Ineffective Vitamin D Synthesis in Cats Is Reversed by an Inhibitor of 7-Dehydrocholesterol-Δ⁷-Reductase¹,²

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ABSTRACT Changes in plasma 25-hydroxyvitamin D (25-OHD) were used as an index of vitamin D status of cats. Plasma 25-OHD concentration of kittens given a purified vitamin D-free diet and exposed to direct summer sun for 15 h/wk declined at a similar rate as kittens given the same diet kept indoors. Similarly, plasma 25-OHD of kittens exposed to ultraviolet (UV) lamps declined at a similar rate as kittens not exposed, and these kittens developed clinical signs of vitamin D deficiency. Eight weaned kittens were given the vitamin D-free purified diet until their plasma concentrations of 25-OHD were < 5 nmol/L. They then had the hair on their backs clipped at weekly intervals and were paired on the basis of skin color and exposed to UV light for 2 h/d. One member of each pair was given an inhibitor of 7-dehydrocholesterol (5,7-cholestradien-3β-ol)-Δ⁷-reductase (EC 1.3.1.21) in the diet. Cats receiving the inhibitor had a progressive increase in 25-OHD concentration of plasma with time to 91 ± 22 nmol/L (mean ± SEM), whereas cats not receiving the inhibitor had plasma 25-OHD concentrations that were not detectable (P < 0.001). Biopsy samples of skin from cats receiving the inhibitor had more than five times the concentration of 7-dehydrocholesterol (P < 0.001) than the skin of control cats. Low concentration of 7-dehydrocholesterol (presumably due to high activity of the reductase) in the skin of cats is the major impediment to effective vitamin D synthesis. Analysis of wild caught potential prey of cats indicated that these animals could supply adequate vitamin D to meet the requirement of growing kittens. J. Nutr. 129: 903–908, 1999.

KEY WORDS: • cholecalciferol • 7-dehydrocholesterol-Δ⁷-reductase • vitamin D synthesis • Cats

Vitamin D is a conditional nutrient for most mammals, as it can be synthesized when skin is exposed to ultraviolet (UV) light. The extent of synthesis in humans depends on season and latitude (Webb et al. 1988), the extent and time of skin exposure (Holick 1989) and skin pigmentation (Clemens et al. 1982). Gershoff et al. (1957) reported vitamin D deficiency in 3- to 6-mo-old kittens given a purified vitamin D-free diet and showed that clinical signs of deficiency could be prevented by oral administration of 6.25 μg of cholecalciferol twice weekly. However, a high incidence of deaths occurred in kittens given the purified diet supplemented with vitamin D, so this estimate of the requirement is open to question. As Gershoff suggested a relatively low requirement for vitamin D in cats, it was subsequently assumed that “the cat kept in a well-lighted room is almost independent of dietary sources of this vitamin: (and) probably vitamin D is synthesized in the cat’s skin and ingested while it is grooming its coat” (Scott and Scott 1967). 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. In 1986 the National Research Council on the basis of these two experiments proposed a minimal vitamin D requirement for growing kittens of 12.5 μg (500 IU)/kg diet dry matter.

Hazewinkel et al. (1987) demonstrated that vitamin D synthesis was defective in dogs that had their backs shaved when exposed to UV light, and these dogs developed clinical signs of vitamin D deficiency. Subsequently, How et al. (1994) reported that they were unable to demonstrate the presence of pre-vitamin D in isolated cat skin exposed to UV light.

The objective of this series of experiments was to determine if cats could effectively synthesize vitamin D in their skin on exposure to UV light, and if synthesis was not effective, to determine the impediment to synthesis. Plasma concentration of 25-hydroxyvitamin D (25-OHD) was used to assess the vitamin D status of kittens. Holick (1990) recommended that plasma concentration of 25-OHD was the preferred index of vitamin D status. Plasma 25-OHD is more stable than that of either vitamin D or calcitriol, as the half-life of 25-OHD in the plasma of humans is about 3 wk. In contrast, the half-life of vitamin D is about 24 h and calcitriol 4 to 6 h (Holick 1990).

