Journal of Cellular Biochemistry 88:387–393 (2003)

Mechanisms of Intestinal Calcium Absorption

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Abstract Calcium is absorbed in the mammalian small intestine by two general mechanisms: a transcellular active transport process, located largely in the duodenum and upper jejunum; and a paracellular, passive process that functions throughout the length of the intestine. The transcellular process involves three major steps: entry across the brush border, mediated by a molecular structure termed CaT1, intracellular diffusion, mediated largely by the cytosolic calcium-binding protein (calbindinD_{9k} or CaBP); and extrusion, mediated largely by the CaATPase. Chyme travels down the intestinal lumen in ~3 h, spending only minutes in the duodenum, but over 2 h in the distal half of the small intestine. When calcium intake is low, transcellular calcium transport accounts for a substantial fraction of the absorbed calcium. When calcium intake is high, transcellular transport accounts for only a minor portion of the absorbed calcium, because of the short sojourn time and because CaT1 and CaBP, both rate-limiting, are downregulated when calcium intake is high. Biosynthesis of CaBP is fully and CaT1 function is approximately 90% vitamin D-dependent. At high calcium intakes CaT1 and CaBP are downregulated because 1,25$(OH)_2D_3$, the active vitamin D metabolite, is downregulated. J. Cell. Biochem. 88: 387–393, 2003. © 2002 Wiley-Liss, Inc.

Key words: transcellular transport; paracellular transport; CaT1; calbindinD_{9k}; CaATPase; vitamin D; Ca entry; intracellular Ca diffusion; Ca extrusion

Calcium is absorbed in the mammal along two routes: a transcellular mechanism, predominant in the duodenum, and regulated by vitamin D, and a paracellular, concentration-dependent diffusional process that takes place throughout the length of the intestine [Bronner et al., 1986; Bronner, 1987]. The amount of calcium transferred paracellularly is a positive function of the sojourn time of the chyme in a given intestinal segment and is inversely proportional to the rate of intestinal propulsion. The maximum sojourn time is in the ileum [Bronner and Pansu, 1999], a site where, under normal calcium intakes, the highest proportion of dietary calcium is absorbed [Marcus and Lengemann, 1962].

Figure 1 illustrates calcium absorption in the rat duodenum and ileum, as evaluated by an intestinal loop technique [Pansu et al., 1981, 1983a.] As shown in Figure 1, duodenal calcium absorption can be analyzed as a sum of two processes, a saturable transport, that is well described by a Michaelis–Menten relationship, and a straight, linear, concentration-dependent function. In the ileum, on the other hand, only the linear, concentration-dependent process, identified as paracellular movement through the tight junctions [Bronner et al., 1986], is in evidence.

Table I shows the rate of movement (in terms of sojourn time), of the chyme in the rat intestine, along with the pH in each segment. If the calcium content of the chyme is relatively low, much of the calcium in solution will be absorbed in the duodenum by the active, transcellular process and relatively little will be available for paracellular absorption in the distal jejunum and ileum [Pansu et al., 1993]. If, however, the calcium content of the chyme is high, as with high calcium intake, most of the calcium will be absorbed by the paracellular process, largely in the distal portions of the

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Received 26 July 2002; Accepted 1 August 2002
DOI 10.1002/jcb.10330

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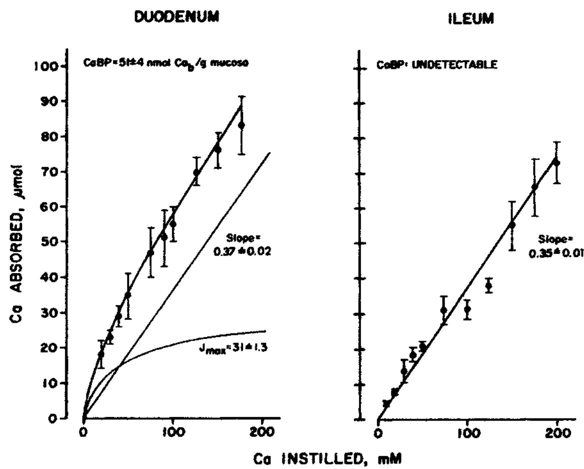

