# Intestine, Bone, and Mammary Gland Contributions to Maternal Plasma Calcium Increase after Abrupt Weaning (43365)

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*Abstract.* **The response of maternal plasma calcium concentration to the abrupt and permanent removal of the suckling pups on Day 13 of lactation was investigated. Maternal plasma calcium did not change at 6 hr or 12 hr following pup removal. At 18 hr and 24 hr after weaning, the maternal plasma calcium concentration increased in mothers consuming either 0.47% calcium or 0.02% calcium diets. At 24 hr after weaning, the plasma calcium increase in mothers consuming low dietary calcium was 55% that of mothers consuming adequate dietary calcium. The contribution of the mammary gland to the plasma calcium increase in rats consuming the low dietary calcium was investigated by removing the mammary glands. Following mammary gland removal, plasma calcium increased 50% compared with mothers that had intact mammary glands. The data suggest that intestinal absorption of calcium and bone calcium mobilization remain stimulated by the lactation process for at least 24 hr after removal of the nursing pups.** [P.S.E.B.M. 1992, Vol 199]

Lactation imposes a severe demand on calcium needed for milk synthesis. This heightened calcium need is reflected in the maternal blood calcium concentration. The lactating rat is hypocalcemia by 0.5–1 mg/dl compared with nonlactating rats (1), but becomes hypercalcemic after abrupt removal of the pups. Blood calcium increases approximately 2 mg/dl in the vitamin D-sufficient (2) or -deficient (3) state. The same phenomenon occurs in the lactating dairy cow following abrupt cessation of milking (4). The accumulation of calcium in the blood presumably reflects ongoing lactation-associated calcium mobilization despite reduced demand for milk calcium following removal of the nursing stimulus. The source of the calcium may be of bone (5, 6) or intestinal (7) origin. Previous reports have not attempted to discriminate between bone and intestine as sources of this accumulated calcium. In addition, the mammary gland may also be a source of calcium, especially as it becomes engorged with milk following weaning. This report provides an estimate of the contribution of intestine, bone, and mammary gland to the increase in maternal plasma calcium following abrupt weaning in the rat.

## Methods

**Time Course Experiment.** Rats of the Holtzman strain were obtained from the Harlan Sprague-Dawley Co. (Madison, WI). Virgin females were mated at 80–90 days of age using proven breeder males. Age-matched lactating and nonlactating female rats were thus produced. They were housed in solid-bottomed plastic cages bedded with wood shavings. Rats were fed a stock diet (Wayne Rodent Blox) until Day 0 of lactation (parturition day). Rats were then fed a purified diet (8) containing either 0.47% calcium or 0.02% calcium for the remainder of the experiment. All diets contained 0.3% phosphorus. Litter size was adjusted to 10 pups per litter on Day 3 of lactation. On Day 13 of lactation, the pups were removed and blood samples were taken at various times following pup removal. Blood samples were taken from the tip of the tail into heparinized tubes under ether anesthesia.

**Mammary Gland Removal Experiments.** Lactating rats with litters were obtained from the supplier on

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Day 6 of lactation. They were fed the 0.02% calcium diet until Day 13 of lactation. The nursing pups were first removed from the mother rats. The mother rats were then anesthetized with ketamine (Ketalar, 60 mg/kg) and xylazine (Rompum, 8 mg/kg) 12 hr later. A single ventral incision was followed by exposure of the thoracic and inguinal mammary glands. An initial blood sample (0.4 ml) was taken from a jugular vein. Mammary tissue was then carefully dissected out, so as not to rupture major blood vessels in the process. This procedure took 15–20 min to complete. Following mammary gland removal, the incision was closed, and the animal was returned to a solid-bottomed cage covered with wood shavings. Supplemental heat was not provided. The sham operation was a ventral incision, but the mammary glands were left intact. Nonlactating control rats were also sham operated. Six hours after the initial blood sample, a final blood sample was obtained from the vena cava, and the animals were then sacrificed. Animals were kept anesthetized throughout the experiment by the administration of supplemental anesthetic when required. In additional experiments, the entire mammary tissue was removed from lactating rats at 0 hr or 12 hr following pup removal immediately following sacrifice. Mammary tissue was minced, homogenized in 50 m*M* Tris-HCl/150 m*M* NaCl buffer (2 ml buffer/g tissue) using a Polytron, and strained through cheesecloth. Aliquots of homogenates were first evaporated and then ashed at 600°C in a muffle furnace. The ash was dissolved in 3 *M* HCl, diluted appropriately, and analyzed for calcium as described below.

