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EXTREMELY HIGH CIRCULATING LEVELS OF 1α,25-DIHYDROXYVITAMIN D_3 IN THE MARMOSET, A NEW WORLD MONKEY

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SUMMARY: Compared to most mammals, the marmoset, a new world monkey, requires particularly large amounts of vitamin D to maintain normal growth. We compared serum concentrations of vitamin D metabolites in marmosets with rhesus monkeys and humans. The circulating levels of 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$] in marmosets were 4 to 10 times higher than those in rhesus monkeys and humans. But none of the marmosets exhibited hypercalcemia. In two marmosets which had suffered bone fractures, the 1α,25-$(OH)_2D_3$ levels were particularly elevated. These results suggest that the marmoset has an end-organ resistance to 1α,25$(OH)_2D_3$.

Vitamin D_3 is metabolized first in the liver to 25-hydroxyvitamin D_3 [25$(OH)D_3$] and then in the kidney mainly to 24,25-dihydroxyvitamin D_3 [24,25$(OH)_2D_3$] and 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$] (1, 2). The latter metabolite has been thought to be the active form of vitamin D_3 in enhancing intestinal calcium transport and bone mineral mobilization (1 - 3). Since excess production of 1α,25$(OH)_2D_3$ induces marked hypercalcemia, its renal biosynthesis must be tightly regulated. Thus, plasma levels of 1α,25$(OH)_2D_3$ are maintained within a very narrow range (20 - 80 pg/ml) in most mammals (4 - 7).

Recently, attention has been focused on a clinical disorder, vitamin D-dependent rickets, type II. The disease is an inheritable disorder characterized by a high circulating level of 1α,25$(OH)_2D_3$ and extreme resistance to treatment with 1α,25$(OH)_2D_3$ (8 - 12). In the course of searching

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Abbreviations used: 1α,25$(OH)_2D_3$, 1α,25-dihydroxyvitamin D_3; 25$(OH)D_3$, 25-hydroxyvitamin D_3; 24,25$(OH)_2D_3$, 24,25-dihydroxyvitamin D_3.

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for an animal model for studying the pathogenesis of vitamin D-dependent type II rickets, we found that the marmoset, a new world monkey, has an extremely high circulating level of $1\alpha,25(OH)_2D_3$ without exhibiting hypercalcemia, suggesting that the marmoset may be such a model.

MATERIALS AND METHODS

Animals: Seven adult common marmosets (Callithrix jacchus), 5 males and 2 females, weighing approximately 300 g, were fed a commercial diet containing 9,100 IU of vitamin D_3/kg of diet (Science diet: "Marmosets", Riviana Food Inc., Topeka, Kansas) and fruit (one eighth of an apple and one fourth of a banana per animal every day). In addition, they were given orally 500 IU of vitamin D_3 twice a week. Six young adult rhesus monkeys (Macaca mulatta), all females, weighing 4 - 6 kg, were fed a commercial diet containing 2,400 IU of vitamin D_3/kg of diet (Japan CLEA, Tokyo, Japan).

Chemicals: Vitamin D_3 was purchased from Wako Pure Chemicals, Osaka, Japan. $25(OH)[26,27\text{-}^3H]D_3$, $24,25(OH)_2[23,24\text{-}^3H]D_3$ and $1\alpha,25(OH)_2[23,24\text{-}^3H]D_3$ were obtained from Amersham International, Bucks., U.K. The $1\alpha,25(OH)_2D_3$ receptor protein was kindly donated by Dr. T. Iijima from Yamasa Biochemicals, Chiba, Japan. All other chemicals were of analytical grade.

Measurement of vitamin D_3 metabolites in serum: Blood samples (2 ml for marmosets and 4 ml for rhesus monkeys) were taken from the femoral vein once a week just before the vitamin D_3 supplementation. Blood was also collected from 6 healthy human male volunteers.

