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Short Communication

Cutaneous Vitamin D Synthesis in Carnivorous Species

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Abstract

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 carnivorous species. To this endskin tissue of 22 different carnivorous species were collected from dead animals from zoo's and our pathology department. Wis tar rat skin served as a positive control.Cholesterol, 7-DHC, and vitamin D content was determined after UVB exposure at 37°C, and compared to non-irradiated skin. Overall, there was a significant effect of species and skin thickness, but not of UVB irradiation, on 7-DHC and vitamin D concentrations of the skin.The relatively low cutaneous levels of the vitamin D precursor 7-DHC observed in this study suggest that most terrestrial carnivores are unable to synthesize sufficient amounts of vitamin D. The results have to be taken into account when preparing food for these species when held under captive conditions.

INTRODUCTION

Dogs and cats cannot synthesize sufficient amounts of vitamin D in the skin under the influence of ultraviolet B light (i.e. 280-315nm) (UVB), and thus for their vitamin D requirement depend solely on dietary vitamin D (How et al. 1994) [1]. Vitamin D is synthesized by photo isomerization of 7-dehydrocholesterol (7-DHC) into pre-vitamin D, followed by heat isomerization into vitamin D (Figure 1). This physicochemical process has been revealed both in vivo as in vitro (How et al. 1994, Morris 1999) [1,2]. The amount of 7-DHC in rat skin proved to be much higher compared to dog and cat skin (How et al. 1994) [1], explaining the lower ability of dogs and cats to synthesize sufficient amounts of vitamin D in the skin. Vitamin D status in man and animals is evaluated by determining plasma 25-hydroxy vitamin D (25OHD) levels. Puppies raised on synthetic vitamin D deficient food, meeting dietary calcium and phosphorus requirements, developed radiological and histological diagnosed rickets and low plasma levels of 25OHD, despite daily UVB irradiation of the skin (Hazewinkel and Tryfonidou 2002) [3]. Furthermore, puppies raised on identical food only differing in vitamin D content (11.4 vs. 1350µg of vitamin D3 per kg diet), the high vitamin D group had 75 times higher 25OHD plasma levels compared to the low vitamin D group. In kittens, plasma 25OHD levels are also dependent on dietary intake and linearly correlate with the amount of vitamin D in the diet, regardless of exposure to sunlight (Morris et al. 1999). [2] A reverse in the cutaneous vitamin D synthesis capacity was demonstrated in cats that were supplemented with an inhibitor of delta 7-DHC reductase, suggesting that over-expression of this enzyme shifts the vitamin D precursor 7-DHC to a different metabolic pathway (i.e. cholesterol synthesis) (Morris 1999) [2]. This led to the conclusion that carnivorous species could lose the their requirements with the vitamin D content present in their prey. To the authors' knowledge, nothing is known about the ability of cutaneous vitamin D synthesis for other carnivorous species, which is important for possibly required adaptations of daily rations of these carnivorous species in captivity. 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 carnivorous species.

MATERIALS AND METHODS

Skin tissue (3x3cm) of different carnivorous species were collected from the back of dead animals from zoo's and our pathology department, and stored at -70°C for further analysis. Laboratory rat skin (8 months old male Wistar) served as a positive control. Cholesterol, 7-DHC, and vitamin D content was determined after UVB exposure at 37°C, and compared to non-irradiated skin. Two pieces of 1x1cm skin were cut from the sample, and weighed (Table 1). The pieces were thawed and heated to 37°C in a water bath for 2 hours prior to extraction (UVB- group), or UVB exposure (UVB+ group) and extraction.All skin samples were freed of subcutaneous tissue, shaved, and the UVB+ samples were irradiated with UVB (Arcadia 12% UVB D3 reptile lamp 15W, peak at 305 nm, Arcadia, Redhill, UK) light for 30 min (only the UBV+ group). Irradiation of the skin samples of the UVB+ group was done at 1200±100µW/cm2 (=12.00±1.00 J/s.m2). With a total exposure time of 1800 seconds, consequently irradiation corresponds with 21600 J/m2 = 2.160 J per cm^2. The amount of UVB administered was measured by the use of an UVB-meter (Solarmeter 6.2UV meter, Solartech Inc., USA). After 2 hours at 37°C, the pieces were cut into very small chunks and transferred into glass tubes and the lipids were extracted by the method as described by Bligh and Dyer (1959) [4]. In short 0.8

