Vitamin D and Primates: Recurring Problems on a Familiar Theme

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Captive primates housed indoors with little access to ultraviolet light have historically been susceptible to metabolic bone disease. The trend in zoological parks toward building large, indoor exhibits has potentially exacerbated this problem. Most skylight materials are not transparent to the wavelengths of ultraviolet light (UV-B) necessary for endogenous production of vitamin D. It is possible that most primates, like humans, produce milk that is low in vitamin D. Thus mother-reared infants without exposure to UV-B would be at risk for rickets. The incidence of metabolic bone disease in callitrichids has been greatly reduced by the addition of large amounts of vitamin D3 to their diet. However, metabolic bone disease still occurs in these primates. Nowadays, osteomalacia in callitrichids is generally found in multiparous females, often occurring during pregnancy. This might reflect the dominance of reproductive females over other group members. Dominant animals often consume a higher proportion of preferred supplemental foods, which can be deficient in vitamin D. However, vitamin D metabolism is poorly understood in New World monkeys. Thus it is possible that dietary vitamin D is not an effective method to meet the requirements of these animals under all conditions. We found wide variation in the serum levels of 25-0H-vitamin D within a colony of common marmosets even though all animals were maintained on a single-item purified diet with a constant level of vitamin D3 (3000 IU/kg). We are currently testing the efficacy of a low wattage UV light source on these animals.

Key words: vitamin D3, primates, ultraviolet light, metabolic bone disease

INTRODUCTION

Captive primates housed indoors with little or no access to ultraviolet (UV) light have historically been susceptible to metabolic bone disease (Boulay and Crawford 1968). This was especially true of New World primates, who, unlike Old World primates, cannot effectively utilize the plant form of vitamin D (ergocalciferol or vitamin D_2) (Hunt et al 1967 , Lehner et al. 1967, Marx et al. 1989). Supplementing the diet of captive primates with vitamin 03, the animal form of the vitamin, has largely proved effective at reducing the incidence of bone disease in primates. However, there are still certain classes of primates that appear to remain at risk, namely mother-reared infants and multiparous females in the family Callitrichidae.

Cholecalciferol, commonly called vitamin D3' is a precursor to the steroid hormone calcitriol (1,25-dihydroxycholecalciferol, or 1,25-(OH)2-vitamin D3). In most vertebrates studied to date, cholecalciferol is produced in the skin by a two-stage process. First provitamin D (7-dehydrocholesterol) is converted to previtamin D. This reaction requires irradiation of the skin by ultraviolet light in the wavelength range of 290-315 nm, or UV-B (Holick et al 1981).Previtamin

D is then converted to cholecalciferol (vitamin D) by a thermally dependent process. Vitamin D then enters the blood and binds to circulating proteins, primarily vitamin D-binding protein.

To form calcitriol, the vitamin D metabolite with the highest biological activity, cholecalciferol is first hydroxylated in the liver to form 25-(OH)-vitamin D3 or calcidiol, which is the main storage form of the hormone. Like vitamin D, calcidiol circulates in the blood primarily bound to vitamin D-binding protein. Hydroxylation of calcidiol in the kidney forms 1,25-(OH)2-vitamin D3. The hydroxylation of 25-(OH)-D to 1,25- (OH)2-D is a tightly regulated physiological process. The known function of 1,25- (OH)2-D is to regulate whole-body calcium metabolism. The hormone enhances intestinal absorption of both calcium and some phosphate, and acts on the kidneys to stimulate renal conservation of calcium and phosphate. It also has direct effects on bone; in concert with parathyroid hormone, calcitriol regulates the mobilization of calcium from bone. Adequate amounts of the hormone are necessary for normal growth and development of bone, and in maintaining mature bone tissue. Although there is little direct evidence that 1,25-(OH)2-D has biological functions in addition to whole-body calcium regulation, many factors suggest the possibility. Most nucleated cells in the body contain receptors for the hormone, including brain cells. Vitamin D deficiency affects more than just bone health. For example, skeletal muscle weakness is associated with vitamin D deficiency (Anderson and Toverud 1994, Fraser 1995). In vitro, vitamin D metabolites can inhibit cell proliferation and promote cell differentiation (Fraser 1995). Epidemiological evidence suggests that vitamin D may reduce the risk of humans for prostate cancer and possibly other cancers (Anderson and Toverud 1994). All these facts suggest that 1,25-(OH)2-D has some, yet unknown, role in cell metabolism (Fraser 1995).

