Jejunal and Ileal Calcium Absorption in Patients with Chronic Renal Disease

EFFECT OF 1α-HYDROXYCHOLECALCIFEROL

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ABSTRACT Calcium absorption in 30-cm segments of small intestine was measured by constant perfusion of test solutions containing different concentrations of calcium gluconate. In both the jejunum and ileum, calcium absorption rates increased progressively as luminal calcium concentration was increased stepwise between 1 and 20 mM. Although calcium transport was not saturable within these limits, unidirectional flux ratios of calcium (^{47}Ca) suggest that calcium absorption is active in both the jejunum and ileum. Calcium absorption in patients with chronic renal disease was markedly depressed in both regions of the small intestine. This was due to decreased flux out of the lumen; flux in the reverse direction was normal. Flux ratios in the renal disease patients showed no evidence for active calcium transport. Treatment of these patients for 1 wk with 2 μg/day of 1α-hydroxycholecalciferol [1α-(OH)-D_3] restored net calcium absorption and unidirectional calcium flux out of the lumen to normal values in the jejunum; in the ileum, 1α-(OH)-D_3 increased calcium absorption 60-83% of normal at the various luminal calcium concentrations. 1α(OH)-D_3 had no effect on unidirectional calcium flux into the lumen or on xylose and electrolyte absorption in either area of the small intestine.

INTRODUCTION

It is known from previous work that calcium is actively absorbed from the proximal small bowel of most animals including man (1–6). This transport system is adaptable to the needs of the body (7–8) and is dependent on vitamin D (2, 4, 9); it is reduced in patients with chronic renal disease (6, 10–12), presumably because of deficient renal synthesis of 1,25-dihydroxycholecalciferol (13-16). It is not known to what extent the distal small bowel absorbs or secretes calcium in normal humans, nor is it known if ileal transport of calcium is defective in patients with chronic renal disease. One study in patients with renal disease suggested that ileal absorption of radioactive calcium is actually higher than normal and that this partially compensates for the proximal intestinal transport defect (17, 18).

The purpose of the present series of experiments was to determine to what extent the ileum absorbs or secretes calcium in normal subjects, to see if calcium absorption is abnormal in the ileum of patients with chronic renal disease, and to obtain more information about the effect of 1α-hydroxycholecalciferol (1α-(OH)-D_3)[1] on jejunal and ileal calcium transport in patients with chronic renal disease. Previous studies have shown that 1,25-dihydroxycholecalciferol and 1α-(OH)-D_3 enhance radioactive calcium absorption in patients with chronic renal disease (15, 19, 20), but the effect of these forms of vitamin D on net and unidirectional calcium fluxes in specific regions of the human small intestine has not been determined. Our studies were carried out with the triple-lumen perfusion technique. This has the advantage that net absorption or secretion and unidirectional calcium flux rates can be determined from segments of jejunum and ileum, under conditions where calcium concentration and other factors can be controlled and systematically varied.

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TABLE I

Clinical Data in Normal Subjects and in Renal Disease Patients before and after 1 wk of Treatment with 1α-(OH)-D_3

	Normals	Renal disease patients		
		Before 1α-(OH)-D_3	After 1α-(OH)-D_3	P*
Age, *yr*	33±3 (22–61)	36±6 (18–58)		
Dietary ca. intake, *mg/day*	1,000±100 (400–3,000)	775±100 (450–1,000)		
Serum sodium, *meq/liter*	139±1	134±1	134±2	NS
Serum potassium, *meq/liter*	4.4±0.05	5.2±0.3	5.4±0.4	NS
Serum phosphorous, *mg 100 ml*	3.9±0.1	6.3±0.6	6.1±0.7	NS
Serum calcium, *mg 100 ml*	9.4±0.1	9.3±0.2	10.0±0.2	0.01
Alkaline phosphatase, *IU*	Not done	257±47	247±47	NS

* By paired statistical analysis.

METHODS

Subjects. 12 patients with end-stage renal disease undergoing hemodialysis triweekly were studied. 10 of them had chronic glomerulonephritis, and 2 had chronic pyelonephritis. Six were studied in the proximal jejunum, and six were studied in the mid-ileum. Their age, previous dietary intake of calcium, and pertinent laboratory data are given in Table I. 26 completely normal people were studied for comparison (12 in the jejunum and 14 in the ileum. All subjects gave informed consent for the performance of these studies, and the experiment was approved by a Human Research Review Committee.

