Plasma 25-Hydroxyvitamin D in Growing Kittens Is Related to Dietary Intake of Cholecalciferol


James G. Morris,*2 Kay E. Earle† and Phillip A. Anderson‡
*Department of Molecular Biosciences, School of Veterinary Medicine, University of California, Davis, CA 95616, and †Waltham Centre for Pet Nutrition, Waltham-on-the-Wolds, Melton Mowbray, LE14 4RT, United Kingdom

ABSTRACT Vitamin D synthesis by growing kittens exposed to ultraviolet light is ineffective. Concentration of 25-hydroxyvitamin D (25-OHD) in plasma (the most useful index of vitamin D status) was measured in six groups each of seven kittens given a purified diet (12 g calcium and 8 g phosphorus/kg, calculated metabolizable energy = 20 kJ/g) that contained either 0.0, 3.125, 6.25, 12.5, 18.75 or 25 mg cholecalciferol/kg diet. All kittens received these diets from 9 to 22 wk of age, and the two groups given the 0.0 and 3.125 μg cholecalciferol/kg treatments continued to receive the diets until they were 34 wk old. Total and ionizable calcium and phosphorus in plasma were not affected by treatments. No adverse clinical changes were observed or found on radiographic examination of the kittens at 22 or 34 wk of age. Plasma concentration of 25-OHD was linearly related (r² = 0.99, P < 0.001) to dietary intake of cholecalciferol. Plasma concentration of 25-OHD in kittens given the diet without added vitamin D was significantly less at 22 wk than at 9 wk, whereas kittens receiving the diet containing 3.125 μg cholecalciferol/kg had significantly higher 25-OHD concentrations at 22 and 34 wk than at 9 wk of age. Kittens given the 6.25 μg cholecalciferol/kg diet had plasma 25-OHD concentrations at 22 wk > 50 nmol/L which is considered replete for humans. An allowance of 6.25 μg (250 IU) of cholecalciferol/kg diet is suggested to provide a margin of safety. J. Nutr. 129: 909–912, 1999.

KEY WORDS: • cholecalciferol • 25-hydroxyvitamin D • kitten • cats

Mammals, including humans, obtain most of their vitamin D from exposure to ultraviolet (UV) radiation (Holick 1994). Hazewinkel et al. (1987) reported that dogs exposed to UV light were not able to synthesize adequate vitamin D to prevent clinical signs of vitamin D deficiency. Morris (1999) also found that plasma concentration of 25-hydroxyvitamin D (25-OHD) of kittens given a vitamin D-free diet and exposed to direct summer sun in California for 15 h/wk declined at the same rate as kittens kept indoors, and they developed clinical signs of vitamin D deficiency. Similarly, no synthesis was observed under laboratory conditions where cats were exposed for 3 h/d to UV lights, and these cats also developed clinical signs of vitamin D deficiency.

How et al. (1994) did not find pre-vitamin D in isolated cat skin exposed to UVB light and attributed the lack of synthesis to a low concentration of 7-dehydrocholesterol (the precursor of pre-vitamin D) in the skin of cats. Morris (1999) demonstrated that vitamin D synthesis could be induced in vitamin D-deficient cats by administering an inhibitor of 7-dehydrocholesterol-Δ-reductase. The inhibitor produced a fivefold increase in skin concentration of 7-dehydrocholesterol, and the cats had increasing levels of 25-OHD in their plasma with time of exposure. Vitamin D-deficient cats exposed to UV light and not given the inhibitor had no increase in the concentration of 25-OHD in plasma.

Vitamin D deficiency in growing kittens was investigated by Gershoff et al. (1957), who gave three- to six-month-old kittens a purified vitamin D-free diet based on casein, corn oil and hydrogenated fat as sources of protein and fat. The diet was supplemented with most vitamins, but did not contain supplemental sources of taurine, vitamin E or arachidonate (or its precursor). These authors reported that the clinical signs of vitamin D deficiency could be prevented by oral administration of 6.25 μg (250 IU) of cholecalciferol twice weekly. A high incidence of deaths occurred in kittens given the diet with added vitamin D, so the requirement is questionable. Rivers et al. (1979) observed no signs of vitamin D deficiency in adult cats fed a vitamin D-free diet for over a year and suggested that the requirement of vitamin D for adult cats may be very low. Based on the experiment of Gershoff et al. (1957), the National Research Council (1986) proposed a minimal vitamin D requirement for growing kittens of 12.5 μg cholecalciferol (500 IU)/kg diet dry matter.

