# Effect of Dietary Calcium and Age on Jejunal Calcium Absorption in Humans Studied by Intestinal Perfusion

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ABSTRACT Jejunal calcium absorption was measured from test solutions containing 1.0, 2.5, 5, and 10 mM calcium (as calcium gluconate). Absorption rates increased progressively as luminal calcium concentration was increased, although there was a tendency toward saturation of the absorptive process at the higher concentrations. Calcium absorption was higher in normal young adults than in normal subjects over age 60. In both groups a 300 mg calcium diet for 4–8 wk enhanced calcium absorption relative to absorption rates after 4–8 wk on a 2,000 mg calcium diet. This adaptation was more definite and dramatic in the young than in the old subjects. Indirect estimates suggest that adaptation to a low calcium diet and the higher absorption in young than old normal subjects are mediated by an increased $V_{max}$ rather than a decreased $K_m$.

## INTRODUCTION

Physiologic variations in intestinal calcium absorption related to age and diet have been repeatedly demonstrated in animal studies. Young growing rats absorb more calcium than mature rats in vivo and in vitro (1–7), and at any age intestinal calcium absorption in animals is enhanced by a low calcium diet (3, 7–11). With regard to humans, an orally ingested bolus of isotopic calcium is absorbed less well in old than in young people (12, 13), and balance techniques have revealed enhanced calcium conservation by the digestive system following weeks to months of reduced calcium intake (14). These studies have indicated that the human digestive system, like that of animals, varies its rate of calcium absorption with respect to diet and age. However, isotopic and balance techniques for studying calcium absorption in man have inherent limitations. For instance, isotopic calcium absorption is not synonymous with net calcium absorption because of calcium diffusion from blood to intestinal lumen, which at times may even exceed the unidirectional flux of calcium from lumen to blood (15). Absorption rates determined on the basis of balance techniques reflect calcium secretion by the digestive glands as well as intestinal absorption. Neither isotopic nor balance methods measure calcium absorption under defined intraluminal conditions (calcium concentration, pH, sodium concentration, flow rate, etc.).

In an attempt to obtain more information about the effect of dietary calcium and age on intestinal calcium absorption in humans, calcium absorption was studied by the triple-lumen perfusion system (16). This method has the advantage that net absorption or secretion is measured from a specified segment of the small bowel under conditions where calcium concentration, sodium concentration, pH, and other factors can be controlled and systematically varied. We studied old and young subjects who were adapted to a 300 and a 2,000 mg calcium diet for 4–8 wk. These intakes were chosen because the 300 mg diet is known to be insufficient to maintain a positive calcium balance, while the 2,000 mg diet greatly exceeds the normal minimum requirement for a positive calcium balance (14). Calcium absorption was measured in a 60 cm segment of proximal jejunum using four test solutions spanning the range of calcium concentrations in the proximal small bowel following the ingestion of normal food.

## METHODS

*Subjects.* The studies were performed in two groups of normal volunteer subjects. The first group consisted of seven young adults, five men and two women, ranging in age from 22 to 31 yr with a mean age of 28. The second group of subjects consisted of six elderly adults, three men and three women, ranging in age from 61 to 75 with a mean age of 68. All were in good health, took no medications on a regular basis, and had no clinical evidence of osteoporosis.

The subjects were initially assigned to either a low or

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a high calcium diet for 4–8 wk. After absorption studies were completed, they were switched to the opposite diet for a corresponding series of studies. One of the young subjects was studied only on the low calcium diet.

*Diet.* Subjects initially filled out a questionnaire that gave an approximation of their previous calcium intake. They were then randomly assigned to either a low (300 mg/day) or a high calcium (2,000 mg/day) regimen. A dietition instructed each subject in modifications of his usual diet necessary to approximate the desired calcium intake. A daily dietary report was submitted by each subject listing all foods and beverages and their amounts, and the total daily calcium intake was calculated according to a reference text listing the calcium content of foods and beverages (17). Subjects were instructed to further revise their diets if their calcium intake varied more than slightly from the assigned level.

Because of the high caloric intake associated with the 2,000 mg calcium diet, subjects assigned to that diet were given the option of a medium diet (800 mg calcium/day) supplemented by 1,200 mg calcium in the form of calcium gluconate powder; one-third of the daily allotment was taken with each meal. Six of the 13 subjects elected the calcium gluconate powder rather than the high calcium diet. Vitamin supplements other than those in prepared foods were prohibited; vitamin D-fortified milks were permitted.

It was found that in general calcium intake adhered closely to the assigned levels. When errors in intake were observed, they occurred in the appropriate direction, so that subjects on a low calcium intake ingested less than 300 mg/day and subjects on a high calcium intake more than 2,000 mg/day. As already stated, when variations from assigned intake were noted, the subjects were quickly reinstructed so that calcium intake over the 4–8-wk study period closely approximated the desired level in each subject.