Fig. 1. Calcium absorption in two intestinal segments of rats. Weanling male rats were placed on a high calcium diet (1.5% Ca, 1.5% P) for 10 days. When their weight averaged 77 g, calcium absorption was analyzed by an in situ loop procedure in the duodenum and ileum. The units for J_{max} are micromoles, for CaBP (calmodulinD_{9k}) nanomoles calcium$_{bound}$/g mucosa. Two nmol Ca_{bound} = 1 nmolCaBP. Values are shown with respective standard errors. Reproduced by permission from Pansu et al. [1983b].

small intestine. This is true not only because of the relatively large mass of calcium, but also because in animals on high calcium intake the vitamin D-dependent transcellular process is downregulated [Pansu et al., 1979]. The regulated, transcellular process assumes functional importance, therefore, only under conditions of low calcium intake. In what follows, I shall describe the transcellular process in detail.

TABLE I. pH and Sojourn Time in Segments of the Intestine of Rats Fed a High-Calcium Diet[a]

Segment	Length (cm)	pH	Sojourn time (min)
Small intestine			
Duodenum	8	6.6	3
Jejunum[b]	36		
J_1		>6.6	4
J_2		>6.6	12
J_3		>6.6>8.0	27
Ileum[b]	36		
I_1		>8.0	39
I_2		>8.0	44
I_3		>8.0	58
Total time			187
Large intestine			
Cecum	4	7.6	92
Colon[c]			
C_1[c]	7	7.0	50
C_2	7	ND	42
Total time			184
Total time, small and large intestine			371

[a]Small intestine data from Duflos et al. [1995]. Large intestine data by Pansu (unpublished).
[b]Segments were 12 cm long.
[c]C_1 refers to the ascending colon segment; C_2 refers to the descending colon segment.
ND, not determined.
Reproduced with permission from Bronner and Pansu [1999].

Transcellular calcium movement involves three major steps: entry, mediated by a dual entry mechanism [Slepchenko and Bronner, 2001], intracellular diffusion, mediated by the vitamin D-dependent calbindinD_{9k} [CaBP; Bronner et al., 1986], and extrusion from the cell, mediated largely by the CaATPase [Carafoli, 1994], with the Na/Ca exchanger playing a very minor role [Nellans and Popovitch, 1981, 1984]. Extrusion is not rate limiting for calcium absorption [Bronner et al., 1986], even though there are indications that extrusion can be upregulated by vitamin D [Wasserman et al., 1992].

CALCIUM ENTRY

Calcium entry at the brush border side of the duodenal cell is mediated by an entry structure, termed CaT1 [Hoenderop et al., 1999a; Peng et al., 1999]. Inasmuch as the calcium content of the luminal fluid considerably exceeds the free intracellular calcium concentration—which is generally in the 50–100 nanomolar range—unhindered entry of calcium across the brush border would very quickly raise the intracellular calcium concentration to unacceptable levels. On the other hand, the transit time at which the luminal contents moves down the length of the duodenum is of the order of minutes (Table I). Therefore, if calcium entry were so restricted that virtually no calcium could enter the cell at luminal calcium concentrations above 5 mM—typical of calcium concentrations at feeding times—not enough calcium would be absorbed.

Slepchenko and Bronner [2001] have recently modeled transcellular calcium transport in the rat duodenum with the aid of extensive experimental data. We showed that if a two-mechanism entry step is postulated, transcellular calcium movement can be accurately simulated. The two-step entry mechanism we postulate consists of a carrier-mediated transport, which, saturates with a $K_m = 0.35$ mM, and a $V_m = 2.2$ μmol/h/g. This corresponds to relatively high membrane permeability and is in accord with findings published for CaT1 [Hoenderop et al., 1999b, 2000; Peng et al.,

1999]. The second component is a channel-like flow. Its permeability is much lower than that of the carrier-mediated transport. It is regulated by the intracellular-calcium concentration, with the cytosolic calcium binding to a channel domain or some other molecule that acts as a channel gate. There is evidence that the apical entry of calcium through CaT1 is regulated by intracellular calcium [Hoenderop et al., 1999b; Peng et al., 1999].

This means that the apical permeability to calcium should be a function of the intracellular calcium concentration at the inner side of the brush border. With channel permeability proportional to the fraction of uninhibited channels, the brush border permeability determines the ratio of V_m to K_m, two parameters of a Michaelis–Menten equation, the equation that describes the relationship between luminal calcium concentration and transcellular absorption (cf. Fig. 1).

