Influence of Vitamin D on *in Vivo* Intestinal Calcium Transport in Normal Rats[1] (36488)

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The effects of vitamin D on intestinal calcium transport have usually been studied in vitamin D-depleted animals since the actions of the vitamin are most clearly defined in this state (1). However, most vitamin D is eaten by nondeficient subjects and vitamin D toxicity from over-dosage usually occurs in subjects previously normal in vitamin D status. The metabolism of vitamin D to biologically active products is also dependent on the metabolic state of the animal with respect to calcium and vitamin D (2). Nevertheless, effects of vitamin D on *in vivo* intestinal calcium transport in the normal state have received little attention (3, 4). In the present study the response of normal animals to vitamin D was examined. High dosages (500 and 10,000 units) were chosen to achieve clear-cut responses. In addition, the response to large doses may be helpful in understanding the mechanisms involved in vitamin D over-dosage in animals previously normal in vitamin D status. We found consistently small, but not statistically significant increases in net calcium absorption in the groups receiving vitamin D. Flux of calcium into the lumen, however, was significantly decreased in the group receiving 10,000 units, but not in the group receiving 500.

Materials and Methods. Normal male albino rats (Carworth Labs) were fed regular Wayne Lab Blox. Forty-eight hours before study one of a pair of weight-matched rats was injected intramuscularly with vitamin D_2 (+D) dissolved in ethanol:propylene glycol (1:5) and the other (control) received the vehicle. For 10 pairs the +D animals received 500 U of vitamin D (+D 500) and for 13 pairs 10,000 U (+D 10,000). Twenty-four hours before study all rats were injected intramuscularly with $^{45}CaCl_2$ (initial sp act, 12 mCi/mg, New England Nuclear Corp., Boston, MA) dissolved in 0.1 *N* HCl (100 μCi/ml). The +D 500 group and its controls received 25 μCi and the +D 10,000 group and its controls received 20 μCi/100 g body weight. All rats were fasted with distilled water *ad libitum* for 24 hr after ^{45}Ca injection. The methods of anesthesia, intestinal perfusion, work-up of intestinal segments, determination of tissue ^{45}Ca and calcium concentration have been described previously (5). Ten to 15 cm of the duodenum–proximal jejunum and terminal ileum were cannulated and the bile duct was ligated. The intestine was tied off just proximal and distal to the cannulated segments. After irrigation with approximately 10 ml of isotonic saline solution and 20 ml of air, the intestine was replaced in the abdominal cavity. The segments were then perfused *in situ* continuously by the single pass technique for 2 hr with 150 m*M* sodium chloride (8.90 g/liter) containing 3.4 m*M* calcium chloride (0.38 g/liter) and 20 mg/liter of phenol red. The pump rate was 0.30–0.32 ml/min and the effluents from each segment of each rat were collected separately at 0–45, 45–60, 60–75, 75–90, 90–105 and 105–120 min after the start of the perfusion. The entry of ^{45}Ca into and the absorption of calcium from the lumen were determined by measuring radioactivity (liquid scintillation counting) and the change in calcium concentrations and

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TABLE I. ^{45}Ca and Calcium Concentrations and ^{45}Ca Specific Activities (mean ± SE[a]).

