more hours to UVB ensured that normal physiological concentrations of plasma 25(OH)D3 and 1,25 (OH)2D3 were maintained. Furthermore, higher growth rates were found for female bearded dragons with UVB exposure (Oonincx, Stevens et al. 2010). In another study the appropriate husbandry recommendations for red-eared slider turtles included sunlight that is not

obstructed by UVB filtering material or an artificial source of UVB. This is due to the fact that the turtles that were exposed to UVB radiation had significantly higher 25(OH)D₃ concentrations than the turtles that did not receive supplemental lighting (Acierno, Mitchell et al. 2006). The same recommendations were also given for corn snakes, as the plasma 25(OH)D₃ concentrations significantly increased in snakes that were exposed to supplemental lighting. However, in contrast to the study with the bearded dragons, there was no increase in weight (Acierno, Mitchell et al. 2008).

In addition to the existing studies, it is interesting to find out how different reptiles and amphibians' skins respond to UVB radiation. Reptiles and amphibians are increasingly kept as pets today. This study can contribute to the prevention of possible diseases and may also give recommendations for husbandry. Therefore, the aim of this study was to investigate the differences of the ability to synthesize sufficient amounts of vitamin D in the skin of different species of reptiles and amphibians.

Materials and methods

Skin tissue of different reptiles and amphibians were collected from dead animals from a terrarium specialist. The samples that were collected contained both back and belly parts in order to make it possible to compare different parts of the skin. Furthermore, skin of the same species were used but in different colour mutations. All the skin tissue was stored at -70 degrees Celsius for further analysis. As a positive control the skin of a laboratory rat (8 months old male Wistar) was used. Two pieces of skin were cut from the sample, the subcutaneous tissue was removed and was weighed. One of the pieces was exposed to 30 minutes UVB radiation (UVB + group) and the other was not (UVB - group). For the radiation a UVB D₃ reptile lamp (Arcadia 12%, 15W, peak at 305 nm, Arcadia, Redhill, UK) was used. Cholesterol, 7-DHC, and vitamin D were determined after UVB exposure at 37°C, and compared to non-irradiated skin. After radiation the pieces were cut into very small pieces and placed into glass tubes. Also the pieces from the UVB - group were cut and placed into glass tubes. For the extraction of lipids, the method was used as described by Bligh and Dyer (1959) (BLIGH, DYER 1959). This method proceeds by adding 0.8 mL millipure water and 3 mL chloroform:methanol 1/2, v/v to the tubes and is then vortexed regularly for 40 minutes. After those 40 minutes the tubes were centrifuged for 2 minutes at 2000 rpm. The supernatant was transferred to new glass tubes and 2 mL millipure water and 2 mL chloroform was

added. Next the tubes were vortexed again for 30 seconds, and then centrifuged for 5 minutes at 2000 rpm. The lower layer was transferred into a new pointed glass tube and evaporated under nitrogen gas. After that, the samples were stored at -20 degrees Celsius. Before the MS-analysis, the samples were suspended in 500 μ L chloroform:methanol 1/1, v/v containing 0.002% butylated hydroxytoluene (BHT) as an anti-oxidant. Next, 20 μ L was injected on a Lichrospher RP18-e column. At a constant flow rate of 1 mL/min a gradient was generated from acetonitrile:water 95/5, v/v, to acetone/chloroform 85/15, v/v. The total run time per sample was 13 minutes. MS of lipids was performed using Atmospheric Pressure Chemical Ionization (APCI) on a Biosystems API-4000 Q-trap (MDS Sciex, Concord, ON, Canada). The system was controlled by Analyst version 1.4.2 software (MDS Sciex, Concord, ON, Canada) and operated in positive ion mode and in the multiple reaction-monitoring mode (MRM). The following settings were used: temperature 420°C, nebulizer gas (GS1) 5, nebulizer current 3 μ A, curtain gas 10, collision gas. For each compound, high and declustering potential and collision energy were empirically optimized. The design of this study is similar to a study done with terrestrial carnivorous species (Cutaneous vitamin D synthesis in carnivorous species by Corbee, 2014) (Corbee 2014)

Data Analysis

Data analysis was performed using Analyst 1.4.2 software (MDS Sciex, Concord, ON, Canada). Quantitation was done relative to standards run separately. All steroid standards were from Sigma-Aldrich (St. Louis, MO, USA). The extraction efficiency of cholesterol is expected to be similar to vitamin D and 7-DHC. Cholesterol is a good indicator of the amount of cellular material present in the skin and therefore the data are expressed as a ratio to cholesterol. All skin samples were analysed in twice. In case of high variations (i.e. >20%) the skin samples were analysed another two times. In the table below the average levels of two samples are demonstrated (Table 1).

