Long-Term Marginal Intakes of Zinc and Retinol Affect Retinol Homeostasis without Compromising Circulating Levels during Lactation in Rats

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ABSTRACT Marginal zinc or vitamin A intake is more common than previously thought in industrialized and developing countries, with pregnant and lactating women believed to be particularly at risk. However, the lack of sensitive indicators of zinc and vitamin A status precludes accurate assessment of marginal nutriture. Concurrent deficiencies in zinc and vitamin A intake often coexist, and the interaction between zinc deficiency and vitamin A metabolism may confound results from epidemiologic or intervention studies. To investigate effects of a maternal diet chronically restricted in zinc or vitamin A intake on indices of vitamin A metabolism, we fed rats a control diet (C) or a diet marginal in zinc (ZD), marginal in vitamin A (AD), marginal in both (DD) or pair-fed to DD (PF), preconception through lactation. Plasma retinol (ROH) was greater and retinol binding protein (RBP) was lower in rats fed ZD, AD and DD compared with those fed C. Hepatic cellular retinol binding protein (CRBP) expression was greater than controls in rats fed ZD and AD and lower in those fed DD, whereas RBP expression was greater in the DD- and PF-fed groups compared with rats C. Mammary gland CRBP and RBP expression were not affected by the diets. Milk ROH was lower in rats fed AD, and milk RBP was lower in those fed ZD and DD compared with rats fed C. In summary, chronic, marginal intake of zinc or vitamin A resulted in alterations in tissue retinol metabolism and milk retinol levels without decreasing plasma zinc, retinol or ROH:RBP during lactation. These observations are of concern because these parameters, which are commonly used to assess zinc and vitamin A status, may lead to misassessment of marginal zinc or vitamin A nutriture in some human populations.

KEY WORDS: mammary gland • zinc • retinol • milk • lactation • rats

The effect of severe vitamin A deficiency on morbidity and mortality has led the WHO to declare vitamin A deficiency an "international crisis," with pregnant and lactating women believed to be particularly at risk (1). However, effects of marginal vitamin A nutriture may also be of concern and may go undiagnosed because routine indicators of vitamin A status, i.e., plasma retinol (ROH)2 and the ratio of ROH:retinol binding protein (RBP), are believed to be maintained over a wide range of dietary intakes. Furthermore, physiologic adaptations to lactation, such as adipose mobilization, may participate in retinol homeostasis by providing an additional source of retinol to the mammary gland for milk secretion. However, effects of chronic, marginal intake of vitamin A during lactation on retinol homeostasis have not been assessed adequately.

Although severe zinc deficiency is uncommon in human populations, marginal intake of zinc is now believed to be more prevalent than once thought in both industrialized and developing countries (2). Zinc deficiency negatively affects vitamin A status by reducing plasma retinol and increasing hepatic retinol (3–5). The situation is further confounded by the existence of concurrent deficiencies in micronutrients such as zinc and vitamin A in many populations (2). Consequently, due to the documented interactions between zinc and vitamin A (3–5), concurrent inadequacies in zinc and vitamin A intake may synergistically affect retinol homeostasis during lactation and thus warrant further consideration.

Although consequences of severe zinc or vitamin A deficiency on nutritional status during pregnancy and lactation have been examined in a number of animal models, effects of long-term marginal zinc and vitamin A intake on indices of retinol metabolism during lactation are largely unknown. We hypothesized that long-term marginal vitamin A or zinc intake would alter retinol metabolism and milk composition during lactation in a rat model without decreasing circulating levels of zinc and retinol. Our results indicate that plasma retinol and the ratio of retinol:retinol binding protein (ROH:RBP) were not reduced despite alterations in tissue retinol metabolism and milk retinol levels, illustrating the difficulty in detecting marginal vitamin A status, which could lead to misassessment of vitamin nutriture in some populations.

MATERIALS AND METHODS

Diets. Rats were fed a casein-based semipurified experimental diet based on the AIN-93 recommendations (6). The diet composi-
tion differed only in vitamin A and/or zinc content as follows: 1) diet marginally low in both vitamin A and zinc (DD); 2) diet marginally low in zinc (ZD); 3) diet marginally low in vitamin A (AD); 4) control diet (C); 5) control diet, pair-fed to group DD (PF) (Table 1).