Groups:	Control		+D 500		Control		+D 10,000	
Intestinal segment:	Duodenum	Ileum	Duodenum	Ileum	Duodenum	Ileum	Duodenum	Ileum
^{45}Ca concns (^{45}Ca cpm × 10^{-3}/ml or g)								
Lumen	0.23 ± 0.03	0.42 ± 0.05	0.23 ± 0.02	0.38 ± 0.02	0.26 ± 0.02	0.37 ± 0.03	0.17 ± 0.02[b]	0.27 ± 0.03[b]
Mucosa	19.7 ± 1.0	44.6 ± 4.8	18.3 ± 0.6	44.8 ± 1.7	13.9 ± 0.5	28.4 ± 0.7	13.7 ± 0.3[b]	29.1 ± 1.0[b]
Plasma	25.2 ± 1.1		24.8 ± 1.3		17.5 ± 0.8		16.9 ± 0.5	
Calcium concns (μmoles/ml or g)								
Lumen	3.26 ± 0.02	3.32 ± 0.02	3.20 ± 0.08	3.32 ± 0.04	3.17 ± 0.03	3.37 ± 0.02	3.14 ± 0.02	3.34 ± 0.02
Mucosa	3.60 ± 0.25	4.60 ± 0.27	3.39 ± 0.24	4.75 ± 0.25	3.15 ± 0.15	4.41 ± 0.14	3.15 ± 0.17	4.41 ± 0.17
Plasma	2.12 ± 0.03		2.03 ± 0.06		1.98 ± 0.03		1.98 ± 0.03	
Sp act (^{45}Ca cpm × 10^{-3}/μmoles of calcium)								
Lumen	0.09 ± 0.01	0.13 ± 0.01	0.07 ± 0.01	0.11 ± 0.01	0.08 ± 0.01	0.11 ± 0.01	0.05 ± 0.01[b]	0.08 ± 0.01[b]
Mucosa	5.5 ± 0.4	9.7 ± 0.7	5.6 ± 0.5	9.6 ± 0.5	5.8 ± 0.3	6.5 ± 0.2	5.2 ± 0.2[b]	5.7 ± 0.3[b]
Plasma	11.9 ± 0.5		12.1 ± 0.5		9.0 ± 0.4		8.6 ± 0.3	

[a] All differences between corresponding duodenal and ileal data are significant ($p < .01$).

[b] Significantly lower than corresponding data in control group ($p < .05$).

volume in the solution perfused. The ^{45}Ca concentrations (cpm/ml) were within ±5% of each other in the effluents collected during the last four periods. Secretion and absorption rates during the last 0.5 hr of the study were considered to represent steady-state conditions and were used for the calculations.

After termination of the perfusion blood was obtained from the inferior vena cava and anticoagulated. Inspection of the perfused intestinal segments showed an intact vascular supply and no tissue damage. Each segment was stripped from the mesentery and its length and weight were measured. The mucosa was scraped from underlying tissues with the edge of a microscope slide. Calcium and ^{45}Ca concentrations were determined in plasma and intestinal mucosa by atomic absorption spectrometry and liquid scintillation counting (5). The Student's t test was used for statistical comparison of differences, and p values (6) of less than .05 were considered to be significant.

Calculations. Flux into the lumen was calculated assuming no reabsorption of secreted ^{45}Ca. This is a reasonable assumption based on low luminal ^{45}Ca concentration relative to ^{40}Ca and is supported by previous studies (5). Data are expressed as rates per 0.5 hr and are the sums of the last two 15 min periods calculated as follows:

$$^{45}\text{Ca flux into the lumen (cpm in 15 min)} = V\cdot[^{45}\text{Ca}],$$

where V is the volume (ml) of the effluent; $[^{45}Ca]$ is ^{45}Ca concentration in the effluent in counts per minute per milliliter. V is calculated as follows:

$$V = V_p\cdot\text{PRR},$$

where V_p is the pump rate (ml/15 min), and PRR is the ratio of the initial to the final phenol red concentrations.

Calcium flux into the lumen was calculated as its plasma-to-lumen flux.

$$\text{Calcium flux into the lumen } (\mu\text{moles/15 min}) = {}^{45}\text{Ca flux into the lumen/plasma } {}^{45}\text{Ca sp act}$$

where plasma ^{45}Ca specific activity is expressed as ^{45}Ca counts per minute per micromole of calcium.

$$\text{Net calcium absorption } (\mu\text{moles/15 min}) = V_p[^{40}\text{Ca}_i] - V_p[^{40}\text{Ca}_f]\text{PRR}.$$

The subscripts i and f refer to initial and final values, $[^{40}Ca]$ is the calcium concentration (μmoles/ml) and the other symbols have the same meaning as above.

Results. At each vitamin D dosage mean body weight (g) was the same in vitamin D injected (+D) and control (C) groups: +D 500 = 172 ±5, C = 163 ± 6, +D 10,000 = 138 ± 3, C = 136 ± 3. Length and weight of the corresponding duodenal and ileal segments and of their mucosa and underlying tissue layers were also the same in the two groups. Concentrations of ^{45}Ca and calcium (^{40}Ca) and ^{45}Ca specific activities in the intestinal lumen, tissues and plasma are shown in Table I. Mean ^{45}Ca concentration in the duodenal and ileal lumen was significantly lower in the +D 10,000 than corresponding control group ($p < .05$). In both groups of vitamin treated and control rats luminal ^{45}Ca concentration was significantly lower in the duodenum than in the ileum ($p < .01$). Plasma and intestinal tissue ^{45}Ca concentrations did not differ in corresponding control and +D groups. In all groups ileal tissue concentrations were about twice as great as duodenal concentrations ($p < .005$). In comparison with plasma, mucosal ^{45}Ca concentration was significantly lower in the duodenum ($p < .05$) and significantly higher in the ileum ($p < .01$).

