Levels of cellular retinol-binding proteins in the small intestine of rats during pregnancy and lactation

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Abstract The movement and metabolism of vitamin A is dependent on a number of specific carrier proteins. The small intestine contains both cellular retinol-binding protein (type two) (CRBP(II)), restricted to the villus-associated enterocytes, and cellular retinol-binding protein (CRBP), present primarily in supporting mesenchymal cells. The content of these proteins in the small intestine of prepartum and postpartum Sprague-Dawley rats was determined by radioimmunoassay. Levels of CRBP(II), but not CRBP, changed dramatically during this period. Total content of CRBP(II) in the small intestine rose precipitously in late pregnancy and continued to rise throughout lactation to a peak at day 21 postpartum more than 300% greater than in nulliparous, nonpregnant controls. In contrast, total small intestinal weight and CRBP content increased only ~100% from late pregnancy to day 21 of lactation. CRBP(II) concentration in the proximal and middle segments of small intestine (expressed on a g wet tissue, mg protein, or mg DNA basis) remained at control levels through day 17 of pregnancy, increased 50–100% in late pregnancy, then rose markedly at parturition to levels two- to threefold greater than controls. CRBP(II) concentration was then maintained at a relatively constant elevated level during the remainder of lactation, but decreased markedly after weaning, approaching control levels within 1 week. The concentrations of CRBP(II) in enterocytes isolated from the proximal two-thirds of the small intestine from rats on day 20 of pregnancy and days 1 and 16 of lactation, expressed on a mg DNA basis, were similar and ~60% greater than controls. The comparable peripartum and postpartum levels of CRBP(II) in the isolated enterocytes suggested that the increase of total CRBP(II) observed during lactation was primarily due to hyperplasia of the small intestinal epithelium, i.e., an increase in the total number of enterocytes expressing CRBP(II).—**Quick, T. C., and D. E. Ong.** Levels of cellular retinol-binding proteins in the small intestine of rats during pregnancy and lactation. *J. Lipid Res.* 1989. **30:** 1049–1054.

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Cellular retinol-binding protein (CRBP) and cellular retinol-binding protein (type two) (CRBP(II)) are closely related cytosolic proteins that are involved in the transport and metabolism of vitamin A (1). In the small intestine of rats, CRBP is present only in cells of the mesenchymal lamina propria underlying the epithelium of the villi and in the gut-associated lymphoid tissue. CRBP(II) is restricted to the absorptive epithelial cells (enterocytes) after they have emerged from the crypts and migrated onto the villi (2).

CRBP(II)-bound retinol is esterified by an intestinal microsomal enzyme, lecithin:retinol acyltransferase, in an acyl CoA-independent reaction (3, 4). Microsomes from small intestine also catalyze the reduction of CRBP(II)-bound retinaldehyde to retinol (5). The existence of such enzymatic activities suggests that CRBP(II) may play an integral role in the intestinal metabolism of vitamin A, derived from dietary β-carotene or retinyl esters, that is required for export of the vitamin from the intestine within chylomicrons. The biochemical role of CRBP in the small intestine is unclear, although studies with other organs indicate it may be involved in the translocation of retinol to specific binding sites on chromatin where retinol may modulate gene expression (6).

Increased absorption of dietary vitamin A in the lactating dam may be essential to supply the requirement of the offspring. Neonatal liver stores of vitamin A are marginal and, consequently, neonates are critically dependent on colostrum and milk to supply the vitamin A required for viability and growth during the suckling period (7). Prepartum dietary vitamin A has little effect on the maternal transfer of vitamin A to fetuses or on the postpartum vitamin A content of the milk. However, postpartum supplemental dietary vitamin A can significantly increase the vitamin A content of the milk (8).

A recent study in collaboration with this laboratory found that CRBP(II) mRNA in the small intestine of rats increases about fourfold between day 16 of pregnancy and day 1 of lactation and remains elevated during the suck-

Abbreviations: CRBP, cellular retinol-binding protein; CRBP(II), cellular retinol-binding protein (type two); PBS, phosphate-buffered saline: 0.13 M NaCl, 8.9 mM Na_2HPO_4, 0.8 mM NaH_2PO_4, 1.6 mM KH_2PO_4, pH 7.2; BSA, bovine serum albumin; DTT, dithiothreitol.