**Analysis.** Blood samples were centrifuged to obtain plasma. Samples were diluted in a 0.1% La, 0.5% HCl, and 32.5 $\mu$g/ml of $SrCL_2 \cdot 6H_2O$ solution, and calcium concentration (plasma or mammary gland homogenate) was determined by atomic absorption spectroscopy.

**Statistics.** Data were analyzed using paired *t* test (9) or one-way analysis of variance for repeated measures (10, 11), where appropriate.

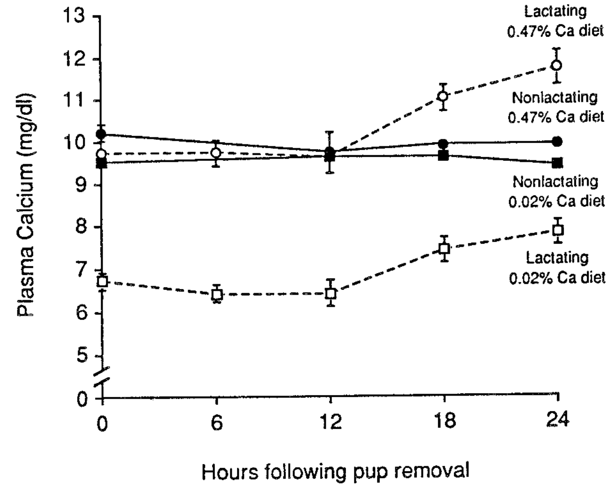

**Figure 1.** Plasma calcium concentrations in lactating rats following abrupt weaning. Values are the mean ± SE for five to six lactating rats and three to four nonlactating rats per time point.

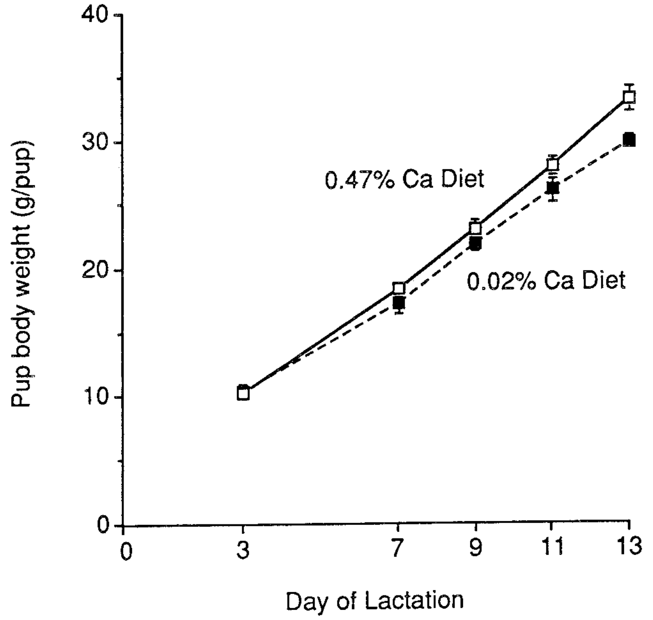

**Figure 2.** Growth of pups from nursing mothers consuming 0.47% calcium or 0.02% calcium diets. Values are the mean ± SE for five to six rats per group.

## Results

The time course of plasma calcium concentration following abrupt weaning is shown in Figure 1. Lactating rats consuming the 0.47% calcium diet had plasma calcium concentrations that were initially about 0.5 mg/dl lower than those of nonlactating rats consuming the same diet. Following pup removal, plasma calcium increased at 18 hr ($P = 0.02$) and 24 hr ($P = 0.005$) above 0-hr levels. Nonlactating rats consuming the 0.47% calcium diet showed no plasma calcium increase at 18 hr or 24 hr. Lactating rats consuming 0.02% calcium diet also showed a significant increase in plasma calcium at 18 hr ($P = 0.04$) and at 24 hr ($P = 0.001$) following pup removal. Nonlactating rats consuming the 0.02% calcium diet did not show changes in plasma calcium concentration at 18 hr or 24 hr.

The effects of dietary calcium content upon litter weight gain during lactation are shown in Figure 2. Body weights of pup-nursing mothers consuming either 0.47% or 0.02% calcium diet increased linearly from Day 9 to Day 13. Simple linear regression of body weights from Day 9 to Day 13 indicated growth rate of 2.5 g/pup/day and 2.0 g/pup/day for pup-nursing mothers consuming 0.47% calcium and 0.02% calcium, respectively.