Corresponding concentrations of calcium were the same in control and +D rats. In the +D 10,000 group luminal calcium concentrations in the duodenum were significantly lower than in the corresponding ileum ($p < .05$). In both groups intestinal tissue calcium concentration was significantly lower in the duodenum than in the ileum ($p < .005$). Calcium concentrations were significantly higher in tissue than in plasma ($p < .01$).

The low luminal ^{45}Ca specific activities were the result of the relatively high luminal

TABLE II. ^{45}Ca and Calcium Flux into the Lumen and Net Calcium Absorption Rates (mean ± SE[a]).

Groups:	Control		+D 500		Control		+D 10,000	
Intestinal segment:	Duodenum	Ileum	Duodenum	Ileum	Duodenum	Ileum	Duodenum	Ileum
Flux into the lumen								
^{45}Ca (cpm × 10^{-3} in 0.5 hr/g)								
Mucosal wet wt	6.9 ± 0.9	12.6 ± 1.3	7.4 ± 0.6	10.2 ± 0.8	5.7 ± 0.5	9.2 ± 0.8	4.5 ± 0.4[b]	7.4 ± 0.5[b]
Mucosal dry wt	38.6 ± 4.7	77.9 ± 8.0	42.0 ± 4.1	55.7 ± 4.3	37.5 ± 3.1	65.0 ± 4.8	27.8 ± 1.8[b]	51.1 ± 3.5[b]
Calcium(μmoles in 0.5 hr/g)								
Mucosal wet wt	0.59 ± 0.09	1.07 ± 0.10	0.62 ± 0.05	0.84 ± 0.06	0.60 ± 0.03	1.10 ± 0.06	0.51 ± 0.02[b]	0.86 ± 0.04[b]
Mucosal dry wt	3.3 ± 0.4	6.6 ± 0.7	3.5 ± 0.7	4.6 ± 0.3	4.1 ± 0.3	7.1 ± 0.5	3.1 ± 0.1[b]	6.0 ± 0.4[b]
Net absorption								
Calcium (μmoles in 0.5 hr/g)								
Mucosal wet wt	5.7 ± 0.7	4.8 ± 0.6	7.8 ± 0.9	5.8 ± 1.0	9.8 ± 0.7	6.5 ± 0.8	11.5 ± 0.9	8.3 ± 0.6
Mucosal dry wt	30.7 ± 3.8	24.9 ± 3.8	44.9 ± 4.5	34.0 ± 5.0	64.4 ± 5.1	48.1 ± 6.2	70.6 ± 5.9	57.7 ± 4.0
Flux out of the lumen								
Calcium (μmoles in 0.5 hr/g)								
Mucosal wet wt	6.3 ± 0.7	5.8 ± 0.6	8.4 ± 0.9	6.6 ± 1.0	10.3 ± 0.8	7.5 ± 0.8	12.0 ± 1.0	9.1 ± 0.7
Mucosal dry wt	34.0 ± 3.9	31.5 ± 3.9	48.4 ± 4.6	38.6 ± 5.1	68.5 ± 5.2	55.3 ± 6.3	73.7 ± 6.0	63.7 ± 4.1

[a] All differences between corresponding duodenal and ileal data are significant ($p < .05$).

[b] Fluxes into the lumen are significantly lower in +D 10,000 group than corresponding data in control group ($p < .05$).

load of exogenous calcium (^{40}Ca) in the perfusion solution. Mucosal ^{45}Ca specific activities were around 50 times those in the lumen in all segments perfused. The specific activity of ^{45}Ca was significantly lower in intestinal tissue than in plasma in all groups ($p < .01$). Plasma ^{45}Ca specific activities were similar in the vitamin D-treated and corresponding control rats.