Rats. This study complied with the Guide for the Use and Care of Laboratory Rats and was administered under the auspices of Animal Resource Services of the University of California, Davis, which is accredited by the American Association for the Accreditation of Laboratory Animal Care. Virgin Sprague-Dawley rats (n = 50; 25 g) were obtained from a commercial source (Simonsen, Gilroy, CA). The rats were maintained in stainless steel hanging cages in a temperature-controlled facility with a 12-h dark-light cycle and allowed to consume purified, deionized water ad libitum. After consumption of standard nonpurified diet (Ralston Purina, St. Louis, MO) for a 7-d acclimatization period, rats (n = 6/diet) were randomly assigned to 1 of 4 experimental diets to consume ad libitum. The remaining 6 rats were pair-fed during the dark cycle to the mean of food consumed by rats fed the DD diet on the previous day. Rats were fed diets for 70 d preconception through d 10 of lactation. Throughout the experiment, food intake was recorded every other day and weight recorded weekly. On postnatal d 2, litters were culled to 10 and pups were cross-fostered to dams receiving the same dietary treatment, if needed. On postnatal d 10, dams were removed from pups for 4 h. After dams were anesthetized with a xylazine/ketamine cocktail (intraperitoneal, 1.6 mg xylazine/kg and 33 mg ketamine/kg), oxytocin (subcutaneous, 10 U/kg) was administered and milk was manually expressed from all mammary glands until all milk was collected (~10 min). Blood was removed by cardiac puncture, dams were killed by asphyxiation with CO₂ and samples of liver and mammary gland were dissected.

Tissue collection. Samples of mammary gland and liver were dissected and immediately homogenized in TRIzol (for RNA extraction, Life Technologies, Rockville, MD) or snap-frozen in liquid nitrogen (for retinol analysis) and frozen at −80°C. Blood obtained by cardiac puncture was collected into heparinized vials. Plasma was separated immediately after hemolysis and hematocrit analysis and frozen at −80°C until analysis.

Hemoglobin/hematocrit. Whole-blood hemoglobin was analyzed after conversion to cytochrome-c by a commercially available kit from Sigma (St. Louis, MO). Hematocrit was measured after whole blood was drawn into a glass capillary tube, sealed and centrifuged for 10 min at top speed in a bench-top microcapillary centrifuge (10 min, 4000 × g).

Mineral analysis. Plasma was digested at room temperature for 5 d with 0.1 mol/L Ultra-pure nitric acid. Mammary glands were minced and rinsed 3 times in fresh isotonic saline at room temperature for 10 min each to remove sequestered milk. Whole milk, liver and blood-dried, minced mammary glands were digested with concentrated nitric acid and wet-ashed using a modification of Clegg et al. (7). Zinc was analyzed by flame atomic absorption spectroscopy (Model Smith-Heififie 4000, Thermo Jarrell Ash, Franklin, MA).

Retinol. Total ROH was quantified by reversed-phase HPLC after dilution with reconstitution solvent (methanol/acetonitrile/isopropanol, 3:1:1) using an aqueous mobile phase [methanol/TAA/ammonium acetate, 75:12:5.125 mol/L, (TAA = tetrathylammoniumacetoni-trile)], 100% methanol, 1% acetic acid, 1 mg/ml of rat plasma (control) samples were injected into a C-18 4-μm reversed-phase column (Waters, Milford, MA) and quantified using a Shimadzu HPLC (Columbia, MD) with N-(4-hydroxyphenyl) retinamide as an internal standard. Total retinol in milk or tissue was quantified after saponification with 30 g/L potassium hydroxide and extraction into hexane (8). Samples were evaporated under nitrogen, diluted with reconstitution solvent and analyzed according to the method above.

Retinol binding protein (RBP). Plasma and milk RBP was quantified by slot blot using human RBP (Sigma) as a standard. Western blot using normal rat serum and defatted milk was used to determine the nonspecificity of RBP antibody (unpublished observations). Whole milk was defatted before analysis by centrifugation at 4°C for 20 min at 10,000 × g. RBP was detected using a rabbit antibody directed against human RBP (Dako, Carpinteria, CA) followed by detection using donkey anti-rabbit immunoglobulin G conjugated to horseradish peroxidase (Amersham Pharmacia Biotech, Piscataway, NJ) and visualization with enhanced chemiluminescence (Amersham Pharmacia Biotech). Densitometry was performed using Chemi-doc Imaging System (BioRad, Hercules, CA), and quantification of plasma RBP was accomplished following linear regression of standard curve. Analyses of human plasma and aged-matched male rat plasma were used to validate each blot.