Table 1: All the samples, including both the English name and Latin name of all the species. Further 7-dehydrocholesterol (7-DHC) and vitamin D (VitD) concentration of the skin samples before (-) and after (+) exposure to UVB (2.16 J per cm²), expressed in pmol per nmol cholesterol are given. The weight of the skin (Sw) is given in mg per cm². Missing information is marked ' - '.

Species	Latin name	7-DHC -	7-DHC +	Vit D -	Vit D +	Sw
Rat	<i>Rattus norvegicus</i>	13	77	3	0,3	160
Tiger-leg monkey frog	<i>Phyllomedusa tomopterna</i>	105	73	0,4	0,5	66
Giant monkey frog	<i>Phyllomedusa bicolor</i>	13	11	0,8	1	70
Giant tree frog	<i>Litoria infrafrenata</i>	60	69	12	2	63
Dyeing poison frog	<i>Dendrobates tinctorius</i>	42	34	1	1	176
Yellow- headed day gecko	<i>Phelsuma Klemmeri</i>	-	5	-	0,6	556
Crested gecko	<i>Correlophus ciliatus</i>	53	57	3	4	79
Leopard gecko	<i>Eublepharis macularius</i>	17	16	1	1	117
Electric blue gecko	<i>Lygodactylus williamsi</i>	6	5	1	0,5	109
Panther chameleon	<i>Furcifer pardalis</i>	190	255	2	0,5	11

Production of cutaneous vitamin D3 after UVB radiation in reptiles and amphibians

Veiled chameleon	Chamaeleo calyptrates	157	131	10	3	25
Long-tailed grass lizard (ventral)	Takydromus sexlineatus	10	15	2	1	155
Long-tailed grass lizard	Takydromus sexlineatus	9	25	2	1	113
Long-tailed lizard (ventral)	Latastia longicaudata	-	10	-	0,5	56
Long-tailed lizard	Latastia longicaudata	9	9	0,3	0,2	25
Green spiny lizard	Sceloporus malachiticus	19	13	2	1	63
Common collared lizard	Crotaphytus collaris	26	23	1	2	22
Common collared lizard	Crotaphytus collaris	56	45	9	10	53
Central bearded dragon	Pagona vitticeps	1	15	0,3	0,1	81
Central bearded dragon (ventral)	Pagona vitticeps	127	81	5	6	92
Central bearded dragon	Pagona vitticeps	162	284	5	8	100

Production of cutaneous vitamin D3 after UVB radiation in reptiles and amphibians

Rankin's dragon	<i>Pagona henrylawsoni</i>	26	147	5	5	51
Common agama (ventral)	<i>Agama Agama</i>	24	45	2	3	23
Common agama	<i>Agama Agama</i>	140	103	9	4	20
Black-necked agama (ventral)	<i>Agama atricollis</i>	425	378	10	33	7
Black-necked agama	<i>Agama atricollis</i>	343	282	9	5	11
Eyed dabb lizard	<i>Uromastyx ocellata</i>	25	27	8	4	37
Mop-headed iguana	<i>Uranoscodon superciliosa</i>	108	278	13	9	28
Green iguana (ventral)	<i>Iguana iguana</i>	33	27	4	3	105
Green iguana	<i>Iguana iguana</i>	35	35	4	4	72
Savannah monitor	<i>Varanus exanthematicus</i>	236	167	2	2	42
Ball python (ventral)	<i>Python regius</i>	27	16	2	1	117
Ball python	<i>Python regius</i>	120	62	0,4	0,7	147
Ball python	<i>Python regius</i>	42	39	0,3	0,3	187
Boa constrictor	<i>Boa constrictor</i>	21	22	3	3	185