To 4 ml of the pooled serum approximately 2,500 cpm of $25(OH)[^3H]D_3$, $24,25(OH)_2[^3H]D_3$ and $1\alpha,25(OH)_2[^3H]D_3$ were added, and the sera were extracted with dichloromethane and methanol (1 : 2, v/v) by the method of Mallon et al (13). The lipid extract was then applied to a Sephadex LH-20 column (0.7 x 14 cm), which was eluted with a mixed solvent of chloroform and n-hexane (60 : 40, v/v). Fractions of 4 - 10 ml, 10 - 20 ml and 20 - 40 ml which contained $25(OH)D_3$, $24,25(OH)_2D_3$ and $1\alpha,25(OH)_2D_3$, respectively, were collected separately. Each fraction was evaporated under nitrogen and injected into a high pressure liquid chromatograph, Waters HPLC Model 204, equipped with a Zorbax-Sil column (4.6 mm x 15 cm, Dupont, Wilmington, Delaware). The solvent systems used were 1.5, 2.0 and 3.0% methanol in dichloromethane for $25(OH)D_3$, $24,25(OH)_2D_3$ and $1\alpha,25(OH)_2D_3$ fractions, respectively. Each resulting fraction was subjected to competitive protein binding (CPBA) or radioreceptor assays. $25(OH)D_3$ and $24,25(OH)_2D_3$ were determined by CPBA using serum obtained from rachitic rats as binding protein. $1\alpha,25(OH)_2D_3$ was measured by a modified method of Eisman et al. (14) using the Yamasa $1\alpha,25(OH)_2D_3$ receptor protein.

Measurement of serum calcium, phosphorus and alkaline phosphatase activity: Serum concentration of calcium was determined with an atomic absorption spectrophotometer (Hitachi, Model 170-50A, Tokyo, Japan). Serum concentration of phosphorus was measured by the method of Fiske-SubbaRow (15). Alkaline phosphatase activity was measured by the method of Bessey-Lowry (16).

RESULTS

Table I shows the serum concentrations of calcium and phosphorus and serum alkaline phosphatase activity in the 7 marmosets. The serum levels of calcium were 7.9 to 9.9 mg/100 ml and of phosphorus, 2.1 to 4.7 mg/100 ml. Marmosets Nos. 6 and 7 showed an apparent hypophosphatemia. Both

Table I. Serum calcium and phosphorus concentrations and alkaline phosphatase activity in individual marmosets.

Animal No.	Sex	Ca (mg/100 ml)	P (mg/100 ml)	Alkaline phosphatase (units)	Bone fracture
1	F	9.0	4.7	1.7	-
2	M	9.7	3.9	1.9	-
3	F	8.4	3.7	3.1	-
4	M	7.9	4.6	2.5	-
5	M	8.1	3.4	1.9	-
6	M	8.8	2.1	9.2	+
7	M	9.9	2.1	12.4	+

The serum calcium and phosphorus concentrations and alkaline phosphatase activity of rhesus monkeys were 9.76 ± 0.25 mg/100 ml, 3.82 ± 0.54 mg/100 ml, and 3.70 ± 0.47 Bessey-Lowry units, respectively (means ± SEM of 6 animals).

marmosets were found by X-ray examination to have osteomalacia and bone fractures. Serum alkaline phosphatase activity in both individuals was 4 to 6 times higher than in the other 5.

Serum levels of 25(OH)D_3 were distributed over a range of 12.4 to 204.1 ng/ml, the lowest in Nos. 6 and 7 (Table II). Circulating levels of 1α,25(OH)$_2D_3$ were 196.1 to 642.4 pg/ml. Note that, in marmosets Nos. 6 and 7, the 1α,25(OH)$_2D_3$ levels were particularly elevated and the 24,25-(OH)$_2D_3$ levels were extremely low (less than 0.2 ng/ml) (Table II).

Figure 1 shows the comparative mean circulating levels of vitamin D metabolites in marmosets, rhesus monkeys and healthy human subjects. The

Table II. Serum concentrations of vitamin D_3 metabolites in individual marmosets.

Animal No.	25(OH)D_3 (ng/ml)	1α,25(OH)$_2D_3$ (pg/ml)	24,25(OH)$_2D_3$ (ng/ml)
1	140.2	499.8	3.11
2	72.3	196.1	0.91
3	204.1	304.9	8.23
4	183.3	259.5	4.65
5	32.8	403.7	0.76
6	16.5	642.4	< 0.2
7	12.4	524.1	< 0.2