Milk fat and protein. Whole milk was drawn into a glass capillary tube, sealed and centrifuged for 20 min at 4°C in a tabletop hematocrit centrifuge at top speed. The percentage of fat was measured gravimetrically. Total milk protein was quantified by the Lowry method (9).

### Table 1

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
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</thead>
<tbody>
<tr>
<td>Casein</td>
<td>200.0</td>
</tr>
<tr>
<td>Vitamin mix² (vitamin A-free)</td>
<td>10.0</td>
</tr>
<tr>
<td>Mineral mix³ (zinc-free)</td>
<td>35.0</td>
</tr>
<tr>
<td>Cerelose</td>
<td>392.0</td>
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<tr>
<td>Cornstarch</td>
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<tr>
<td>Corn oil</td>
<td>70.0</td>
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<tr>
<td>DL-Methionine</td>
<td>3.0</td>
</tr>
<tr>
<td>Alphacel</td>
<td>50.0</td>
</tr>
<tr>
<td>Choline chloride (70%)</td>
<td>1.1</td>
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</tbody>
</table>

1 DD, diet marginally low in both vitamin A and Zinc; AD, diet marginally low in vitamin A; ZD, diet marginally low in Zinc; C, control diet; PF, control diet, pair-fed to group DD.

2 The vitamin mix (g/kg) contained: 25.0 inositol, 5.0 ascorbic acid, 2.5 calcium chloride, 1.5 thiamine hydrochloride, 1.5 pyridoxine hydrochloride, 1.5 nicotinic acid, 1.5 menadione, 0.5 riboflavin, 0.5 p-aminobenzoic acid, 0.03 folic acid, 0.0125 biotin, 7.8 Rovi-xim E-50% (Hoffman-La Roche, Nutley, NJ), 0.23 Rovimix AD325/325, 1.5 B-12 (Model Smith-Heififie 4000, Thermo Jarrell Ash, Franklin, MA).

3 The mineral mix (g/kg) contained: 500 dicalcium phosphate, 74 sodium chloride, 220 potassium sulphate, 52 potassium sulphate, 24 magnesium oxide, 3.5 manganese carbonate, 6 ferric sulfate, 0.3 copper carbonate, 0.01 potassium iodate, 0.01 sodium selenite, 0.55 chromium potassium sulphate, 119.5 powdered sugar to replace zinc carbonate. Diets DD and ZD contained 10 mg Zn/kg as zinc carbonate. Diets AD, C and PF contained 25 mg Zn/kg as zinc carbonate.

### Northern blotting

Preparation of cDNA probes. cDNA for rat RBP and rat cellular RBP (CRBP) were generous gifts from Dr. Michael Saret (University of California, Davis). Rat glyceroldehyde phosphate dehydrogenase (GAPDH) cDNA was used as a normalization control. GAPDH (POEM-T Easy), RBP or CRBP (pBluescript II) cDNA was transfected into competent Escherichia coli (DH5α) and selected for on ampicillin (100 mg/L) Luria-Bertani (LB) plates. E. coli containing the rat cDNA insert was grown in ampicillin-containing (100 mg/L) LB broth and total DNA was purified using Midi Prep Kit (Qiagen, Valencia, CA). Total DNA was digested with EcoRI (RBP and GAPDH cDNA) and EcoRI and Hind III (CRBP cDNA) overnight at 37°C, and digests were electrophoresed on a 1% low melt agarose gel containing ethidium bromide (Sigma). Rat cDNA was purified from agarose gel using Gene-Clean Kit (BIO101, Vista, CA) and stored in Trizma Base-EDTA buffer, pH 8, at 4°C. cDNA was labeled with 32P using a DNA radiolabeling kit (Amersham Pharmacia Biotech) and unincorporated nucleotides were removed (S-200 MiniSpin Columns, Amersham Pharmacia Biotech). Probes were denatured at 95°C for 3 min before hybridization.

Isolation of mRNA and Northern blot. Total RNA was isolated following a modification of the Trizol procedure (Life Technol-
FIGURE 1  Effect of diets low in zinc and vitamin A (DD), zinc (ZD) or vitamin A (AD) on food intake before conception compared with control rats. Values represent means ± sd, n = 6. Asterisk indicates significant difference from control rats, P < 0.05.

RESULTS

Food intake. During the initial 70-d prepregnancy period, food intake was reduced by 8.5% in rats fed DD (P < 0.05) (Fig. 1). Although the reduction in food intake of rats fed DD disappeared throughout pregnancy, differences in food intake resumed throughout lactation with reductions in food intake of 11% (DD, P < 0.05), 3.5% (ZD, P = 0.37) and 1% (AD, P = 0.76), relative to rats fed control diet.