Production of cutaneous vitamin D3 after UVB radiation in reptiles and amphibians

Belize Crawl-Cay (ventral)	<i>Boa constrictor</i> imperator 'crawl-cay'	11	9	3	2	109
Belize Crawl-Cay	<i>Boa constrictor</i> imperator 'crawl-cay'	31	32	0,8	3	57
Corn snake	<i>Pantherophis</i> guttata	14	17	3	4	68
Corn snake	<i>Pantherophis</i> guttata	10	12	2	3	28
Corn snake	<i>Pantherophis</i> guttata	7	8	6	11	31
Corn snake	<i>Pantherophis</i> guttata	9	11	5	3	24
Corn snake	<i>Pantherophis</i> guttata	17	10	4	2	47
Corn snake	<i>Pantherophis</i> guttata	12	7	3	6	19
Olive house snake	<i>Lamprophis</i> olivaceus	11	15	2	2	3
Common garter snake	<i>Thamnophis sirtalis</i>	38	41	3	4	5
White-lipped python	<i>Leiopython albertisii</i>	72	52	0	0	21
False map turtle	<i>Graptemys</i> pseudogeographica	12	14	4	3	44

Leopard tortoise	Stigmochelys pardalis	24	30	6	14	104
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Results

Skin samples were analyzed and 7-DHC and vitamin D concentrations of the skin in pmol expressed per nmol cholesterol. These measurements were performed before and after irradiation with UVB and are listed in Table 1. In total 78 skin samples were collected from 32 different species (Figure 1). All the samples were collected from the dorsal part of the animals, with the exception of the turtle skin, which was collected from the legs. For some species there was enough skin to exam differences in 7-DHC and vitamin D concentrations of the skin from the ventral in contrary to the dorsal skin. The skin samples are described with first the ventral skin sample and then the dorsal skin sample from the same animal. Those species were the long-tailed grass lizard, long-tailed lizard, central bearded dragon, common agama, black-necked agama, green iguana, ball python and Belize *Crawl-Cay*. From some species several skin samples were collected. Those skin samples were from different animals with differences in skin color. In captivity a lot of color mutations have emerged, therefore we have used especially skin samples from the corn snake. In the table the skin samples are listed form light to darker skin colors. Not only corn snake skin, but also from the common collared lizard, central bearded dragon and ball python. The differences in skin levels of samples from the same species of animal will be discussed separately.

Wistar rat skin served as control, but the vitamin D levels after irradiation were lower than before irradiation. Most species had relatively low, but detectable levels of 7-DHC in their skin, although the tiger-leg monkey frog, panther chameleon, veiled chameleon, central bearded dragon, common agama, and mop-headed iguana had higher levels of 7-DHC, in comparison to those of rats. The black-necked agama even had really high levels of 7-DHC. After UVB irradiation, tiger-leg monkey frog, giant monkey frog, common collared lizard skin 7-DHC levels decreased, together with an increasing level of vitamin D. In this analysis rat, giant tree frog, panther chameleon, eyed dabb lizard, and false map turtle skin vitamin D levels dropped after UVB exposure, which coincided with an increase in 7-DHC. In crested gecko, rankin's dragon, boa constrictor, olive house snake, common garter snake, and leopard tortoise skin 7-DHC levels also

increased after UVB exposure. The European badger had higher levels of vitamin D in the skin after UVB exposure without a detectable decrease of 7-DHC.

Differences and similarities were found in the skin samples collected from the ventral and dorsal part. In long-tailed grass lizard skin 7-DHC levels increased and vitamin D levels decreased after UVB exposure for both samples collected from the ventral and dorsal region. We are missing the information of the long-tailed lizard ventral skin from before the UVB irradiation. However, after UVB irradiation the dorsal skin vitamin D levels slightly decreased, together with an consistent level of vitamin D. The 7-DHC and vitamin D levels of the common agama are different from each other at all the possibilities when you compare ventral and dorsal skin. The black-necked agama has higher levels of 7-DHC before irradiation in both ventral and dorsal skin, but even higher in ventral skin. Only one sample had the exact levels of 7-DHC and vitamin D before and after UVB exposure that was the dorsal skin from the green iguana. This is in contrast to the ventral skin where both levels of 7-DHC were lower. In ventral green iguana skin vitamin D level before UVB exposure equals vitamin D level of dorsal skin. After irradiation the level of vitamin D decreases of ventral green iguana skin.

