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Vitamin D Intakes by Cotton-Top Tamarins (*Saguinus oedipus*) and Associated Serum 25-Hydroxyvitamin D Concentrations

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Rickets and osteomalacia have been reported frequently in captive callitrichids. Some have assumed that these conditions are a consequence of unmet, unusually high requirements for vitamin D and that these high requirements are characteristic of all New World primates. As a consequence, certain commercial diets formulated for New World primates contain such high concentrations of vitamin D that their consumption by other species has resulted in signs of vitamin D toxicity. This study was conducted to assess the vitamin D status of captive cotton-top tamarins consuming diets providing either 2,500 or 26,000 IU of vitamin D3/kg dry matter. These diets had been consumed for at least 2 years before the study, with the lower vitamin D intakes by six tamarins (0.5 to 9 years old) in a zoo colony and the higher vitamin D intakes by 24 tamarins (2 to 12 years old) in a pharmaceutical research laboratory. Although not measured in this study, none of the dietary ingredients has been shown to contain vitamin D2. Serum 25-hydroxyvitamin D (25(OH)D) concentrations in the captive tamarins were compared with serum 25(OH)D concentrations (range, 25.5 - 120 ng/mL; 64 - 300 nmol/L) reported by others in healthy wild tamarins in Colombia, South America. Concentrations of 25(OH)D in serum from zoo tamarins consuming 2,500 IU vitamin D3/kg dietary dry matter ranged from 48 to 236 ng/mL (120 - 590 nmol/L), whereas those in serum from laboratory tamarins fed 26,000 IU vitamin D3/kg dietary dry matter ranged from 11 to 560 ng/mL (28 - 1,400 nmol/L), with no significant ($P>0.05$) association between serum 25(OH)D concentration and sex or age. However, in the laboratory tamarins, serum 25(OH)D concentrations

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ranged from 46 to 60 ng/mL (115 - 150 nmol/L) in one 8-year-old male and four 12-year-old females that had four to nine pregnancies each. Younger females (2 - 5 years old) that had zero or one pregnancy and the other males (3 - 12 years old) generally had serum 25(OH)D concentrations above 126 ng/mL (315 nmol/L). None of the individuals in the zoo colony showed signs of colitis. Of the two tamarins in the laboratory group with 25(OH)D levels below 50 ng/mL (125 nmol/L), one was a 4-year-old male with anorexia and cachexia associated with severe colitis. The second was a 7-year-old clinically normal, multiparous (five) female with normal hematology and clinical chemistry but histologic evidence of severe colitis. Because all other individuals in this group had histologic evidence of moderate to severe colitis but were normal in other respects, an unequivocal association between low serum 25(OH)D concentrations and colitis was not apparent. A dietary vitamin D3 concentration of 2,500 IU/kg dry matter was more than sufficient to support serum 25(OH)D concentrations equivalent to those found in the wild and, although the number of observations was small, supported apparently normal growth and adult weights, reproduction through five parities, and general health in a zoo colony showing no evidence of colitis. Zoo Biol 18:473 - 480, 1999.

Key words: vitamin D status; vitamin D requirement; callitrichids

INTRODUCTION

Primates in captivity have exhibited rickets or osteomalacia on numerous occasions [Allen et al., 1995]. These conditions have been reported more often in platyrrhines (New World primates) than in catarrhines (Old World primates), a difference proposed to be a consequence of a higher vitamin D requirement by New World primates or of their limited ability to use vitamin D2 [Hunt et al., 1966]. The vitamin D needs of relatively few of the more than 230 extant species of non-human primates have been studied, but evidence that vitamin D2 has less biological activity than vitamin D3 (based on calcium absorption, prevention or reversal of metabolic bone disease, or toxicity of excess vitamin D) in the squirrel monkey (*Saimiri sciureus*), white-fronted capuchin (*Cebus albifrons*), and rhesus macaque (*Macaca mulatta*) is quite convincing [Hunt et al., 1967a,b, 1972; Lehner et al., 1967]. Indeed, in addition to the well-recognized superiority of vitamin D3 over vitamin D2 in meeting the dietary vitamin D requirements of poultry, vitamin D3 appears biologically more active for several mammalian species, although the magnitude of the difference is commonly less [Horst et al., 1988]. Nevertheless, it may be presumptuous to generalize from limited species evidence to broad species groups. It is noteworthy that vitamin D-binding and vitamin D metabolite-binding transport proteins and vitamin D-metabolite binding affinities vary among New World and Old World primate species as well as between New World and Old World groups [Hay and Watson, 1976, 1977; Gacad and Adams, 1992].