Vitamin D can be toxic. The exact mechanism of the toxicity is not well understood, but in mammals is associated with an increase in serum levels of 25- (OH)-D, hypercalcemia, and eventually calcification of soft tissue. It is not clear whether the toxic effects are a result of 25-(OH)-D binding to the receptor sites and mimicking the action of 1,25-(OH)2-D, or if the high serum levels of 25-(OH)-D result in a displacement of 1 ,25-(OH)2-D from vitamin D-binding protein, causing an increase of free calcitriol in the blood. Complicating this picture is the fact that rats suffering from acute toxicity (vitamin D is used as a rodenticide) die before developing hypercalcemia (Fraser 1995).

What has become apparent, however, is that vitamin D toxicity is unlikely to arise due to endogenous production from UV-B exposure (Fraser 1995). The slow rate of diffusion of vitamin D from skin and the susceptibility of vitamin D to further photochemical change limit the supply to safe levels. No such protection exists for oral or injected doses, however .

Vitamin D and Captive Animals

For most animals under natural conditions vitamin D is not a nutrient. Rather I it is an endogenously produced component of an endocrine-like hormonal system. However, many captive animals are kept under conditions in which they are exposed to little or no UV-B radiation. Thus they require an exogenous source of vitamin D. For most, but not all, animals, this is easily supplied by the diet. However, in green iguanas, giant day geckos, and perhaps other basking reptiles dietary vitamin D may not protect against the development of D deficiency (Bernard et a! 1991).

Primates at Risk

There are two categories of captive primates for which it appears to be difficult to maintain adequate vitamin D status by dietary means: mother-reared, nursing infants, and multiparous, callitrichids (specifically common marmosets). The reasons for the difficulties in these two groups are quite different.

Nursing Infants

Little is known about vitamin D levels in milk. However, the concentration of vitamin D in human milk (25 IU/I) (Hollis et al 1981) is too low to supply the suggested daily requirement for human infants (400 IU/day). Human infants fed solely on breast milk and without access to UV-B radiation would be at risk of developing rickets (Anderson and Toverud 1994, Fraser 1995). This appears to be true of non- human primates as well. Clinical rickets has been diagnosed in several colobus monkeys and a Francois langur (Morrisey et al. 1994), silvered leaf monkeys and sakis (R. Cambre pers. Comm.), and gorillas (T. Meehan pers. Comm. In all cases the animals were infants or young juveniles that had been mother-reared in indoor enclosures. The diets offered to the adults were apparently adequate in vitamin D, and there were no known bone health problems in other group members. Although for these species we do not know the relationship between maternal vitamin D status and the amount of vitamin D in milk, it appears that maternal sufficiency does not guarantee adequate vitamin D in milk.

This result can be understood when viewed in an evolutionary perspective. Under natural conditions, vitamin D is not a nutritional requirement for primates, young or old. Neonates would depend on stored vitamin D obtained before birth across the placenta, and later, on endogenous production due to UV-B exposure. There is no evidence for an evolutionary "need" for vitamin D in mother's milk.

Mother-reared infants of species with long lactation periods, where infants derive the vast majority of their nutrients from milk for a considerable length of time, appear to be at risk for vitamin D deficiency in the absence of UV-B exposure. It is possible that maternal vitamin D status during pregnancy could affect the amount of stored vitamin D in the neonate, and thus have some effect on the latency of deficiency. However, there is no evidence that supplementing mothers after the births of their infants is likely to have any beneficial effect on the infant.

Common Marmosets

Most New World monkeys (owl monkeys, genus *Aotus,* appear to be the only exception) differ from other primates in vitamin D metabolism. They cannot effectively utilize vitamin D2 (Hunt et al 19671 Lehner et al. 19671 Marx et al. 1989). Circulating levels of both 25-(OH)-D and 1 ,25-(OH)2-D are substantially higher than in humans (Adams et al. 1985, Yamaguchi et al. 1986, Gacad et al. 1992). The serum levels of 25-(OH)-D are quite variable, but generally exceed the "safe" range for humans. Marmosets suffering from clinical metabolic bone disease have levels of 25-(OH)-D at the low end of the normal range of variation for humans (Yamaguchi et al. 1986). In addition, New World monkeys are tolerant of extremely high doses of dietary D3, and can be fed diets containing levels of vitamin D that would be toxic to most other animals without adverse effect.

Historically, bone disease has been a substantial problem in captive New World monkey populations. The incidence of bone disease has dramatically declined, however, especially among young, growing animals. This may be due to the large quantities of vitamin D3 that have been added to commercial diets for New World monkeys.