A second experiment was done to determine whether dietary calcium depletion for 7 days instead of

13 days affected the rise in plasma calcium. The rats ($n = 5$) were given the 0.02% calcium diet beginning on Day 7 of lactation and pups were removed on Day 13 of lactation. Plasma calcium increased from 8.2 ± 0.2 mg/dl at 0 hr to 9.3 ± 0.1 mg/dl at 24 hr following pup removal. This increase was the same as that for the lactating rats consuming 0.02% calcium diet for 13 days (1.1 ± 0.2 mg/dl).

Table I shows the effect of maternal dietary calcium consumption upon the increase in maternal plasma calcium at 24 hr following pup removal. Consumption of the 0.02% calcium diet resulted in a significantly lower plasma calcium increase than did consumption of the 0.47% calcium diet.

The plasma calcium concentration changes of anesthetized rats in the presence or absence of mammary gland tissue are shown in Table II. Mammary gland removal significantly reduced the plasma calcium increase compared with mammary gland-intact lactating rats. The nonlactating, sham-operated rats showed no change in plasma calcium concentration.

### Discussion

The accumulation of calcium in the blood of lactating rats and cows following abrupt cessation of milk removal suggests that there is a substantial transfer of calcium from tissue(s) to the blood, and that this process continues for several hours following removal of the milking or nursing stimulus. The possibility that some of this transfer was from bone tissue was of special interest. These experiments were done to examine this possibility.

**Table I.** Effect of Dietary Calcium upon the Increase in Maternal Plasma Calcium Concentration after Pup Removal[a]

| Group (% calcium in diet) | $n$ | Increase in plasma calcium (mg/dl) |
|---|---|---|
| 0.47 | 10 | 1.9 ± 0.2 |
| 0.02 | 11 | 1.1[b] ± 0.1 |

[a] Values are the mean ± SE of the difference between plasma calcium measured at 0 hr and at 24 hr after pup removal.
[b] Significantly different from 0.47% calcium group ($P < 0.005$).

**Table II.** Maternal Plasma Calcium Increase after Pup Removal in the Presence or Absence of Mammary Tissue[a]

| Group | $n$ | Initial plasma calcium (mg/dl) | Increase in plasma calcium (mg/dl) |
|---|---|---|---|
| Lactating, mammary gland intact | 5 | 8.1 ± 0.2 | 1.2 ± 0.1 |
| Lactating, mammary gland removed | 5 | 7.9 ± 0.2 | 0.6[b] ± 0.1 |
| Nonlactating | 5 | 9.6 ± 0.2 | 0.0 ± 0.1 |

[a] Values are the mean ± SE of the differences between initial and final calcium concentrations of individual rats.
[b] Significantly different from lactating, mammary gland intact group ($P < 0.002$).

The pup growth data indicate that lactation occurs at about 80% of the level attained when adequate dietary calcium is available. Thus, while restriction of dietary calcium impairs lactation to some degree, the lactating rat is still able to maintain substantial milk production when all milk calcium is derived from bone. The lowered lactation was probably due to a general decrease in nutrient intake, since food consumption during the 2 days prior to pup removal by the lactating rats consuming low calcium diet (52 ± 1 g/day) was also 80% that of the lactating rats consuming adequate dietary calcium (66 ± 2 g/day).

The lack of plasma calcium accumulation in the first 12 hr suggests that during this time calcium efflux from bone and/or intestine is matched by calcium uptake into mammary gland. The accumulation of lactose in the mammary gland during the first 12 hr following abrupt weaning indicates that milk synthesis continues for several hours after the suckling stimulus is removed (12). Mammary gland calcium uptake is presumably associated with this ongoing milk synthesis. The mammary gland acts as a calcium sink, since the non-milk-engorged mammary gland epithelium is impermeable to calcium transfer from alveolar lumen to blood (13).

The rapid accumulation of plasma calcium from 12 to 24 hr indicates a tissue calcium efflux to blood greater than tissue influx from blood. The calcium build-up occurred with or without intestinal calcium absorption, although the magnitude of accumulation was 0.8 mg/dl lower when no dietary calcium was absorbed. The remaining 1.1-mg/dl accumulation could only come from bone or mammary tissue. Thus, while dietary calcium absorption undoubtedly contributed to the accumulation of blood calcium, a substantial portion was also derived from nonintestinal sources. The major nonintestinal sources of calcium are bone and mammary tissue. The relative contribution of each tissue to blood calcium accumulation was estimated under conditions in which intestinal absorption was eliminated by the feeding of a low calcium diet. The severe hypocalcemia induced by 13 days of low calcium feeding was largely ameliorated by feeding low calcium for 7 days. The extent of blood calcium accumulation (1.1 mg/dl) was identical whether the mothers were initially severely (6.5 mg/dl) or slightly (8.2 mg/dl) hypocalcemic. Thus, the lower plasma calcium accumulation of rats consuming low calcium was not merely a reflection of severe hypocalcemia. Removal of the mammary glands reduced blood calcium accumulation by 50% during the period of most rapid plasma calcium increase (12–18 hr after weaning). The effects were lactation specific, since nonlactating sham controls maintained stable blood calcium concentrations over the 6-hr experimental period. The residual plasma calcium accumulation in the absence of mammary gland must result from bone calcium efflux. Even at several hours following removal of pups, bone continues to mobilize calcium. The lactational stimulus for bone calcium mobilization must, therefore, be long-lived.