Blood indices. Dietary treatments did not affect hemoglobin or hematocrit. There were no significant differences in plasma zinc except in the PF group, which was significantly lower than all other groups (P < 0.05) (Fig. 2). Plasma retinol was significantly higher in rats fed DD, AD and ZD (P < 0.05) compared with those fed C and PF (Table 2). Plasma RBP was significantly lower in rats fed DD, AD and ZD (P < 0.01) than in rats fed C and PF.

Mammary gland. Pair-fed rats had significantly higher mammary gland zinc than rats fed C (P < 0.05) (Fig. 3). Mammary gland retinol was significantly higher in rats fed ZD compared with DD (P < 0.05), and the ratio of mammary gland ROH:milk ROH was significantly elevated in rats fed AD compared with the other groups (P < 0.05) (Table 3). RBP mRNA was detected in the mammary gland of lactating rats except in the PF group, independent of length of exposure to radionuclide probe to radiography film. Expression of RBP and CRBP mRNA relative to controls was not significantly affected by diet (data not shown).

Milk composition. No effect of dietary treatment was observed for milk fat or total milk protein except for PF rats, which had significantly higher fat and protein compared with all other groups (P < 0.01, data not shown). PF rats had significantly higher milk zinc than all other groups (P < 0.001; Fig. 2). Rats fed AD (P < 0.05) had lower milk retinol than rats fed all other diets (Table 3). Rats fed DD, ZD and PF had significantly (P < 0.05) lower milk RBP compared with controls and those fed AD. The ratio of ROH:RBP has been used as an indicator of vitamin A status or retinyl esters present in both milk and plasma. The ratio of ROH:RBP in milk of rats was significantly higher in rats fed DD, AD and ZD (P < 0.05) compared with those fed C and PF (Table 2). Plasma RBP was significantly lower in rats fed DD, AD and ZD (P < 0.01) than in rats fed C and PF.

Table 2

<table>
<thead>
<tr>
<th>Diet</th>
<th>pROH</th>
<th>RBP</th>
<th>ROH:RBP</th>
<th>hROH</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>1.64</td>
<td>2.08</td>
<td>1.3</td>
<td>1.49</td>
</tr>
<tr>
<td>ZD</td>
<td>2.36</td>
<td>0.90</td>
<td>2.8</td>
<td>1.37</td>
</tr>
<tr>
<td>AD</td>
<td>2.13</td>
<td>0.81</td>
<td>2.7</td>
<td>0.98</td>
</tr>
<tr>
<td>DD</td>
<td>2.43</td>
<td>0.81</td>
<td>3.1</td>
<td>2.28</td>
</tr>
<tr>
<td>PF</td>
<td>1.34</td>
<td>2.47</td>
<td>0.7</td>
<td></td>
</tr>
</tbody>
</table>

1 Values are means ± SD, n = 6. Means in a column without a common letter differ, P < 0.05.
fed DD, ZD and PF was significantly higher than in those fed C and AD (Table 3).

Liver. There was no effect of diet on hepatic zinc (Fig. 3). Rats fed DD and AD had lower hepatic retinol than control rats (P < 0.05), whereas PF rats had higher hepatic retinol than ZD, C (P < 0.05), AD and DD (P < 0.01) (Table 2). Compared with control rats, the relative expression of CRBP mRNA was lower in DD-fed rats, and greater in rats fed ZD and AD, and the relative expression of RBP mRNA was greater in rats fed DD and PF diets, P < 0.05 (Fig. 4).

TABLE 3

<table>
<thead>
<tr>
<th>Diet</th>
<th>Gland ROH</th>
<th>Milk ROH</th>
<th>Gland ROH:Milk ROH</th>
<th>Milk RBP</th>
<th>Milk ROH:Milk RBP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nmol/g</td>
<td>μmol/L</td>
<td></td>
<td>μmol/L</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0.44 ± 0.11ab</td>
<td>0.78 ± 0.25a</td>
<td>0.6 ± 0.2a</td>
<td>0.23 ± 0.02a</td>
<td>3.4 ± 0.2c</td>
</tr>
<tr>
<td>ZD</td>
<td>0.61 ± 0.23a</td>
<td>1.44 ± 0.46a</td>
<td>0.4 ± 0.2a</td>
<td>0.16 ± 0.05b</td>
<td>9.2 ± 0.4ab</td>
</tr>
<tr>
<td>AD</td>
<td>0.42 ± 0.04ab</td>
<td>0.42 ± 0.11b</td>
<td>1.0 ± 0.2b</td>
<td>0.23 ± 0.01a</td>
<td>2.0 ± 0.1c</td>
</tr>
<tr>
<td>DD</td>
<td>0.37 ± 0.07b</td>
<td>0.80 ± 0.22b</td>
<td>0.5 ± 0.2b</td>
<td>0.11 ± 0.01bc</td>
<td>7.6 ± 0.2b</td>
</tr>
<tr>
<td>PF</td>
<td>0.46 ± 0.10ab</td>
<td>0.99 ± 0.22a</td>
<td>0.5 ± 0.3a</td>
<td>0.08 ± 0.02c</td>
<td>12.3 ± 0.2a</td>
</tr>
</tbody>
</table>

