Effects of ultraviolet radiation produced from artificial lights on serum 25-hydroxyvitamin D concentration in captive domestic rabbits (*Oryctolagus cuniculi*)

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Objective—To determine the effects of UVB radiation produced by artificial lights on serum 25-hydroxyvitamin D concentrations in domestic rabbits (*Oryctolagus cuniculi*).

Animals—9 juvenile domestic rabbits.

Procedures—After an acclimation period, rabbits were anesthetized with isoflurane, and an initial blood sample was collected for determination of serum 25-hydroxyvitamin D concentration. Rabbits were randomly assigned to receive 12-hour exposure to UVB radiation produced by 2 compact fluorescent lights daily (n = 5) or no UVB supplementation (4) commencing on day 1. The UVB radiation emitted into the cage was measured at 9 points approximately 34 cm from the surface of the UVB light sources (representing the position of the rabbits in the cage) after 10 hours of exposure on days 1, 8, and 14. On day 14, another blood sample was collected from anesthetized rabbits for determination of serum 25-hydroxyvitamin D concentration.

Results—The UVB radiation level was 8.3 to 58.1 μW/cm^2 for the exposed rabbits and consistently < 0.001 μW/cm^2 for the control rabbits. Mean ± SD serum 25-hydroxyvitamin D concentrations in the rabbits that were or were not provided supplemental UVB radiation for 14 days differed significantly (66.4 ± 14.3 nmol/L and 31.7 ± 9.9 nmol/L, respectively).

Conclusions and Clinical Relevance—Exposure to UVB radiation produced by artificial light significantly increased serum 25-hydroxyvitamin D concentration in juvenile rabbits. Because vitamin D is an essential hormone in vertebrates, these findings suggest- ed that the provision of supplemental UVB radiation to captive rabbits may be important. (*Am J Vet Res* 2014;75:380–384)

Vitamin D is an important hormone and is essential to many different processes within the body, including calcium homeostasis (through active intestinal and renal absorption of calcium), regulation of parathyroid hormone concentrations, and stimulating osteoclast maturation in the bones.[1] In vertebrates, 2 primary methods are used to obtain vitamin D: via ingestion of prey or plant matter containing vitamin D or via photochemical synthesis from exposure to UVB radiation (wavelength, 290 to 315 nm).[1] Although various vertebrates, including humans, are known to have the capacity to acquire vitamin D through their diet, dogs and cats are the only species known to use this method as their sole source of acquiring vitamin D.[2] The production of vitamin D through exposure to UVB radiation is a multistage process.[1] Because UVB exposure is an important catalyst to vitamin D synthesis in many vertebrates, including rabbits,[3] it may be essential that they are provided exposure to UVB radiation. Under natural conditions or for animals housed outdoors, this occurs with exposure to sunlight; however, for animals housed indoors, this may not occur because glass or acrylic windows form an effective barrier to UVB radiation.[4] It is possible that captive indoor pet rabbits rely on exposure to UVB radiation to generate vitamin D and may be deficient if not provided UVB exposure.

In domestic rabbits, calcium metabolism is somewhat independent of vitamin D because rabbits are known to passively absorb calcium from the intestine and maintain a total serum calcium concentration that is 30% to 50% higher than that reported for other mammals.[5] Rabbits' efficiency with calcium absorption is thought to be related to the increased calcium demand required for the lifelong growth of their teeth.[5] Acquired dental disease is a common finding in pet rabbits[5–7] and can present a major health risk to the rabbits and financial burden to the owners. It has been proposed that hypovitaminosis D may play a role in the development of nutritional osteodystrophy of the skull and acquired dental disease in pet rabbits.[6–9] Given these concerns, it

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is important to investigate the physiologic relationship between husbandry and vitamin D for captive indoor rabbits. More specifically, because there are currently no recommendations to provide captive indoor rabbits exposure to UVB radiation, there is a need to evaluate whether serum vitamin D concentrations in these animals are influenced by the provision of UVB light.

