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Introduction

Rickets and osteomalacia, classic conditions of calcium and vitamin D deficiency, are common problems in sun-basking reptiles, such as the green iguana, when housed indoors. Vitamin D deficiency may occur because exposure to ultraviolet (UV) radiation of appropriate wavelength and intensity is inadequate or because intake and/or absorption of dietary vitamin D3 is insufficient. The principal benefit of artificial light for captive herpetofauna, apart from photoperiodic effects, is the promotion of vitamin D synthesis, provided that artificial light includes appropriate ultraviolet (UV) wavelengths. Dermal and epidermal conversion of 7-dehydrocholesterol to cholecalciferol requires irradiative exposure between 280 and 315 nm (Webb and Holick, 1988). Most artificial lights sources do not emit a significant level of radiation in this range.

The objective of these projects was to determine the effectiveness of oral and injectable supplementation of vitamin D3 as well as experimental UV-emitting lamps as means of improving the vitamin D status of captive green iguanas.

Materials and Methods

Prior to the initiation of these projects, all animals were fed diets containing approximately 2,000 IU vitamin D3 per kg, 1.4% calcium and 0.7% phosphorus. None of the animals were provided with exposure to natural or artificial UV radiation for at least 6 months. However, many of the animals had developed pathologic fractures and classic signs of rickets, suggesting problems with calcium and/or vitamin D metabolism.

Serum levels of 25-hydroxyvitamin D for all animals were determined by the radiorecptor assay technique of Chen et al. (1990). Whole body radiographs, taken prior to and immediately after the experimental period, were used as a gross measure of skeletal calcification.

Project 1: Twenty-five green iguanas (11 adults, 14 juveniles) were each allotted by age and weight to one of five treatment groups. Animals receiving treatment 1 served as the control and were provided with no additional vitamin D and no UV lights. Iguanas on treatment 2 were given IM injections of vitamin D3 (cholecalciferol) at the rate of 1,000-3,000 IU/kg body weight at beginning of the study (day 0), and again on day 46. The initial injection was crystalline cholecalciferol (Sigma Chemicals #C-9756, St Louis, MO) in a glycerol suspension, and the subsequent injection was an emulsifiable base containing 10,000 IU cholecalciferol/ml in 95% ethanol (Henkel Corporation, LaGrange, IL). Animals in treatments 3 through 5 had exposure for 12 hours per day to a daylight phosphor lamp, a 35% Sylvania 2096 phosphor experimental lamp or a 100% Sylvania 2096 phosphor experimental lamp, respectively. The animals were able to regulate their distance from the lamp from 5 cm to 70 cm [2-28 in.] but were provided with no shaded area. Blood was collected from each animal on day 0, 27, 46, 67, 89, 166 and 204 for determination of 25-hydroxyvitamin D.

Project 2: Eight hatchling green iguanas, blocked by initial weight and whole body bone mineral concentration, were allotted to one of two treatment groups. On day 0, animals in treatment group 1 were administered an oral dose of vitamin D3 suspended in corn oil (34,000 IU/ml) at the rate of 0.25 µl (8.5 IU)/g body weight. Doses were administered to the closest µl using an adjustable Eppendorf ® micropipettor. Additionally, the animals on treatment 1 were exposed to General Electric Cool White fluorescent lamps for 12 hours daily.[a] Animals in treatment group 2 were given no supplemental vitamin D but were exposed to an experimental lamp, the Sylvania Experimental Reptile Light (modification of the 25% 2096 phosphor lamp with a color rendering index of at least 91 and a correlated color temperature of 5500°K), for 12 hours each day. All

lamps were mounted 61 cm [24.4 in.] from the floor of the reptile enclosures. Although the iguanas were able to regulate their distance from the lamp, between 30 cm and 61 cm [12-24 in.], they were provided no completely shaded area. Blood samples were obtained from each animal on day 0, 7, 21 and 35 for determination of serum 25-hydroxyvitamin D.

Results and Discussion

It is not clear why animals that consume diets high in calcium and vitamin D3 should show evidence of calcium and/or vitamin D deficiency. It was, however, clearly demonstrated that bone metabolism in these green iguanas was severely compromised prior to initiation of this study. Prior to beginning treatments, no differences in any measure were apparent between treatment groups in either project. Before treatment, serum concentrations of vitamin D metabolites were extremely low or immeasurable in all animals. Likewise, initial radiographs indicated mild to moderate osteomalacia, a general lack of radiographic definition and thin cortices in long bones. Radiographs taken after treatment revealed improved radiographic definition and displayed signs of healing in old fracture sites. No new fracture sites were seen post-treatment m any of the animals.