At luminal calcium concentrations in the tens of millimolar range, apical calcium entry is dominated by the channel flux mechanism. Our model predicts that in the total absence of vitamin D, calcium entry is reduced to less than 10% of what it is in the presence of vitamin D. Experimental findings [Van Cromphaut et al., 2001; Wood et al., 2001], published subsequent to our analysis, have shown that in vitamin D-receptor knock-out mice CaT1 expression is downregulated by about 90%. In other words, some 10% of calcium entry seems to be vitamin D-independent.

Our analysis cannot distinguish between two possibilities: whether in the absence of vitamin D some 90% of the entry structures have become inoperative, or, whether only the channel flow mechanism is subject to vitamin D control, with the saturable transport mechanism independent of vitamin D regulation. The latter possibility, namely that only the channel component is vitamin D-dependent, is attractive, inasmuch as at higher luminal calcium and under conditions of maximum vitamin D expression some 90% of the calcium that traverses the duodenal cell has come into the cell by the channel mechanism [Slepchenko and Bronner, 2001].

INTRACELLULAR CALCIUM DIFFUSION

When one calculates the rate of calcium self-diffusion in a duodenal cell, it is apparent that the rate does not match the much higher rate of transcellular absorption that has been established experimentally [Bronner et al., 1986]. The increased rate of intracellular diffusion is insured by the presence of the cytosolic calcium-binding protein, CaBP, which binds calcium and thereby raises the total cytosolic calcium concentration. We evaluated the effect of CaBP on calcium diffusion [Slepchenko and Bronner, 2001]. For example, at a luminal calcium concentration of 20 mM, some 11% of the total calcium flux of ~7 μmol/h/g is unbound, the remainder is bound to and carried by CaBP (assumed to be at maximum, 0.25 mM). At higher luminal calcium concentrations, intracellular self-diffusion of calcium accounts for only 2% of total calcium flux.

Figure 2 shows simulation results for steady-state transcellular calcium transport in duodenal loop preparations of rats on a high calcium diet. The data points, shown as open circles, represent simulation results superimposed on a Michaelis–Menten relationship, with a $V_m = 23.3$ μmol/h/g and a $K_m = 48.7$ mM, values similar to those derived previously by analysis of the experimental data [Bronner et al., 1986].

When experimentally derived values of maximum calcium transport through the duodenal cell are plotted as a function of the experimentally determined CaBP content, it is apparent that transport is a function of the CaBP content, even though the diffusion coefficient of calbindin in the cytosol is only about 1/5th of the calcium diffusion coefficient (Fig. 3).

The slope of the linear relationship between maximum calcium transport and CaBP content

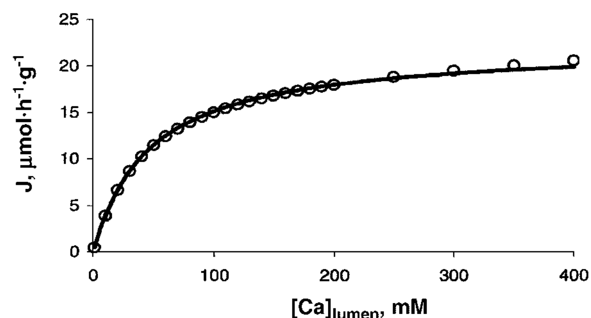

Fig. 2. Simulation results for steady-state transcellular calcium transport as measured in duodenal loops at high luminal calcium concentrations ($[Ca]_{lumen}$; data from Bronner et al. [1986]). *J*, transcellular flux. Data points, open circles, represent simulation results imposed on a Michaelis–Menten relationship, with $V_m = 23.3$ μmol/h/g and $K_m = 48.7$ mM. V_m is the maximum rate of luminal calcium efflux and K_m is the luminal calcium concentration at $V_m/2$. Reproduced with permission from Slepchenko and Bronner [2001].

