Vitamin A Intake Affects the Contribution of Chylomicrons vs. Retinol-Binding Protein to Milk Vitamin A in Lactating Rats

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ABSTRACT To investigate the influence of vitamin A intake on the contribution of chylomicrons vs. holo retinol-binding protein to milk vitamin A, female rats were fed diets containing either 10 (n = 6) or 50 μmol vitamin A/kg body (n = 4) during pregnancy and through d 13 of lactation. [3H]Vitamin A was incorporated into each diet beginning on d 6 of lactation. Vitamin A concentrations on d 13 were significantly higher in dam liver (× 3), pup liver (× 2.6), milk (× 2.5) and mammary tissue (× 1.3) in rats consuming the higher level of vitamin A. In both groups, vitamin A specific activities in plasma and milk reached apparent plateaus by 2.33 d after addition of [3H]vitamin A to the diets. Vitamin A specific activity in milk was higher than in plasma at all times in both groups. The estimated minimum contribution of chylomicrons to milk vitamin A was 32 ± 3% in rats fed the lower level of vitamin A vs. 52 ± 10% at the higher level (P = 0.014). We concluded that dietary vitamin A, like triglycerides, may be directed to mammary tissue during lactation for preferential secretion into milk; thus, increasing vitamin A intakes will increase the contribution of dietary vitamin A to milk. In contrast to milk, mammary tissue vitamin A turns over very slowly. J. Nutr. 131: 1279–1282, 2001.

KEY WORDS: chylomicrons • vitamin A • milk • mammary tissue • rats

Increases in vitamin A intake are associated with increased levels of vitamin A in milk in both humans (1–3) and experimental animals (4,5). However, the mechanistic relationships between dietary vitamin A and milk vitamin A have not been studied extensively. Thus, the quantitative contributions of the two physiologic carriers of vitamin A, chylomicrons and retinol-binding protein (RBP), to milk vitamin A are not yet known.

Scow and colleagues (6,7) hypothesized that during hydrolysis of chylomicron triglycerides by lipoprotein lipase (LPL), a membrane continuum develops among chylomicrons, the endothelial cells to which LPL is anchored and the underlying tissue parenchymal cells. This continuum may facilitate transfer of fatty acids, partial glycerides, and some unesterified cholesterol and fat soluble vitamins to the parenchymal cells. Further, Blaner et al. (8) showed that LPL can hydrolyze [3H]retinyl esters in lipid emulsions and that the enzyme increases [3H]uptake into cultured adipocytes. Because LPL activity decreases in white adipose tissue and increases in mammary tissue during lactation (9), dietary fatty acids and presumably other lipid-soluble nutrients are directed into milk fat. It is thus reasonable to hypothesize that higher vitamin A intakes are associated with an increased contribution of dietary (chylomicron) vitamin A to milk.

Here we used the kinetic technique of “continuous infusion” (10) to examine the contribution of newly absorbed dietary vitamin A to milk vitamin A during lactation in rats. By preloading the liver with unlabeled vitamin A and then labeling the ingested vitamin A in rats fed two levels of vitamin A, we were able to estimate the quantitative importance of chylomicrons vs. holoRBP to milk as a function of dietary vitamin A intake.

MATERIALS AND METHODS

Animals and diets. Female (60-d-old) and adult male Sprague-Dawley rats were purchased from Harlan Teklad, Madison, WI. Rats were housed individually in shoe-box cages in a room controlled for temperature (22–24°C), humidity (50%) and light (0700–1900 h). Animals had free access to food (see below) and water throughout the studies; females were weighed twice weekly. Animal procedures were approved by The Pennsylvania State University’s Animal Care and Use Committee.