The objective of this study was to determine the dietary concentrations of cholecalciferol that maintained plasma 25-OHD at a concentration normal for other mammals. An additional aim of the study was to avoid overt clinical signs of vitamin D deficiency and the possible animal discomfort associated with it.

MATERIALS AND METHODS

Animals and housing. Kittens used in the study were produced from British domestic shorthair queens given an expanded (dry) diet containing only the vitamin D derived from the natural ingredients. The diet was given to the queens before mating, during gestation (approximately 64 d) and lactation. This procedure was followed to reduce maternal transfer of vitamin D, so vitamin D status of the kittens entering the study would not be high. Kittens were given a purified diet without supplemented vitamin D at 3 wk of age to accustom them to a purified diet. Weaning began at 7 wk of age, and
the kittens continued to receive the vitamin D-free diet until 9 wk of age when they were randomly assigned to the experimental diets containing the graded levels of vitamin D. After weaning, kittens were housed in individual enclosures (1.15 m x 0.60 m x 0.55 m) with food and water available at all times. The temperature and light/dark cycle in the room was 21 ± 2°C and a 12/12 h, respectively. The experimental protocol adhered to the Guide for the Care and Use of Laboratory Animals (National Research Council 1985).

**Diet.** A purified vitamin D-free diet was prepared from the ingredients given in Table 1. Because the diet was devoid of animal fat and because the synthesis of arachidonate by cats is limited, evening primrose oil was added as a source of γ-linoleic acid. The basal diet was formulated to provide all essential nutrients (other than vitamin D) at levels in excess of those recommended by the National Research Council (1986) and was confirmed by analysis. Concentration of calcium and phosphorus on analysis was 12 and 8 g/kg diet dry matter, respectively. The basal diet was analyzed for vitamin D by a method adapted to pet foods (Morris 1999). To the basal diet, 0.0, 3.125, 6.25, 12.5, 18.75 or 25 μg/kg of cholecalciferol (Sigma Reference Standard; Sigma Chemical Co., St. Louis, MO) in sunflower oil was added. The added cholecalciferol was equivalent to 0, 125, 250, 500, 750 or 1000 IU of cholecalciferol/kg.

**Experimental design.** As kittens were weaned, they were assigned to the dietary treatments on the basis of sex, such that each treatment contained four female and three male kittens. All kittens received the diets containing various levels of vitamin D until they were 22 wk of age. The two groups of kittens assigned to the 0.0 and 3.125 μg/kg diets continued these dietary treatments until 34 wk of age. Samples of blood were taken in heparinized syringes from unanesthetized kittens at 7, 9, 10, 14, 18 and 22 wk of age, and also from the kittens in the 0.0 and 3.125 μg groups at 26, 30 and 34 wk of age. The following measurements were made on the samples collected at wk 9 and 20: total calcium and phosphorus (Coulter CPA Analyzer; Coulter Electronics, Luton, Beds) and ionized potassium and ionized calcium by a Ciba-Corning 228 Analyzer (Ciba-Corning Medfield, MA); at 8 and 20 wk of age: hemoglobin, packed-cell volume, red and white cell numbers (Seronox-Baker Diagnostics System Analyzer 9000; Allentown, PA), total plasma protein, albumin, urea and cholesterol concentrations, and alanine and aspartic aminotransferase activity (Coulter CPA Analyzer). At wk 12 and 20, plasma taurine concentration was measured by an amino acid analyzer. The concentration of 25-OHD in the plasma of kittens was measured by a protein-binding assay (Chen et al. 1990) when the kittens were 7, 10, 14, 18 and 22 wk of age and at 34 wk of age for kittens in the 0.0 and 3.125 μg treatments. Body weight was measured weekly and food intake recorded daily.

Radiographs were taken of the forelimbs of all kittens at 22 wk of age and again at 34 wk of age for the kittens in the 0.0 and 3.125 μg/kg treatment groups and were read by a Board Certified Veterinary radiologist who was not aware of the treatments. Observations were subjected to a three-way analysis of variance using a general linear model, and P < 0.05 was taken as significant. Pair-wise multiple comparisons were made by the Student-Newman-Keuls method. Values are means ± SEM.