Three samples from both central bearded dragon and ball python were collected in this study. Each is composed of one ventral skin sample and two dorsal skin samples. The first dorsal skin sample in the table of the central bearded dragon has significantly lower levels of 7-DHC and vitamin D than the other skin samples of the darker colored central bearded dragon. If we compare the ventral and dorsal skin from the same central bearded dragon it shows higher levels of 7-DHC and vitamin D. What is particularly noticeable is the contrast in 7-DHC levels. Ventral 7-DHC levels decreased after UVB exposure. Dorsal skin sample vice versa gave an increase in 7-DHC level after irradiation.

All the 7-DHC levels of ball python skin decreased after UVB exposure. But what especially stood out in these samples were the higher levels of vitamin D in the ventral skin sample. There are also two species with several skin samples all from the dorsal skin. Two samples were taken from the common collared lizard. There was not much color difference between these two samples. In both of these samples the 7-DHC levels decreased and vitamin D levels increased after UVB exposure.

In addition 6 corn snakeskin samples were taken. These sample colors varied from a yellowish-orange to a dark brown color. In the table we can see that the 7-DHC

levels in the first 4 samples increased after irradiation. In contrary to the two darker colored skin samples, where the 7-DHC levels decreased after UVB exposure. It also showed an increase in vitamin D levels in the three lighter skin samples as well as in the darkest skin sample. The variations in the effects of UVB irradiation might be real, but, due to the limited sample number and differences in samples from the same species, we cannot exclude the possibility that it reflects biological variation between the samples. Overall, there was no significant effect of UVB irradiation on 7-DHC and vitamin D concentrations of the skin in different reptile and amphibian species.

Discussion

The 7-DHC concentration of the skin is a precursor that needs photo isomerization by UVB irradiation to get pre-vitamin D. To complete the synthesis of vitamin D the pre-vitamin D needs heat isomerisation (Tian, Chen et al. 1993, Tian, Chen et al. 1994, Kasian, Vashchenko et al. 2012). This does not require UVB irradiation, but several hours at 'body temperature'. Reptiles and amphibians are poikilothermic, therefore they need to use basking in sunlight in order to increase their body temperature (Holick, Tian et al. 1995, Allen, Chen et al. 1998, Carman, Ferguson et al. 2000). When pre-vitamin D is not heat-isomerized it can be reconverted in 7-DHC, and subsequently converted to lumisterol or tachysterol (Kasian, Vashchenko et al. 2012).

The 7-DHC concentration of the skin is regarded as the indicator for sufficient cutaneous vitamin D synthesis (Kohler, Leiber et al. 2013). This means that the presence of higher levels of 7-DHC in the skin in comparison to the control sample could enable these species to form sufficient amounts of vitamin D in the skin (Kasian, Vashchenko et al. 2012). In vivo, the vitamin D formed in the skin is readily bound to abundantly present vitamin D binding proteins for transportation to the target organs (Tian, Chen et al. 1994, Hazewinkel, Tryfonidou 2002). When the vitamin D is not taken up by this mechanism, as was not the case in this in vitro study, it might be that the synthesized vitamin D was reconverted into 7-DHC, or further photo isomerized to lumisterol or tachysterol (Kasian, Vashchenko et al. 2012). This can explain the decrease of vitamin D and increase of 7-DHC after UVB irradiation as was demonstrated in skin samples of some of the investigated species. This was also seen in the rat skin, which serves as a control in this study.

The target organ, where the vitamin D is transported to, is the liver where it is

hydroxylated to 25-hydroxyvitamin D3. And the final step of this mechanism will take place in the kidneys and 25-hydroxyvitamin D3 is converted into 1,25-dihydroxyvitamin D3, which is the active form of the hormone (Ganong W. 2001, Kasian, Vashchenko et al. 2012).