The purpose of the study reported here was to determine the effects of UVB radiation produced by artificial lights on serum 25-hydroxyvitamin D concentrations in domestic rabbits (*Oryctolagus cuniculi*). Because exposure to UVB radiation has been shown to increase plasma 25-hydroxyvitamin D concentration in reptiles,[10–13] we were interested to determine whether such treatment could have a similar effect on captive indoor rabbits. The biological hypothesis tested was that captive juvenile rabbits exposed to UVB produced by commercial fluorescent light bulbs would have significantly higher serum 25-hydroxyvitamin D concentrations than captive juvenile rabbits that were not exposed to the supplemental UVB.

Materials and Methods

Animals—The study was performed in accordance with the regulations established by the Institutional Animal Care and Use Committee at the University of Illinois.

Nine 6-week-old rabbits (6 females and 3 males) acquired from a private source[a] were used for the study. The rabbits were dwarf mixed-breed rabbits ranging in weight from 220 to 493 g, and all of them had pigmented fur and irises (ie, there were no albino animals). The hair coat color varieties of the rabbits were diverse; because of sample size, it was not possible to further categorize them on the basis of pigmentation. The rabbits were housed singly or doubly in 71 × 44 × 41.5-cm plastic-bottomed wire cages[b] on pine bedding.[c] Fresh water was provided in a sipper bottle ad libitum. The cage substrate and water were replaced daily. The rabbits' daily diet consisted of timothy hay[d] (unlimited access) and 1 cup of alfalfa-based pelleted diet[e] each. The temperature in the room in which the rabbits were housed was approximately 23° to 27°C, and a 12-hour light-dark cycle was maintained. General room lighting was provided with non–UVB-producing fluorescent lighting. The rabbits were acclimated for 72 hours prior to the start of the study.

Procedures—After the initial 72-hour acclimation period, each rabbit was anesthetized with isoflurane[f] delivered via a facemask to collect a blood sample (day 0). Blood sample collection was performed between 4 PM and 6 PM. Each rabbit was placed in dorsal recumbency, and a blood sample was collected from a jugular vein with a 25-gauge needle[g] attached to a 3-mL syringe.[g] The total blood sample volume (2 mL) was < 1% of each rabbit's body weight. The blood sample was placed into a 3-mL tube without anticoagulant[g] and centrifuged[h] for 20 minutes within 90 minutes after collection. The serum was then removed, placed into a cryovial,[i] and frozen at –17°C.

After the initial blood sample was obtained, rabbits were allocated to 1 of 2 groups by means of a random number generator. During the 14-day study period, 5 rabbits were exposed to supplemental UVB lighting (treatment group), and 4 rabbits were not provided with supplemental UVB lighting (control group). Supplemental UVB lighting was provided by use of 2 bulbs[j] placed approximately 24 cm apart at a distance of 46.5 cm from the cage bottom. The lights rested directly on top of the cages. Supplemental UVB light was provided continuously for 12 hours daily. For both groups, UVB radiation was measured by use of a radiometer[k] at a distance of 34 cm from the lights (at the level of the rabbits) at 9 points in a grid pattern along the center and perimeter of the cage on days 1, 8, and 14 after 10 hours of light activity.

Between 4 PM and 6 PM on day 14, each rabbit was again anesthetized, and a blood sample was collected as described for day 0. All serum samples (obtained on days 0 and 14) were placed on frozen gel packs and sent to a veterinary diagnostic laboratory[l] for measurement of serum 25-hydroxyvitamin D concentration.

Statistical analysis—The sample size selected for the study was based on the following assumptions: an expected difference in 25-hydroxyvitamin D concentration between the treatment and control groups of at least 50%, α of 0.05, and power of 0.99. The distribution of the data was evaluated with a Shapiro-Wilk test. Data were normally distributed and are reported as mean ± SD and range. A Levene test was used to determine that the data met the assumption of homogeneity of variance. An independent-samples *t* test was used to determine whether weight was independent of sex. Paired-sample *t* tests were used to determine whether the mean values for weight or 25-hydroxyvitamin D concentration differed between days 0 and 14. If there was a significant change over time, the difference between days 0 and 14 was calculated. An independent-samples *t* test was then used to determine whether the difference in serum 25-hydroxyvitamin D concentration or body weight between the sample days was affected by treatment (UVB exposure) or no treatment (no UVB exposure). An independent-samples *t* test was also used to determine whether the rabbits' sex had an effect on serum 25-hydroxyvitamin D concentration. Commercial statistical software[m] was used to analyze the data; α was set at 0.05.