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ling period (9). We report here that the expression of CRBP(II) in the small intestine of rats in late pregnancy and lactation similarly increased and suggest that this may be an adaptive response prerequisite to greater absorption and transfer of vitamin A from the small intestine to the mammary gland in the postpartum period.

EXPERIMENTAL PROCEDURES

Animals

Nonpregnant and timed pregnant Sprague-Dawley rats (200–250 g) were obtained from Sasco, Inc. and maintained on a standard rat chow diet available ad libitum (Wayne #8604-00, Continental Grain Co.). Litters were culled to eight pups per dam on day 3 of lactation. Dams were injected with 0.4 ml sodium pentobarbital (50 mg/ml), laparotomized, and the small intestine was quickly removed and flushed with ~50 ml ice-cold saline. The small intestine was divided into three segments of equal length and frozen at −70°C.

Preparation of intestinal cytosol

Intestinal segments were homogenized in 4 volumes (w/v) ice-cold PBS for 90 sec using a Tissumizer (Tekmar Co.). Homogenates were centrifuged at 20,000 *g* for 15 min (4°C) and the supernatant fraction was centrifuged at 113,000 *g* for 1 h (4°C). The supernatant fraction (cytosol) from the second centrifugation was decanted, aliquoted, and frozen at −70°C.

Radioimmunoassay of CRBP and CRBP(II)

Radioimmunoassay of CRBP was conducted essentially as described by Ong, Crow, and Chytil (10) and of CRBP(II) as described by Ong (11). The radioiodinated CRBP and CRBP (II) were isolated using disposable PD-10 columns (Sephadex G-25, Pharmacia LKB Biotechnology, Inc.) equilibrated with PBS, 10 mM Na_2EDTA, 10 mM benzamidine HCl, pH 7.5, and 10 mg/ml ovalbumin (A-5503, Sigma Chemical Co.). Peak fractions of ^{125}I-labeled protein were pooled and 10 μl/ml of a protease inhibitor cocktail (PBS, 5 mg/ml aprotinin, 5 mg/ml chymostatin, 5 μM leupeptin, 5 μM pepstatin) was added. The mixture was combined with an equal volume of glycerol, vortexed, and stored at −20°C. ^{125}I-Labeled proteins were routinely used in the assays up to 6–8 weeks following radioiodination.

The radioimmunoassays were modified from those previously reported by using 100 μl per reaction tube of a 10% (v/v) suspension of *Staphylococcus aureus* cells (#507861 Pansorbin Cells, Calbiochem Corp.) in assay buffer to precipitate the protein-antibody complexes instead of using a goat anti-rabbit IgG second antibody immunoprecipitation.

Protein and DNA assays

Cytosolic protein was determined by the BCA protein assay (Pierce Chemical Co.) using bovine serum albumin (Sigma A-7030) in PBS as a standard. DNA in tissue homogenates was analyzed by a modification of the Burton assay (12) using calf thymus DNA (Sigma D-1501) in distilled, deionized water as a standard.

Enterocyte dissociation

In a separate experiment, nulliparous nonpregnant controls and primiparous rats at day 20 of pregnancy and days 1 and 16 of lactation (two animals per group) were killed and the small intestines were removed, segmented, and flushed as described above. The enterocytes, the epithelial cells that line the villi and crypts of the small intestine, were selectively detached and isolated by the method of Pinkus (13). Briefly, segments of small intestine were clamped at one end, filled by syringe with dissociation buffer (PBS, 1.5 mM Na_2EDTA, 0.1% (w/v) BSA, 1 mM DTT), clamped at the opposite end, immersed in PBS, and incubated in a shaking water bath at 37°C. At the end of each incubation period (consecutively: 4 min, 4 min, 7 min, 10 min, 10 min, 10 min), one clamp was removed and the dissociated cell suspension was lightly stripped from the segment into a collection tube to which an equal volume of ice-cold Kreb's-Ringer bicarbonate buffer (KRB with 2.5 mM $CaCl_2$, 1 mM DTT, 0.5% (w/v) BSA) was added. The segment was immediately refilled, clamped, and incubated for the next period. Cells were collected by centrifugation at 800 *g* for 5 min (4°C), resuspended in 0.5 ml PBS, and homogenized by 8–10 strokes in a hand-held Elvehjem glass–Teflon homogenizer. Cytosol was isolated as described above and frozen at −70°C.