*Intestinal perfusion studies.* The triple-lumen intestinal perfusion system was adapted to the study of a slowly absorbed solute by lengthening the distance between sampling sites to 60 cm and the sampling periods to 2 h from each site. Collection from the distal site was staggered 30 min after the corresponding collection from the proximal site, based on volume-flow relationships previously reported (18). The infusion tip of the triple-lumen tube was positioned at the ligament of Treitz. Solutions were prewarmed to approximately 37°C and perfused at a constant rate of 11 ml/min with a Holter pump (Tthe Holter Co., Mt. Laurel Township, N. J.). The rate of sampling from the proximal and distal ends of the test segment was 1 ml/min. Collections were made anaerobically in 30-ml plastic syringes.

Each study consisted of an analysis of calcium absorption from four calcium gluconate solutions having calcium concentrations of 1, 2.5, 5, and 10 mM, respectively. The concentration of ionized calcium in these solutions, determined on an Orion model 99-20/serum calcium flow-through system (Orion Research, Inc., Cambridge, Mass.), was 0.9, 2.4, 4.7, and 9.1 mM, respectively. All solutions contained 95 mM NaCl, 5 mM KCl, 17.5 mM $Na_2SO_4$, and 17.5 mM mannitol, with 5 g/liter polyethylene glycol (PEG) [1] as a nonabsorbable marker. This ionic makeup was selected in order to simulate plasma sodium, chloride, and potassium concentrations and to keep net water absorption near zero, so that the flow rate at all levels of the 60-cm jejunal segment would be approximately equal (19). Bicarbonate was omitted from the test solution so that the pH of the perfusates would be slightly acid and thus simulate the pH of fasting (20) and postcibal (21) jejunal contents.

The four test solutions were perfused in random sequence over a 2 day period, two solutions on each of the test days. Each solution was perfused for 3½ h—1 h for equilibration, then a 2 h collection period. (The samples from the distal tube were started and finished 30 min after those from the proximal tube, accounting for the added 30 min of time for the entire perfusion.) At the end of the first day's perfusions, the tube was pulled back until the distal aspirating site reached the proximal duodenum. The tube was then allowed to advance overnight to its previous position for the second day's perfusion.

*Serum and urine studies.* Two 24 h urine collections were obtained during the 48 h immediately preceding each study. Subjects were required to fast for 10 h preceding the perfusions, and a blood sample was obtained at the start of each study for calcium, phosphorus and alkaline phosphatase determinations.

*Analysis of samples.* pH of intestinal samples, performed directly from the collecting syringe, was determined promptly (Duomatic Model 123, Instrumentation Laboratory, Inc., Lexington, Mass.). The contents of the four syringes comprising each total proximal or distal collection were then pooled in plastic containers, and an aliquot was centrifuged and immediately frozen. After thawing, samples for calcium determination were diluted with an automatic diluter (Micromedic Systems, Inc., Wellesley, Mass.) set to aspirate 100 μl of test material and dilute with 5 ml of diluent. (Evaluation of the diluter for the combined aspirate-and-disperse cycle revealed a coefficient of variation of 0.035%.) The diluted solution was delivered into a polystyrene sample cup, loaded on a Beckman automatic sample changer, and aspirated into a tectronic nebulizer and burner mounted on a Beckman Model 1301 atomic absorption accessory (Beckman Instruments, Inc., Fullerton, Calif.) attached to a Beckman Model DB spectrophotometer with output to a Sargent Model S.R.L. recorder (Sargent Welch Co., Skokie, Ill.). All samples were run in duplicate and were rerun if the result varied by more than 1%.

Electrolyte and PEG concentrations of the perfusates were analyzed according to methods previously described (19–22). Serum and urinary calcium concentrations were determined by the method already described for intestinal fluid calcium analysis. Serum alkaline phosphatase concentration was determined by the method of Klein, Read, and Babson (23). Serum phosphorus was done according to the method of Fiske and Subbarow (24).

*Calculations.* Absorption rates for calcium, sodium, potassium, and water were calculated from the perfusion rate, the change in concentration of the nonabsorbable marker (PEG), and the change in concentration of the respective solute (19–22). Results are expressed as the mean±1 SE. In order to represent conditions within the test segment as closely as possible, the calcium concentrations of the perfusate are expressed as the arithmetic mean of the concentrations determined at the proximal and distal collecting sites.

*Statistics.* Results for the high and low calcium diets evaluated alternatively in one group of subjects were compared statistically by means of the paired Student's *t* test. The grouped Student's *t* was used to compare the old and young subjects.

[1] *Abbreviations used in this paper:* $K_m$, Michaelis constant; PD, potential difference; PEG, polyethylene glycol; $V_{max}$, maximum velocity of transport.

## RESULTS

*Estimated previous calcium intake.* By dietary survey it was estimated that calcium intake prior to institution of this study was 1,050±265 mg/day (range 695–1,569) in the young subjects and 943±133 mg/day (range 681±1697) in the old subjects.

*Estimated calcium intake during the study.* On the assigned 300 mg calcium diet the young subjects were estimated to ingest 224±21 and the old subjects 250±27 mg calcium/day. On the assigned 2,000 mg calcium diet the young subjects were estimated to ingest 2,030±120, and the old subjects 2,134±92 mg of calcium/day.