Bone disease does still occur sporadically in laboratory colonies of common marmosets. Some of these incidences may be dietary related. Breeding female marmosets are generally dominant in food situations, and can monopolize access to supplemental foods which may not contain sufficient vitamin D. However, there appears to be a syndrome in which multiparous females that have given birth four or more times in rapid succession (common marmosets often become pregnant within weeks of parturition) develop severe bone disease during the later stages of pregnancy (D. Abbott, pers. comm.). These incidences have occurred in females fed a diet high in vitamin D3 (5000 or more IU/kg dry weight).

We are currently maintaining a colony of common marmosets on single-item, purified diets that have been the sole food for more than 14 months. The level of vitamin D3 in the diet is about 3000 IU/kg dry weight, which is considered high for most animals, but low for marmosets. Accordingly, we have monitored 25-(OH)-D status in our colony.

Blood samples were taken from animals during two sampling periods. The first samples were taken after the animals had been exclusively fed the diets for 3- 7 months. Animals had been exclusively fed the purified diets for 9-11 months by the second sampling period. Sixteen adult animals (six males and ten females) had samples from each time period. About two months before the second sampling period, unshielded, low wattage UV light emitting florescent lights (Chroma 50, GE) were placed above the cages. The efficacy of vitamin D production due to these lights was estimated using ampules of provitamin D placed at different locations within empty cages. The serum samples were assayed for 25-(OH)-D and the ampules assayed for provitamin D, previtamin D and other photo-products by the Vitamin D, Skin, and Bone Research Laboratory at Boston University School of Medicine, under the direction of Dr. Michael F. Holick. The serum levels of 25-(OH)-D were compared between time periods using paired-sample t-tests.

There was wide variation among individuals in serum levels of 25-(OH)-D in both sampling periods. The median value for the first samples was 78 ng/ml (range = 12 -320 ng/ml); the median value for the subsequent samples was 37.5 ng/ml (range = 7.5 -105 ng/ml). For reference purposes, the median serum level of 25- (OH)-D in wild cotton-top tamarins was 79 ng/ml (M. Power, D. Oftedal and A. Savage, unpublished data). Serum levels were significantly lower in the second sampling period ($p = 0.005$). However, if the data for males and females were considered separately, there was no difference between periods for males ($p = 0.205$), but female 25-(OH)-D status was significantly reduced in the second period ($p = 0.008$). No evidence of clinical bone disease was observed during this time interval, and three females produced offspring.

The Chroma 50 lights did apparently produce effective UV-B radiation as evidenced by the measurable conversion of provitamin D to previtamin D. At the top of the cage the estimates of 12 hour conversion were from 6.5% to 13%. At the height of the food bowl the conversion rate estimates ranged from 1.6% to 8%. There was minimal, but detectable conversion at the bottom

of the cage (0.4% to 2%). For comparative purposes, full exposure of the ampules to sunlight would result in 40% to 100% conversion of provitamin D in 12 hours, depending on atmospheric conditions (M. Allen pers. comm.).

Our results suggest that dietary D3 at the level of 3000 IU/kg dry matter is not sufficient to maintain constant serum levels of 25-(OH)-D in common marmosets, at least not in breeding females. Although Flurer and Zucker (1987) estimated dietary requirement of saddle-back tamarins to be as low as 2000 IU/kg dry weight, their subjects included no breeding females. The large range of serum values among individuals on identical diets may indicate interindividual variation in vitamin D metabolism or in the ability to utilize dietary D, although other potential causes of variation need to be examined. Serum 25-(OH)-D levels in wild cotton-top tamarins also varied among individuals, but not as widely as in the captive common marmoset data (M. Power, 0. Oftedal and A. Savage, unpublished data).

We are continuing to monitor 25-(OH)-D status in these animals. We are preparing to test the effects of additional UV-B light supplementation on serum D status in the near future.

Strategies for Avoiding Vitamin D Deficiency

The ease of providing vitamin D through the diet for most animals has perpetuated the classification of this substance as a nutrient. For the many animals housed indoors at zoos and laboratory colonies, this classification has become the practical truth. However, we are beginning to appreciate that there may be significant variation among species in the efficiency with which dietary vitamin D can meet requirements. With the increase in large, indoor, multispecies exhibits in zoos this issue becomes increasingly germane. Levels of cholecalciferol necessary for meeting the requirements of callitrichids present the danger of toxicity for other animals in the same exhibit (Kenny et a! 1993). Nursing infants that are not yet eating other foods may not benefit from maternal dietary supplementation. Thus there are many classes of animals that zoos will exhibit for which dietary D may be a problematic solution to maintaining bone health.