RESULTS AND DISCUSSION

The small intestine undergoes a number of adaptive changes in response to increased nutrient demands in pregnancy and lactation. In this study, small intestinal weight increased ~20% in late pregnancy and ~100% during lactation (**Fig. 1**) relative to nulliparous, nonpregnant animals killed at the outset of the trial (controls). These values were very similar to previous reports (14, 15). The pattern of change of total intestinal CRBP, associated primarily with the mesenchymal lamina propria,

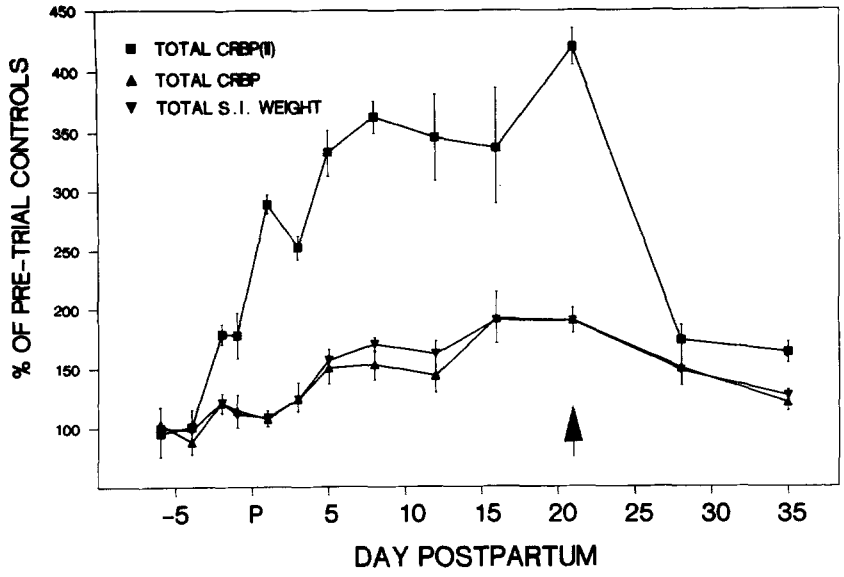

Fig. 1. Total small intestinal weight and content of CRBP and CRBP(II) in prepartum and postpartum rats. Small intestines were removed from Sprague-Dawley rats (200–250 g) on days 15, 17, 19, and 20 of pregnancy, days 1, 3, 5, 8, 12, 16, and 21 of lactation, and days 28 and 35 postpartum following weaning on day 21 (three animals per day). P indicates parturition and the arrow marks the time of weaning (day 21 postpartum). Values are expressed as a percentage of pretrial nulliparous controls. Cytosol was isolated from homogenates of proximal, middle, and distal segments of small intestine and analyzed for CRBP and CRBP(II) by radioimmunoassay as described in Methods.

was virtually identical to changes observed in small intestinal weight. Thus, these parameters together provide a basis for an estimation of general growth and hypertrophy of the small intestine during pregnancy and lactation.

In contrast to the parallel changes in small intestinal weight and CRBP during the pre- and postpartum periods, the total content of CRBP(II), also shown in Fig. 1, rose dramatically from day 17 of pregnancy, increasing ~80% prior to parturition and more than 250% by day 8 postpartum. At day 21 of lactation, prior to weaning, total CRBP(II) content in the small intestine was more than 300% greater than in control animals.

Within a week of weaning, CRBP(II) content had fallen precipitously to ~50% of the day 21 postpartum peak. A separate group of dams were left with their litters between days 21 and 28 postpartum and had levels of CRBP(II) intermediate between those found on day 21 and those in dams 1 week post-weaning (data not shown). The post-weaning content of CRBP(II) remained constant between days 7 and 14 post-weaning and was similar to the content in nonpregnant nulliparous animals which were maintained during the trial (7 weeks) to assess the changes in the small intestine associated with maturity (data not shown). CRBP(II) content was also similar in multiparous nonpregnant controls, suggesting that parity may have little effect on the normal levels of CRBP(II) in the intestine (data not shown).