Table 1: 7-dehydrocholesterol (7-DHC) and vitamin D (VitD) concentration of the skin samples before (-) and after (+) exposure to UVB (2.16 J per cm^2), expressed in pmolper nmol cholesterol. The weight of the skin (Sw) is given in mg per cm^2.

Species	Latin name	7-DHC -	7-DHC +	VitD -	VitD +	Sw
Rat	*Rattusnorvegicus*	270	256	3	4	177
Gray wolf	*Canis lupus*	11	6	10	11	464
Dog	*Canis lupus familiaris*	7	7	11	7	117
African wild dog	*Lycaonpictus*	32	30	4	5	186
Fox	*Vulpesvulpes*	30	27	6	3	245
Arctic fox	*Vulpuslagopus*	35	20	1	1	481
Polar bear	*Ursusmaritimus*	27	27	1	1	239
Red panda	*Ailurusfulgens*	0	11	7	7	165
European badger	*Melesmeles*	0	0	1	4	425
European polecat	*Mustelaputorius*	12	25	6	2	487
Otter	*Lutralutra*	24	23	6	7	350
Raccoon	*Procyon*	32	28	1	1	130
Seal	*Phocavitulina*	164	123	1	1	1186
Ringed seal	*Pusahispida*	108	106	1	1	389
Californian sea lion	*Zalophuscalifonianus*	176	378	8	13	1492
Lion	*Pantheraleo*	6	6	5	7	124
Ocelot	*Leoparduspardalis*	16	17	2	3	186
Bobcat	*Lynx rufus*	21	21	1	1	88
Fishing cat	*Prionailurusviverrinus*	5	6	9	10	75
Cat	*Feliscatus*	16	15	1	1	55
Yellow mongoose	*Cynictispenicillata*	12	7	1	10	150
Meerkat	*Suricatasuricatta*	254	188	1	2	136

mL millipure water and 3 mL chloroform:methanol 1/2, v/v, were added to the tubes and were mixed for 40 min by regular vortexing. Then the tubes were centrifuged for 2 min at 2000 rpm. The supernatant was transferred to new glass tubes and 2 mL millipure water and 2 mL chloroform were added. After vortexing for 30 sec, the tubes were centrifuged for 5 min at 2000 rpm. The lower layer was transferred to a new tube and evaporated under N_2 gas. These samples were stored at -20°C prior to MS-analysis. Before MS-analysis, the samples were resuspended in 500 µL chloroform:methanol 1/1, v/v containing 0.002% butylated hydroxytoluene (BHT) as an anti-oxidant, and 20 µL was injected on a Lichrospher RP18-e column. A gradient was generated from acetonitrile:water 95/5, v/v, to acetone/chloroform 85/15, v/v, at a constant flow rate of 1 mL/min. Total run time per sample was 13 min. 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 (MRM) mode using the following settings: source temperature 420°C, nebulizer gas (GS1) 5, nebulizer current 3 µA, curtain gas 10, collision gas. High and declustering potential and collision energy were empirically optimized for each compound. The MRM transitions (m/z) used were: 367.3 → 159.1 for 7-DHC, desmosterol and vitamin D (species were identified with regard to retention times 7.2, 7.5, and 6.45 min, respectively), and 369.3 → 287.3 for cholesterol.

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)). As cholesterol is expected to be extracted with similar efficiency as vitamin D and 7-DHC, and cholesterol is a good indicator of the amount of cellular material present in the skins, the data are expressed as a ratio to cholesterol. All skin samples were analysed in duplicate. In case of high variations (i.e. >20%) the skin samples were analysed for another two times. The average levels of two samples are demonstrated.

