# Calcium Uptake by Duodenal Epithelial Cells Is Increased during Lactation (43182)

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*Abstract.* **It is known that duodenal absorption of calcium is increased during lactation, and, more specifically, it has been shown that duodenal active transport of calcium is increased. The energy-requiring step for this active transport process is thought to occur at the basolateral membrane of the duodenal epithelial cells, where calcium is pumped from the cell into the extracellular fluid. The present experiments were carried out to determine whether calcium uptake into duodenal epithelial cells is also altered during lactation. Uptake of $^{45}Ca$ into isolated duodenal epithelial cells from lactating rats or age-matched controls was measured. It was found that cells from lactating animals showed markedly enhanced calcium uptake when compared with cells from control animals. Uptake was calcium concentration dependent for both groups. Since duodenal epithelial cells are known to contain increased calbindin$_{9k}$, a calcium-binding protein, during lactation, calcium uptake was also measured in cells that were preincubated in medium containing calcium. In these experiments, the total $^{45}Ca$ uptake was reduced, but the difference between lactating and control remained. These experiments show that the uptake of calcium into duodenal epithelial cells is among the components of the transcellular calcium transport process which is enhanced during lactation.**
[P.S.E.B.M. 1991, Vol 196]

Intestinal absorption of calcium is markedly increased during lactation (1). The intestine enlarges significantly during lactation (2, 3), which may contribute to the overall increase in fractional absorption of calcium (4). In addition, duodenal active transport of calcium has been shown to be increased during lactation, even in the absence of circulating 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] (4, 5). It remains unknown what factor is the direct stimulus for increased duodenal calcium transport during lactation, or what the exact tissue and cellular alterations are which result in increased calcium transport.

Intestinal absorption of calcium occurs by both "passive" paracellular movement which predominates when lumenal calcium concentrations are high and by energy-requiring, transcellular "active" transport which predominates when lumenal calcium concentrations are lower than that of the general extracellular fluid (6). During the transcellular active transport process, calcium is thought to enter the cell down a concentration gradient, be moved across the cell, and then actively pumped across the basolateral membrane into the extracellular fluid compartment. The primary regulation of this process is thought to occur at the basolateral membrane (7–9). However, we have hypothesized that the duodenal epithelial cells must exercise some degree of control over the amount of calcium which is allowed to cross the brush border membrane and enter the cellular cytoplasm. Otherwise, the cell would be vulnerable to very large amounts of calcium when food sources high in calcium were ingested. As an extension of this hypothesis, it would seem useful for the cell to be able to take up more calcium when the basolateral calcium-pumping process was stimulated. Therefore, the present experiments were carried out to determine whether duodenal epithelial cell calcium uptake is altered during lactation, a condition in which calcium transport is known to be elevated.

Received March 15, 1990. [P.S.E.B.M. 1991, Vol 196]
Accepted October 17, 1990.

0037-9727/91/1962-0214$2.00/0


## Materials and Methods

**Animals.** Sprague-Dawley rats, either lactating mothers with 8–12 pups, or age-matched, virgin females were used. Lactating animals were utilized on Days 13–15 of lactation. All animals were maintained on a 12-hr light/12-hr dark cycle and free access to Purina Chow and tap water.

**Cell Isolation.** Duodenal epithelial cells were isolated from the proximal 10 cm of the small intestine

using a modification of the method described by Weiser (10). The proximal 10 cm of duodenum were rinsed with saline and everted over a disposable plastic Pasteur pipette. The pipette was then immersed in a tube containing Solution A (1.5 m*M* KCl, 96 m*M* NaCl, 27 m*M* sodium citrate, 8 m*M* $KH_2PO_4$, 5.6 m*M* $Na_2HPO_4$, 0.5 m*M* dithiothreitol, pH 7.4) for 15 min in a 37°C water bath. The pipette was twirled within the tube every 5 min. The pipette was then transferred to a second tube containing Solution B (1.5 m*M* EDTA, 0.5 m*M* dithiothreitol, 10 m*M* phosphate-buffered saline, pH 7.4) and maintained at 37°C, twirled every 5 min. Cells elute into Solution B from the villus tip first and then with time, cells are eluted sequentially from the tip to the crypt. At 30-min intervals, the pipette was moved into a new tube containing Solution B. Once the pipette was removed from each tube, the eluted cells were collected by centrifugation and washed in phosphate-buffered saline twice followed by a wash and resuspension in the calcium-uptake buffer. All of the data shown in the figures were obtained from the first fraction of cells. In some experiments, cells were counted using a hemocytometer. In general, cells were retrieved as small clumps of four to six cells rather than as individual cells.