MATERIALS AND METHODS

Animals and diets. Specific-pathogen-free cats or kittens from the Feline Nutrition and Pet Care Center, University of California-Davis, Davis, CA, were used in all experiments. Kittens with the...
exception of those exposed to sunlight were housed in temperature-
controlled rooms (20 ± 2°C), and food and water were available at all
times unless stated. A purified diet (Table 1) was used in all
experiments. This diet contained major nutrient and vitamin con-
tents that require extensive clean up preparatory to chromatogra-
phy (HPLC) procedures of Holick et al. (1992). Foods,
purified by HPLC chromatography was used to determine recovery
samples were analyzed by a modification of the high-performance liquid
chromatography (HPLC) procedures of Holick et al. (1992). Foods,
especially those containing animal tissue, contain interfering sub-
stances that require extensive clean up preparatory to chromatogra-
phy. Either 14C- or 1H-labeled cholecalciferol tracer that had been
purified by HPLC chromatography was used to determine recovery
rates.
Sample preparation for HPLC. A sample of food or diet (−10 g of
dry matter) was weighed and combined with the tracer (−500 Bq),
3 g of ascorbic acid, 90 mL of ethanol and 10 mL of 800 g/L KOH
in water in a round-bottom flask with a stirring bar and refluxed
over a hot plate-stirrer for 1 h. After cooling, the digest was
filtered in a 11-cm Buchner funnel with a coarse (Whatman No. 1)
filter paper under partial vacuum. The residue on the filter paper
was washed with 20 mL of ethanol then 30 mL of hexane, the
combined filtrate was transferred to a 500-mL separating funnel,
and 80 mL of water was added. After tumbling (to prevent for-
motion of an emulsion) the funnel about 100 times, the organic
layer was allowed to separate and drained into a clean separating
funnel. The filtrate was extracted two more times with 50 mL
aliquots of hexane, the three extracts were pooled and 0.1 mL of
butylated hydroxy toluene solution (10 g/L hexane) was added.
The combined hexane extracts were washed three or more times
with 100 mL of water or until the water was neutral to phenol-
phthalein. The hexane extract was then dried over 10 g of anhy-
drous sodium sulfate, transferred to a 250-mL round-bottom flask
and reduced to about 5 mL in a rotary evaporator. The solution
was then transferred along with washings to a 15-mL Teflon-lined
scREW-capped test tube and dried under nitrogen. The residue was
taken up in 1 mL of 0.4% isopropanol in hexane and placed on a
prepared silica Sep-Pak Vac RC 500-mg cartridge (Waters part
# 51900). The column was washed with 10 mL of 0.4% isopropa-
nol in hexane which was discarded to waste and then eluted with
a further 10 mL of 0.4% isopropanol in hexane, which was col-
clected and dried under nitrogen. The dry residue was then dis-
solved in 1 mL of methanol and transferred to a Bond Elut LR
500-mg cartridge (Varian Associates Inc., Harbor City, CA) that
had been previously prepared by sequential washings of 5 mL of
hexane, 5 mL of methanol and 5 mL of water. The C-18 column
was sequentially eluted with 5 mL of water, 15 mL of a mixture of
methanol, tetrahydrofuran and water (1:1:2 parts), 10 mL of a
mixture of 70% methanol 30% water and 5 mL of acetonitrile (all
to waste). The column was then eluted with 5 mL of methanol,
which was collected and dried under nitrogen. A HPLC separation.
Two HPLC separations were used, a straight-phase separation to isolate ergocalciferol and cholecalciferol in
a single peak, followed by a reversed-phase separation to isolate ergocalciferol from cholecalciferol. The straight-phase separation used a silica column (Econosil 5 at 250 × 4.6 mm; Alltech, San Jose, CA) with isocratic 0.6% isopropanol in hexane mobile phase at a flow rate of
2 mL/min and the UV detector set to 265 nm and AUFS of 0.02.
The reversed-phase separation used a C-18 column (Vydac C-18, 5
μm; The Separations Group, Hesperia, CA) with an isocratic mobile phase of methanol/acetonitrile (25:75) at a flow rate of
1 mL/min and the UV detector set at 265 nm. Dried samples were
taken up in the mobile phase before chromatography. Recoveries of
the isotope were generally >0.6. Corrected recoveries of diet samples containing 25 μg of cholecalciferol/kg were 1.03 and 1.05 times the theoretical values.
Measurement of 7-dehydrocholesterol (7-DHC) in skin. Samples of skin after removal of hair and subcutaneous tissue were
weighed (and/or the area measured). They were saponified as for food
samples (except that only 5 mL instead of 10 mL 100 gL of KOH was
used, and pyrogallol replaced ascorbic acid), and the same procedure
followed up to the drying after rotary evaporation. The dried samples
were taken up in the mobile phase of 0.6% isopropanol in hexane,
and no clean up procedures were used before the samples were
chromatographed. An Econosil silica column using an isocratic mobile
phase of 0.6% isopropanol in hexane at flow rate of 1 mL/min and
detector settings of 271 nm and AUFS (absorbance units full scale) of
0.05 was used. Mean ± so recovery of 7-DHC added in place of skin
was 0.89 ± 0.07 which is comparable to that reported by Takada et
al. (1981).
25-Hydroxyvitamin D (25-OHD). Plasma concentrations of 25-
hydroxyvitamin D was measured by the protein-binding assay de-
scribed by Chen et al. (1990), using either rat or cat binding protein.
This assay does not distinguish between 25-hydroxyergocalciferol and 25-hydroxycholecalciferol.
Experimental procedures. Four experiments were conducted to
investigate vitamin D synthesis in cats.
(i) Experiment 1. In this experiment, the ability of kittens to
synthesize vitamin D when exposed to natural sunlight was tested.