Results

Of the 9 rabbits used in the study, 5 were provided with supplemental UVB lighting (treatment group), and 4 were not provided with supplemental UVB lighting (control group). Among all 9 rabbits, mean ± SD weight at day 0 was 329.8 ± 96.1 g (range, 220.0 to 493.0 g); at day 14, mean weight was 536.4 ± 139.6 g (range, 391.0 to 794.0 g). This difference in weight was significant ($P < 0.001$); the mean change in weight was 206.6 ± 52.1 g (range, 130.6 to 301.0 g). Weight was found to be independent of sex on both day 0 ($P = 0.22$) and day 14 ($P = 0.31$).

After 10 hours of light activity on days 1, 8, and 14, the UVB radiation levels were measured at the level of the cage substrate for the treatment and control groups. The UVB radiation level was 8.3 to 58.1 $\mu W/cm^2$ for the

Table 1—Serum 25-hydroxyvitamin D concentrations in juvenile domestic rabbits (*Oryctolagus cuniculi*) before (day 0) and after (day 14) exposure to supplemental UVB radiation produced by 2 compact fluorescent lights for 12 hours daily (n = 5) or no exposure to supplemental UVB radiation (control group; 4).

	Serum 25-hydroxyvitamin D concentration (nmol/L)	
Variable	**Mean ± SD**	**Range**
Day 0		
No UVB exposure	29.7 ± 14.9	14 to 44.0
UVB exposure	38.8 ± 21.4	15.0 to 63.0
Day 14		
No UVB exposure	31.7 ± 9.9	22.0 to 45.0
UVB exposure	66.4 ± 14.3*	44.0 to 81.0
Difference in 25-hydroxyvitamin D concentration between time points		
No UVB exposure	2.0 ± 11.7	−14.0 to 13.0
UVB exposure	27.6 ± 14.8†	10.0 to 49.0

For all rabbits, the daily diet consisted of timothy hay (unlimited access) and 1 cup of alfalfa-based pelleted diet each. General room lighting was provided with non–UVB-producing fluorescent lighting. The UVB radiation emitted into the rabbits' cages was measured at 9 points approximately 34 cm from the surface of the UVB light sources (representing the position of the rabbits in the cage) after 10 hours of exposure on days 1, 8, and 14. The UVB radiation level ranged from 8.3 to 58.1 μW/cm² for the UVB-exposed rabbits and was consistently < 0.001 μW/cm² for the control rabbits.

*Mean value for the treatment group is significantly (P = 0.005) greater than that for the control group. †Mean value for the treatment group is significantly (P = 0.026) greater than that for the control group.

rabbits that were exposed to supplemental UVB lighting and consistently < 0.001 μW/cm² for the rabbits that were not exposed to supplemental UVB lighting.

Over the study period, weight gain among rabbits exposed to supplemental UVB lighting and rabbits that were not exposed to supplemental UVB lighting did not differ (P = 0.52). Examination of data for the treatment and control groups revealed no significant difference in serum 25-hydroxyvitamin D concentration on day 0 (P = 0.5); however, a significant (P = 0.005) difference was evident on day 14 (**Table 1**). There was a significant (P = 0.026) difference in serum 25-hydroxyvitamin D concentration by treatment over time.

Discussion

Findings of the present study indicated that 14-day exposure of juvenile rabbits to UVB radiation produced from artificial light resulted in a significant increase in serum 25-hydroxyvitamin D concentration. To gauge the importance of this, it would be necessary to compare these results to a reference interval for serum 25-hydroxyvitamin D concentration in domestic rabbits, but such a reference interval has not been established, to our knowledge. In humans, vitamin D deficiency is defined as serum 25-hydroxyvitamin D concentration < 50 nmol/L; a concentration > 374 nmol/L is considered toxic.[1] On the basis of these human data, the untreated control rabbits in the present study would be considered deficient in vitamin D. To confidently determine a reference interval for serum 25-hydroxyvitamin D concentration in rabbits, a cross-sectional or prospective study would have to be done in healthy animals housed outdoors and indoors.