*Intestinal calcium secretion when calcium-free solutions are infused.* In order to estimate the rate of net calcium diffusion into the jejunal lumen, isotonic saline containing PEG as a marker was infused at the ligament of Treitz at a rate of 11 ml/min in 15 normal subjects. This test solution contained no calcium. In fluid aspirated 10 cm beyond the infusion site, the calcium concentration was 0.03±0.003 mM. In fluid collected 20 cm distally (distal aspirating site located 20 cm beyond proximal aspirating site), the concentration of calcium was 0.05±0.004 mM. The arithmetic mean calcium concentration in the test segment was 0.04±0.003 mM, and the rate of calcium secretion was 0.008±0.002 mmol/h per 20 cm. These results are used later in the calculation of $V_{max}$ and $K_m$ for jejunal calcium absorption. The data are also of interest since they indicate the degree of permeability of the human jejunum to net passive calcium secretion down chemical concentration gradients (lumen calcium 0.04 mM, plasma diffusible calcium assumed to be 1.5 mM [25]). For instance, if this rate of calcium secretion applies throughout the 300 cm of small intestine, calcium secretion in the small bowel could be 0.123 mmol (4.92 mg)/h or 2.95 mmol (118 mg)/24 h. (This estimate would apply only to the conditions of our experiment and would not necessarily be valid after subjects ingest a meal.) Under similar experimental conditions, Cramer noted approximately the same rate of calcium secretion in jejunal and ileal loops of dog intestine (26) that we observed in the human jejunum.

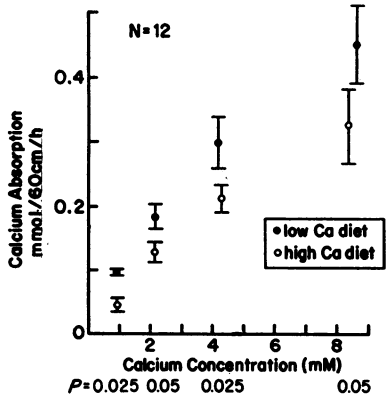


FIGURE 1 Calcium absorption rate at different luminal calcium concentrations. 12 subjects were studied twice, once on a 300 mg and once on a 2,000 mg calcium diet. *P* values are by paired analysis.

*Effect of calcium diet on intestinal calcium absorption.* Paired studies were performed in 12 normal individuals after comparable periods of time on the 2,000 and 300 mg calcium diets. The ages of the subjects ranged from 24 to 75 yr with a mean age of 50. Mean duration on the low calcium diet was 6.0 wk and on the high calcium diet 5.6 wk. As shown in Fig. 1, mean calcium absorption was higher when the subjects were on the low than on the high calcium diet at all four luminal calcium concentrations ($P \leq 0.05$). With both the high and low calcium diets absorption rate increased with increasing luminal calcium concentration, although there was a tendency for absorption rate to plateau at the higher calcium concentrations. Calcium absorption against a concentration gradient on both the low and high calcium diets is demonstrated by the fact that absorption occurred at the 1 mM calcium concentration, which is below the 1.5 mM diffusible and 1.2 mM ionized calcium concentration in plasma (25).

Table I shows that the rates of water, sodium, and potassium absorption were slightly higher on the low than on the high calcium diet and that these small differences were statistically significant by paired analysis. The pH of the collected perfusate was slightly lower on the low calcium diet than on the high calcium diet, and this difference was also statistically significant by paired analysis. We have no explanation for these unexpected findings, but the magnitude of the differences are so small that they are unlikely to have any direct effect on the rate of calcium absorption.

Potassium concentration at the distal collecting site of the triple-lumen tube averaged 5.0 meq/liter on both diets. This value is of interest because potassium equilibrates passively across the human small intestine in accord with electrochemical gradients, and potential difference (PD) can be indirectly estimated from luminal potassium concentration (27). Measured serum potassium concentration in our subjects was 4.3±0.1 meq/liter, and by the Nernst equation these results indicate that the PD across the test segment was about 3.0 mV (lumen negative). Since luminal potassium concentration in the distal sample was not statistically different on the two diets, the change in calcium absorption induced by calcium diet is probably not related to a change in PD across the jejunal mucosa.

The rate of water and sodium absorption and pH were approximately equal after perfusion of the four different test solutions, indicating that changing the calcium concentration of jejunal contents did not affect these results.

TABLE I

*Effect of Calcium Diet in 12 Subjects*

| Dietary calcium | pH | Sodium movement* | Potassium movement* | Water movement* | Final [K] | Serum [Ca] | Urine calcium excretion | Serum [P] | Serum alkaline phosphatase |
|---|---|---|---|---|---|---|---|---|---|
| | | *meq/60 cm/h* | *meq/60 cm/h* | *ml/60 cm/h* | *meq/liter* | *mg/100 ml* | *mg/24 h* | *mg/100 ml* | *King-Armstrong U* |
| 300 mg | 6.13 ±0.04 | −1.1 ±0.9 | −0.3 ±0.04 | −31 ±7 | 5.0 ±0.05 | 9.7 ±0.2 | 145 ±10 | 3.6 ±0.2 | 7.4 ±0.8 |
| 2,000 mg | 6.23 ±0.04 | +1.0 ±0.9 | −0.2 ±0.03 | −27 ±6 | 5.0 ±0.05 | 9.8 ±0.1 | 202 ±23 | 3.7 ±0.1 | 7.1 ±0.9 |
| *P* | 0.02 | 0.02 | 0.005 | 0.01 | NS | NS | 0.05 | NS | NS |

* Negative values signify net absorption, positive values indicate net secretion.