With respect to suggestions of a difference between New and Old World primates in their quantitative requirements for vitamin D (by implication, vitamin D3), little published research has been designed to answer this question. There is an anecdotal report of fibrous osteodystrophy in squirrel monkeys (*Saimiri sciureus*) fed a commercial diet containing vitamin D2 that was not seen "in thousands of rhesus macaques and other *Macaca* species" fed commercial diets containing this vitamin D form [Hunt et al., 1967]. Unfortunately, no information on dietary vitamin D concentrations or husbandry was provided.

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Captive New World primates are commonly fed much higher dietary vitamin D3 levels than are Old World primates. Marmosets and tamarins are often fed commercial diets containing 7,000 to 22,000 IU vitamin D3/kg dry matter [Kenny et al., 1993]. Whether such high levels are needed to meet vitamin D requirements has not been established. Such high vitamin D levels may produce signs of hypervitaminosis D in certain Old World primates [Knapka et al., 1995] and appeared to be responsible for frank toxicity in pacas (*Cuniculus paca*) and agoutis (*Dasyprocta aguti*) consuming primate diets dropped on the floor by New World primates occupying the same multi-species exhibit [Kenny et al., 1993].

Concentrations of serum or plasma 25-hydroxyvitamin D (25(OH)D) have been found to be particularly useful in assessing the vitamin D status of humans and several other animal species [Holick, 1990]. The present study was conducted to permit comparison of serum 25(OH)D concentrations found in captive cotton-top tamarins with those found by Power et al. [1997] in serum of free-ranging cotton-top tamarins in Colombia, South America. Blood serums were collected in late August or early October 1996 from two groups of captive tamarins and were analyzed for 25(OH)D. The two groups of captive tamarins were consuming diets with measured and very different concentrations of vitamin D3, and no vitamin D2 (based on analyses of ingredients by others), and had been consuming those diets for at least 2 years. Since the captive tamarins had no exposure to sunlight nor to artificial light with significant ultraviolet radiation below 320 nm, it was presumed that serum 25(OH)D would reflect differences in dietary vitamin D3 intakes. Comparisons with values from apparently normal wild tamarins should indicate whether the captive diets provided amounts of this nutrient equivalent to the vitamin D obtained from food and solar irradiation in the natural environment.

MATERIALS AND METHODS

Six cotton-top tamarins at the Potter Park Zoo, Lansing, MI, were given a morning diet of a freshly prepared Tamarin Mix (containing [as fed basis] 55% of ground Scenic Psittacine Diet extrusions [Marion Zoological Inc., Plymouth, MN], 34% mashed banana, 5.5% of Mazuri Marmoset Jelly powder [#5041, PMI Feeds, St. Louis, MO], and 5.5% added water), plus intact extrusions of Scenic Psittacine Diet, and wax moth (*Galleria mellonella*) larvae. The afternoon diet contained the Tamarin Mix and fresh fruit, predominantly pears, apples, and grapes. Occasionally, sweet potatoes, oranges, bananas, or tomatoes were substituted for one or more of these fruits.

These six tamarins constituted a family group and were housed together in one exhibit. The senior male (#1277) was born 7/12/87 and, at the time of serum sampling, weighed 519 g. The senior female (#1637) was born 9/14/89 and weighed 685 g. The other tamarins were sired by the senior male and were born to the senior female. Two were twin females (#1908, #1909), born 10/24/94 and weighed 499 and 501 g, respectively. One was a male survivor (#1973) of triplets born 6/1/95 and was not weighed. The youngest was an unweighed female survivor (#2069) of triplets born 5/13/96.