1α-(OH)-D_3. This analog was synthesized by the method of Holick et al. (21). A stock solution containing 50 μg was dissolved in 25 ml of propylene glycol. 1 ml (2 μg) was administered orally 30 min before breakfast for 7 days.

Intestinal perfusion. Subjects were intubated with a triple-lumen polyvinyl tube, and intestinal perfusion was carried out as previously described (6, 8, 22–24). The infusion aperture of the tube was either at the ligament of Treitz (90 cm from the teeth) or in the mid-ileum (infusion aperture 200 cm from the teeth). The distance between the proximal and distal collecting sites was 30 cm.

Test solutions were infused at a rate of 11 ml/min. Four test solutions containing different concentrations of calcium were infused in random sequence. A given solution was infused ¾ h for equilibration followed by a 1-h collection period.

Constituents of test solutions. The osmolality and sodium chloride concentrations of the solutions were adjusted so that water and sodium absorption would be near zero. This was necessary to prevent absorption of large amounts of sodium chloride and water, which might be hazardous to the patients. The test solutions contained 50 mM NaCl, 5 mM KCl, 0.5% polyethylene glycol (the nonabsorbable volume maker), 10 mM *D*-xylose, and either 0,1,5,15 or 20 mM calcium gluconate. 1 μCi of radioactive calcium (^{47}Ca) was added to the 1- and 5-mM calcium test solutions to measure unidirectional calcium flux rates. Mannitol was added in amounts required to give an osmolality of 200 mosmol/kg in the jejunal studies and 280 mosmol/kg in the ileal studies. This change was made to account for the different permeability characteristics of the upper and lower small intestine (25).

Analysis of samples. Intestinal samples were analyzed for pH, electrolytes, polyethylene glycol, calcium, xylose, and ^{47}Ca activity by previously described methods (6, 8).

Calculations. Absorption or secretion rates of water and solutes were calculated from the perfusion rate, the change in polyethylene glycol concentration, and the change in solute concentration. The calcium concentration of the perfusates are expressed as the arithmetic mean of the concentration at the proximal and distal collecting sites. Unidirectional calcium flux was calculated from the ^{47}Ca results by the method of Berger and Steele (26). Potential difference between blood and intestinal lumen was estimated from the concentrations of potassium (which equilibrates passively across gut mucosa in accord with electrochemical gradients [27–29]) in serum and in fluid aspirated from the distal end of the intestinal segment, using the Nernst equation as previously described (30, 31). The unidirectional calcium flux ratios which would be expected if calcium movement was determined entirely by electrochemical gradients were calculated by the Ussing equation (32). For the purpose of this calculation the serum diffusible calcium concentration was assumed to be 1.5 mM (33).

RESULTS

Net calcium absorption. As shown in Fig. 1, calcium absorption in the normal jejunum increased rapidly as luminal concentration was raised from 1 to 18 mM. In patients with renal disease, calcium absorption was less than normal at all four luminal calcium concentrations. These findings are similar to our previous results (6). Therapy with 1α-(OH)-D_3 restored calcium absorption in the renal disease patients to approximately normal values in the jejunum. It should be noted that before 1α-(OH)-D_3, the patients were secreting calcium into the jejunal lumen during perfusion with the 1-mM calcium solution, whereas after 1α-(OH)-D_3, they absorbed calcium from this solution.

In the ileum of 14 normal subjects, calcium was absorbed in direct linear proportion to luminal calcium concentration. Absorption rates in the normal ileum were slightly less than in the normal jejunum with the 1- and 5-mM calcium test solutions and slightly higher than in the jejunum with the 15- and 20-mM solutions. In the

[1] *Abbreviation used in this paper:* 1α-(OH)-D_3, 1α-hydroxycholecalciferol.

ileum, as in the jejunum, calcium absorption was markedly depressed in patients with chronic renal disease This defect was improved but not completely corrected by 1α-(OH)-D_3.

Unidirectional calcium flux. ^{47}Ca was added to the 1-mM and 5-mM calcium solutions, and the results are shown in Table II. In both the normal jejunum and ileum, the flux out of the lumen exceeded the flux into the lumen, and the observed flux ratios were higher than the calculated flux ratios for passive diffusion according to Ussing's criteria (32). The flux out of the lumen was higher with the 5- than with the 1-mM calcium test solutions, which is expected. The flux into the lumen is also higher with the 5- than with the 1-mM calcium solution; although difficult to explain, this same observation has been made previously by Dumont et al. (34) and by Wasserman and Kallfelz (35), and these authors have speculated on its meaning.