Raising the calcium concentration from 1 to 10 mM was associated with a slight fall in final luminal potassium concentration (5.09–4.85), which was statistically significant and suggests that the higher calcium concentration is associated with a slightly lower PD across the jejunal mucosa.

Table I shows that serum calcium, phosphorus, and alkaline phosphatase were approximately equal on the low and high calcium diets. Urinary calcium excretion was, however, 39% higher on the high than on the low calcium diet, and the difference was statistically significant.

*Effect of an acute calcium load on calcium absorption.* The data reported in Fig. 1 indicate that the human jejunum adapts its rate of calcium absorption to the level of calcium in the diet. These studies were conducted after 4–8 wk on a low or a high calcium diet, and it is not known how rapidly intestinal adaptation occurs. In order to see if the jejunum could adapt more or less immediately in response to the luminal calcium concentration, five subjects who for 8 wk had been on a low calcium diet were studied with the 2.5 mM calcium solution. This was perfused before and immediately after 1 liter of 20 mM calcium was infused over a 90 min period. Absorption before the 20 mM calcium infusion was 0.17±0.02 mmol/60 cm per h, compared with 0.15±0.02 after the high calcium infusion ($P > 0.5$ by paired analysis). Thus, the human jejunum does not reduce its calcium absorption rate immediately in response to a high calcium concentration in its luminal contents.

*Effect of age.* As shown in Fig. 2, on the low calcium diet the young subjects absorbed about 45% more calcium than the older subjects, and the differences were statistically significant ($P < 0.05$) at the two highest

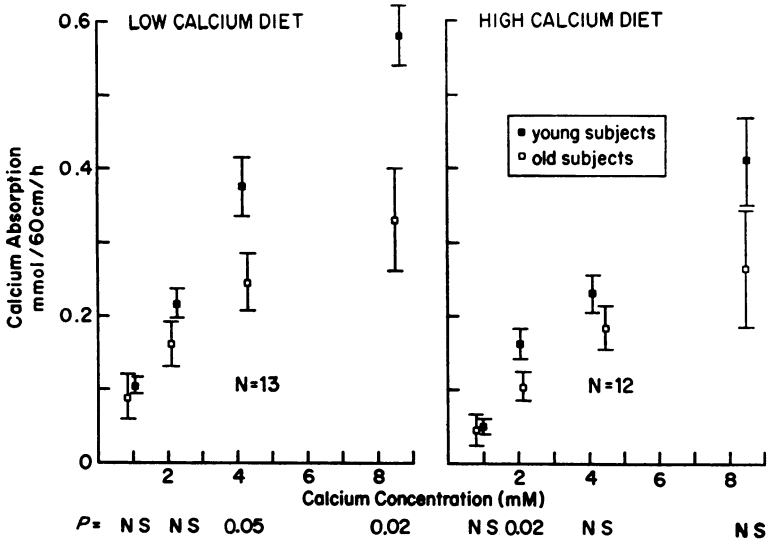


FIGURE 2 Effect of age on calcium absorption. Seven young and six old subjects were studied on the low calcium diet, and six old and six young subjects were studied on the high calcium diet. *P* values are by grouped Student's *t* test.

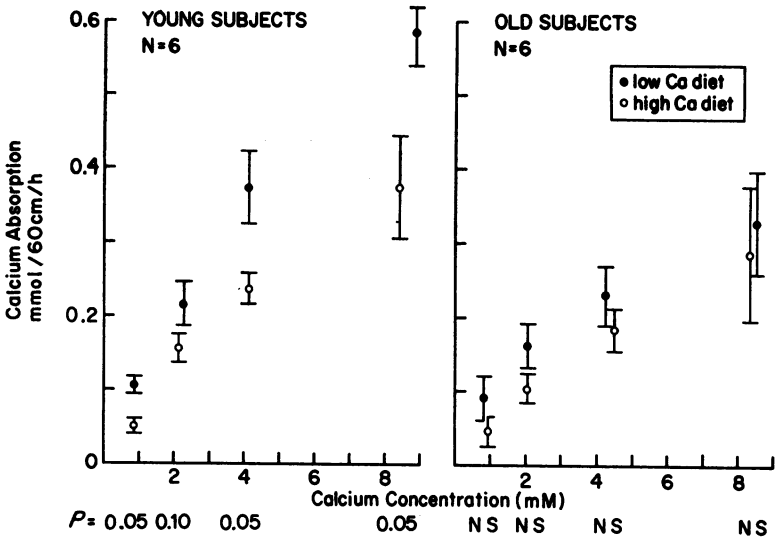


FIGURE 3 Effect of calcium diet in young and old subjects. Each subject was studied twice, once on the low and once on the high calcium diet. $P$ values are by paired analysis.

luminal calcium concentrations. On the high calcium diet young subjects absorbed about 35% more calcium than did the old subjects, but the difference was satistically significant at only one of four luminal calcium concentrations. On both diets the old and young subjects absorbed calcium against a concentration gradient.