Table II shows flux into the lumen of ^{45}Ca and calcium, and net calcium absorption rates in the perfused segments. Rates of ^{45}Ca and calcium flux into the lumen were significantly lower in the ileum of the +D 500 and in both intestinal segments of +D 10,000-treated rats than in the corresponding segments of the control rats ($p < .05$). In all groups the flux into the lumen of ^{45}Ca and calcium was significantly greater in the ileum than in the duodenum ($p < .01$). Although mean net calcium absorption was greater in the vitamin D-treated animals, the differences between corresponding segments were not statistically significant. Lumen-to-plasma fluxes calculated as the sum of net calcium absorption and plasma-to-lumen calcium flux are shown at the bottom of the Table II.

Discussion. These studies were designed to examine the effects of large doses of vitamin D on both flux of calcium into the lumen and net absorption in normal rats. When measured 48 hr after administration, vitamin D did not affect calcium or ^{45}Ca concentrations or specific activities in plasma or intestinal tissue. Because plasma and tissue ^{45}Ca and calcium concentrations were the same in each group of control and vitamin D-injected rats, flux into the lumen could be compared directly in each set of experiments. Net calcium absorption was measured as loss of perfused calcium from the lumen. Net calcium absorption was not significantly increased by vitamin D (Table II, net absorption). This finding agrees with results of previous *in vivo* studies in normal animals (3) and man (4). Although vitamin D increases absorption and lumen-to-plasma flux of calcium in the duodenum of vitamin D-depleted rats (1, 7), no such effects have been demonstrated in normal rats. The phenomenon of a greater response in the deficient than in the normal animal is found with many hormones. The exogenous ^{40}Ca in the perfusion solution minimized reabsorption of secreted ^{45}Ca from the lumen. Therefore, the ^{45}Ca flux into the lumen was a direct measure of the unidirectional plasma-to-lumen calcium flux. Both duodenal and ileal ^{45}Ca flux into the lumen and calcium plasma-to-lumen flux were significantly decreased in the +D 10,000 group.

A biochemical basis for the different effects of vitamin D in normal and deficient animals is probably found in the metabolism of vitamin D in these animals. A metabolite of vitamin D believed to be the metabolically active form in the intestine has been isolated and identified as 1,25-dihydroxycholecalciferol (1,25-DHCC) (8, 9). The metabolism of 25-dihydroxycholecalciferol to 1,25-DHCC is dependent on the vitamin D and calcium status of the animal (2). The formation of 1,25-DHCC is enhanced in vitamin D-deficient rats and in hypocalcemic rats on low calcium diets. There is a striking decrease in 1,25-DHCC formation in rats with a normal serum calcium on high calcium diets. In these animals, 21,25-dihydroxycholecalciferol, a metabolite lacking the activity of 1,25-DHCC, appeared in large amounts (2). It is possible that this difference in vitamin D metabolism between normal and deficient animals explains our findings in the normal animal, *i.e.*, no effect on calcium absorption, decreased calcium secretion. Indeed, in our studies of hypocalcemic vitamin D-deficient rats (10) we found no effect of vitamin D on plasma-to-lumen flux of calcium, although net calcium absorption was increased. Thus the effects of vitamin D on intestinal calcium transport differ in normal and vitamin D-depleted rats, just as the metabolism of the vitamin differs in the two states. The decrease in calcium secretion in normal rats treated with vitamin D could also contribute to the hypercalcemia from vitamin D intoxication.

Summary. Small intestinal calcium transport was compared in normal controls and

in body weight matched rats injected with vitamin D_2 (either 500 or 10,000 U). The vitamin was injected 48 hr before study. Flux into the lumen of ^{45}Ca (injected 24 hr before study) and calcium and net calcium absorption were measured under steady-state conditions in duodenum–proximal jejunum and in terminal ileum. Flux of ^{45}Ca and calcium into the lumen was significantly lower in the rats treated with vitamin D_2 (10,000 U) than in controls, in both proximal and distal intestinal segments. However net calcium absorption was not significantly increased in the vitamin-treated groups compared with controls, in contrast to the behavior of vitamin D-deficient animals in which the main effect of this vitamin is to increase absorption. Thus, the effects of vitamin D differ in normal and depleted rats and this may be explained by differences in vitamin D metabolism in normal and deficient rats. The finding that vitamin D decreased flux of calcium into the lumen in normal rats suggests that this effect, if prolonged, may play a role in enhancing or maintaining the hypercalcemia of vitamin D toxicity.

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