The patients with chronic renal disease had reduced rates of unidirectional calcium flux out of the lumen and near normal rates of unidirectional flux rates into the lumen, and the observed calcium flux ratios were similar to what is expected for passive calcium diffusion. 1α-(OH)-D_3 therapy in these patients elevated the flux out of the lumen, did not significantly alter flux into the lumen, and caused the observed flux ratios to exceed what was expected if calcium absorption were passive. All of the changes with 1α-(OH)-D_3 were statistically significant except with the 5-mM calcium solution in the ileum.

Water, electrolyte, an xylose absorption. The absorption of water, sodium, chloride, and xylose was normal in the dialysis patients. Treatment with 1α-(OH)-D_3 had no statistically significant effect on the absorption of these substances in either area of the small intestine.

Effect of 1α-(OH)-D_3 on serum calcium and phosphorus. Table I shows the serum calcium and phosphorus concentration before and after 1 wk of therapy with 1α-(OH)-D_3. Serum calcium increased from 9.3 to 10.0 ($P < 0.01$). This was the only change noted that was statistically significant.

DISCUSSION

In most species the ileum does not actively absorb calcium unless animals are stressed by placing them on a low calcium diet; in others, such as the hamster, active calcium absorption is greater in the ileum than in the jejunum (2). The rat ileum is also capable of active secretion of calcium (36, 37), and it has been suggested that this may be an important source of endogenous calcium secretion. The studies reported in this paper reveal that calcium is absorbed at approximately the same rates in the ileum as in the jejunum of normal human subjects. We found that calcium absorption in the ileum is a linear function of luminal calcium concentration, at least between 1 and 20 mM. (It is, of course, possible that ileal calcium absorption might become saturated if solutions containing more than 20 mM calcium were perfused, but such an experiment would be difficult to perform because of the limited solubility of calcium salts.) Although this type of kinetic relationship is most characteristic of absorption by passive diffusion, the calcium unidirectional flux ratios with a 1-mM luminal calcium concentration (Table II) are higher than those expected for passive calcium diffusion (32); this sug-

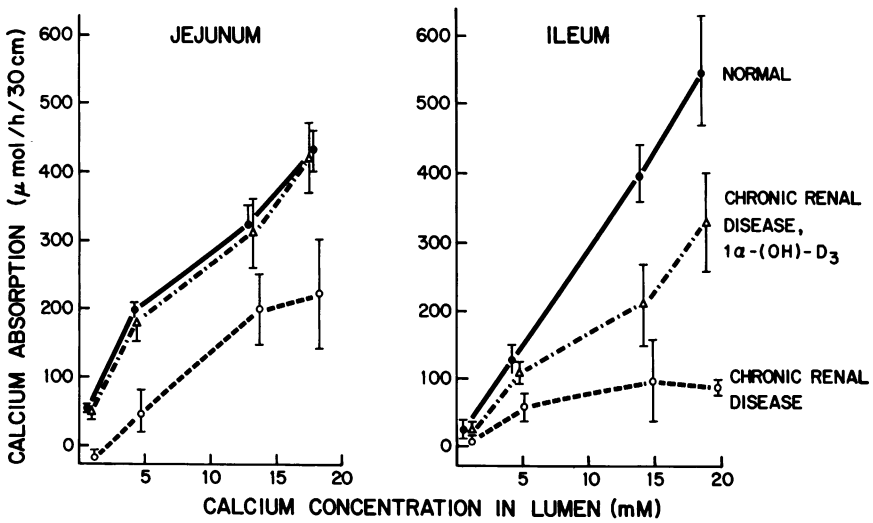

FIGURE 1 Calcium absorption rates at different luminal calcium concentrations in the jejunum and ileum of normal subjects and in patients with chronic renal disease before and after treatment with 1α-(OH)-D_3 (2 μg/day by mouth for 7 days).