The reduced accumulation of plasma calcium in the absence of the mammary gland indicates a decreased influx of calcium into the extracellular fluid under these conditions. Either of two factors may explain the lowered calcium influx. First, calcium may be transferred from the engorged mammary gland back to the extracellular fluid compartment. The calcium content of the entire mammary gland tissue increased from 8 ± 1 (*n* = 3) mg in mothers suckling pups to 51 ± 3 (*n* = 3) mg at 12 hr following pup removal. The extracellular fluid compartment is calculated to contain only about 0.4 mg of additional calcium due to a 0.5-mg/dl increment in calcium concentration. The incremental amount of extracellular fluid calcium is obviously only a small fraction of that stored by the mammary gland. Direct evidence that mammary calcium influx occurs under these experimental conditions is lacking; however, the mammary gland certainly contains sufficient calcium to make this a likely possibility. Second, removal of mammary tissue could also remove the source of a factor that stimulates bone calcium mobilization, and part or all of the decreased plasma calcium accumulation may result from decreased bone calcium mobilization. One such factor, the parathyroid hormone-related peptide, has been detected in milk (14) and mobilizes bone calcium in nonlactating animals (15). Whether parathyroid hormone-related peptide or another factor of mammary gland origin is released into the systemic circulation and stimulates bone calcium mobilization during lactation has yet to be elucidated. In addition, which of the two above-mentioned possibilities accounts for the observed decrease in plasma calcium accumulation in the absence of mammary gland remains to be determined.

The renal effects upon plasma calcium were not directly assessed in these experiments. However, measurable amounts of calcium did not accumulate in the plasma of nonlactating rats consuming a low calcium diet, even though such animals display enhanced renal calcium conservation (16), as do lactating rats (7). Thus, it would appear that changes in renal conservation would not have contributed appreciably to the increase of plasma calcium observed under these conditions.

The estimated contributions of the tissues are summarized in Table III. Of the total increment of the 1.9-mg/dl increase observed at 24 hr after weaning, 1.1 mg/dl could be obtained upon removal of dietary calcium. The difference, 0.8 mg/dl, represents the contribution from diet. Assuming that the 0- to 24-hr increment in blood calcium is derived from bone and mammary gland in a proportion similar to that measured between 12 and 18 hr, the 1.1 mg/dl is composed equally of calcium derived from bone and mammary gland efflux. Interestingly, the increase in blood calcium occurs in the presumed absence of elevated 1,25-$(OH)_2D_3$ in vitamin D-deficient lactating rats (3). Thus, some of the transport processes described herein must also be independent of vitamin D.

**Table III.** Estimated Contribution of Diet, Bone, and Mammary Gland Calcium to the Accumulation of Calcium in Blood Plasma after Abrupt Weaning

| Tissue component | Contribution to increased plasma calcium caused by pup removal | |
|---|---|---|
| | (mg/dl) | (% of total) |
| Diet + Bone + Mammary Gland | 1.9 | 100 |
| Diet | 0.8 | 42 |
| Bone | 0.55 | 29 |
| Mammary Gland | 0.55 | 29 |

It may be argued that the increment obtained under conditions of low dietary calcium may not accurately reflect the bone contribution when dietary calcium is absorbed. The current estimation may be considered a maximal value, since bone calcium flux is certainly greater when all calcium must come from bone. However, it is unlikely that increased bone flux will account for all of the accumulation observed.

The abrupt weaning of lactating rats results in an accumulation of calcium in blood plasma that is derived from bone, intestine, and, possibly, recycled mammary gland calcium stores. These results show that the stimulus for mobilization of calcium from intestine and bone remains for at least 24 hr after the suckling stimulus for lactation is removed, and argue for a humoral factor released into the circulation by the lactation process that results in the mobilization of calcium.

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