1 Values are means ± s.d, n = 6. Means in a column without a common letter differ, P < 0.05.

DISCUSSION

Long-term marginal intakes of zinc and vitamin A before conception and throughout pregnancy and lactation resulted in different homeostatic mechanisms to compensate for effects of these nutritional imbalances on retinol homeostasis, despite no indication of nutritional “deficiency” as assessed by conventional indicators such as plasma zinc, retinol and the plasma ROH:RBP ratio. In fact, plasma retinol levels were higher in lactating rats fed diets marginally low in zinc and vitamin A compared with control rats. Additionally, the ratio of plasma ROH:RBP has been used as an indicator of vitamin A status with a ratio of <1 indicating deficiency and >1, adequacy (10). Although the plasma levels of ROH in rats fed ZD, AD and DD were significantly higher than controls, the level of plasma RBP in these rats was lower, thereby increasing the ratio of ROH:RBP, and masking the effects of marginal intake of zinc and vitamin A on this putative indicator of vitamin A status. These results are of concern because investigations of vitamin A or zinc nutriture in human populations routinely use these parameters (i.e., plasma zinc and retinol and ROH:RBP) as indices of an individual’s zinc or vitamin A status, respectively, and may therefore underrepresent the magnitude of marginal maternal zinc and vitamin A nutriture, thus compromising maternal and infant health worldwide.

This study demonstrates that during lactation, the liver responded to marginally decreased vitamin A intake with increased expression of CRBP, whereas the expression of RBP was unaffected. Although the precise role of CRBP is still under investigation, increased CRBP production is presumably required for transport of increased cellular retinol to esterification enzymes for storage during adequate or supplemented intake; it is reduced during severe vitamin A deficiency and is regulated by nuclear retinoic acid receptors (11–14). Other studies have shown that both vitamin A deficiency in rats (13) and apo-CRBP in vitro (15) inhibit lecithin:retinol acyltransferase (LRAT), the major retinol esterification enzyme in the liver, which requires the presentation of retinol bound to CRBP as a substrate (16–18). Therefore, the increased hepatic CRBP expression may functionally increase the apo-CRBP concentration, thus decreasing retinol esterification and enhancing mobilization of intracellular retinol before tissue depletion of retinol.

Although Dawson et al. (13) observed that vitamin A deficiency increased hepatic RBP expression in rats, and subsequent vitamin A supplementation decreased RBP expression, their model of deficiency resulted in a depletion of hepatic and plasma retinol that was not observed in our study. Marginal intake of vitamin A during lactation resulted in
lower hepatic retinol without altered RBP expression concurrently with reduced plasma RBP, suggesting decreased mobilization of RBP-bound retinol from the liver. This again indicates that current indicators of vitamin A status, such as plasma ROH and ROH:RBP ratio may not detect women suffering from marginal vitamin A intake during lactation.

The mammary gland is the physiologic link between circulating and milk micronutrients, and thus plays an integral role in regulating the transport and export of nutrients into milk (19). A diet marginally restricted in vitamin A did not affect retinol concentration in the mammary gland; however, the amount of retinol exported into milk decreased. This suggests that dietary vitamin A and not circulating RBP-bound retinol is responsible for vitamin A levels in milk, resulting in separate intracellular pools of retinol, i.e., one for milk export and the other for intracellular functions. Rats fed this diet showed a trend (P = 0.10) toward increased expression of CRBP in the mammary gland relative to controls. During adequate vitamin A status, mammary gland LRAT is not believed to play a role in retinol esterification in the mammary gland because the concentration of CRBP is far below the Km needed for LRAT activity in this tissue (20). However, if dietary restriction of vitamin A increases apo-CRBP production, retinol esterification in the mammary gland may be altered, leading to changes in retinol concentration or partitioning in milk.