**RESULTS**

No cholecalciferol or ergocalciferol was detected in the basal diet, and duplicate samples of the diet containing 25 μg cholecalciferol/kg gave recoveries of 1.03 and 1.05 times the theoretical concentration. A veterinarian unaware of the allocation of the treatments examined the kittens at regular intervals and found no clinical signs compatible with vitamin D deficiency. Body weight of the 42 kittens at 8 wk was 0.750 ± 0.034 kg. Neither body weight gain nor body weight at 22 wk was significantly affected by dietary treatments. However, there was a significant (P < 0.003) sex effect on the 22 wk body weight (females, 2.00 ± 0.06 kg; males, 2.28 ± 0.07 kg) but no sex × vitamin D interaction. The body weights of the two groups given the 0.0 and 3.125 μg/kg cholecalciferol diets at 34 wk were 2.76 ± 0.10 and 3.05 ± 0.10 kg, respectively, and were not significantly different. Again there was a significant sex effect (P < 0.001), but no significant interaction of sex and dietary treatments. Similarly, no significant differences in food intake or energy intake among groups were apparent (results not presented).

Neither plasma total nor ionizable calcium was significantly affected by dietary treatments, sex or age. Plasma total and ionizable calcium at 9 and at 20 wk of age were 2.82 ± 0.23 and 2.79 ± 0.04 mmol/L and 1.33 ± 0.012 and 1.30 ± 0.012 mmol/L, respectively. Plasma inorganic phosphorus concentration was not significantly affected by treatments, or sex at 95 and 20 wk of age (2.09 ± 0.10 mmol/L). Similarly, ionized potassium, hemoglobin, packed-cell volume, red and white cell numbers, plasma total protein, albumin, urea and taurine were not affected by treatments. Alkaline phosphatase was not significantly different due to treatments, sex or age (8 and 20 wk values were 174 ± 8.9 and 266 ± 12.0 units/L, respectively), and the activities of alanine and aspartic aminotransferases were also not significantly affected by these variables. Radiographs of the kittens at 22 wk of age, examined by a radiologist, did not reveal any treatment differences in width of the growth plate.

Concentration of 25-OHD in the plasma of kittens at 7 wk of age was 20 ± 1.9 nmol/L, indicating that their reserves were depleted. There was no significant difference in plasma concentration of 25-OHD before dietary treatments were applied

---

**TABLE 1**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>g/kg diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolated soy protein¹</td>
<td>171.0</td>
</tr>
<tr>
<td>Vitamin-free casein²</td>
<td>141.0</td>
</tr>
<tr>
<td>Lactalbumin²</td>
<td>133.0</td>
</tr>
<tr>
<td>Rape seed oil³</td>
<td>200.0</td>
</tr>
<tr>
<td>Sunflower oil⁴ (carrier for vitamin D)</td>
<td>95.0</td>
</tr>
<tr>
<td>Evening primrose oil⁵</td>
<td>5.0</td>
</tr>
<tr>
<td>Sucrose</td>
<td>100.0</td>
</tr>
<tr>
<td>Starch (wheat)</td>
<td>40.5</td>
</tr>
<tr>
<td>Choline chloride⁶</td>
<td>3.0</td>
</tr>
<tr>
<td>Taurine⁵</td>
<td>1.5</td>
</tr>
<tr>
<td>Mineral mixture⁶</td>
<td>100.0</td>
</tr>
<tr>
<td>Vitamin mixture⁸,⁹ (without vitamin D)</td>
<td>10.0</td>
</tr>
</tbody>
</table>