**Calcium Uptake.** Calcium uptake experiments were performed at room temperature using the same buffer we have previously used for measuring calcium transport in everted duodenal sacs (11): 125 m*M* NaCl, 10 m*M* fructose, 30 m*M* Tris (pH 7.4). The calcium content of the buffer varied with the experiment, as indicated. Calcium uptake experiments were carried out in 96-well plates using a cell harvester (Titertek; Flow Laboatories). Each well contained approximately $10^4$ cells in 50 $\mu$l of uptake buffer. To initiate the measurement of uptake, 50 $\mu$l of uptake buffer containing the appropriate amount of $^{45}$Ca-labeled $CaCl_2$ ($10^5$ cpm/well) were added to each well. After incubation for the time indicated for each experiment, the cell harvester was used to collect the cells onto glass fiber filters while being washed for 10 sec with 30 m*M* Tris-buffered saline containing 1 m*M* EGTA. The EGTA was added to the wash buffer to minimize the amount of calcium bound to the outside of the cells. The filters were then removed and the $^{45}$Ca retained in the cells on the filter was counted using a scintillation counter. Filter binding of $^{45}$Ca was very low, but was determined in each experiment and subtracted from each experimental determination. Parallel tubes were pipetted for protein determination using Bio-Rad reagents following NaOH digestion of the cells.

## Results

Calcium uptake from media containing three different concentrations of calcium, 1, 3, and 5 m*M*, was measured. It was found that after 5 min, cells from lactating animals had accumulated significantly more $^{45}$Ca than cells from nonlactating animals, regardless of the calcium concentration (Fig. 1). These data were obtained from Day 13 of lactation, when milk production is at or near peak levels.

For the experiment shown in Figure 1, the cells had been maintained without added calcium until the uptake experiment was initiated. We were interested in knowing whether the differences in calcium uptake between lactating and nonlactating animals might be due in part to differences in intracellular retention of calcium. Therefore, in the next experiment, cells were preincubated for 10 min in the presence of 1 m*M* calcium. The uptake measurements were initiated by the addition of $^{45}$Ca, and uptake was determined 2, 5, 10, and 20 min later (Fig. 2). It was found that preincubation with calcium decreased the total amount of $^{45}$Ca in the cells for each condition and time, but the

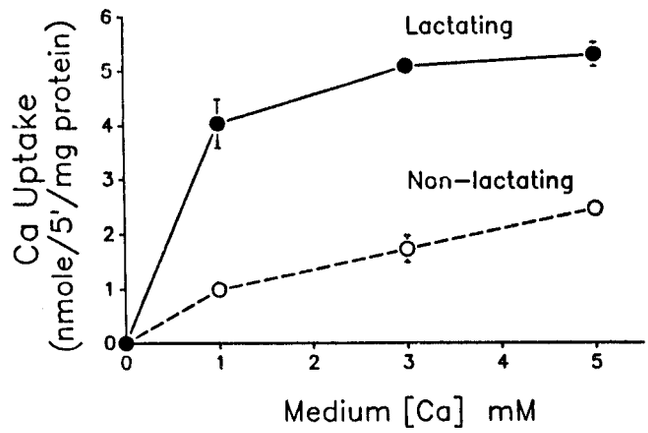


**Figure 1.** Calcium uptake by duodenal epithelial cells isolated from either lactating or nonlactating female rats. Calcium uptake was measured following a 5-min incubation in the indicated concentrations of calcium. Lactation cells were obtained on Day 13 of lactation. Each point represents the mean ± SE from three samples.

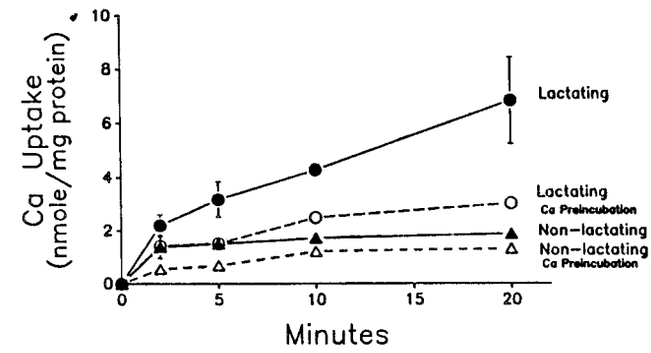


**Figure 2.** Calcium uptake was measured in cells maintained in either calcium-free medium (no added calcium), or after a 10-min preincubation in 1 m*M* calcium. Calcium uptake was initiated by the addition of $^{45}$Ca such that the final calcium concentration was 1 m*M*, and calcium uptake and retention were measured at the indicated time points. Lactation cells were obtained from Day 15 of lactation. Each point represents the mean ± SE from three samples.

cells from lactating animals still contained more $^{45}Ca$ than did those from nonlactating animals. Cells from lactating animals that were not preincubated with calcium continued to exhibit a net accumulation of calcium throughout the 20-min period. All of the other groups appeared to approach a steady state by 10 min.