The capacity for increased calcium absorption in response to a low calcium diet in old and young subjects is compared in Fig. 3. Mean absorption rates increased by about 66% in the young subjects and by about 50% in the old subjects when they switched from a high to a low calcium diet. These differences are statistically significant in the young but not the old subjects when analyzed by paired analysis.

Table II shows the absorption data and the serum and urinary values in the old and young subjects on the low calcium diet. Water, sodium and potassium were absorbed at slightly higher rates in the young than in the old subjects, although absorption rates in both groups were near zero (a maximum of about 5% of the fluid perfused was absorbed), in accord with the design of the test solutions. The luminal fluid pH was slightly but significantly higher by statistical analysis in the young than in the old subjects. The younger subjects had a slightly higher average serum calcium concentration than the old subjects ($P < 0.02$).

It is of interest that the older subjects had somewhat higher values for urinary calcium (difference not significantly different) than the young subjects in spite of the fact that they absorbed les calcium in the intestinal perfusion experiments. Similar data for the high calcium diet are shown in Table III.

*Effect of sex.* There was no significant difference in calcium absorption rate at any luminal calcium concentration between the eight men and five women in this study. This generalization held true in both the young and old subjects on both levels of previous dietary calcium intake.

TABLE II

*Effect of Age in Subjects on a Low Calcium Diet*

| Subjects | pH | Sodium movement* | Potassium movement* | Water movement* | Final [K] | Serum [Ca] | Urine calcium excretion | Serum [P] | Serum alkaline phosphatase |
|---|---|---|---|---|---|---|---|---|---|
| | | *meq/60 cm/h* | *meq/60 cm/h* | *ml/60 cm/h* | *meq/liter* | *mg/100 ml* | *mg/24 h* | *mg/100 ml* | *King-Armstrong U* |
| Young | 6.23 | −3.9 | −0.4 | −44 | 5.0 | 10.1 | 130 | 3.8 | 5.7 |
| n = 7 | ±0.05 | ±1.2 | ±0.05 | ±9 | ±0.06 | ±0.2 | ±14 | ±0.2 | ±0.3 |
| Old | 6.01 | +1.3 | −0.3 | −19 | 5.1 | 9.4 | 152 | 3.3 | 9.2 |
| n = 6 | ±0.05 | ±0.9 | ±0.06 | ±8 | ±0.05 | ±0.1 | ±15 | ±0.2 | ±1.3 |
| $P$ | 0.005 | 0.005 | NS | NS | NS | 0.02 | NS | NS | 0.025 |

* Negative values signify net absorption, positive values indicate net secretion.

TABLE III

*Effect of Age in Subjects on a High Calcium Diet*

| Subjects | pH | Sodium movement* | Potassium movement* | Water movement* | Final [K] | Serum [Ca] | Urine calcium excretion | Serum [P] | Serum alkaline phosphatase |
|---|---|---|---|---|---|---|---|---|---|
| | | *meq/60 cm/h* | *meq/60 cm/h* | *ml/60 cm/h* | *meq/liter* | *mg/100 ml* | *mg/24 h* | *mg/100 ml* | *King-Armstrong U* |
| Young | 6.25 | +0.1 | −0.2 | −20 | 5.0 | 9.8 | 167 | 3.9 | 6.5 |
| n = 6 | ±0.06 | ±1.2 | ±0.06 | ±7 | ±0.07 | ±0.2 | ±39 | ±0.2 | ±1.0 |
| Old | 6.14 | +3.3 | −0.1 | +9 | 5.1 | 9.6 | 202 | 3.5 | 7.8 |
| n = 6 | ±0.05 | ±1.2 | ±0.04 | ±6 | ±0.05 | ±0.1 | ±34 | ±0.2 | ±1.3 |
| *P* | NS | NS | NS | 0.05 | NS | NS | NS | NS | NS |

* Negative values signify net absorption, positive values indicate net secretion.

*Kinetic analysis.* As already noted, absorption rates of calcium exhibited a tendency to plateau as increasing luminal calcium concentrations were perfused. However, an analysis of these data in terms of transport constants ($V_{max}$ and $K_m$) is not possible unless a correction is made for passive calcium absorption or secretion in response to concentration gradients. It is extremely difficult to obtain the information necessary for such corrections in an in vivo human system due to the fact that plasma calcium concentration cannot be varied over a wide range. A method by which kinetic constants can be calculated is illustrated in Fig. 4. One correction point is obtained by assuming that passive calcium transport is zero when luminal and plasma concentrations are equal at 1.5 mM, which is the concentration of diffusible calcium in plasma (25). A second correction point was obtained by measuring the rate of net calcium secretion into the jejunal lumen when luminal concentration was near zero. When a test solution containing no calcium was infused into the jejunal lumen of 15 normal subjects, the rate of calcium secretion was 0.025±0.002 mmol/60 cm per h, and the average calcium concentration between the two collecting points was 0.04±0.003 mM.[2] These

[2] These studies are described in more detail in the third paragraph of Results. The experiments were done with a 20 cm test segment, but the results are expressed per 60 cm here for comparison with absorption data carried out in 60-cm jejunal segments.