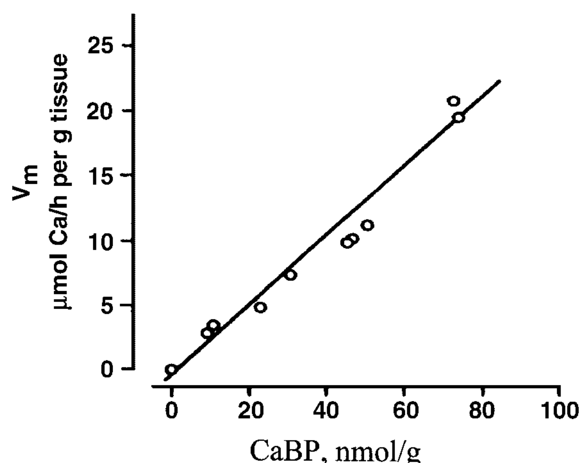

Fig. 3. Relationship between intestinal calcium transport, Vm, and CaBP content. V_m was calculated from in situ duodenal, jejunal, and ileal loop experiments [Pansu et al., 1983a,b] and plotted as a function of the CaBP content. The equation describing the relationship is $V_m = -0.59 + 0.26$ CaBP, units as shown. Reproduced with permission from Bronner et al. [1986].

(Fig. 3) is largely determined by the CaBP diffusion coefficient. We have calculated the numerical value of this coefficient by two approaches: from the experimentally established Michaelis–Menten relationship between V_m and the cellular CaBP content; and from a published diffusion coefficient for calcium and the mass relationship between the protein and the mineral. Both approaches yielded a similar value, i.e., $\sim$60 μm^2/sec [Slepchenko and Bronner, 2001].

The function of calbindin thus is two-fold: It acts as a calcium buffer, so that the free intracellular calcium ion concentration remains acceptably low. At the same time, being a relatively small, cytosolic molecule, it functions as a transport protein. The biosynthesis of calbindin is totally vitamin D-dependent [Buckley and Bronner, 1980; Pansu et al., 1983b; Christakos et al., 1992]. In a vitamin D-deficient animal, transcellular calcium transport is diminished and becomes nil in the total absence of calbindin. In fact, the CaBP content of the intestine is a direct measure of vitamin D deficiency or sufficiency [Bronner and Freund, 1975].

In intestinal loop experiments [Pansu et al., 1981, 1983a,b], the intestine of an anesthetized, laparotomized animal is exteriorized, the most proximal 10 cm of the small intestine is tied proximally and distally, and a calibrated quantity of a calcium-containing buffer is instilled into the rinsed loop. The drop in calcium concentration as a function of time is established on the basis of measurements in several loops, with a given time point generally the mean of three to six loop measurements. Figure 4a represents typical results.

Figure 4b shows the results of simulation with the model [Slepchenko and Bronner, 2001]. As can be seen, at a low and relatively high luminal calcium concentrations, there is good agreement between results of the simulation studies and the experimental findings shown in Figure 4a. In the intermediate concentration range, agreement between model and experiment is relatively poor. The reason for this is that in the modeling studies we ignored intracellular calcium uptake. When the initial luminal calcium concentration is low, the process of absorption is brief and the relatively slow process of intracellular calcium uptake is therefore likely to have only little influence on calcium transport. At luminal calcium concentrations of above 50 mM, uptake by intracellular fixed binding sites is small compared to the total transport and can therefore also be ignored. This is not true at the intermediate luminal calcium concentrations, i.e., between 5 and 50 mM.

Interestingly, the discrepancy between simulation and experiment gives an indication of the rate and capacity by which duodenal cells take up calcium intracellularly. For example, our modeling experiments predict that at 25 mM,

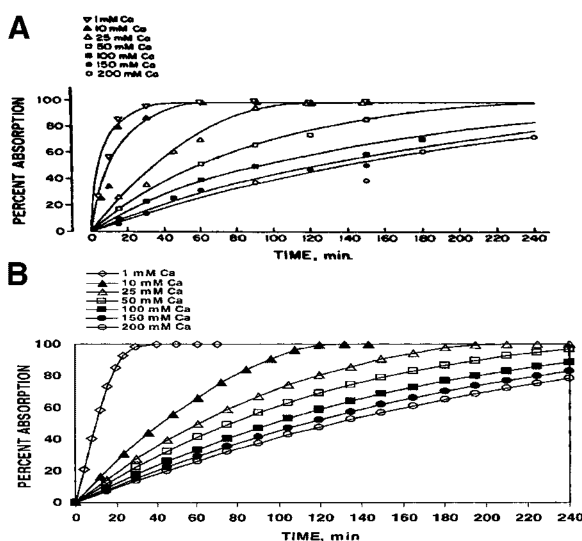

Fig. 4. Time dependence of luminal calcium transport in duodenal loops. **A**: Experimental findings [Bronner et al., 1986]. **B**: Simulation results [Slepchenko and Bronner, 2001]. Reproduced with permission from Slepchenko and Bronner [2001].