STATISTICS

Multivariate analysis was performed to determine effects of UVB, species, and skin thickness (determined by the weight of the piece of skin of 1x1cm) on skin 7-DHC, and skin vitamin D concentrations, the latter two expressed per nmol cholesterol.

RESULTS

A total of 44 skin samples from 22 different species were analyzed and 7-DHC and vitamin D concentrations of the skin in pmol expressed per nmol of cholesterol before and after irradiation with UVB are demonstrated in Table 1. Wistar rat skin, as non-carnivorous control, contained the highest amount of 7-DHC. Most carnivorous species had relatively low, but detectable levels of 7-DHC in their skin, although the meerkat and sea-carnivores had higher levels of 7-DHC, comparable to

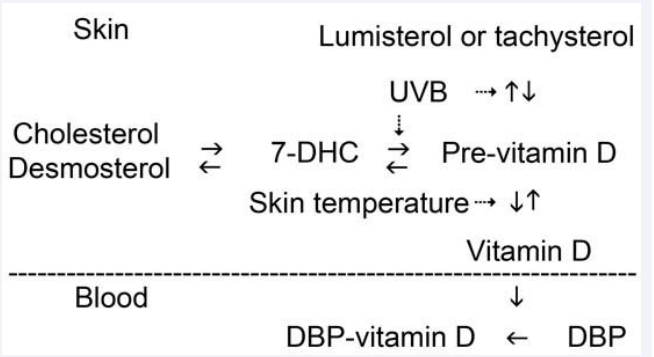

Figure 1 Metabolic pathways of 7-dehydrocholesterol (7-DHC). Pre-vitamin D can be synthesized from 7-DHC in the skin under the influence of ultraviolet B light (UVB). Under the influence of UVB, pre-vitamin D can be converted into lumisterol or tachysterol. Under influence of the skin temperature, pre-vitamin D can be converted into vitamin D, which is bound to vitamin D binding protein (DBP) in the blood. If 7-DHC is not used for vitamin D synthesis, it can be converted to cholesterol and desmosterol.

those of rats (Table 1). The relatively low levels of 7-DHC seemed not to be related to a lower cholesterol synthesis as, the levels of desmosterol, another precursor of cholesterol were not dissimilar between rat and the various carnivorous species (result not shown). All carnivorous species had detectable levels of vitamin D in their skin before and after irradiation with UVB. After UVB irradiation, rat, African wild dog, gray wolf, meerkat, otter, and yellow mangooseskin 7-DHC levels decreased, together with an increasing level of vitamin D. In the European polecat skin vitamin D levels dropped after UVB exposure, which coincided with an increase in 7-DHC. In fishing cat, ocelot, red panda, and sea lion skin 7-DHC levels also increased after UVB exposure. The European badger has higher levels of vitamin D in the skin after UVB exposure without a detectable decrease of 7-DHC. The variation in the effects of UVB irradiation might be real, but, due to the limited sample number, we cannot exclude the possibility that it reflects biological variation between the samples. Overall, there was a significant effect of species and skin thickness, but not of UVB irradiation, on 7-DHC and vitamin D concentrations of the skin.

