Impact of maternal dietary n-3 and n-6 fatty acids on milk medium-chain fatty acids and the implications for neonatal liver metabolism

Elizabeth M. Novak and Sheila M. Innis
Nutrition and Metabolism Program, Department of Pediatrics, Child and Family Research Institute, University of British Columbia, Vancouver, British Columbia, Canada

Submitted 5 May 2011; accepted in final form 21 July 2011

Novak EM, Innis SM. Impact of maternal dietary n-3 and n-6 fatty acids on milk medium-chain fatty acids and the implications for neonatal liver metabolism. Am J Physiol Endocrinol Metab 301: E807–E817, 2011. First published July 26, 2011; doi:10.1152/ajpendo.00225.2011.—Levels of n-6, n-3, and medium-chain fatty acids (MCFA) in milk are highly variable. Higher carbohydrate intakes are associated with increased mammary gland MCFA synthesis, but the role of unsaturated fatty acids for milk MCFA secretion is unclear. This study addressed whether n-6 and n-3 fatty acids, which are known to inhibit hepatic fatty acid synthesis, influence MCFA in rat and human milk and the implications of varying MCFA, n-6, and n-3 fatty acids in rat milk for metabolic regulation in the neonatal liver. Rats were fed a low-fat diet or one of six higher-fat diets, varying in 16:0, 18:1n-9, 18:2n-6, 18:3n-3, and long-chain (LC) n-3 fatty acids. Higher maternal dietary 18:2n-6 or 18:3n-3 did not influence milk MCFA, but lower maternal plasma triglycerides, due to either a low-fat or a high-fat high-LC n-3 diet led to higher milk MCFA. MCFA levels were inversely associated with 18:1n-9, 18:2n-6, and 18:3n-3 in human milk, likely reflecting the association between dietary total fat and unsaturated fatty acids. High LC n-3 fatty acid in rat milk was associated with lower expression of fatty acid synthase in mammary glands of rats and mice fed di
t high in corn oil (33, 34), which is rich in 18:2n-6 but not n-3 fatty acids (10).

Our focus is the effect of maternal diet fat quantity and composition on milk fatty acids and their relevance to hepatic gene expression in the milk-fed neonate. In the present study, we addressed whether the proportion of maternal dietary energy from fat or carbohydrate, or n-6 and n-3 fatty acids, at constant fat intake influences MCFA secretion in milk. To accomplish this, we custom prepared diets to enable rigorous control of dietary protein and essential nutrient density relative to dietary energy and combined this with studies to address the relevance of the milk fatty acids for growth, liver fatty acids, and hepatic gene expression in the milk-fed pup. Parallel studies addressed associations between MCFA and n-6 and n-3 fatty acids in mature human milk and give insight into the similarities and differences between milk lipid synthesis in rodents and humans.

MATERIALS AND METHODS

Animals and diets. All animal procedures were conducted as approved by the Animal Care Committee of the University of British Columbia and conformed to the guidelines of the Canadian Council on Animal Care. Female Sprague Dawley rats (Charles River Laboratories) were housed in a temperature-controlled animal facility with a 12:12-h light-dark cycle and free access to food and water. The animals were randomly assigned to one of seven semisynthetic diets on day 10 of gestation and maintained on the same diet throughout lactation, n = 6–7/group. The diets were prepared to provide constant protein, vitamins, and minerals per kilocalorie (kcal) of diet, as described in detail (17) and provided in Table 1, with additional well-absorbed fatty acids that can be transported directly to the liver for ß-oxidation (2, 5, 7, 23).

Large numbers of studies make it clear that human milk levels of MCFA vary widely, from ~6 to 20% of milk fatty acids, with large variability also in milk levels of unsaturated fatty acids (22). While the fatty acid composition of the maternal diet appears to be the major factor contributing to variability in the milk unsaturated fatty acids, 18:1n-9, 18:2n-6, 18:3n-3, 20:5n-3, and 22:6n-3 (4, 15, 16, 22, 36), little attention has been given to the role of the dietary fatty acid composition on secretion of MCFA in milk, with most studies focused on the increased secretion of MCFA among lactating women with high-carbohydrate diets (13, 21, 29, 31, 36). In the liver, which like the mammary gland has the capacity for fatty acid synthesis and triglyceride secretion, the n-6 and n-3 fatty acids inhibit fatty acid synthesis at the level of gene transcription via interaction with several transcription factors, including sterol regulatory element-binding protein (SREBP) (14, 24, 27, 40, 41). Recent studies have shown a central role of SREBP in regulating mammary gland lipogenesis (35) and decreased expression of fatty acid synthase in mammary glands of rats and mice fed diets high in corn oil (33, 34), which is rich in 18:2n-6 but not n-3 fatty acids (10).