We also compared the effects of preincubation with varying concentrations of calcium on calcium uptake. In this experiment, cells were preincubated with either 1, 3, or 5 m*M* calcium for 10 min. $^{45}Ca$ was then introduced, and net accumulation was measured after 5 min. Nonpreincubated cells exposed to increasing calcium concentrations exhibited increased calcium uptake. Cells preincubated with 3 m*M* or 5 m*M* calcium did not take up any more calcium than did cells preincubated in 1 m*M* calcium (Fig. 3). This was particularly noticeable in cells from lactating animals.

## Discussion

It has been shown previously that duodenal active transport of calcium is increased during lactation (4, 5). In addition, it is known that lactating animals have increased duodenal epithelial calbindin$_{9k}$ content compared with nonlactating animals, an effect of lactation which is not dependent on 1,25(OH)$_2$D (12). The present data show that an additional component of the transcellular calcium transport process, calcium uptake, is also enhanced during lactation. In these experiments, $^{45}Ca$ accumulation represents the net uptake of calcium during the experimental period. The cells are continuing to extrude calcium across the basolateral membrane via an active transport process throughout the experiment. Intact cells were used for these experiments so that cellular polarity and intracellular components, such as calbindin$_{9k}$, which may impact on calcium uptake would be maintained.

Circulating levels of 1,25(OH)$_2$D are increased during lactation (13). It has been shown previously that repletion of vitamin D-deficient rats to a D-sufficient state increased calcium uptake (14). Whether or not the increased 1,25(OH)$_2$D of lactation accounts for the increased calcium uptake during lactation is not addressed by the present experiments. However, it has been shown that increased active transport of calcium during lactation takes place even in the absence of 1,25(OH)$_2$D (4, 5).

The knowledge that lactation results in increased calbindin$_{9k}$ in duodenal epithelium (12) led us to measure calcium uptake in both calcium-deprived and calcium-preincubated cells. Preincubation of cells in calcium-containing medium did decrease the total accumulation of radioactive calcium during the time periods studied, but did not eliminate the difference between accumulation by cells from lactating and nonlactating animals. This observation is consistent with the hypothesis that the increased calbindin$_{9k}$ content of cells from lactating animals results in increased calcium accumulation relative to control animals, and may allow these cells to have a greater transport capacity for calcium. The present findings in combination with previous findings from other laboratories (e.g., 4, 5, 12) are consistent with a model that during lactation, duodenal epithelial cell calcium handling is altered such that (i) increased calcium is allowed to enter the cell, (ii) calbindin$_{9k}$ capacity is increased to prevent damagingly high intracellular Ca levels, and (iii) active pumping of calcium across the basolateral side of the cell into extracellular fluid is increased. The data presented here are consistent with the first step in this process and with the evidence provided by others (12) that calbindin$_{9k}$ is increased during lactation. Additional studies will be required to determine both what the direct effectors are for each of these processes, and exactly what characteristics of the brush border membrane are altered to enhance calcium uptake.

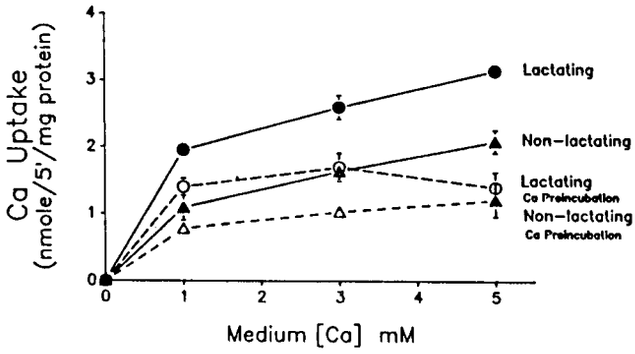


**Figure 3.** Calcium uptake was measured 5 min after addition of $^{45}Ca$. Preincubation was carried out for 10 min in the presence of the same calcium concentration used to measure uptake as indicated on the abscissa (calcium preincubation, open symbols), or in medium containing no added calcium (closed symbols). Lactation cells were obtained from Days 14–15 of lactation. Each point represents the mean ± SE for three cell samples.


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