¹ Ardex The British Arkady Co Ltd., Old Trafford, Manchester, M16 ONJ, UK.  
² Cambridge Biosciences, UK.  
³ Pura Foods, Orchard Place, London 14QUH, UK.  
⁴ Flora, Van Den Burgh Foods Ltd. Claylands Workshops, Notts, UK.  
⁵ Anglia Oils Ltd., King George Dock, Kingston-upon-Hull, Yorks, UK (to supply γ-linoleic acid).  
⁶ BDH Laboratory Supplies, Poole, Dorset, UK.  
⁷ Provided (g/kg diet): calcium phosphate dibasic (anhyd.) 39.0; potassium phosphate dibasic (anhyd.) 9.0; calcium carbonate 11.0; magnesium sulfate (anhyd.) 4.8; calcium chloride 10.0; potassium bicarbonate 10.0; sodium bicarbonate 14.0; (mg/kg diet) zinc sulfate · 7 H₂O 445 manganese sulfate · 5H₂O 384; copper sulfate · 3H₂O 80; ferric citrate 1000; potassium iodide 3.5; stannous chloride · 2H₂O 100; sodium selenate 30; ammonium molybdate · 4H₂O 4.0; chromic chloride · 6H₂O 26; nickel chloride · 6H₂O 30; sodium fluoride 14; ammonium vanadate · 4H₂O 2.0.  
⁸ Roche Products Ltd., H Eleanor Gate, H Eleanor, Derbys, UK.  
⁹ Provided (mg/kg diet): retinyl acetate 6.88; DL-α-tocopherol acetate 160; menadione sodium bisulfite complex 15; thiamin hydrochloride 25; riboflavin 10; pyridoxine · HCl 10; nicotinic acid 100; calcium pantothenate 20; myo-inositol 200; pteroylmonoglutamic acid 10; cyanocobalamin 50 μg; d-biotin 1.0; ascorbyl phosphate, 400 (as a preservative).
The absence of clinical signs of vitamin D deficiency in the kittens given the diet containing no added cholecalciferol could mean that kittens do not require vitamin D, that body reserves were sufficient until the kittens were 34 wk of age or that the basal diet contained an adequate level of the vitamin. We reject these possibilities for the following reasons. Gershoff et al. (1957) reported that "rickets produced (in the kittens) on the 1:1 ratio (Ca/P) was more severe than that on the 2.05 ratio," and all of the six kittens given the vitamin D-deficient diet with the 2.06 Ca/P ratio survived to 21 mo, whereas only one of seven kittens given the 1:1 ratio diet was alive at 21 mo.

The rectilinear relationship we observed between plasma 25-OHD, and dietary intake of vitamin D was of the same form as reported by Holick and Clark (1978) for vitamin D-deficient rats given various oral doses of cholecalciferol. The response of 25-OHD in rats to oral cholecalciferol extends well into the pharmacological range (Holick 1989). Therefore, there is no plateau value of 25-OHD that can be used to assess adequacy of this vitamin. But, because the relationship between the concentration of vitamin D in the diet and the 25-OHD concentration in plasma is very close, circulating levels of 25-OHD could be used as a bioassay for the available vitamin D in foods. It appears that cats, like other animals, have a large reserve activity of vitamin D-25-hydroxylase in liver.

The lack of an elevation in alkaline phosphatase in kittens given the 0.0 μg cholecalciferol/kg diet is consistent with observations of kittens exhibiting frank clinical signs of vitamin D deficiency (Morris, unpublished data). Alkaline phosphatase in kittens does not appear to be a sensitive indicator of vitamin D deficiency, especially in the early stages of the disease when the diet is well supplied with calcium. In humans, alkaline phosphatase increases in active rickets, and Gershoff et al. (1957) suggested on limited data that alkaline phosphatase may be the most sensitive chemical means (then available) for evaluating the state of rickets in cats.

Definition of a requirement for an essential nutrient requires that some physiological function be selected which is
optimized or maximized, or some metabolic variable, such as circulating concentration of a nutrient, be maintained at a certain level (Morris and Rogers 1994). For the vitamin D requirements of kittens, we suggest that the circulating level of 25-OHD could be used to estimate a requirement. The concentration of 25-OHD in plasma is more stable than the concentration of either vitamin D or calcitriol. The half-life of vitamin D in the plasma of humans is about 24 h, that of 25-OHD about 3 wk and that of calcitriol 4 to 6 h (Holick 1990). Because the concentration of 25-OHD increased in the group given the 3.125 µg/kg diet and significantly decreased in the group given 0.0 µg cholecalciferol/kg diet, it could be inferred that 3.125 µg cholecalciferol/kg diet may be adequate for kittens. However, the concentration in the plasma of three kittens in the 3.125 µg/kg group was less than 10 ng/mL (25 nmol/L) which has been suggested by Holick (1990) as indicating impending or frank vitamin D deficiency in humans. In contrast, the concentration of 25-OHD in the plasma of all kittens in the group receiving the diet containing 6.25 µg cholecalciferol/kg at 22 wk of age was in excess of 20 ng/mL (50 nmol/L) which is regarded as normal for humans. Therefore a conservative estimate of requirement including a margin of safety is 6.25 µg of cholecalciferol/kg diet with a metabolizable energy value of 20 kJ/g. This value is half that of the National Research Council (1986) recommendation.

ACKNOWLEDGMENT

The rape seed oil was a gift from Pura Foods, Orchard Place, London, 140JH England.

LITERATURE CITED


National Research Council (1985) Guide for the Care and Use of Laboratory Animals, Publication no. 85–23 (rev.), National Institutes of Health, Bethesda, MD.