The ideal solution, of course, is to give animals access to unfiltered sunlight. This ideal solution is neither always practical nor possible, however. Many animals must be housed in enclosed exhibits. Thus any sunlight they will be exposed to passes through potentially UV-B blocking materials. Most forms of glass and plastic are not transparent to UV-B radiation, however commercially available UV-B transparent materials are marketed. Zoos should give thought to testing these materials for use in enclosed exhibits.

The use of artificial UV-B light sources could be effective under certain circumstances. However, the potential eye and skin hazards of high intensity UV light for both animals and keepers must be considered, as well as the rapid decline in UV intensity that occurs with distance from the source. Episodic exposure to high intensity UV-B should be tested, as this strategy might be effective for certain species. Clinical intervention with the administration of either oral or injected vitamin D is often disruptive and labor intensive, but may be the only practical approach for nursing infants of some primate species it is not possible to ensure adequate exposure of the infant to UV-B.

REFERENCES

Adams, J.S., Gacad, M.A., Baker, A.J., Gonzales, B., Rude, R.K. Serum concentrations of 1,25-dihydroxyvitamin D3 in Platyrrhini and Catarrhini: a phylogenetic appraisal. AMERICAN JOURNAL OF PRIMATOLOGY 9:219-224, 1985.

Anderson, J.J.B., Toverud, S.U. Diet and vitamin D: A review with an emphasis on human function. J. NUTRI. BIOCHEM. 5:58-65, 1994.

Bernard, J.B., Oftedal, O.T., Barboza, P.S., Mathias, C.E., Allen, M.E., Citino, S.B., Ullrey, D.E., Montali, R.J. The response of vitamin D- deficient green iguanas *(Iguana iguana)* to artificial ultraviolet light. PROCEEDINGS OF THE AMERICAN ASSOCIATION OF ZOO VETERINARIANS, pp. 147-150, 1991.

Boulay, G.H. du, Crawford, M.A. Nutritional bone disease in captive primates. SYMP. ZOOL. SOC. LOND. 21 :223-236, 1968.

Flurer, C.I., Zucker, H. Evaluation of serum parameters relevant to vitamin D status in tamarins. J. MED. PRIMATOLOGY 16: 175-184, 1987.

Fraser, D.R. Vitamin D. THE LANCET 345: 104-107, 1995.

Gacad, M.A., Deseran, M.W ., Adams, J.S. Influence of ultraviolet B radiation on vitamin D3 metabolism in vitamin D3-resistant New World monkeys. JOURNAL OF PRIMA TOLOGY , 28:263-270, 1992

Holick, M.F., MacLaughlin, J.A., Doppelt, S.H. Regulation of cutaneous previtamin photosynthesis in man: Skin pigment is not an essential regulator . 211 :590-593, 1981.

Hollis, B.W., Roos, B.A., Draper, H.H., Lambert, P.W. Vitamin D and its I
in human and bovine milk. JOURNAL OF NUTRITION 111: 1240-1248,

Hunt, R.D., Garcia, F.G., Hegsted, D.M., Kaplinski, N. Vitamins D2 and D3 in World primates: influence on calcium absorption. SCIENCE 157:943- 1967.

Kenny, D., Cambre, R.C., Lewandowski, A., Pelto, J.A., Irlbeck, N.A., Wilson, H., Mierau, G.W., Sill, F.G., Garcia, A.P. Suspected vitamin D3 toxicity inh - *(Cuniculus paca)* and agoutis *(Dasyprocta aguti)*. JOURNAL OF ZOO AND WILDLIFE MEDICINE 24:129-139, 1993.

Lehner, N.D.M., Bullock, B.C., Clarkson, T.B., Lofland, H.B. Biological activities of vitamin D2 and D3 for growing squirrel monkeys. LAB. ANIM. CARE 17:483- 493, 1967.

Marx, S.J., Jones, G., Weinstein, R.S., Chrouse, G.P ., Renquist, D.M. Differences in mineral metabolism among nonhuman primates receiving diets with only vitamin D3 or only vitamin D2. J. CLIN. ENDOCRINOL. METAB. 69:1282-1290, 1989.

Morrisey, J.K., Reichard, T., Janssen, D.D., Llyod, M., Bernard, J.B. Vitamin D deficiency rickets in Colibinae monkeys. PROCEEDINGS OF THE AMERICAN SOCIETY OF ZOO VETERINARIANS, pp. 381-383, 1994.

Yamaguchi, A., Kohno, Y., Yamazaki, T ., Takahashi, N., Shinki, T ., Horiuchi, N., Suda, T ., Koizumi, H., Tanioka, Y ., Yoshiki, S. Bone in the marmoset: a resemblance to vitamin-D dependent rickets, type II. CALCIFIED TISSUE INT. 39:22-27, 1986.