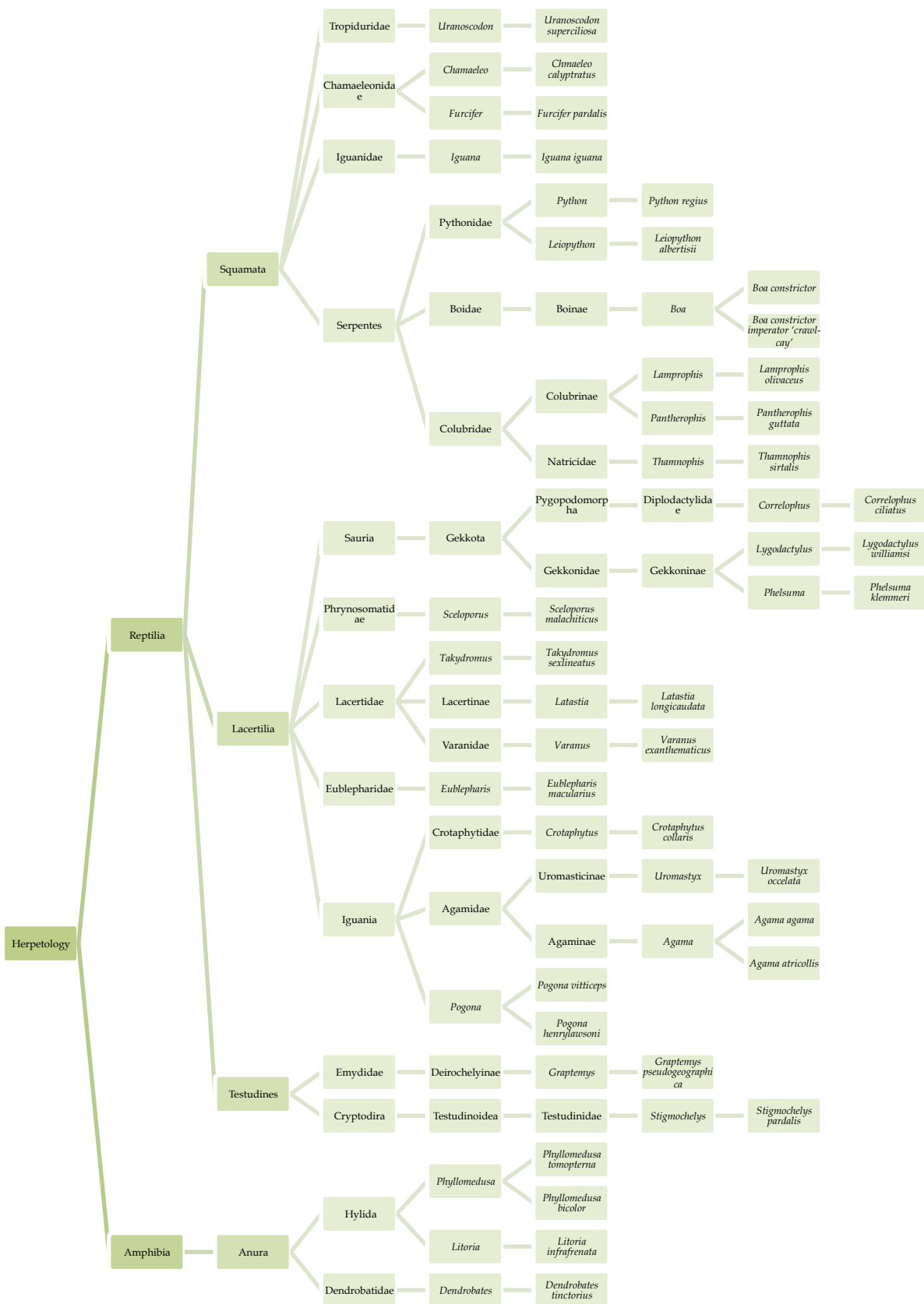
The dosage of UVB used in our study (2.16 J per cm²) is similar to the UVB dosage of How et al. (1994) (2.25 J per cm²), excluding differences in findings due to different dosage of UVB. No effective vitamin D synthesis was demonstrated in the skin in most species in this study after UVB exposure.

The 32 carnivorous species investigated here differ in their 7-DHC content of the skin, and some of them had even higher 7-DHC content of the skin compared to the rat. It seems that some species are able to synthesize sufficient amounts of vitamin D in their skin. From the investigated species, both chameleons (panther and veiled), 2 out of 3 Rankin's dragon skins, the dorsal skin of the common agama, both ventral and dorsal skin of black-necked agama, mop-headed iguana, and savannah monitor had higher levels of 7-DHC in their skin than the control rat.