DISCUSSION

Up till now, 7-DHC concentration of the skin is regarded as the indicator for sufficient cutaneous vitamin D synthesis (Kohler et al. 2013) [5]. The 21 carnivorous species investigated here differ in their 7-DHC content of the skin, and all of them had lower 7-DHC content of the skin compared to the omnivorous rat. Most carnivorous species are thus unlikely to be able to synthesize sufficient amounts of vitamin D in their skin. From the investigated carnivorous species, meerkat and sea-carnivores had the highest levels of 7-DHC in their skin. The presence of this vitamin D precursor in higher levels in the skin compared to other carnivores could enable these species to form sufficient amounts of vitamin D in the skin. Synthesis of vitamin D from 7-DHC in the skin demands photo isomerization by UVB irradiation to pre-vitamin D, followed by heat isomerisation into vitamin D (Kasian et al. 2012) [6]. This final step requires several hours at body temperature and does not require the presence of UVB. When pre-vitamin D is not heat-isomerized it can be reconverted in 7-DHC, and subsequently converted to lumisterol or tachysterol (Kasian et al. 2012) [6]. The theoretical potential of large amounts of 7-DHC to form vitamin D after long sun exposure is not realized due to further isomerization of pre-vitamin D by these reactions as well as by degradation of vitamin D by UVA (Jablonski and Chaplin 2013) [7]. 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 et al. 1994, Hazewinkel and Tryfonidou 2002) [3,8,]. 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 et al. 2012) [6]. This can explain the decrease of vitamin D and increase of 7-DHC as was demonstrated in skin samples of some of the investigated carnivorous species. The dosage of UBV used in our study (2.16 J per cm^2) is similar to the UVB dosage of How et al. (1994) [1] (2.25 J per cm^2), excluding differences in findings due to different dosage of UVB. No effective vitamin D synthesis was demonstrated in the skin in most carnivorous species after UVB exposure, while in rat skin significant vitamin D synthesis was demonstrated. This does not rule out the possibility of cutaneous vitamin D synthesis by carnivores, because it might be that the skin of the carnivores is thicker or more pigmented (Libon et al. 2013) [9], and therefore needs longer exposure to UVB or a higher amount of UVB to become effective. However, exposure of the skin samples with the highest weight and pigmentation (i.e. Californian sea lion and seal) for 2 hours did not result in an increase in skin vitamin D content (data not shown). In humans, the main function of epidermal pigmentation is protection of DNA against UVB (Jablonski and Chaplin 2013) [7]. In the natural habitat, the amount of UVB and the amount of vitamin D in the diet may be different from current housing conditions in zoo animals, which might result in vitamin D deficiency (Pye et al. 2013) [10]. Body temperature also influences isomerization, which may be different from the standardized 37°C that we used in our study. It is not known what the diet was of the animals that were used in this study. 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, as was possibly the case in European polecat, dog, and fox skin in this study. 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 et al. 1998) [11]. However we did not find differences in desmosterol levels, the other cholesterol precursor. From human studies it is known that ageing lowers the 7-DHC concentration in the skin (Gallagher et al. 2013) [12]. Elderly people and people are therefore more prone to vitamin D deficiency and need longer sun exposure for adequate synthesis of vitamin D. We did not know the age of the animals used in this study, so we can only speculate on the influence of ageing. From the skin samples only parts of the skin from the back were available. In chickens, 7-DHC concentrations were 30 times higher in leg skin compared to back skin. After UVB exposure of the whole body (0.5 J per cm2), pre-vitamin D was only present in uncovered skin of legs and

feet, while no pre-vitamin D was demonstrated in back skin (Tian et al. 1994) [13]. 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 and Jensen 2010) [14]. Feathers of chickens provide protection to UVB, while the pigmented skin in the legs and feet are capable of vitamin D synthesis. Although we assumed that in carnivores there are no differences as a result of their fur, we shaved all the skin samples prior to measurement. Because of all the variables that influence cutaneous vitamin D synthesis (both in vivo and in vitro) as were presented in this study, it is hard to draw firm conclusions from this in vitro study. It seems unlikely that terrestrial carnivores are capable of synthesizing sufficient amounts of vitamin D in their skin due to the low levels of 7-DHC. Furthermore, the 7-DHC concentration in the skin of carnivores found in this study are 4-40 times lower compared to skin of sheep and goats (Kohler et al. 2013) [5]. In vivo studies, using vitamin D deficient diets are needed to prove if the carnivorous species are able to synthesize vitamin D in the skin of biological significance as demonstrated in dogs (Hazewinkel and Tryfonidou 2002) [3] and cats (Morris 1999) [15].

CONCLUSION

This in vitro study demonstrated that carnivorous species differ in 7-DHC and vitamin D concentrations in the skin. The relatively low cutaneous levels of the vitamin D precursor 7-DHC observed in this study suggest that most terrestrial carnivores are unable to synthesize sufficient amounts of vitamin D [16-18]. The results have to be taken into account when preparing food for these species when held under captive conditions.

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