### Table 1
Composition of the basal diet

<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>Lactic casein²</td>
<td>141.0</td>
</tr>
<tr>
<td>Lactalbumin²</td>
<td>133.0</td>
</tr>
<tr>
<td>Partially hydrogenated vegetable oil³</td>
<td>295.0</td>
</tr>
<tr>
<td>Borage oil⁴</td>
<td>5.0</td>
</tr>
<tr>
<td>Sucrose⁵</td>
<td>100.0</td>
</tr>
<tr>
<td>Starch (corn)⁶</td>
<td>90.5</td>
</tr>
<tr>
<td>Choline chloride⁷</td>
<td>3.0</td>
</tr>
<tr>
<td>Taurine⁸</td>
<td>1.5</td>
</tr>
<tr>
<td>Mineral mixture⁹</td>
<td>50.0</td>
</tr>
<tr>
<td>Vitamin mixture (without vitamin D)¹⁰</td>
<td>10.0</td>
</tr>
</tbody>
</table>

1) SPI Group, San Leandro, CA.
2) New Zealand Milk products.
3) HUNT Wesson Inc., Fullerton, CA.
4) Traco Labs, Seymour, IL (contained 23% γ-linolenic acid).
5) Holly Sugar Corp., Colorado Springs, CO.
6) National Starch and Chemical Corp., Bridgewater, NJ.
7) DuPont, Highland Park, IL.
8) Taiho Pharm Co., Torrence, CA.
9) Provided (g/kg diet): calcium phosphate dibasic (anhyd.) 19.50; po-
tassium phosphate dibasic (anhyd.) 4.50; calcium carbonate 5.50; potas-
sium chloride 5.00; potassium bicarbonate 5.00; sodium bicarbonate 7.00;
magnesium sulfate (anhyd.) 2.25; (mg/kg diet) zinc sulfate 7.1; O 222.5;
manganese sulfate H₂O 192; copper sulfate 5H₂O 40; ferric citrate 500;
potassium iodide 1.75; stannous chloride 2H₂O 5; sodium selenate 1.5;
ammonium molybdate 4H₂O 2; chromic chloride 6H₂O 13; nickel chloride 6H₂O 15; sodium fluoride 7; ammonium vanadate 4H₂O 1.0.
10) Provided (mg/kg diet): retinyl acetate 6.88; α-L-α-tocopherol acetate 1330; menadione sodium bisulfite complex 15; thiamin hydrochlo-
ride 25; riboflavin 10; pyridoxine.HCI 10; nicotinic acid 100; calcium pantothenate 20; myo-inositol 200; pteroylmethoglutamic acid 10; cy-
anocobalamin 50 μg; β-biotin 1.0; ascorbyl phosphate, 400 (as a pre-
servative).
Nine newly weaned kittens were given the vitamin D-free diet and divided into two groups of four (control) and five (exposed) kittens. The control group was kept indoors and received no exposure to UV light. The exposed group was placed in a wire cage without shade and received full summer (June-July) sun at Davis, CA (lat 38°N, long 121°W) from 0900 to 1200 h (summer time) for 5 d/wk. Exposed kittens had water, but no food was available in this period, and were under continual surveillance for hyperthermia. One kitten in the exposed group had the hair clipped on its back to increase the area of skin exposed. All kittens were housed in the same cage except when one group was exposed to sunlight.