An interesting observation made in the present study was that the serum 25-hydroxyvitamin D concentration in the control group did not change between days 0 and 14 despite the fact that the rabbits were provided an acceptable diet of timothy hay and pellets with adequate vitamin D supplementation (1.1 U/g feed). For ethical reasons, food was not withheld from these rabbits and they were not provided a vitamin D–deficient diet to assess the dietary influence of vitamin D, as has been done in other species.[11] Instead, it was expected (and confirmed by the study data) that serum 25-hydroxyvitamin D concentrations in rabbits fed a diet with supplemental vitamin D would be maintained, whereas serum 25-hydroxyvitamin D concentrations in rabbits fed a diet with supplemental vitamin D and exposed to supplemental UVB lighting would increase.

Although the findings of the present study confirmed that UVB radiation produced by artificial lighting can increase serum 25-hydroxyvitamin D concentration in rabbits, it is possible that the role of dietary vitamin D may have a stronger influence on circulating 25-hydroxyvitamin D concentrations if the dietary concentrations are altered. A previous study[14] evaluating rabbits of various ages, including pregnant females, revealed much higher serum concentrations of 25-hydroxyvitamin D in rabbits housed on a breeding farm, compared with values in laboratory-housed rabbits (587.8 ± 238.2 nmol/L and 284.1 ± 76.6 nmol/L, respectively). In the report[14] of that study, there was no mention of natural or artificial UVB exposure, but the dietary vitamin D content of the pelleted diet at the breeding farm was approximately 3 times that of the diet at the laboratory and almost 6 times that of the diet fed to the rabbits in the present study. This suggests that dietary supplementation with vitamin D can also lead to increases in serum 25-hydroxyvitamin D concentrations. Thus, it appears that vitamin D concentrations in rabbits will increase in response to ingestion of supplemental vitamin D in the diet or exposure to supplemental UVB radiation. It should be noted, however, that the serum 25-hydroxyvitamin D concentrations described in the aforementioned report[14] would be considered toxic in humans.

In another study,[8] rabbits kept under free-range conditions with access to direct sunlight had higher plasma 1,25-dihydroxyvitamin D concentrations than did rabbits that were mainly confined to hutches. These findings suggest that exposure to natural sources of UVB radiation stimulates the photochemical synthesis of vitamin D in rabbits. The results of the present study supported this, but also indicated that UVB-producing artificial lights can be used to increase circulating 25-hydroxyvitamin D concentrations in rabbits. This is important because many pet rabbits are housed exclusively indoors. On the basis of the data obtained in this study, there is concern that dietary supplementation of vitamin D alone may not be adequate for juvenile rabbits. In addition, although increasing the amounts of vitamin D in rabbit diets may prove useful in increasing circulating concentrations of vitamin D in rabbits fed those diets, it may also increase the risk of toxicosis.

Because the photochemical synthesis of vitamin D associated with UVB radiation can be regulated by the needs of the host,[15] the best way to ensure rabbits have appropriate circulating concentrations of vitamin D would be to provide them with a dietary source that is considered to provide adequate but not toxic circulating concentrations along with exposure to UVB radiation (natural or artificial). Additional studies to determine appropriate husbandry guidelines are needed.

In the present study, the weight gain (62.6%) in the rabbits between days 0 and 14 was not surprising because of the young age of these animals.[16] However, given the fact that an increase in serum 25-hydroxyvitamin D concentration was not evident in the control group, there was concern that these rabbits would be more susceptible to the clinical effects of vitamin D deficiency as they continued to grow. In humans, vitamin D deficiency is evidenced as failure of mineralization of growing bone or osteoid tissue and associated osteomalacia, which result in bowing of the long bones, swelling of the ends of long bones, pathological fractures, poor growth, delayed dentition, and slow motor development.[17] Additionally, humans with hypophosphatemic rickets frequently have dental disease involving enamel hypoplasia, occlusion defects, and dental abscesses.[18] Chronic low-level deficiency of vitamin D during growth and adulthood may be a contributing factor to dental disease in rabbits as well, and dental disease is a common reason that domestic rabbits are evaluated at veterinary clinics.[5–7,9] A study of 40 pet rabbits with acquired dental disease revealed that poor tooth and bone quality were the most important etiologic factors, but no obvious underlying cause for the apparent osteodystrophy was elucidated.[9] However, it is important that any similarity of the pathophysiologic effects of vitamin D deficiency in humans and rabbits is not oversimplified, because rabbits are more efficient in passive intestinal absorption of calcium and can actively excrete calcium via their kidneys. Future studies to evaluate the specific role of vitamin D in rabbits with dental disease may help further elucidate the underlying pathological processes associated with this condition.