It can also be possible that the skin thickness and amount of pigmentation is of importance for cutaneous vitamin D synthesis (Libon, Cavalier et al. 2013)

The solution for this can be longer exposure to UVB or a higher amount of UVB before it becomes effective. The yellow-headed day gecko, the species with the highest skin thickness, unfortunately is lacking the information from before irradiation. Therefore it cannot be used in the context of vitamin D synthesis after UVB irradiation. To assess whether the amount of pigmentation is of importance for cutaneous vitamin D synthesis, this study incorporated skin samples with different color mutations. The amount of vitamin D is not increasing with darker skin. The second darkest skin even has the lowest concentration vitamin D. Furthermore, the third-lightest skin even has the highest concentration of vitamin D after irradiation. In the table it can be seen that the central bearded dragon does have the highest amount of vitamin D concentration in the darkest skin. It should be taken into account that the sequence of the skin coloring is made visible to the naked eye.

Figure 1. Phylogenetic tree of the 32 species used in this study



As has been described above body temperature also influences isomerization, which may be different from the standardized 37°C that we used in this study. Reptiles and amphibians use basking in sunlight to get a higher body temperature. Possibly they apply different temperatures or length of basking, instead of the 30 minute UVB radiation. While basking primarily the dorsal skin will be irradiated, which leads to the question of whether there is a difference in the cutaneous production of vitamin D in different parts of the skin.

In this study we made the difference between ventral and dorsal skin. The concentration found after UVB irradiation when we made the comparison of ventral and dorsal skin could either be the same as or could be higher or lower. In dairy cows and sheep, just like in humans, the whole skin is capable of vitamin D synthesis, even when it is covered with hair, fur, or wool (Hymøller, Jensen 2010).

Many of the animals that have been used in this study are covered with scales. In order to exclude the influence of scales we first made a test between a skin sample with and without scales. This made it clear that there was no difference in the vitamin D concentration. All the samples in the table have been processed with scales.

In the natural habitat, the amount of UVB and the amount of vitamin D in the diet may be different from current housing conditions for these animals, which might result in vitamin D deficiency (Pye, Ellis et al. 2013). Diet may also affect 7-DHC concentration of the skin before UVB exposure due to the need for cholesterol, as well as after UVB exposure by the reconversion of the vitamin D present before UVB irradiation in the skin into 7-DHC. Cholesterol content of the skin influences 7-DHC concentration, as was demonstrated by up-regulation of 7-DHC reductase in human fibroblast cultures in a cholesterol deficient medium (Wassif, Maslen et al. 1998). However, we did not find differences in desmosterol levels, the other cholesterol precursor. Besides that we know nothing about their diet, we also know nothing about their age. From human studies it is known that ageing lowers the concentration 7-DHC in the skin (Gallagher, Peacock et al. 2013). Therefore, elderly people are more likely to get vitamin D deficiency and need longer sun exposure for adequate synthesis of vitamin D. The animals used in this study have died in a natural way. There were no data on their medical history and possible cause of death.

Because of all the variables that influence cutaneous vitamin D synthesis (both in vivo and in vitro) as was presented in this study, it is hard to draw firm conclusions from

this in vitro study. It seems possible that some reptiles and amphibians are capable of synthesizing sufficient amounts of vitamin D in their skin, but for most of the species it seems unlikely due to the low levels of 7-DHC.

Conclusion

This study demonstrated that reptile and amphibian species differ in 7-DHC and vitamin D concentrations in the skin. However, no significant effect of UVB irradiation was found on these concentrations. The relatively low cutaneous levels of the vitamin D precursor 7-DHC observed in this study suggest that most reptiles and amphibians are unable to synthesize sufficient amounts of vitamin D. But some reptiles and amphibians species could be capable of synthesizing sufficient amounts of vitamin D in their skin. The results have to be taken into account when appropriate husbandry and food recommendations are given for these species when held under captive conditions.