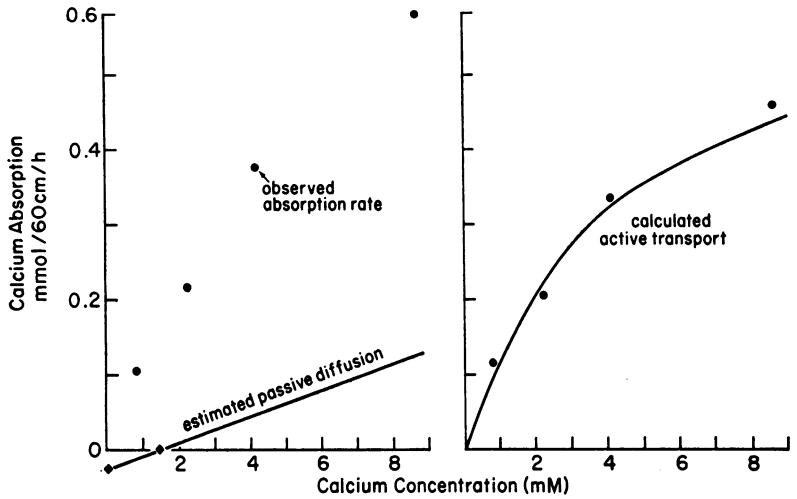


FIGURE 4 Method of estimating passive calcium diffusion. On the left side the observed rates of calcium absorption at different luminal calcium concentrations are plotted (circles). The diamond shaped symbols show the rate of calcium diffusion into the lumen when luminal calcium concentration was near zero, and the rate of calcium diffusion (assumed to be zero) when luminal and plasma diffusible calcium concentrations are equal at 1.5 mM. A straight line through these points is assumed to approximate the rate of passive calcium diffusion at any given luminal calcium concentration. On the right side of the diagram, estimated passive diffusion has been subtracted from observed absorption rates to give the calculated active transport. The line through the points is calculated by the formula for nonlinear regression.

TABLE IV

*Kinetic Constants Calculated by Nonlinear Regression**

| Group | $V_{max}$ | Apparent $K_m$ | Correlation coefficient |
|---|---|---|---|
| | *mmol/60 cm/h (mean ±SEM)* | *mM (mean ±SEM)* | |
| All ages—paired | | | |
| Low calcium diet | 0.44±0.04 | 3.1±0.65 | 0.999 |
| High calcium diet | 0.29±0.02 | 3.5±0.48 | 0.999 |
| Low calcium diet | | | |
| Young | 0.76±0.07 | 5.6±1.10 | 0.999 |
| Old | 0.25±0.01 | 1.3±0.26 | 0.999 |
| High calcium diet | | | |
| Young | 0.48±0.14 | 5.5±3.10 | 0.994 |
| Old | 0.19±0.01 | 2.0±0.32 | 0.999 |
| Young—paired | | | |
| Low calcium diet | 0.74±0.08 | 5.4±1.10 | 0.999 |
| High calcium diet | 0.38±0.06 | 4.0±1.40 | 0.997 |
| Old—paired | | | |
| Low calcium diet | 0.25±0.008 | 1.4±0.15 | 0.999 |
| High calcium diet | 0.22±0.018 | 2.9±0.60 | 0.999 |

* Passive diffusion was estimated and subtracted from observed absorption rates according to the method described in Fig. 4 and in the text.

two correction points are plotted in Fig. 4; a line extended through them is drawn, assuming that passive calcium absorption or secretion is linearly related to the concentration gradient between intestinal lumen and plasma between zero and 10 mM calcium concentration. Data in rats in vitro suggest that this assumption is valid for both a high and a low calcium diet (10).

If this correction is made, calcium transport secondary to passive calcium diffusion can be subtracted from the observed calcium absorption rates, and kinetic constants for active calcium absorption can be obtained by the Michaelis-Menten equation for nonlinear regression:

$$V = \frac{(V_{max} \times \text{Conc})}{(K_m + \text{Conc})},$$

where $V$ is the transport rate at any given concentration, $V_{max}$ the maximum velocity of transport, and $K_m$ the concentration at which transport rate is one-half maximal.

Table IV shows the estimated kinetic constants for the experimental results depicted in Figs. 1–3. It will be noted that higher absorption in young than old subjects and adaptation to a low calcium diet is associated with an increase in $V_{max}$ rather than a decrease in apparent $K_m$.[3] The agreement between the corrected points and the formula for nonlinear regression was excellent, with $r$ values approaching unity. This indicates that the formula for nonlinear regression is an excellent description of our corrected experimental results.

[3] Apparent $K_m$ is used to signify that the results have not been corrected for the effect of unstirred layers (28, 29). Such corrections are probably impossible in vivo and have never been estimated in vitro for calcium transport.

## DISCUSSION

Previous studies have shown that the pH of intestinal contents after normal food varies from 3.5 to 6.7 in the proximal jejunum, with most values below 6.0. Beyond the mid-small bowel the pH rises to an average level of 7.6, and no samples have a pH lower than 6.5 (21). These values are of significance because in the presence of phosphorus and other dietary constituents calcium tends to precipitate above a pH of 6.1 (30). Therefore, under normal conditions dietary calcium is probably maximally soluble (and therefore more absorbable) in the duodenum and proximal jejunum and in most experimental animals the proximal small bowel has a greater capacity for active calcium transport than more distal segments of the small bowel. Previous studies also indicated the calcium concentrations normally found in the proximal small bowel after eating normal food. After a meal of steak, toast, butter, and salad (which if ingested three times a day would correspond to a calcium intake of 216 mg of calcium per day), calcium concentration in the proximal small bowel varied between 0.3 and 2 mM, whereas after a meal containing 250 ml of milk (which would correspond to 939 mg of calcium per day), the calcium concentrations in the proximal bowel were between 3 and 8.5 mM (21). Thus, the physiologically important calcium concentrations in the proximal small bowel vary between 0.3 and 8.5 mM when subjects ingest a low and moderately high amount of dietary calcium.

