Vitamin D and the Dual Processes of Intestinal Calcium Absorption

R. H. Wasserman

Department of Biomedical Science, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853

It is well accepted that the intestinal absorption of calcium occurs by 2 distinct mechanisms, a saturable active transport process and a nonsaturable passive diffusion process. The relative importance of these 2 processes and their vitamin D dependency was the subject of recent communications to this Journal. McCormick (1), in an Issues and Opinion statement, put forth the view that calcium absorption under normal conditions occurs primarily by a saturable process requiring vitamin D. Bronner et al. (2) countered with the view that the major mode of calcium absorption is by vitamin D–independent passive diffusion and not by active transport. Bronner et al. (2) further stated that in the ileum all calcium is absorbed by the passive route which, as stated before, they consider to be a vitamin D–independent process. McCormick (1) agreed that absorption by passive diffusion is independent of vitamin D. Contrary to the aforementioned views, evidence is presented suggesting that the ileum, where most of dietary calcium is absorbed, can absorb calcium by a vitamin D–dependent active process, and that the passive diffusion process of calcium absorption can be positively affected by vitamin D.

A brief description of the processes of intestinal calcium absorption. Active calcium transport, prominent in the mammalian and avian duodenum, is by way of the saturable transcellular path, with 3 primary components participating in series in the calcium absorption process. These include the recently identified epithelial calcium channels (CaT1 and ECaC), which enhance the downhill (energetically speaking) transfer of luminal calcium into the absorbing enterocyte (3,4); the intracellular calbindins (mammalian calbindin-D9k and avian calbindin-D28k), which bind calcium at high affinity and may increase the overall rate of trans-cytosolic diffusion of calcium (5,6); and the ATP-activated basolateral membrane calcium pump (7), which transports cytosolic calcium uphill into the extracellular fluid of the lamina propria. The vitamin D hormone, 1,25-dihydroxycholecalciferol [1,25(OH)2D3], stimulates the synthesis of the epithelial calcium channels (8) and the plasma membrane calcium pumps (9,10), and induces the formation of the calbindins (11).

In contrast, calcium absorbed by the diffusional mode traverses the intestinal cellular membrane by way of the paracellular path, the “microspace” between adjacent enterocytes of the epithelial cellular membrane. Net absorption of calcium by the diffusional process requires a luminal free Ca concentration of ~2-6 mmol/L to overcome the energy barrier comprised of a positive transtransmural electropotential (approximately +6 mV), and the free calcium concentration in the extracellular fluid of the lamina propria of ~1.25 mmol/L (12,13). At concentrations >2–6 mmol/L, the absorption of calcium via this route is directly related to luminal calcium concentration.

Tang and Goodenough (14) recently summarized the characteristics of paracellular tight junctions of epithelial membranes purported to have biophysical properties similar to conventional ion channels showing selectivity, competition between ions, dependency on concentration, and modifiable by pH.

Intestinal sites of calcium absorption. An important consideration is the relative contribution of the different segments of the intestinal tract to overall calcium absorption, with the small intestine accounting for about 90%. Despite the vigor of the active transport process by the duodenum, most of the absorption of ingested calcium occurs in the lower segment of the small intestine, the ileum. This was shown by Marcus and Lengemann (15) who reported that, for the rat small intestine, 88% of calcium absorption in this segment occurs in the ileum, 4% in the jejunum, and 8% in the duodenum. Using radiostrontium as a calcium tracer, Cramer and Copp (16) found that 65, 17, and 7% of the absorption of calcium in the rat intestine occurs in the ileum, jejunum, and duodenum, respectively. In dogs, the respective values in the ileum, jejunum, and duodenum are 80, 16, and 4% (17). An important factor determining the contribution of the ileum to overall calcium absorption is the relatively long transit time of calcium in that segment relative to the other segments of the small intestine. The transit half-time in rat ileum, as determined by Marcus and Lengemann (18), is 102 min and in the duodenum, 6 min; Duflos et al. (19) estimated that sojourn times in rat ileum and duodenum are 121.5 and 2.2 min, respectively.

The colon accounts for <10% of the total amount of calcium that is absorbed (17,20).

The question of vitamin D–dependent active ileal calcium absorption. An indication of the importance of vitamin D on ileal calcium absorption comes from experiments comparing total calcium absorption in vitamin D–deficient subjects before and after vitamin D treatment, realizing that ~70–80% of the ingested calcium is absorbed by the ileal segment. A case in point is the study of Sheikh et al. (21) in which the effect of 1,25(OH)2D3 on net calcium absorption in dialysis patients with renal disease was investigated. That report was commented upon by both McCormick (1) and Bronner et al. (2). Reference is made only to the dialysis patients consuming a normal calcium meal. The serum levels of 1,25(OH)2D3 in the untreated dialysis patients was 9 pg/mL (22 pmol/L). After oral intake of 1.0 µg (2.4 nmol) 1,25(OH)2D3 twice daily for 9 d, the serum level of vitamin D hormone was 91 pg/mL (218 pmol/L). Each group consumed 301 mg of calcium in the test...
meal. As displayed in Table 1, the net calcium absorption in the untreated group was 43 mg; in the treated group, it was 175 mg, an increase of ~300% due to treatment. With about 90% of ingested calcium absorbed in the small intestine and with the reasonable assumption that the relative absorption of calcium in the different segments of the small intestine of humans is similar to that of monogastric animals, it follows that most of the 1,25(OH)2D3-dependent increase must have occurred in the ileum, as shown in Table 1. The relative absorption of calcium by segments of the small intestine could be affected by treatment but, when the relative segmental sojourn times are considered, this is not of sufficient magnitude to alter the view that most of the calcium absorption takes place in the ileum, both in the presence and absence of vitamin D.