Weighed amounts of food were presented twice a day for 13 days, and uneaten items were removed and weighed before the next feeding. Consumption was determined by difference. After 13 days, one of the twin females (#1909) was removed from the exhibit, and feed consumption of the remaining five tamarins was determined for another 13 days.

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Twenty-five cotton-top tamarins (13 males and 12 females), ranging in age from 2 to 12 years and weighing 365 to 540 g, were housed and fed individually or in pairs at Parke-Davis Pharmaceutical Research, Ann Arbor, MI. Caging was stainless steel, and the facility and care were AALAC approved as prescribed by ILAR [1996]. Eighty-five to 106 g of ZuPreem Canned Marmoset Diet (#6920, Premium Nutritional Products, Inc., Topeka, KS), plus 10 mg Na ascorbate and 20–30 g of fruit (bananas, blueberries, grapes, kiwi fruit, or strawberries) were fed each day. Although most of the diet was consumed before the next feeding, refused food was not weighed. Thus, it was not possible to precisely assess dry matter or vitamin D consumption.

Vitamin D concentrations in the commercial feeds used in this study were determined by modified AOAC [1980] procedures [Chen et al., 1990]. Vitamin D2 was not present. Although not analyzed, vitamin D concentrations in the fruits and vegetables used have been reported to be zero [Holland et al., 1992]. Likewise, the wax moth larvae were not analyzed, but were not fed from time of receipt until they were offered to the tamarins, were exposed neither to sunlight nor to artificial light with ultraviolet B irradiance, and contributed little to the average dry matter intake of the tamarins (<0.9%). Average daily vitamin D3 intakes at the Potter Park Zoo were calculated by dividing the vitamin D3 provided in daily group food intakes by the number of tamarins in the group. Average vitamin D3 concentrations in the diet offered at Parke-Davis Pharmaceutical Research were calculated from vitamin D3 concentrations in diet ingredients and ingredient proportions in the diet offered. Serum concentrations of 25(OH)D were determined as described by Chen et al. [1990].

RESULTS

Assays of the commercial feeds revealed vitamin D3 concentrations (as is basis) of 1,160 IU/kg in Mazuri Marmoset Jelly powder, 3,060 IU/kg in Scenic Psittacine Diet (average of ground [3,000] and unground [3,120] samples), and 11,760 IU/kg in ZuPreem Canned Marmoset Diet.

Average daily intakes of food during the first 13 days by six Potter Park tamarins were 162 g of Tamarin Mix, 31 g Scenic Psittacine Diet (as air-dry extrusions), 72 g of fruits and vegetables, and 2.9 g wax moth larvae. This diet provided 145 g of dry matter and 37.6 IU of vitamin D3, assuming that the fruits, vegetables, and wax moth larvae provided no vitamin D. This was the equivalent of 2,593 IU/kg of dry matter.

Average daily intakes of food during the second 13 days by five Potter Park tamarins were 137 g of Tamarin Mix, 20 g of Scenic Psittacine Diet (as air-dry extrusions), 72 g of fruits and vegetables, and 2.6 g wax moth larvae. This diet provided 119 g of dry matter and 29.9 IU of vitamin D3, or the equivalent of 2,513 IU/ kg of dry matter.

If one assumes that the difference between dry matter and vitamin D3 intakes of six versus five tamarins represents consumption by cotton-top tamarin #1909, this 2-year-old female consumed 26 g of dry matter and 7.7 IU of vitamin D3, or 2,962 IU D3/kg of dry matter. This tamarin weighed 501 g, and estimated average daily dry matter and vitamin D3 consumption per 100 g of body weight would have been 5.2 g and 1.54 IU, respectively.

Average daily food intakes of the 25 cotton-top tamarins at Parke-Davis were not determined, but the offered proportions of fruit mix and ZuPreem Canned Marmoset Diet (#6920) would have provided approximately 26,000 IU of vitamin D3/kg of dry matter.