Using the triple-lumen perfusion system we therefore studied calcium absorption in 60 cm of proximal jejunum, starting at the ligament of Treitz, and used four different test solutions containing 1.0, 2.5, 5, and 10 mM calcium as calcium gluconate. The sodium, potassium, and chloride concentrations in the jejunal lumen were maintained constant at levels approximating those of the extracellular fluid, and the composition of the test solutions was designed to maintain a flow rate of about 10 ml/min throughout the 60 cm jejunal segment. The normal jejunum adjusts its luminal contents to a pH near 6.2, probably by Na:H exchange (19, 20), so that the intestinal fluid pH was constant at a level near 6.2 without the addition of buffer to the test solutions. At this pH calcium gluconate is about 99% ionized.

By our method, disappearance of calcium from the intestinal lumen is assumed to represent absorption of calcium. Theoretically, however, calcium might be taken up by the intestinal cells, accumulate there, and later be resecreted into the intestinal lumen. Under this condition, disappearance of calcium from the lumen is not equal to absorption of calcium into the body. We think this possibility is unlikely for two reasons. First, all our calcium absorption studies lasted 2 h and were begun after a 1 h equilibration period. It seems likely that a steady state would have been reached after these relatively long per-

fusion periods and that calcium disappearing from the lumen would be balanced by calcium entering the body. Second, the absorption studies before and after a 20 mM calcium infusion argue against the accumulation-resecretion theory. Calcium accumulation within the mucosa would be expected to be greater during a 20 mM calcium perfusion than in any of our other absorption studies since the highest concentration of calcium infused during the absorption studies was 10 mM. If calcium accumulated in intestinal cells is later resecreted into the intestinal lumen, a 20 mM calcium solution infused into the lumen should reduce the subsequent rate of calcium disappearance from the lumen. However, the data revealed that calcium disappeared from the jejunal lumen (2.5 mM perfusion) at the same rate before and after the jejunum was perfused for 90 min with a 20 mM calcium solution. For these reasons, we think it is likely that calcium absorption rates as measured by intestinal perfusion are true measures of net intestinal absorption into the body. Admittedly, definitive proof is not available.

In both young and old subjects on both high and low calcium diets, calcium was absorbed from the jejunum when the luminal calcium concentration was less than plasma diffusible calcium concentration (1.5 mM) and less than plasma ionized calcium concentration (1.2 mM) (25). Thus, jejunal calcium absorption occurred against a calcium concentration gradient, in accord with the results of previous studies by Wensel, Rich, Brown, and Volwiler (15) and by Ewe (31). Since the test solutions in the present study were designed so that water absorption would be near zero (water absorption rates by actual determinations were less than 5% of the infused test solution), this calcium absorption against a concentration gradient cannot be attributed to solvent drag. In previous studies we have shown that the normal jejunum has a potential difference (PD) of near zero (20) when it is perfused with solutions similar to those used in the present studies; therefore, calcium absorption rates reported herein were probably not influenced by an electrical gradient. Although PD was not determined in the present studies, it can be indirectly estimated from the final (distally collected) potassium concentration in the jejunal lumen relative to plasma potassium concentration (27). The results suggested that the jejunal PD was about 3 mV, lumen side negative. Thus, calcium was absorbed against both an electrical and a concentration gradient, when water flow was near zero. These findings are indicative of active intestinal transport, which has been demonstrated previously in animal preparations in vitro and in vivo.

The rate of calcium absorption increased progressively as luminal calcium concentration was increased from 1 to 10 mM. These results are in contrast to those reported by Ewe, who studied calcium absorption in a 30 cm segment of human jejunum isolated between two balloons and found that calcium absorption did not increase as luminal calcium concentration was increased above 2.25 mM (31). The reason for this discrepancy between our study and the results of Ewe is not clear.

Although jejunal calcium transport did not attain a level of saturation within the range of concentrations studied, the rate of absorption did begin to plateau at the higher calcium concentrations (Figs. 1–4). This would be compatible with both active carrier mediated calcium transport and passive calcium diffusion secondary to concentration gradients. That there is some passive calcium diffusion in response to concentration gradients is evident from the fact that when the luminal contents were initially calcium free, calcium was secreted into the jejunal lumen. The magnitude of this passive secretion, in relation to absorption rates at higher luminal concentrations, is depicted in Fig. 4.