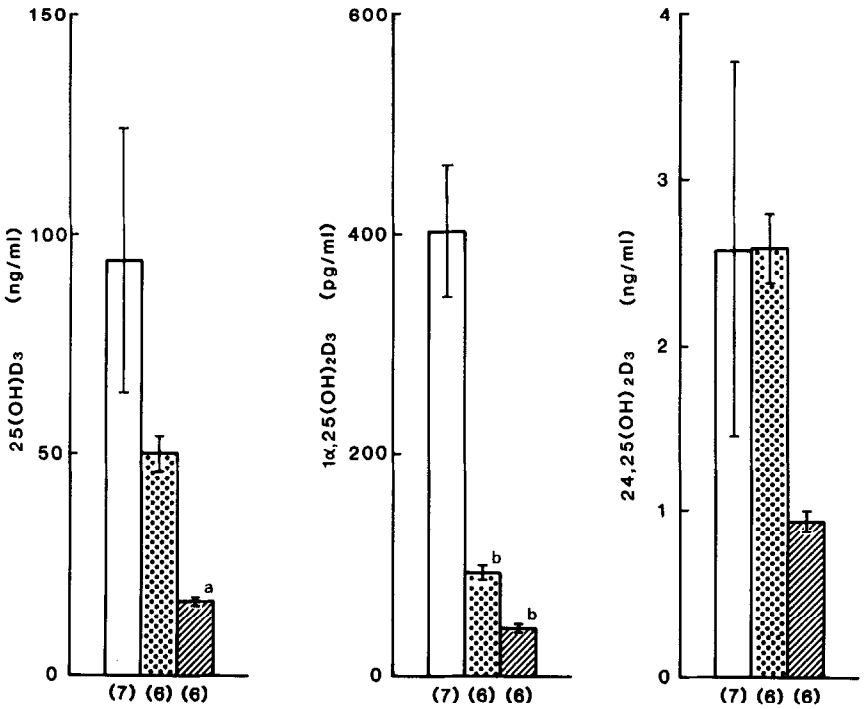

Fig. 1. The mean levels of circulating 25(OH)D_3, 1α,25(OH)$_2D_3$ and 24,25-(OH)$_2D_3$ in marmosets (□), rhesus monkeys (▣) and healthy human subjects (▨). Vertical bars show means ± SEM. Numbers of animals used in parentheses.
Significance of difference from marmosets: a, $p < 0.05$; b, $p < 0.005$.

mean level of 25(OH)D_3 in marmosets was about 2 and 4 times higher than in rhesus monkeys and humans, respectively. The mean 1α,25(OH)$_2D_3$ level in marmosets was also 4 times higher than in rhesus monkeys and 10 times as high as humans. But the mean level of 24,25(OH)$_2D_3$ in marmosets was not significantly different from rhesus monkeys, though the levels in individual marmosets varied considerably.

DISCUSSION

The marmoset requires exceptionally large amounts of vitamin D to maintain its normal growth compared with most mammals, including rhesus monkeys and humans. In this experiment, marmosets were maintained on a diet containing 9,100 IU of vitamin D_3 per kg of diet. The mean daily food intake of marmosets was 20 ± 5 g. In addition, the marmosets were given 500 IU of vitamin D_3 twice a week. Thus the daily intake of vitamin D_3

can be calculated to be 300 IU/head. This amount is about 18 times more than for rhesus monkeys on a body weight basis.

In spite of the high intake of vitamin D_3, all marmosets had normocalcemia or even moderate hypocalcemia. Furthermore, two (Nos. 6 and 7) of the 7 marmosets had severe osteomalacia with hypophosphatemia. X-ray examination showed bone fractures in both individuals. The serum levels of $1\alpha,25(OH)_2D_3$ in these two marmosets were particularly elevated, whereas $24,25(OH)_2D_3$ levels were too low to detect (less than 0.2 ng/ml). Their $25(OH)D_3$ levels were also rather low. These results suggest that the renal $25(OH)D_3$-1α-hydroxylase activity is markedly stimulated in marmosets, especially in Nos. 6 and 7. In fact, the circulating $1\alpha,25(OH)_2D_3$ levels in marmosets were 4 - 10 times higher than in rhesus monkeys and humans.

It is of great interest that none of the marmosets had hypercalcemia. These findings are very similar to those in vitamin D-dependent type II rickets (8 - 12). It has also been reported that the circulating levels of glucocorticoids are extremely high in marmosets (17). However, marmosets are resistant to high levels of glucocorticoids. Since $1\alpha,25(OH)_2D_3$ is thought to act by a mechanism similar to that proposed for the classical concept of steroid hormone action, the marmoset may have resistance to a variety of steroid hormones. The nature of the receptor proteins in the target tissues in marmosets specifically bound to $1\alpha,25(OH)_2D_3$ is of considerable interest and is now under investigation in our laboratories.

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