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References

- ACIERNO, M.J., MITCHELL, M.A., ROUNDTREE, M.K. and ZACHARIAH, T.T., 2006. Effects of ultraviolet radiation on 25-hydroxyvitamin D3 synthesis in red-eared slider turtles (*Trachemys scripta elegans*). *American Journal of Veterinary Research*, **67**(12), pp. 2046-2049.
- ACIERNO, M.J., MITCHELL, M.A., ZACHARIAH, T.T., ROUNDTREE, M.K., KIRCHGESSNER, M.S. and GUZMAN, D.S.-, 2008. Effects of ultraviolet radiation on plasma 25-hydroxyvitamin D3 concentrations in corn snakes (*Elaphe guttata*). *American Journal of Veterinary Research*, **69**(2), pp. 294-297.
- ALLEN, M., CHEN, T.C. and HOLICK, M.F.E.A., 1998. Evaluation of vitamin D status in the green iguana (*Iguana iguana*): oral administration vs UVB exposure. (Biologic effects of light. Berlin: Walter de Gruyter), pp. 99-101.
- BLIGH, E.G. and DYER, W.J., 1959. A rapid method of total lipid extraction and purification. *Canadian journal of biochemistry and physiology*, **37**(8), pp. 911-7.
- BOYER, T.H., 1996. Metabolic bone disease. *Reptile Medicine and Surgery*, , pp. 385-392.
- CARMAN, E.N., FERGUSON, G.W., GEHRMANN, W.H., CHEN, T.C. and HOLICK, M.F., 2000. Photobiosynthetic opportunity and ability for UV-B generated vitamin D synthesis in free-living house geckos (*Hemidactylus turcicus*) and Texas spiny lizards (*Sceloporus olivaceus*). *Copeia*, (1), pp. 245-250.
- CORBEE, R.J., 2014. *Nutrition and the skeletal health of dogs and cats*.
- FERGUSON, G.W., GEHRMANN, W.H., CHEN, T.C., DIERENFELD, E.S. and HOLICK, M.F., 2002. Effects of artificial ultraviolet light exposure on reproductive success of the female panther chameleon (*Furcifer pardalis*) in captivity. *Zoo biology*, **21**(6), pp. 525-537.
- FERGUSON, G.W., JONES, J.R., GEHRMANN, W.H., HAMMACK, S.H., TALENT, L.G., HUDSON, R.D., DIERENFELD, E.S., FITZPATRICK, M.P., FRYE, F.L., HOLICK, M.F., CHEN, T.C., LU, Z., GROSS, T.S. and VOGEL, J.J., 1996. Indoor husbandry of the panther chameleon *Chamaeleo* [Furcifer] pardalis: Effects of dietary vitamins A and D and ultraviolet irradiation on pathology and life-history traits. *Zoo biology*, **15**(3), pp. 279-299.
- GALLAGHER, J.C., PEACOCK, M., YALAMANCHILI, V. and SMITH, L.M., 2013. Effects of vitamin D supplementation in older African American women. *Journal of Clinical Endocrinology and Metabolism*, **98**(3), pp. 1137-1146.
- GANONG W., 2001. Hormonal control of calcium metabolism and the physiology of bone. (Review of medical physiology. New York: Lang Medical Books/McGraw-Hill Medical Publishing Division), pp. 369-382.
- HAZEWINKEL, H.A.W. and TRYFONIDOU, M.A., 2002. Vitamin D3 metabolism in dogs. *Molecular and cellular endocrinology*, **197**(1-2), pp. 23-33.
- HOLICK, M.F., TIAN, X.Q. and ALLEN, M., 1995. Evolutionary importance for the membrane enhancement of the production of vitamin D3 in the skin of poikilothermic

animals. *Proceedings of the National Academy of Sciences of the United States of America*, **92**(8), pp. 3124-3126.

HOW, K.L., HAZEWINKEL, H.A.W. and MOL, J.A., 1994. Dietary Vitamin D Dependence of Cat and Dog Due to Inadequate Cutaneous Synthesis of Vitamin D. *General and comparative endocrinology*, **96**(1), pp. 12-18.

HYMØLLER, L. and JENSEN, S.K., 2010. Vitamin D₃ synthesis in the entire skin surface of dairy cows despite hair coverage. *Journal of dairy science*, **93**(5), pp. 2025-2029.