It is evident in **Fig. 2** that most of the increase in the content of CRBP(II) in late pregnancy and lactation occurred in the proximal two-thirds of the small intestine, particularly in the proximal segment, while levels in the distal segment remained low. In control animals, approximately equal amounts of CRBP(II) were present in the proximal and middle segments of the small intestine, while less than 7% of the total intestinal CRBP(II) was contained in the distal segment. By day 21 of lactation, 64% of the total small intestinal CRBP(II) was found in the proximal segment, 32% in the middle segment, and less than 4% in the distal segment. While CRBP(II) content in the distal segment was ~250% and ~125% greater than controls on days 12 and 21 of lactation, respectively, it remained less than 7% of the total intestinal CRBP(II). The greater content of CRBP(II) in the jejunum relative to the ileum was observed previously by Ong (11). CRBP(II) content in the proximal, middle, and distal segments of small intestine from post-trial controls was 40.3, 21.8, and 4.5 nmoles, respectively— quite similar to values in animals post-weaning shown in Fig. 2. Thus, a steeper proximal-distal gradient of CRBP(II) was established as animals matured, due to an 82% increase in the concentration of CRBP(II) in the proximal segment, relative to pre-trial controls, while the concentrations in the middle and distal segments increased only 18% and 37%, respectively.

The profile of CRBP(II) concentration, expressed on a nmoles per g wet tissue basis, in the proximal and middle segments of the small intestine (**Fig. 3**) showed a late gestational rise and then a sharp increase between day 20 of pregnancy and day 1 of lactation. The concentrations

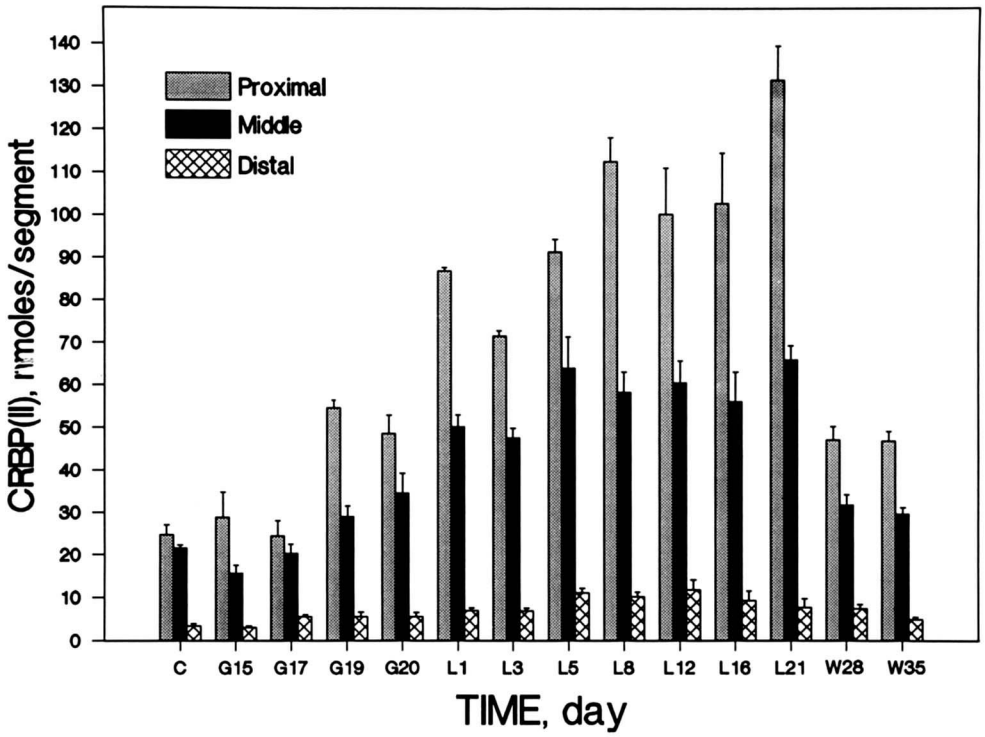

Fig. 2. Total content of CRBP(II) in proximal, middle, and distal thirds of small intestine from prepartum and postpartum rats. Animals and analysis are the same as described in Fig. 1; C, control; G, gestation; L, lactation; W, weaned; (bars represent the mean of three animals ± SEM).