During lactation, the liver responds to decreased zinc intake with increased expression of CRBP compared with control rats, similar to observations made in rats fed diets marginal in vitamin A; however, the mechanisms responsible for this regulation remain unknown. Mobaran et al. (21) showed that zinc deficiency reduced CRBP concentration in the liver of rats. However, these authors utilized a liquid diet and a relatively insensitive method for quantifying CRBP; thus, their results cannot be separated from alterations in protein metabolism. Additionally, their model resulted in significant decreases in both serum retinol and serum zinc compared with control rats, indicating severe effects of the diet. In our study, total hepatic retinol was not affected by reduced zinc intake; therefore it is likely that subtle alterations in intracellular retinol partitioning or transcription factor regulation by zinc or vitamin A are responsible for these changes (14). Additionally, retinol dehydrogenase (RDH) activity is decreased and retinol oxidase (RO) activity is increased by zinc deficiency. The combination of increased liver CRBP and altered retinol enzymatic activity (decreased LRAT or RDH or increased RO) could result in altered retinol esterification or mobilization of retinol stores; however, effects on the activity of these enzymes during marginal zinc intake have not been addressed. Although the concentration of total retinol and the expression of RBP mRNA in the liver were unaffected in the rats fed the zinc-restricted diet in this study, levels of circulating retinol increased and RBP decreased compared with controls. This resulted in an increased ratio of ROH:RBP from 1.3 in control rats to 2.8 in zinc-restricted rats, suggesting that when mild zinc restriction is incurred during lactation, mobilization of retinol bound to RBP from the liver is impaired. Alternatively, increased intestinal retinol absorption or mobilization of retinol from extrahepatic tissues such as adipose, which is catalyzed to provide nutrients to the mammary gland for milk synthesis, may play an important role in maintaining retinol homeostasis during lactation, and remains to be investigated. Interestingly, the ratio of milk ROH:milk RBP increased from 3.4 in control rats to 9.2 in rats fed a marginal zinc diet, suggesting increased milk retinyl esters in these animals and possibly an effect of marginal zinc intake on esterification enzymes in the mammary gland.

Contrary to observations in rats fed either zinc or vitamin A-restricted diets, the combined reduction of both vitamin A and zinc intake significantly decreased CRBP expression in the liver, indicating that the consumption of a diet marginally deficient in both micronutrients results in changes similar to those that have been observed with moderate or severe deficiencies of these micronutrients (14). Additionally, we observed increased expression of RBP and decreased ROH in liver concurrently with decreased RBP and increased ROH in the circulation of rats fed the zinc and vitamin A-restricted diet, suggesting that hepatic mobilization of retinol bound to RBP was impaired. Interestingly, rats fed the diet low in zinc and vitamin A had milk retinol levels similar to control rats, possibly indicating that milk retinol levels may not be useful as an indicator of vitamin A status as has been suggested by Stoltzfus and Underwood (22). Although milk RBP levels were reduced, the ratio of milk ROH:RBP increased in the rats fed the diet restricted in zinc and vitamin A, suggesting increased milk retinyl ester concentration compared with control rats. However, neither the relevance of altered ROH:RBP levels in milk nor the physiologic importance of RBP in milk has been established. Taken together, these observations parallel effects of more moderate deficiencies of these micronutrients on retinol metabolism and indicate that the alterations observed are not necessarily an effect of food restriction because these rats responded differently from the pair-fed rats with respect to many of the variables analyzed. Interestingly, the pair-fed rats responded dramatically and differently from control rats with respect to most of the variables assessed. The applicability of utilizing a food-restricted control group during lactation is itself questionable due to the profound effects of stress on prolactin and cortisol, which may serve to alter metabolism more severely during lactation and warrants further investigation.

In summary, marginal intake of zinc or vitamin A during pregnancy and lactation resulted in significant alterations of retinol metabolism without compromising circulating levels of zinc or retinol or ROH:RBP ratio. This observation is of concern because these parameters are used to assess zinc and vitamin A status, and may lead to misassessment of zinc or vitamin A nutriture in humans, in addition to potentially confounding epidemiologic studies. Interestingly, in rats fed diets marginal in zinc and vitamin A, alterations in mammary gland retinol metabolism reflected zinc intake, whereas alterations in hepatic retinol levels reflected marginal vitamin A intake. However, the mechanisms responsible for these alterations remain to be elucidated.

ACKNOWLEDGMENTS

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LITERATURE CITED