Concentrations of 25(OH)D in serum from Potter Park tamarins ranged from 48 to 236 ng/mL (120 - 590 nmol/L), with the senior male having the lowest value. The senior female had 129 ng/mL (322 nmol/L), and values for the younger tamarins (oldest to youngest) were 148, 150, 236, and 150 ng/mL (370, 375, 590, and 375 nmol/L), respectively.

One serum sample from the Parke-Davis tamarins was lost. The other 24 values ranged from 11 to 560 ng/mL (28 - 1,400 nmol/L). Two were 11 and 12 ng/mL (28 and 30 nmol/L), five ranged from 46 to 60 ng/mL (115 - 150 nmol/L), three were between 126 and 176 ng/mL (315 - 440 nmol/L), and the remaining 14 were ≥224 ng/mL (≥560 nmol/L). There was no significant ($P > 0.05$) association between serum 25(OH)D concentration and age or sex. However, in the Parke-Davis tamarins, serum 25(OH)D concentrations ranged from 46 to 60 ng/mL (115 - 150 nmol/L) in one 8-year-old male and four 12-year-old females that had four to nine pregnancies each. Younger females (2 - 5 years old) that had zero or one pregnancy and the other males (3 - 12 years old) generally had serum 25(OH)D concentrations >126 ng/mL (>315 nmol/L).

DISCUSSION

Although the tamarin populations at the two institutions were not randomly assigned to the two different dietary concentrations of vitamin D3, there was a trend for higher average 25(OH)D concentrations in serum of tamarins fed a diet containing 26,000 IU D3/kg dry matter than in serum of tamarins consuming a diet containing approximately 2,500 IU D3/kg dry matter. Although Power et al. [1997] observed a serum 25(OH)D level of 25.5 ng/mL (64 nmol/L) in an apparently healthy freeranging male cotton-top tamarin, they proposed that a range of 50–120 ng/mL (125 - 300 nmol/L) could be considered normal for this species. They also suggested that the very high levels of 300 - 600 ng/mL (750 - 1,500 nmol/L) reported in some captive callitrichids [Yamaguchi et al., 1986] may be above normal.

All the serum 25(OH)D levels of the six zoo tamarins in this study consuming a diet with 2,500 IU vitamin D3/kg dry matter were near or moderately above the normal range (50 - 120 ng/mL; 125 - 300 nmol/L) proposed by Power et al. [1997]. None of the tamarins in this colony exhibited colitis clinically, nor was colitis evident at necropsy in other animals that died for reasons unrelated to vitamin D supply.

Of the 24 laboratory tamarins fed 26,000 IU D3/kg dry matter, seven were essentially within the proposed normal range, 15 were substantially above it, and two were below it. Because some of the cotton-top tamarins at the Parke-Davis Pharmaceutical Research laboratory periodically exhibited colitis clinically, and all exhibited moderate to severe colitis on colonic biopsy, there was concern that gastrointestinal pathology and decreased transit time might have impaired vitamin D absorption and influenced 25(OH)D concentrations in the serum of the two individuals with low values. The health of all animals was assessed by weighing them 40 and 26 days before and on the day of bleeding, by daily visual appraisal of behavior and fecal consistency, and by performing standard hematologic and clinical chemistry examinations at the time blood was drawn for 25(OH)D assays. Determinations included erythrocyte, leukocyte, and platelet counts; hemoglobin concentrations; hematocrits; mean corpuscular volumes;

mean corpuscular hemoglobins; mean corpuscular hemoglobin concentrations; and serum concentrations of glucose, cholesterol, total protein, albumin, globulin, creatinine, urea nitrogen, total bilirubin, calcium, inorganic phosphorus, sodium, chloride, potassium, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, creatine kinase, and lactate dehydrogenase. If leukocyte counts exceeded 20,000 cells/ mL, differential leukocyte counts were conducted.