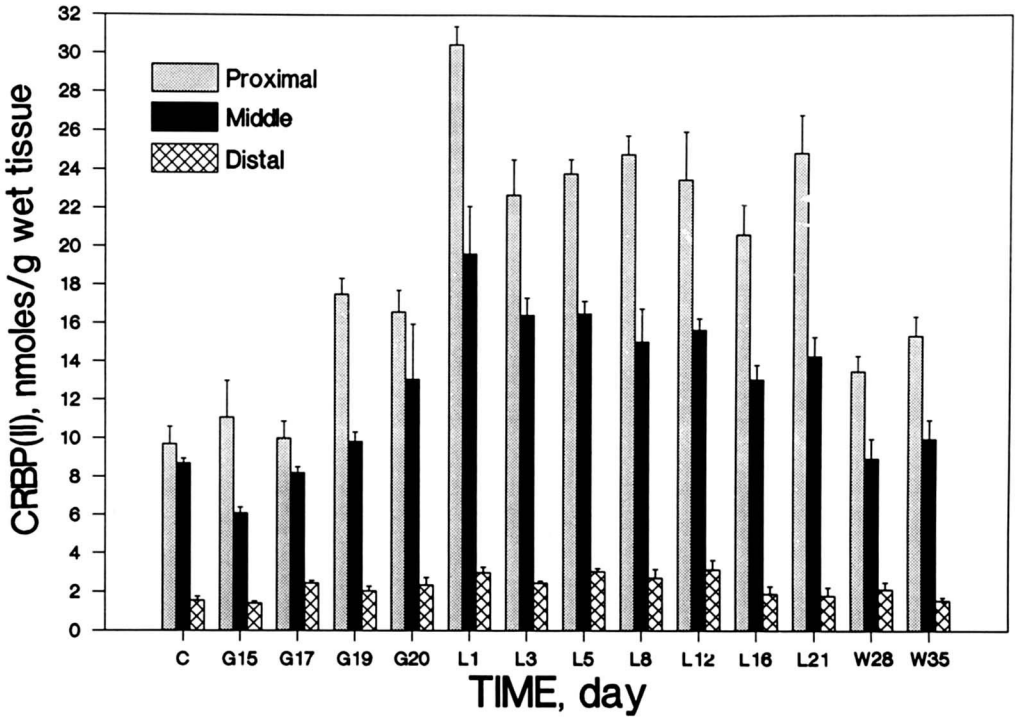

Fig. 3. Concentration of CRBP(II) in proximal, middle, and distal segments of small intestine from prepartum and postpartum rats. Animals and analyses are the same as described in Fig. 1; C, control; G, gestation; L, lactation; W, weaned; (bars represent the mean of three animals ± SEM).

of CRBP(II) in the proximal and middle segments of small intestine from dams on day 1 postpartum were ~210% and ~125% greater than corresponding segments in pre-trial controls. CRBP(II) concentrations decreased somewhat between days 1 and 3 of lactation, consistent with the postpartum changes in CRBP(II) mRNA levels described earlier by Levin et al. (9). CRBP(II) was then maintained at an elevated level for the duration of lactation. The reason for the decrease of CRBP(II) concentration following the parturient peak is not clear. After weaning, CRBP(II) concentration in the proximal and middle segments fell to approximate control levels. In the distal segment, CRBP(II) concentration rose in late pregnancy to a plateau ~70% greater than controls, which was maintained through day 12 of lactation followed by a decline in late lactation and the postweaning period. The pattern of pre- and postpartum changes of CRBP(II) concentration in segments of the small intestine, expressed on a percent soluble protein or on a mg DNA basis, was essentially the same as that presented here on a g wet tissue basis (data not shown).

In sharp contrast to the changes observed in CRBP(II) levels during the pre- and postpartum periods, the concentration of CRBP, shown in **Fig. 4,** remained relatively constant throughout pregnancy and lactation. Whereas, CRBP(II) constituted ~1% of the total soluble protein in the proximal segment of small intestine from day 1 postpartum dams, the amount of CRBP was ~180-fold less. No proximal to distal concentration gradient of CRBP was apparent as was evident with CRBP(II).

The increases in CRBP(II) above that due to general intestinal growth, as reflected in changes in the small intestinal weight and CRBP shown in Fig. 1, would presumably be due to an increase in CRBP(II) per cell or in the total number of cells expressing CRBP(II). The CRBP(II): DNA ratios in enterocytes isolated from the proximal two-thirds of small intestines from rats on day 20 of pregnancy and days 1 and 16 of lactation were 58%, 48%, and 53%, respectively, greater than in controls. These data indicate that cell expression of CRBP(II) had increased but was maximal by day 20 of pregnancy and did not increase further during lactation.