The present results demonstrate that calcium absorption at all four luminal calcium concentrations was enhanced when subjects had been on a 300 mg calcium diet for 4–8 wk, compared with calcium absorption rates when the same subjects had ingested a 2,000 mg calcium diet for a comparable period. This confirms previous work in laboratory animals (3, 7–11) and in humans (12, 14, 32) studied by calcium balance and isotopic techniques. The mechanisms whereby the intestine adapts its rate of absorption to meet the needs of the body is unknown, although it has been suggested that the effect may be mediated by an increased level of calcium binding protein in the intestinal mucosa (33) and/or by the stimulus of hypocalcemia, resulting in secretion of parathyroid hormone, which in turn stimulates the synthesis in the kidney of 1,25-dihydroxycholecalciferol (34, 35).

Some insight into the adaptation mechanism might result from an analysis of the kinetics of calcium transport on a high and a low calcium diet. Walling and Rothman conducted such a study in rats in vitro, and they found that animals on a low calcium diet had the same $V_{max}$ but a much lower $K_m$ than animals on a high calcium diet (10). They postulated that the low calcium diet enhanced the affinity of the active transport site for calcium in some manner, possibly by an increase in calcium-binding protein within mucosal cells. They concluded that since $V_{max}$ was unchanged by diet, the total amount of active transport carrier was presumably unchanged.

Our studies, on the contrary, suggest that a change in $V_{max}$ rather than $K_m$ is associated with the intestinal adaptation to a low calcium diet. However, it must be pointed out that it is difficult to measure accurately $V_{max}$ and apparent $K_m$ of the active calcium transport carrier in vivo. This is because it is impossible to measure directly the passive transport of calcium as intraluminal

calcium concentration is raised above plasma calcium concentration. A method for estimating the passive component was presented in the Results section, and when calculated net passive absorption or secretion is subtracted from observed absorption or secretion, the active component of transport is derived indirectly. Due to the uncertainty involved in estimating the diffusion component, the kinetic constants derived from this analysis must be regarded as approximations. The results are of interest, however, in that they suggest that adaptation to a low calcium diet is associated with an increase in $V_{max}$ rather than a fall in apparent $K_m$. As already noted, this is in conflict with the results of Walling and Rothman in rats in vitro, where a low calcium diet led to a lower apparent $K_m$ but no change in $V_{max}$ (10). Until better methods are devised for measuring calcium diffusion rate at different luminal calcium concentration in vivo, it will be impossible to say if this discrepancy is due to species variation, to differences in in vivo and in vitro preparations, or to inaccuracy in our estimate of the passive calcium transport rate.

Two other observations are of interest in regard to adaptation of the jejunum in response to dietary calcium intake. First, whatever the mechanism of enhanced absorption after a period of low dietary calcium intake, our results indicate that calcium absorption cannot be suppressed acutely by intrajejunal infusion of a high concentration of calcium. Second, the fact that our subjects on a low calcium diet absorbed slightly but significantly more sodium, potassium and water than when they were on the high calcium diet suggests that other jejunal transport mechanisms than calcium may be enhanced by a low calcium diet. Studies specifically designed to test this hypothesis should be carried out.

Our comparative calcium absorption studies in young adult and old normal subjects revealed that young people absorb more calcium at any given luminal calcium concentration than old people. This was true for both the high and low calcium diets but was more marked on the low calcium diet. When the absorption rates from the high and low calcium diets were compared, the data suggested that young subjects adapt better to a low calcium diet than old subjects. For instance, with the 10 mM calcium solution the young subjects previously on a low calcium diet absorbed calcium at a rate 56% higher than when they had been on a high calcium diet. By contrast, the corresponding value in the older subjects was only 16%.

Previous animal studies in vivo and in vitro as well as human studies using isotopic calcium to estimate calcium absorption have also shown higher calcium absorption in young than in old subjects, but the mechanism is not clear. There is no reason to attribute our results to differences in diet or sunlight. Our young subjects were all employed full-time or enrolled in graduate programs that kept them indoors much of the time, whereas most of our elderly subjects were active, spending more daytime hours out of doors than their younger counterparts. The intake of calcium and vitamin D by the young and old subjects prior to beginning our study, as estimated by dietary survey, did not differ in the two groups.

Our kinetic analysis suggests that young people have a higher $V_{max}$ and a similar apparent $K_m$ compared with the older subjects. This in turn suggests that young people simply have more active transport carrier than older people. As already emphasized, the reliability and significance of calcium kinetic constants in vivo must be accepted with caution.

It is of interest to compare the calcium absorption rates observed here with the jejunal calcium concentrations after ingestion of normal food (21). After a low calcium meal jejunal calcium concentration varies from 0.3 to 2 mM, whereas after a moderately high calcium meal the luminal calcium concentration increases to as high as 8.5 mM. Figs. 2 and 3 show that jejunal absorption rate would increase fivefold in young subjects and fourfold in old subjects as luminal calcium concentrations increased from 0.7 to 8.5 mM, provided they were adapted to a low calcium diet. If they were adapted to a high calcium diet, absorption rate increased 7.5-fold and 6-fold in young and old subjects as luminal calcium concentration increased from 0.7 to 8.5 mM. These results illustrate the critical importance of luminal calcium concentration (i.e., dietary calcium) in determining the amount of calcium absorbed from the human jejunum, especially in older subjects. It should be noted that this conclusion would not have been reached had jejunal calcium absorption become saturated at the very low level (2.25 mM) reported by Ewe (31).

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