KARSTEN, K.B., FERGUSON, G.W., CHEN, T. and HOLICK, M.F., 2009. Panther chameleons, *furcifer pardalis*, behaviorally regulate optimal exposure to UV depending on dietary vitamin D status. *Physiological and Biochemical Zoology*, **82**(3), pp. 218-225.

KASIAN, N.A., VASHCHENKO, O.V., GLUHOVA, Y.E. and LISETSKI, L.N., 2012. Effect of the vitamin D photosynthesis products on thermodynamic parameters of model lipid membranes. *Biopolymers and Cell*, **28**(2), pp. 114-120.

KIK, M.J. and BEYNEN, A.C., 2003. Evaluation of a number of commercial diets for iguana (*Iguana iguana*), bearded dragons (*Pogona vitticeps*), and land and marsh tortoises. *Tijdschrift voor diergeneeskunde*, **128**(18), pp. 550-554.

KOHLER, M., LEIBER, F., WILLEMS, H., MERBOLD, L. and LIESEGANG, A., 2013. Influence of altitude on vitamin D and bone metabolism of lactating sheep and goats. *Journal of animal science*, **91**(11), pp. 5259-5268.

LAING, C.J. and FRASER, D.R., 1999. The vitamin D system in iguanian lizards. *Comparative Biochemistry and Physiology - B Biochemistry and Molecular Biology*, **123**(4), pp. 373-379.

LAING, C.J., TRUBE, A., SHEA, G.M. and FRASER, D.R., 2001. The requirement for natural sunlight to prevent vitamin D deficiency in iguanian lizards. *Journal of Zoo and Wildlife Medicine*, **32**(3), pp. 342-348.

LIBON, F., CAVALIER, E. and NIKKELS, A.F., 2013. Skin color is relevant to vitamin D synthesis. *Dermatology*, **227**(3), pp. 250-254.

MADER, D.R., 2006. Metabolic Bone Diseases. *Reptile Medicine and Surgery*. pp. 841-851.

MORRIS, J.G., 1999. Ineffective vitamin D synthesis in cats is reversed by an inhibitor of 7-dehydrocholesterol-d7-reductase. *Journal of Nutrition*, **129**(4), pp. 903-908.

OONINCX, D.G.A.B., STEVENS, Y., VAN DEN BORNE, J.J.G.C., VAN LEEUWEN, J.P.T.M. and HENDRIKS, W.H., 2010. Effects of vitamin D₃ supplementation and UVB exposure on the growth and plasma concentration of vitamin D₃ metabolites in juvenile bearded dragons (*Pogona vitticeps*). *Comparative Biochemistry and Physiology - B Biochemistry and Molecular Biology*, **156**(2), pp. 122-128.

PYE, G., ELLIS, W., FITZGIBBON, S., OPITZ, B., KEENER, L. and HOLLIS, B., 2013. Serum vitamin D levels in free-ranging koalas (*Phascolarctos cinereus*). *Journal of zoo and wildlife medicine*, **44**(2), pp. 480-3.

TIAN, X.Q., CHEN, T.C., LU, Z., SHAO, Q. and HOLICK, M.F., 1994. Characterization of the translocation process of vitamin D3 from the skin into the circulation. *Endocrinology*, **135**(2), pp. 655-661.

TIAN, X.Q., CHEN, T.C., MATSUOKA, L.Y., WORTSMAN, J. and HOLICK, M.F., 1993. Kinetic and thermodynamic studies of the conversion of previtamin D3 to vitamin D3 in human skin. *Journal of Biological Chemistry*, **268**(20), pp. 14888-14892.

WASSIF, C.A., MASLEN, C., KACHILELE LINJEWILE, S., LIN, D., LINCK, L.M., CONNOR, W.E., STEINER, R.D. and PORTER, F.D., 1998. Mutations in the human sterol delta7-reductase gene at 11q12-13 cause Smith-Lemli-Opitz syndrome. *American Journal of Human Genetics*, **63**(1), pp. 55-62.

WEBB, A.R. and HOLICK, M.F., 1988. The role of sunlight in the cutaneous production of vitamin D3. *Annual Review of Nutrition*, **8**, pp. 375-399.