(ii) Experiment 2. Four weaned male kittens were given the vitamin D-free diet and when their body weight was 1.23 ± 0.014 kg (mean ± SEM), they were randomly allocated to a control group of two kittens (no UV exposure) and a group that received 3 h/d exposure to UV lights (FS40 or FS60 fluorescent sun lamps supplied by Universal Light Source, San Francisco, CA) for 173 d. The efficacy of the lamps was confirmed by exposing vials containing alcoholic solution of 7-dehydrocholesterol and by measuring the formation of pre-vitamin D. Plasma concentration of 25-OHD was measured at intervals during the subsequent 173 d.

(iii) Experiment 3. Ten male kittens were given the vitamin D-free diet at weaning and continued to receive the diet until their plasma concentration of 25-OHD was less than 5 nmol/L. Four pairs of cats were selected based on skin color and divided into two groups. One group of cats received the purified diet alone, while the other group of cats received the purified diet to which 250 mg of BM 15.766 (4-[2-[1-(4-chlorocinnamyl)piperizin-4-yl]ethyl]benzoic acid) Boehringer Mannheim GmbH Mannheim, Germany) was added/kg diet. The compound BM 15.766 is an inhibitor of 7-dehydrocholesterol-d7-reductase (EC 1.3.3.21) that catalyzes the conversion of 7-dehydrocholesterol to cholesterol. All eight cats had the hair clipped from the scapula to the tuber coxae and about 5 cm down the mid-side skin biopsy was taken and the concentration of 7-DHC in their skin compared to other species is presented in Fig. 3.

(iv) Experiment 4. Ten male kittens were given the vitamin D-free purified diet at weaning and continued to receive the diet until their plasma concentration of 25-OHD was less than 5 nmol/L. Four pairs of cats were selected based on skin color and divided into two groups. One group of cats received the purified diet alone, while the other group of cats received the purified diet to which 250 mg of BM 15.766 (4-[2-[1-(4-chlorocinnamyl)piperizin-4-yl]ethyl]benzoic acid) Boehringer Mannheim GmbH Mannheim, Germany) was added/kg diet. The compound BM 15.766 is an inhibitor of 7-dehydrocholesterol-d7-reductase (EC 1.3.3.21) that catalyzes the conversion of 7-dehydrocholesterol to cholesterol. All eight cats had the hair clipped from the scapula to the tuber coxae and about 5 cm down the lateral surface at weekly intervals. Pairs of cats were housed in adjacent metabolism cages (61W × 61D × 90H cm) so they were exposed to the same pair of fluorescent UV lamps suspended immediately above their cages for 9 wk. During UV exposure, food but not water was removed from the cages of all cats. Samples of blood were taken at approximately weekly intervals and the 25-OHD concentration measured. At the end of the 9-wk experimental period, a mid-side skin biopsy was taken and the concentration of 7-DHC in the skin was measured.

Analysis of potential prey of feral cats. To determine if potential prey animals could supply the cat’s dietary requirement for vitamin D, seven wild caught roof rats (Rattus rattus L); three mice (Mus musculus) and two birds were analyzed. In addition, four laboratory-raised rats were analyzed for vitamin D content.