Female rats were fed a modification of the AIN-93G diet (11) containing the following (g/kg): vitamin-free casein, 200; cornstarch, 397; maltodextrin, 132; sucrose, 50; cellulose, 50; mineral mix, 33 (AIN93GMX, Teklad); vitamin A–free vitamin mix, 10 (TD94161, Teklad); l-cystine, 3; choline bitartrate, 2.4; t-butylhydroquinone, 0.014; and soybean oil, 70 to which had been added 10 μmol of retinyl palmitate (Sigma Chemical, St. Louis, MO) per kg. Male rats were the same diet when they were being used for breeding and a commercial cereal-based diet (Laboratory Rodent Diet 5001; PMI Nutrition International, St. Louis, MO) at other times.

Specific batches of purified diet were labeled with [3H]vitamin A, [10,11-3H]Retinyl acetate (sp. act. 3,168 TBq/mmol; generously donated by Hoffman-La Roche, Nutley, NJ) was dissolved in a small amount of soybean oil; this oil was premixed with the total amount of oil required to formulate the diet. Vitamin A–labeled diets were fed during lactation as indicated below.

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**TABLE 1**  
**Vitamin A levels in plasma and milk of lactating rats fed two levels of vitamin A**

<table>
<thead>
<tr>
<th>Lactation day</th>
<th>Plasma</th>
<th>Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LO</td>
<td>HI</td>
</tr>
<tr>
<td></td>
<td>µmol/L</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>1.67 ± 0.17</td>
<td>1.52 ± 0.19</td>
</tr>
<tr>
<td>8</td>
<td>1.60 ± 0.12</td>
<td>1.43 ± 0.15</td>
</tr>
<tr>
<td>10</td>
<td>1.57 ± 0.26</td>
<td>1.50 ± 0.15</td>
</tr>
<tr>
<td>12</td>
<td>1.50 ± 0.21</td>
<td>1.29 ± 0.28</td>
</tr>
</tbody>
</table>

1 Data are means ± s.e for rats fed 10 (LO; n = 6) or 50 µmol vitamin A/kg (HI; n = 4) during pregnancy and through d 13 of lactation. Within each dietary group, there were no effects of time on either plasma or milk vitamin A levels. * HI mean significantly different from LO mean (P < 0.005; t test).

**RESULTS**

The breeding success rate was ~65%; litter sizes ranged from 9 to 16 pups at parturition. Body weights at the time of mating averaged 255 ± 14 g in the LO group (n = 6) and 235 ± 14 g in the HI (n = 4); rats in the latter group were ~12 d younger. On d 13 of lactation (111–132 d of age), body weights were 348 ± 16 g in the LO group and 303 ± 21 g in the HI group (n = 3); liver weight as a percentage of body weight averaged 5.3 ± 0.16 and 4.2 ± 0.42, respectively.

Plasma and milk vitamin A concentrations were not significantly affected by time between d 6 and 13 of lactation in either dietary group (Table 1). Although plasma vitamin A concentrations were not significantly influenced by diet, milk retinol levels were ~1.5 times higher in dams with the higher vitamin A intake.

As was the case for milk, vitamin A levels in liver and mammary tissue were significantly affected by diet on d 13 of lactation (Table 2). Liver vitamin A concentrations in lactating dams in the HI group were 4 times those in the LO group; values were 3.6 times in pup livers. Vitamin A concentrations in mammary tissue of dams in the HI group were 2.3 times those of the LO group dams. In the LO group on d 6 and 9, liver and mammary tissue vitamin A concentrations were similar to the values shown in Table 1 for d 13 as follows: for liver, concentrations were 546 ± 47 (n = 4) on d 6 and 52 ± 74 nmol/g (n = 4) on d 9; for mammary tissue, corresponding concentrations were 546 ± 47 (n = 4) on d 6 and 52 ± 74 nmol/g (n = 4) on d 9.