TABLE II

Unidirectional Calcium Flux Rates and Ratios Measured with ^{47}Ca

	Flux out	Flux in	$[Ca]_L$	Potential difference	Flux ratio		
					Observed	Calculated	*P*
	μmol/h/30 cm	*μmol/h/30 cm*	*mM*	*mv*			
1 mM Calcium test solution							
Jejunum							
Normal	91±11	43±10	0.77±0.05	−1±1	2.9±1.0	0.5±0.1	0.025
Chronic renal disease untreated	38±11	61±16	1.11±0.07	+2±2	0.8±0.3	0.9±0.2	0.8
Treated (1 α-(OH)-D_3)	114±25	72±20	0.76±0.07	−1±1	1.7±0.2	0.5±0.1	0.02
Ileum							
Normal	62±5	34±5	0.88±0.03	+4±1	2.5±0.5	0.8±0.1	0.01
Chronic renal disease untreated	36±11	30±21	1.17±0.17	+4±2	1.3±1.7	1.1±0.3	0.4
Treated	45±11	26±12	0.97±0.03	+7±1	2.3±0.7	1.2±0.1	0.05
5 mM Calcium test solution							
Jejunum							
Normal	284±21	82±15	4.32±0.17	0±1	5.3±1.3	3.1±0.4	0.05
Chronic renal disease untreated	125±29	63±20	4.88±0.20	−1±1	2.9±1.0	3.2±0.3	0.7
Treated (1 α-(OH)-D_3)	271±40	54±14	4.45±0.10	−2±2	6.4±1.5	2.7±0.3	0.05
Ileum							
Normal	174±12	45±7	4.74±0.10	+5±1	5.0±1.2	4.8±0.3	0.5
Chronic renal disease untreated	104±23	36±6	5.03±0.14	+4±2	3.6±0.9	4.4±0.6	0.5
Treated (1 α-(OH)-D_3)	146±26	31±10	4.75±0.09	+4±3	8.0±2.0	4.5±0.6	0.1

Unidirectional flux rates calculated by the formula of Berger and Steele (26). Potential difference was calculated from the concentration of potassium in plasma and fluid from the distal end of the test segment, using the Nernst equation (29, 30). Negative sign preceding potential difference means the lumen is negative with respect to blood; positive sign means the lumen is positive with respect to blood. The Ussing equation (32) was used to determine the calculated flux ratio for passive calcium diffusion. The concentration of diffusable calcium in plasma was assumed to be 1.5 mM (32). $[Ca]_L$ is the mean luminal calcium concentration. Statistical analysis compares observed and calculated flux ratios.

gests active calcium absorption from the ileum as well as from the jejunum.

Therefore, it can be concluded that the ileum of normal subjects, who had been ingesting an average of about 1,000 mg of calcium per day (Table I), absorbs calcium approximately as well as the jejunum and that this is mediated at least in part by active transport. We found no evidence of calcium secretion by the normal ileum, even when the luminal concentration of calcium was 0.9 mM (which is less than the 1.5-mM diffusible calcium ion concentration in normal plasma [33]).

Our results show that ileal calcium absorption in patients with chronic renal disease is markedly depressed, even more than it is depressed in the jejunum of these patients. (This cannot be attributed to some nonspecific effect of uremia on ileal function, since xylose, water, and electrolytes were absorbed normally.) Therefore, the suggestion (17, 18) that ileal hyperabsorption of calcium partially compensates for a proximal small bowel defect in calcium absorption seems unlikely. In fact, malabsorption of calcium in the ileum probably contributes to negative gastrointestinal calcium balance in patients with chronic renal disease.

The cause of jejunal and ileal malabsorption of calcium in chronic renal disease appears to be due, at least in part, to the absence of active calcium absorption, since experiments with radioactive calcium in these patients revealed a reduced flux out of the lumen and unidirectional flux ratios suggesting only passive calcium diffusion. The unidirectional flux of calcium into the lumen was normal in the jejunum and ileum of the renal disease patients, providing no evidence for an excessive leak of calcium from plasma into the gut lumen.

One of the purposes of the present series of experiments was to examine the change in intestinal transport of calcium that occurs when patients with chronic renal disease are treated with a vitamin D metabolite whose action on the small intestine does not depend on renal metabolism. Our patients were therefore studied before and after 7 days of oral therapy with 2 μg/day of 1α-(OH)-D_3. This is about one-fifth the dose previously used by most investigators and shown to increase radio-

active calcium absorption in such patients (19, 20). Therapy with this vitamin D analog restored jejunal calcium absorption to normal. 1α-(OH)-D_3 also caused an increase in ileal calcium absorption in the renal disease patients, but the ileal defect was not corrected completely to normal. Assuming that all of the chronic renal disease defect in intestinal calcium absorption is due to vitamin D deficiency, these results suggest that the ileum responds more slowly than the jejunum to vitamin replacement. In both areas of the small bowel, 1α-(OH)-D_3 exerted its effect via an increase in the unidirectional flux out of the lumen, which produced flux ratios significantly higher than expected for passive calcium diffusion. There was no change in the unidirectional flux of calcium into the gut lumen. These results suggest that 1α-(OH)-D_3 therapy restores active calcium absorption in the jejunum and ileum of patients with chronic renal disease and that it has no effect on passive calcium diffusion from blood to lumen.

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