What then is the process of calcium absorption in the ileum that is stimulated by 1,25(OH)2D3? Wasserman et al. (12) and Karbach (35), using similar Ussing chamber procedures, demonstrated, using in vitro transport chambers, that adaptation of rats to low-calciu diets, which stimulates the formation of 1,25(OH)2D3, increases the ileal mucosal-to-serosal (absorptive) flux of calcium. Also, Vergne-Marini et al. (26), in a study of patients with chronic renal disease, provided evidence for the 1,25(OH)2D3-mediated active calcium transport in human ileum. Further, calbindin-D9K and the basolateral membrane calcium pump were detected and quantified in rat ileum by immunological procedures, and their dependency on vitamin D was documented (27,28). Thus, rat ileum contains 2 important proteins required for active calcium transport, i.e., calbindin-D9K and the calcium pump. However, epithelial calcium channels recently identified in rat duodenum and jejunum have not yet been found in rat ileum but are present in the lower intestinal segments of humans (29) and mice (30). Even without the microvillar calcium channels, calcium can enter the rat enterocyte by simple diffusion and then actively transported from the cell interior to extracellular fluid by the actions of calbindin-D9K and the basolateral calcium pump.

The information presented above provides strong evidence that rat ileum has the capacity to actively transport calcium by a vitamin D–mediated process, although at a slower rate compared with the duodenum. The slower rate is due to the apparent absence of an entry calcium channel in rat ileum (but present in the ileum of humans and mice) and to the lower concentrations of calbindin and the membrane calcium pump in the ileum compared with the duodenum. Despite this slower rate, the active transport process in the ileum would have a considerable effect on overall calcium absorption because of the relatively long transit time of calcium in the ileum compared with the duodenum and jejunum.

**The question of the vitamin D dependency of paracellular calcium diffusion.** Earlier transport studies on chick and rat intestine suggested that vitamin D not only increases active calcium absorption, but also increases the diffusional, nonsaturable absorption of calcium (31,32). Since then, a number of reports appeared in support of the notion that vitamin D has the ability to enhance paracellular calcium transport. These include studies by Hurwitz and Bar (33) on the intact chick; by Karbach (34) on flux measurements of all segments of the rat small intestine by the Ussing chamber procedure; by Jungbluth and Binswager (35), using similar Ussing chamber procedures, which provided “further evidence that the calcium transport in intestinal Ca transport is to increase cell-mediated active mucosal-to-serosal transport and paracellular diffusion in Ca transport” and by Chihrayath et al. (36) who, from their studies with the Caco-2 intestinal cell line, gave evidence of a vitamin D–dependent paracellular diffusion path at confluence. The Caco-2 cell line experiments of Fleet et al. (37,38) showed that 1,25(OH)2D3 increases the bidirectional fluxes of calcium, a criterion often used to indicate transport via a paracellular path. 1,25(OH)2D3 was also reported to increase the Na+, K+, and Rb+ paracellular permeability of embryonic chick small intestine (39). Further, Dostal and Toverud (40), using the in situ loop technique in 35-d-old rats, determined the relation between duodenal calcium absorption and intraluminal calcium concentration. They showed (40) that the nonsaturable diffusion component of the calcium absorption curve was significantly greater in the vitamin D–replete rats than in the vitamin D–deficient rats.

Although this cited information provides considerable support for absorption of calcium by a vitamin D–dependent diffusional process, additional studies on this matter are warranted because reports and/or views of others (1,2,24,25) do not support this contention. Even so, consideration should be given to possible mechanisms by which vitamin D might affect the diffusional permeability of the tight junctional complexes of the intestinal cellular membrane to calcium. Possibly involved are 1,25(OH)2D3-mediated second messengers, which might influence paracellular permeability by directly or indirectly affecting cytoskeletal activity (41,42). It should also be noted that the hormonal control of paracellular permeability was demonstrated in other systems as exemplified by the studies of Chen et al. (43), Kelly and Wood (44), Gorodeski (45), and Yu and Beyenbach (46).

**TABLE 1**

1,25-Dihydroxycholecalciferol and the relative segmental absorption of calcium by the small intestine of dialysis patients.

<table>
<thead>
<tr>
<th>Calcium absorption2</th>
<th>Relative absorption of calcium by segment3</th>
<th>Untreated group %</th>
<th>Treated group %</th>
<th>1,25D-dependent mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duodenum</td>
<td>7</td>
<td>12</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Jejunum</td>
<td>13</td>
<td>23</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Ileum</td>
<td>80</td>
<td>140</td>
<td>106</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>43</td>
<td>175</td>
<td>132</td>
<td></td>
</tr>
</tbody>
</table>

1 Modified from Sheikh et al. (21). See text for details.
2 Intake by both groups was 301 mg Ca in a test meal.
3 Based on studies described in the text (14–16).

**LITERATURE CITED**

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