Increases in small intestinal mucosal area, elongation and thickening of villi, and greater numbers of enterocytes in late pregnancy and particularly during lactation have been widely reported (15–17). Cairnie and Bentley (18) estimated that the number of enterocytes per villus in the small intestine of rats on day 14 of lactation is ~40% greater than in controls. Thus, the residual increase in total intestinal CRBP(II) during lactation above that which can be attributed to the elevated cellular expression of the protein was probably due to a progressive increase in the total number of enterocytes within the small intestine.

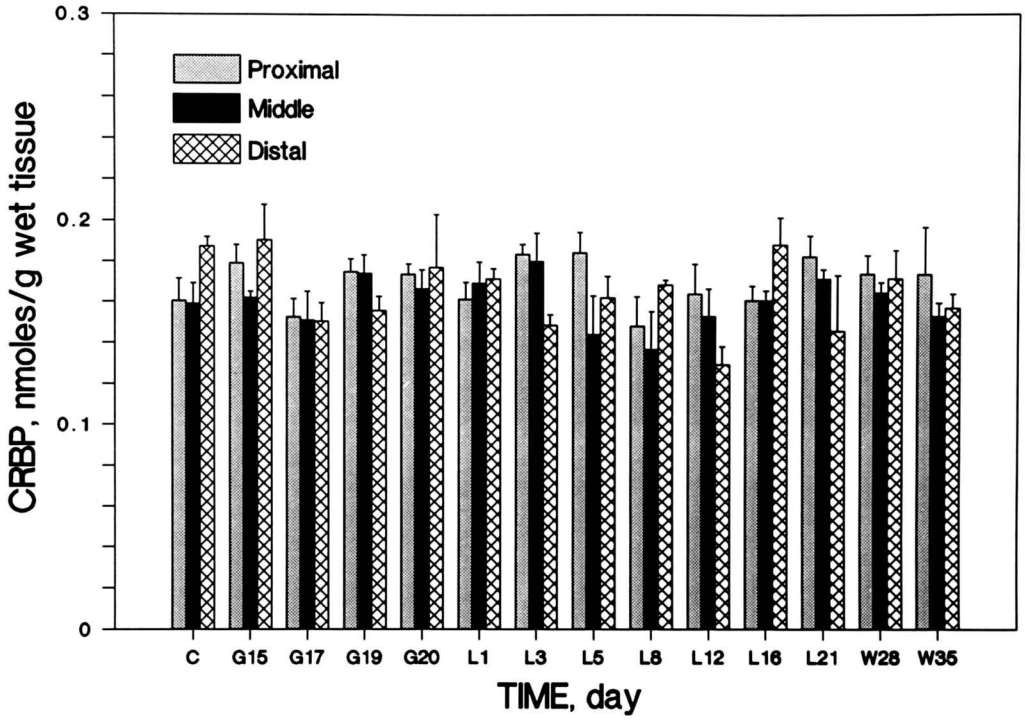

Fig. 4. Concentration of CRBP in proximal, middle, and distal segment of small intestine from prepartum and postpartum rats. Animals and analyses are the same as described in Fig. 1; C, control; G, gestation; L, lactation; W, weaned; (bars represent the mean of three animals ± SEM.

The factors that modulate these changes in the small intestine and may specifically regulate CRBP(II) expression are not established. Cripps and Williams (15) reported that food intake in rats increased ~60% in late pregnancy, fell slightly postpartum, and then increased rapidly from day 3 to day 18 of lactation to a peak 300% greater than controls—strikingly similar to the pattern of CRBP(II) accumulation in this study. However, it appears that these changes are due to hormonal factors or nutrient flux in the small intestine rather than dry matter intake per se (18, 19). The rapid increase in intestinal CRBP(II) in late pregnancy, elevated levels maintained during lactation, and precipitous decline at weaning suggested that prolactin may have a role, as has been demonstrated with fluid and ion transport in the small intestine of pregnant and lactating rats (20, 21). Injection of prolactin in vitamin D-deficient male and female rats rapidly stimulated intestinal calcium absorption (22). However, in a preliminary study in this laboratory, injecting male and female rats two times per day with 500 μg of ovine prolactin (Sigma L-4876) for 2 days was without apparent effect on CRBP(II) levels in the intestine (data not shown). Thus, the regulation of CRBP(II) expression may be modulated by other or a complex of endocrine and nutrient factors associated with changes in the prepartum and postpartum periods.

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