Analyzed data for serum 25-hydroxyvitamin D are presented in Figures 1and 2. In Project 1, significant differences were not evident between the control treatment and the daylight treatment at any time ($P > 0.05$). Differences between the control treatment and the injection treatment were seen only at day 27 ($P < 0.005$) and at day 67 ($P < 0.0003$), each of which were approximately 3 weeks subsequent to vitamin D injections. Both the 35% and 100% 2096 treatments produced highly significant differences from other treatments at all sampling times after baseline ($P < 0.0001$). In Project 2, baseline concentrations were not significantly different ($P > 0.05$) between treatments, nor were there significant differences between the oral dose treatment and the UV light treatment at 7 days ($P > 0.05$). By days 21 and 35, however, differences between the treatments were significant ($P < 0.004$ and $P < 0.0007$, respectively).

The iguanas on Project 1 demonstrated a serum response in 25-hydroxyvitamin D to both forms of injectable cholecalciferol. It appeared that the injection in the emulsified base produced a higher response in serum 25-hydroxyvitamin D concentrations and sustained elevated serum concentrations of this vitamin D metabolite for a longer period. Each injected dose was roughly equivalent to the total amount of cholecalciferol an iguana would consume in a diet containing 2,000 IU/kg in a 5-month period. Yet, serum concentrations were still significantly lower than those attained by exposure to UV light.

The percentage of 2096 phosphor was a significant factor in producing elevated levels of 25-hydroxyvitamin D in animals exposed to experimental lamps, with higher concentrations of this vitamin D metabolite associated with higher percentages of the phosphor. Serum concentrations of 25-hydroxyvitamin D in all animals exposed to the 2096 phosphor were maintained consistently in excess of 200 ng/ml, and ranged as high as 1,200 ng/ml.

In Project 2, all dosed animals exhibited a serum response to the oral cholecalciferol. The peak response in the absorption curve to this oral dose may have been missed due to the intervals between blood collections. However, by day 21 serum concentrations of 25-hydroxyvitamin D in the orally dosed iguanas already were on the decline. The amount of cholecalciferol administered to each animal (8.5 IU/g BW) was an extremely high dose, yet, serum concentrations of 25-hydroxyvitamin D were significantly lower than those attained by exposure to UV radiation. Serum concentrations of 25-hydroxyvitamin D in all animals exposed to the Experimental Reptile Light rose relatively rapidly, and were maintained after day 21 in excess of 330 ng/ml, ranging as high as 575 ng/ml.

Serum concentrations of 25-hydroxyvitamin D of the magnitude seen in these projects have not been reported in other species, but concentrations previously reported in captive green iguanas housed outdoors were > 400 ng/ml (Allen et al., 1994). There may be several potential problems

associated with highly elevated serum concentrations of vitamin D metabolites. In those species which have been studied, concentrations such as those seen here, might be associated with pathology. However, no clinical signs or radiographic evidence of vitamin D toxicity were seen in these iguanas.[b]

To attain such extraordinary serum concentrations of 25-hydroxyvitamin D, the iguana must have a remarkable capacity to convert cutaneous precursors to previtamin D3. Additionally the thermal conversion of previtamin D3 to vitamin D3 also must be highly efficient. The regulation of cutaneous production of vitamin D3 precursors in human skin precludes excess conversion of previtamin D3 to vitamin D3 by shifting photoisomerization of previtamin D3 to its biologically inert isomers, lumisterol and tachysterol, in lieu of vitamin D3 (Holick et al., 1981 and Webb and Holick, 1988). The extraordinarily elevated concentrations of serum 25-hydroxyvitarnin D found in iguanas exposed daily for 12 hours to UV radiation may challenge the effectiveness of this method of regulation. It is quite possible, however, that if iguanas are provided with access to shaded areas, as they would be in the wild, they may regulate vitamin D production by behaviorally limiting their UV exposure.[c]

Further studies are necessary if the mechanism of vitamin D production and regulation in iguanas are to be identified. It is critical, however, to continue research with lamps such as the Sylvania Expeimetal Reptile Light to establish long term safety and effectiveness for maintaining the health of captive herpetofauna. It is obvious that any UV source identified for intended use with captive animals should support adequate vitamin D3 photobiogenesis without causing tissue damage or other adverse effects.

Related Articles

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