**TABLE 2**  
**Tissue weights and vitamin A concentrations on d 13 of lactation in liver and mammary tissue of lactating rats fed two levels of vitamin A in pup liver**

<table>
<thead>
<tr>
<th></th>
<th>LO</th>
<th>HI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weight, g</td>
<td>18.3 ± 0.66</td>
</tr>
<tr>
<td></td>
<td>Vitamin A, nmol/g</td>
<td>485 ± 26</td>
</tr>
<tr>
<td>Pup liver2</td>
<td>Weight, g</td>
<td>0.76 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>Vitamin A, nmol/g</td>
<td>24.6 ± 19.8</td>
</tr>
<tr>
<td></td>
<td>Mammary tissue</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Weight, g</td>
<td>26.8 ± 3.01</td>
</tr>
<tr>
<td></td>
<td>Vitamin A, nmol/g</td>
<td>3.54 ± 1.35</td>
</tr>
</tbody>
</table>

1 Data are means ± s.e for rats fed 10 (LO; n = 6) or 50 µmol vitamin A/kg (HI; n = 4) during pregnancy and through d 13 of lactation. * HI mean significantly different from LO mean (P < 0.01; t test).

2 Values are for means for 6 (LO) or 4 litters (HI) of 7 pups/litter.
TABLE 3

Plasma and milk vitamin A relative specific activities in lactating rats fed two levels of vitamin A

<table>
<thead>
<tr>
<th>Lactation day2</th>
<th>Days on [3H]A</th>
<th>Plasma LO</th>
<th>HI</th>
<th>Milk LO</th>
<th>HI</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>0.33</td>
<td>8.76 ± 2.96</td>
<td>18.87 ± 4.92*</td>
<td>10.15 ± 2.82</td>
<td>36.10 ± 2.61*</td>
</tr>
<tr>
<td>8</td>
<td>2.33</td>
<td>13.92 ± 3.39</td>
<td>24.12 ± 4.49*</td>
<td>19.75 ± 2.16</td>
<td>42.40 ± 5.17*</td>
</tr>
<tr>
<td>10</td>
<td>4.33</td>
<td>12.99 ± 1.75</td>
<td>21.46 ± 5.08*</td>
<td>21.13 ± 2.18</td>
<td>41.89 ± 5.25*</td>
</tr>
<tr>
<td>13</td>
<td>7.33</td>
<td>13.50 ± 2.80</td>
<td>23.20 ± 9.20*</td>
<td>22.54 ± 2.35</td>
<td>41.35 ± 8.06*</td>
</tr>
</tbody>
</table>

1 Data are means ± SD, n = 6 for female rats fed 10 μmol vitamin A/kg diet (LO) and n = 4 for those fed 50 μmol/kg (HI) on d 6, 8, 10 and 13 of lactation (0.33, 2.33, 4.33 and 7.33 d after start of feeding vitamin A-labeled diet ([3H]A), respectively). * For plasma or milk, HI mean significantly different from LO mean (P < 0.001, Kruskal-Wallis test).

2 Within each group, there was no effect of time on relative specific activities in plasma or milk from 2.33 to 7.33 d.

3 Values are [plasma or milk retinol specific activity (dpm/nmol)/diet vitamin A specific activity (dpm/nmol)] × 100.

DISCUSSION

As reported in other studies of humans (1–3) and rats (4–5), our results confirm the observations that increases in dietary vitamin A intake are associated with increases in milk vitamin A. Although these data suggest that dietary vitamin A may be directed to milk, they provide no information on the quantitative contributions of chylomicrons vs. holoRBP to milk vitamin A. Presumably holoRBP is able to deliver vitamin A to lactating mammary tissue because vitamin A is present in milk even if rats are fed a vitamin A–free diet (5). In such animals, all of the milk vitamin A must be derived from holoRBP because chylomicrons would contain almost no vitamin A.