To our knowledge, the present study is the first to provide evidence that supplemental UVB radiation produced by artificial lights can significantly increase serum 25-hydroxyvitamin D concentration in juvenile rabbits, compared with findings in rabbits that were not exposed to supplemental UVB radiation but that were fed the same diet. The clinical importance of this increase in serum 25-hydroxyvitamin D concentration is unknown at this time; however, we speculate that it is clinically relevant. In vertebrates, it is important to be aware that excessive supplementation with vitamin D can have toxic effects. However, toxicosis was expected to be of minimal concern in the rabbits in the present study because excessive exposure to UVB radiation degrades vitamin D_3 into an inactive photoproduct before it can become active vitamin D in the body.[1] However, it should be noted that UVB radiation has been associated with skin neoplasia, especially in fair-haired humans and light-colored animals, such as white cats.[19,20] For rabbits, this could be especially important when evaluating certain breeds, such as the New Zealand white rabbit. Another potential adverse effect of UVB exposure to consider would be retinal damage.[20] In general, it might be expected that the potential for damage may vary depending on the amount and quantity of UVB exposure and degree of iris pigmentation. New Zealand white rabbits or other rabbit breeds that lack pigment in the iris may be useful in experiments to determine the potential pathological effects associated with UVB exposure. Finally, excess calcium in the diet has been associated with urolithiasis in rabbits.[21] It would be essential in the further evaluation of the metabolic effects of vitamin D in domestic rabbits to determine the minimum effective UVB radiation amount to prevent adverse effects.

On the basis of the information obtained from the present and previous studies, further evaluation of the physiologic effects of vitamin D in rabbits is warranted. It is important when designing future studies to consider not only the role of UVB exposure but also the effect of dietary forms of vitamin D (D_2 and D_3) on circulating 25-hydroxyvitamin D concentrations. In addition, the physiologic effects of increased 25-hydroxyvitamin D concentration as a result of UVB exposure on calcium metabolism (eg, passive and active absorption from the intestines), phosphorus metabolism, and endocrine function (eg, parathyroid hormone) should be further characterized. As well as evaluating the potential physiologic impacts of UVB exposure in rabbits, it will also be necessary to evaluate potential pathological effects associated with UVB exposure. Rabbits used in such studies should be assessed for development of skin neoplasia (eg, squamous cell carcinoma), retinal disease, and dystrophic mineralization, thereby providing further insight into the value of UVB exposure for these animals. Prospective clinical trials to assess the potential benefits of UVB exposure are also necessary, and investigations designed to determine the effects of increased circulating 25-hydroxyvitamin D concentration on bone mineralization and the dental condition should be pursued. Changes in husbandry recommendations to include full-spectrum lighting may become a standard of care to improve the overall health of pet rabbits.

a. Sailfin Pet Shop, Champaign, Ill.
b. 72 × 44 × 41.5 cm, Marchioro S.p.A., Isola Vicentina, Italy.
c. Sunseed Co, Bowling Green, Ohio.
d. Western Timothy Hay, Oxbow Animal Health, Murdock, Neb.
e. Wool Formula 17-19, Heinold Feeds, Kouts, Ind.
f. Butler Animal Health Supply Co, Dublin, Ohio.
g. Tyco Healthcare Group LP, Mansfield, Mass.
h. IEC HN-SII Centrifuge, Thermo Electron Corp, Milford, Mass.
i. CryoTube vials, 1.8 mL, Nunc A/S, Roskilde, Denmark.
j. Sun-Glow Coil Lantern, 20 W, 5.0 UVB, Fluker Farms, Port Allen, La.
k. UV meter No. 1400, International Light Inc, Newburyport, Mass.
l. Diagnostic Center for Population and Animal Health, East Lansing, Mich.
m. SPSS, version 19.0, SPSS Inc, Chicago, Ill.

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