Statistical analysis. A paired t-test was used to compare treatments in experiment 4. All values are mean ± SEM and a value of P < 0.05 was considered significant.

RESULTS

No vitamin D (ergocalciferol or cholecalciferol) was found in the basal diet. The 25-OHD concentration in the plasma of control kittens was maintained at undetectable levels by d 87 in both groups and remained so until 173 d, when all kittens were showing clinical signs of vitamin D deficiency. These results support the observations that cats exposed to sunlight have ineffective vitamin D synthesis. As the duration of UV exposure caused an initial erythematous reaction in the kittens and produced thickening of exposed skin over the course of exposure, it appeared unlikely that greater UV exposure would have elicited production of vitamin D.

The concentration of total 7-DHC in the skin of cats and other species is presented in Fig. 2. Cats had very low concentrations of 7-DHC in their skin compared to other species known to synthesize vitamin D. Therefore, in the following experiment, a low concentration of 7-DHC was examined as a cause of ineffective vitamin D synthesis in cats.

In experiment 4, both groups had similar initial concentrations of 25-OHD (Fig. 3). However, after 1 wk delay, cats receiving the inhibitor of 7-DHC reductase in the diet and exposure to UV light had a steady increase in concentration of 25-OHD in plasma, attaining levels that would be regarded as normal in other species. However, cats not receiving the inhibitor in the diet and exposed to UV light had no increase in plasma 25-OHD concentration. Cats receiving the inhibitor had concentrations of 7-DHC in the skin that were five times that of cats in the control group (P < 0.001) (Table 2), indicating the inhibitor reduced the
conversion of 7-DHC to cholesterol, permitting effective vitamin D synthesis.

The analysis of potential prey animals of cats is presented in Table 3. Considerable variation in whole-body vitamin D occurred among individuals, especially among the roof rats. Laboratory rats that had been fed a commercial nonpurified diet had higher concentrations than wild roof rats, which may reflect a dietary rather than a breed difference. They also had less variation among individuals, which tends to support diet as the primary determinant of individual vitamin D content. The higher concentration of cholecalciferol in deer mice than roof rats trapped at the same location was not anticipated, but could be due to different feeding patterns of the two species.

**DISCUSSION**

Vitamin D synthesis by cats was ineffective as exposure of kittens neither to sunlight for 15 h/wk nor to UV lights for 2 h/d (experiments 1 and 2) decreased the rate of decline in plasma 25-OHD, nor prevented the appearance of clinical signs of vitamin D deficiency (results not reported here). Similarly, in experiment 4, depleted cats exposed to UV light for 2 h/d did not have an increase in plasma 25-OHD. The lack of effective vitamin D synthesis did not appear to be the result of limited skin exposure due to the cover of pelage, as the kitten clipped in experiment 1 had a similar rate of decline in plasma 25-OHD as the other kittens. In addition, control cats in experiment 4 with the hair clipped so the skin was directly exposed to UV light did not have a rise in 25-OHD concentration in plasma. The availability of 7-DHC in skin appears to be the primary impediment to effective synthesis, as increasing the concentration of 7-DHC in skin in experiment 4 resulted in effective vitamin D synthesis.

De novo sterol synthesis occurs in all animal tissues that have been investigated, but the relative rates in individual tissues vary greatly among species. In rats, monkeys and humans, the liver and intestines appear to have the highest rates, but in pigs the intestine contributes only a minor amount while the liver and adipose tissue account for 67 and 29%, respectively (Nes and McKean 1977). There do not appear to have been any studies on the pathway of cholesterol synthesis in the skin of cats, but it would be reasonable to suggest that the metabolic pathway first proposed by Kandutsch and Russell as the primary determinant of individual vitamin D content.

### TABLE 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>nmol/cm²</th>
<th>nmol/g</th>
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<tbody>
<tr>
<td>Control</td>
<td>0.57 ± 0.05</td>
<td>4.55 ± 0.44</td>
</tr>
<tr>
<td>Inhibitor</td>
<td>3.17 ± 0.34</td>
<td>22.85 ± 2.39*</td>
</tr>
</tbody>
</table>

*Significantly different from control, P < 0.001.