1 g of mucosal tissue, equivalent to 10^8 cells, will have taken up 25 nmol calcium by 45 min when uptake appears complete [Slepchenko and Bronner, 2001]. This value accords with the experimental value of 10 nmol/10^6 cells/h at a time, 30 min, when uptake was not yet complete [Bronner et al., 1983].

In an attempt to compare overall calcium permeability of duodenal cells with their calbindin content, we calculated permeability as the ratio V_m/K_m, having obtained numerical values for experimental K_m's from the literature [Pansu et al., 1983a,b] and permeability values of the modeling function from the modeled linear relationship between calcium flux and CaBP. All values were then normalized to their respective maxima.

As can be seen (Fig. 5), the solid line derived by modeling well describes the relationship between permeability, expressed as the ratio of V_m/K_m, and CaBP content. The relationship is quite similar to the experimental plot showing a linear relationship between V_m and CaBP content (Fig. 3). This correspondence moreover importantly confirms two assumptions made in the modeling analysis, namely (1) that the vitamin D-insensitive component of calcium entry amounts only to some 7%, and (2) that transcellular calcium transport is a linear function of the CaBP content.

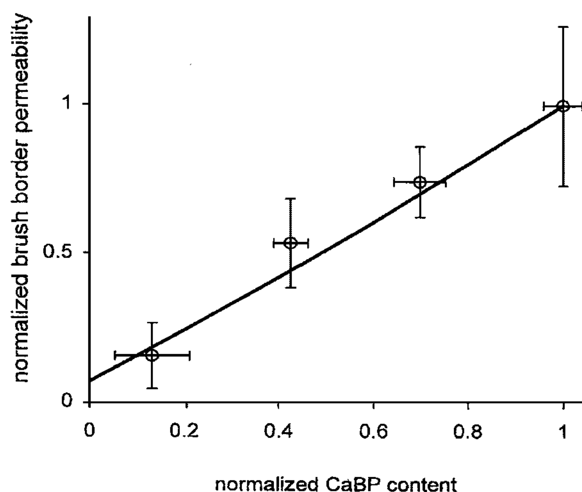

Fig. 5. Relationship between calmodulin D_{9k} (CaBP) content and calcium entry, with both parameters vitamin D-dependent. Experimental values (open circles) with associated standard errors are from animals of varying ages [Pansu et al., 1983a,b]. Permeability was estimated as equal to V_m/K_m. To permit comparison with modeling results (solid line), all values were normalized to their respective maxima. Reproduced with permission from Slepchenko and Bronner [2001].

What is the physiological significance of the dual entry mechanism? The channel mechanism becomes important under conditions of calcium deficiency. When an organism on a low calcium intake is faced with an opportunity to increase calcium intake, for example, by coming across a food source rich in calcium, the relatively low level of saturation associated with the transporter would limit the amount of calcium that can be absorbed. However, the channel mechanism permits calcium to enter in proportion to the luminal calcium concentration. Because the channel mechanism is vitamin D-dependent, its contribution to calcium entry increases as the amount of circulating $1,25(OH)_2D_3$ goes up. The amount of circulating $1,25(OH)_2D_3$ in turn varies inversely with the calcium status, i.e., it is low when there is ample calcium available and it is high under conditions of calcium deficiency [Edelstein et al., 1978].

When calcium intake and, therefore, luminal calcium is high, the need to bar excess calcium entry becomes overriding. The drop in circulating $1,25(OH)_2D_3$ will lead to a shutting down of the channel entry system, with the duodenal cell increasingly relying on the low saturation transport system for exogenous calcium entry from the lumen. When calcium intake rises, the plasma level of $1,25(OH)_2D_3$ drops, causing less CaBP to be synthesized. In addition, an intracellular rise in calcium may also depress CaBP expression [Buckley and Bronner, 1980]. As a result, transcellular calcium transport diminishes.