The tamarin with 12 ng 25(OH)D/ml serum (30 nmol/L) was a 4-year-old male with 32,900 leukocytes/mL blood. Colonic biopsies at 1 year of age revealed moderate chronic colitis. By 3 years of age, the colon was thickened sufficiently to be evident by abdominal palpation, and biopsies revealed severe colitis, which was still evident at 4 years of age. When blood was drawn for 25(OH)D assay, in addition to an elevated leukocyte count with a preponderance of neutrophils (88%), total protein (5.5 g/dL) and glucose (64 mg/dL) levels were low, possibly related to the anorexia and cachexia typical of acute exacerbation of colitis. This animal never regained vigor, lost weight and body condition, and was euthanized the following year.

The tamarin with 11 ng 25(OH)D/mL serum (28 nmol/L) was a 7-year-old female that had produced five litters with a total of eight living young. There was no clinical evidence of unthriftiness or illness despite histologic evidence of severe colitis in annual colonic biopsies. All hematologic and biochemical measures for this female and all other tamarins were within the limits previously seen in clinically healthy individuals within this colony, except for a 7-year-old male with 23,200 leukocytes/mL blood and a 12-year-old male with 25,200 leukocytes/mL blood. These individuals had 176 and 126 ng 25(OH)D/mL serum (440 and 315 nmol/L), respectively.

Flurer and Zucker [1987] concluded that serum 25(OH)D concentrations of 30–300 nmol/L were indicative of adequate vitamin D supplies for brown-headed or saddle-back tamarins (*Saguinus fuscicollis*). In their study, dietary concentrations of 2,000 IU of vitamin D3/kg dry matter were sufficient to support those levels. Although the present study was not designed to determine the vitamin D3 requirement of cotton-top tamarins, consumption of a diet containing 2,500 IU of vitamin D3/kg dry matter resulted in serum 25(OH)D levels (120–590 nmol/L) that were within or above the range proposed by Flurer and Zucker [1987] as adequate for this other tamarin species.

Whether colitis and possible infection by a *Helicobacter* species [Saunders et al., 1999] might influence dietary vitamin D requirements of cotton-top tamarins is not clear. Certainly, two of the lowest serum 25(OH)D levels were found in the Parke-Davis tamarins, but even with histologic evidence of moderate to severe colitis in all individuals, intakes of 26,000 IU of vitamin D3/kg dietary DM also produced some of the highest serum 25(OH)D concentrations. Genetic diversity was greater among the Parke-Davis tamarins than among those at Potter Park, but to suggest that greater variations in serum 25(OH)D values were due to greater genetic diversity would be speculative.

It is noteworthy that the analyzed values (as is basis) for vitamin D3 concentrations in Scenic Psittacine Diet (3,060 IU/kg) and ZuPreem Canned Marmoset Diet (11,760 IU/kg) were somewhat higher than the respective manufacturers' specifications (2,000 and 9,000). In contrast, the concentration of vitamin D3 determined by analysis (1,160 IU/kg) was much lower than specified for Mazuri Marmoset Jelly (30,000 IU/kg). Although only one sample of this product was analyzed, the close agreement between analyses of two samples of the Scenic Psittacine Diet (3,000 and

3,120 IU D3/kg) suggests that the assay procedure was repeatable. If the specified concentration of vitamin D3 had been present in the Mazuri Marmoset Jelly, vitamin D3 concentrations in the total daily diet would have been approximately 4,000 IU/kg dry matter instead of the 2,500 IU/kg dry matter actually found.

CONCLUSIONS

Power et al. [1997] found serum 25(OH)D concentrations of 25 - 120 ng/mL in wild cotton-top tamarins in Colombia and proposed that serum 25(OH)D concentrations of 50 - 120 ng/mL (125 - 300 nmol/L) could be considered normal. Assuming that the vitamin D assays of the feeds used in this study were correct and that serum 25(OH)D concentrations are appropriate indicators of vitamin D status, a diet containing approximately 2,500 IU D3/kg dry matter was more than sufficient to support serum 25(OH)D concentrations equivalent to those found in the wild. Although this study was not designed to establish the minimum vitamin D requirement of cotton-top tamarins, a dietary concentration of approximately 2,500 IU of vitamin D3/kg dry matter was sufficient to support apparently normal growth, adult weights equivalent to those previously reported [Silva and Downing, 1995], reproduction through five parities, and general health in a small captive colony showing no evidence of colitis.

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