Investigating the source of milk vitamin A in monkeys, Vahlquist and Nilsson (15) concluded that RBP is the primary vehicle for delivery of vitamin A to milk. However, these investigators compared isolated RBP and a plasma lipoprotein preparation that presumably did not contain significant amounts of chylomicrons. They speculated that if plasma retinyl ester levels were increased (as would occur transiently during chylomicron metabolism), the contribution from lipoproteins would increase. In a later study, Davila et al. (4) found that when rats were fed 105 μmol vitamin A/kg diet, milk vitamin A on d 14 of lactation was 7 times that of rats fed 2 μmol/kg diet. These authors speculated that a higher vitamin A intake resulted in an enrichment of retinyl esters in chylomicrons and that these could be delivered to milk during lipolysis of chylomicron triglycerides by lactating mammary...
tissue. In our experiment, when intake was increased 4 times (from 10 to 50 μmol/kg), milk vitamin A on d 13 of lactation increased 1.5 times. Because plasma retinol concentrations did not differ in the LO vs. HI groups, we conclude that in the HI group, all of the increase in milk vitamin A was due to chylomicrons. In addition, our calculations indicated that chylomicrons contributed at least 32% of the milk vitamin A in rats fed 10 μmol/kg diet and ~74% in the HI group. That is, as dietary vitamin A was increased, chylomicrons became an increasingly important source of milk vitamin A. We hypothesize that the extra dietary vitamin A is delivered as chylomicron vitamin A to mammary tissue alveolar cells, the site of milk synthesis and secretion. Because relative vitamin A specific activity in milk was substantially higher than that in plasma in both dietary groups, we speculate that a large portion of incoming vitamin A is directed to the mammary gland for secretion into milk rather than to liver as occurs in the nonlactating state. In view of the differing specific activity responses that we observed in milk vs. mammary tissue, we postulate that the vitamin A secreted into milk comes from a pool that is kinetically distinct from that which we measured in mammary tissue.

Until now, little was known about the effects of vitamin A intake on mammary tissue vitamin A levels. In this study and in related work (5), we have shown that dietary vitamin A level positively affects mammary tissue vitamin A concentrations in pregnant/lactating/postlactating rats, even though plasma retinol levels were not changed. The data indicate that the increase comes either from the higher vitamin A level in chylomicrons or from an increased rate of uptake of holoRBP. In a related study (5), we also reported that in age-matched rats that did not conceive, mammary tissue vitamin A levels were not affected by diet, implying that reproductive state may influence vitamin A uptake by mammary tissue.

Ross et al. (16) evaluated the ability of lactating mammary gland to take up chylomicron vitamin A by measuring recovery of [3H]vitamin A in lactating rat mammary tissue after injection of [3H]vitamin A-labeled chylomicrons. Of the dose given, 15–30% was recovered in mammary tissue 2–3 min after chylomicron injection (A.C. Ross, Penn State University; personal communication). Further, tritium uptake increased directly as chylomicron [3H]vitamin A was increased over a 25-fold range. Because there was little uptake of label in postlactating rats, the authors speculated that chylomicron vitamin A uptake by lactating mammary tissue depends on local binding of chylomicrons and lipolysis of chylomicron triglycerides. This effect is likely related to the increase in LPL activity in mammary tissue that occurs at parturition and during lactation (9,17). Analogous to the situation in cultured adipocytes studied by Blaner et al. (8), in which cellular uptake of [3H]retinoids was facilitated by LPL, it may be that LPL in lactating mammary tissue is responsible for hydrolysis of chylomicron-derived retinyl esters, thus allowing retinol uptake by alveolar cells. Because lactating mammary tissue also contains acyl CoA:retinol acyltransferase (18), an enzyme which esterifies retinol, vitamin A delivered during hydrolysis of chylomicron lipids could be reesterified for secretion into milk or storage in the epithelial cells. The observation that increased vitamin A intake facilitates vitamin A secretion into milk has important implications for improving the vitamin A status of neonates.

LITERATURE CITED

5. Green, M.H., Snyder, R.W., Akohoue, S.A. & Green, J.B. Increased mammary tissue vitamin A levels associated with increased vitamin A intake during lactation are maintained after lactation in female rats. J. Nutr. 2001 (in press).