### TABLE 3

<table>
<thead>
<tr>
<th>Animal</th>
<th>Cholecalcifer (nmol/kg dry matter)</th>
</tr>
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<tbody>
<tr>
<td>Mammals</td>
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</tr>
<tr>
<td>Roof rats (Rattus rattus L.)</td>
<td>9.88 ± 2.6</td>
</tr>
<tr>
<td>Deer mice (Mus musculus)</td>
<td>52 ± 6.5</td>
</tr>
<tr>
<td>Rats (laboratory-raised)</td>
<td>78 ± 9.9</td>
</tr>
<tr>
<td>Birds</td>
<td></td>
</tr>
<tr>
<td>Pine siskin (Carduelis pinus)</td>
<td>39</td>
</tr>
<tr>
<td>Scrub jay (Aphelocoma coerulescens)</td>
<td>13</td>
</tr>
</tbody>
</table>

*1 Values are means ± SEM (n = 4).
*2 1 nmol/kg is equivalent to 15.38 IU/kg.
(1960) from lanosterol to cholesterol would be the same in cats as in other mammals.

While the inhibitor of 7-DHC reductase increased the concentration of total 7-DHC in the skin fivefold and permitted vitamin D synthesis to occur, some synthesis would be expected to have occurred from the endogenous 7-DHC present in the untreated cats. An increase of one-fifth of the final plasma 25-OHD concentration in the treated cats (91 nmol of 25-OHD/L) would have been detected in the control cats. By saponification, we measured the total 7-DHC, both in the free and esterified forms. In rat skin, most (78–93%) of the 7-DHC is in the esterified form (Takada et al. 1981). Possibly, the 7-DHC in the untreated cats was in a conformational state that did not permit it to be converted to pre-vitamin D, or the pre-vitamin D formed was not free to be transported to the liver for hydroxylation to 25-OHD.

The absence of vitamin D from the diet of cats results in clinical signs of deficiency, but the time before signs appear is related to both the initial stores of vitamin D and the calcium level in the vitamin D-free diet. In experiments not reported here, kittens from queens receiving a diet containing 400 μg of cholecalciferol/kg during pregnancy and lactation were given a vitamin D-free diet. These kittens completed their growth phase (>26 wk of age) without clinical signs of deficiency. Similarly, when weaned kittens were given a vitamin D-free diet containing 14 g of Ca/kg dry matter, clinical signs were either not expressed or delayed due to nonvitamin D-mediated calcium absorption (Lee et al. 1991).

Cats before domestication must have derived sufficient vitamin D from their natural food to meet their needs. To assess the possibility that diet could have supplied sufficient vitamin D, it is necessary to have an estimate of the vitamin D requirement of cats. Earle et al. (1994) and Morris et al. (1999) reported that kittens given purified diets containing 3.125 μg of cholecalciferol/kg dry matter did not show clinical signs of vitamin D deficiency when the diet had a high (12 g/kg of dry matter) calcium concentration, but the plasma concentrations of 25-OHD were below those considered normal for other mammals. However, a diet with 6.25 μg of cholecalciferol/kg resulted in plasma 25-OHD in excess of 50 nmol/L (20ng/mL), which is regarded as adequate in humans (Holick 1990). If this value is taken as adequate in cats, and the wild trapped animals in Table 3 are representative of the diet of pre-domesticated cats, the diet alone could supply sufficient vitamin D. For cats consuming this diet of whole animals, the ability to synthesize vitamin D is redundant. Ineffective synthesis of vitamin D is the most recent example of the many nutritional peculiarities of cats. This metabolic change has presumably occurred over the 35 million years following the divergence of felds from the general fissipid progenitors and has occurred in response to an all-animal tissue diet. As administration of the inhibitor of 7-DHC reductase allowed vitamin D synthesis to occur, it suggests that the activity of the 7-DHC reductase is high, not that cholesterol synthesis is limited. To our knowledge, no examples of effective vitamin D synthesis in cats is due to high activity of the 7-DHC reductase enzyme.

ACKNOWLEDGMENTS

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LITERATURE CITED