Even luminal calcium concentrations of 1 mM far exceed the free intracellular calcium concentration of 100 nM or less. Under those conditions fixed intracellular buffering undoubtedly helps intracellular calcium homeostasis.

A few final words about the extrusion mechanism, mediated by the CaATPase. The essential mechanism, as depicted in Figure 6, consists of a calcium-binding domain on the cytoplasmic side of the pump molecule, which spans the entire membrane. There is also a calmodulin-binding domain. Calcium is expelled through a channel-like opening formed by the transmembrane elements. For this to occur, phosphorylation is thought to bring about a conformational change such that calcium, bound to the ATPase, is propelled through the opening. The estimated V_m of the intestinal enzyme is 20–30 nmol Ca/min/mg protein [Bronner, 1996], a value that appears adequate to extrude calcium even at the

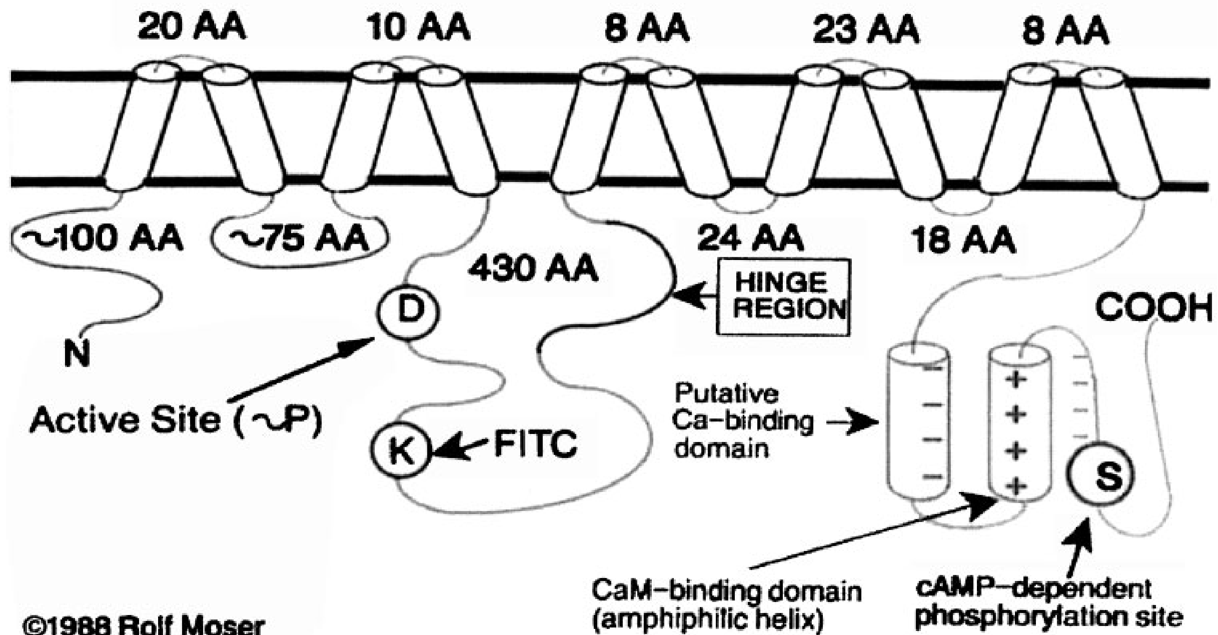

Fig. 6. Cartoon of the CaATPase. Reproduced with permission from Bronner et al. [1986].

highest rates of calcium transit [Slepchenko and Bronner, 2001]. Data suggest that the number of CaATPases can be increased with vitamin D treatment [Wasserman et al., 1992], but there is no indication that enzyme biosynthesis is vitamin D-dependent.

SUMMARY

In summary, calcium transcellular transport involves a two-stage entry mechanism at the brush border, mediated by CaT1; intracellular calcium transport, mediated by the cytosolic, vitamin D-dependent CaBP; and cellular extrusion from the basolateral pole of the duodenal cell, effected largely by the CaATPase. Maximum transcellular transport is directly proportional to the cellular content of CaBP, to which over 90% of the transported calcium is bound. Calcium extrusion, largely by the CaATPase, is not a limiting rate. Transcellular calcium absorption is functionally important at low calcium intakes, but is downregulated at high calcium intakes, when paracellular calcium transport predominates.

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