Fatty acids in milk provide the infant with a concentrated energy source and fatty acids for developing membrane lipids and storage in adipose triglycerides. The major sources of fatty acids for secretion in milk triglycerides are fatty acids derived by uptake from the maternal plasma and fatty acids synthesized de novo in the mammary gland epithelial cells (9, 15, 28, 29, 35). Typically, microsomal fatty acid synthesis leads to the saturated fatty acid palmitic acid (16:0), which can be further elongated to stearic acid (18:0) and desaturated to stearoyl-CoA desaturase to oleic acid (18:1n-9) (38). Fatty acid synthesis in the mammary epithelial cells, in contrast, is terminated at fatty acid chain lengths of 8 to 14 by the mammary specific enzyme thioesterase II, and this gives rise to medium-chain fatty acids (MCFA) (7, 13, 28, 29, 35, 37). The reason why the mammary epithelial cells synthesize MCFA rather than 16:0 is incompletely understood but may involve the need to secrete milk fat as liquid droplets, a process enabled by higher proportions of MCFA, and benefits to the infant that include...
information in Tables 2 and 3. The diets were designed for three comparisons, with no MCFA in any diet. First, fat and carbohydrate were exchanged by isocaloric substitution to give, as a percent of kilocalories, 78% carbohydrate and 4% fat, or 62% carbohydrate and 20% fat, each with a similar 2–3% energy from 18:2n-6. Because replacement of carbohydrate with fat necessitated higher fat, this was accomplished with the monounsaturated fatty acid 18:1n-9. Hence the high-carbohydrate and comparative high-fat diet are designated as low-fat and high-fat 18:1n-9, respectively. Next, to address the effects of saturated, monounsaturated, n-6, and n-3 fatty acids, an additional three diets with 20% kcal from fat were prepared using oils high in 16:0, 18:2n-6, or 18:3n-3, and these are designated as high-fat 16:0, high-fat n-6, and high-fat n-3, respectively. Finally, the effects of long-chain (LC) n-3 fatty acids, 20:5n-3 and 22:6n-3, were addressed in high-fat n-6, and high-fat n-3, respectively. 18:1n-9 had similar 18:2n-6 and 18:3n-3 to the high-fat 18:1n-9 diet, thus enabling comparison with a diet with no 20:5n-3 and 22:6n-3. In all cases, fresh diet was provided each day in excess of each animal’s needs. All litters were reduced to a standard 10 pups at birth. Offspring were weighed on postnatal day 1 and blood from one pup per litter were taken on postnatal day 3 for studies of hepatic gene expression and biochemical analysis. Livers were flash-frozen in liquid nitrogen and then transferred to storage at −70°C. Blood samples were centrifuged at 3,700 rpm for 15 min to separate plasma. Approximately 500 μl of milk were collected from each dam at 4 days postpartum, following intraperitoneal administration of 0.2 ml oxytocin (10 IE/ml) under isofluorane anesthesia. Day 4 postpartum was chosen to be close to the day the neonatal livers were analyzed. Nonfasting blood samples were collected from rat dams and centrifuged as above to separate plasma. All samples were stored at −70°C until analyzed.

**Human milk.** The protocol and procedures involving the women who provided their milk were approved by the Ethical Review Boards for Research Involving Human Subjects at the University of British Columbia and the British Columbia Children’s and Women’s Hospital. All subjects provided written informed consent before participation. Milk samples were obtained from 175 women at 1 mo postpartum and 149 women at 2 mo postpartum, with 131 women providing milk at both 1 and 2 mo postpartum. All of the women were breastfeeding one full-term gestation infant, all were over 18 yr of age, and 56% were breastfeeding their first infant. At the time of milk collection, none of the women reported that they were taking any supplemental oils containing n-3 fatty acids, and none followed a vegan diet. All of the women were participants in a prospective study that involved random assignment to 400 mg/day 22:6n-3 or a placebo from 16 wk gestation until the infant was born (11, 12, 19), with supplementation ending 4–8 wk before milk collection. Milk (60–100 ml) was collected in prepared, labeled vials, frozen on collection, and stored at −70°C until analysis.

**Milk fatty acid analyses.** For analyses of rat and human milk fatty acids, the milks were thawed in ice-cold water, 9:0, 13:0, and 17:0 were added as internal standards, and then the fatty acids were directly transmethylated (20, 25, 26). The fatty acid methyl esters were separated and quantified by GC using a SP-2560 capillary column (100 m × 0.25 mm internal diameter, 20-μm film thickness) (Supelco, Bellefonte, PA). The GC was programmed to separate and quantify the fatty acids from 16:0 to 22:6n-3 and 24:1. The fatty acids were separated by their carbon number and carbon chain length. The fatty acids were separated and quantified by GC using a SP-2560 capillary column (100 m × 0.25 mm internal diameter, 20-μm film thickness) (Supelco, Bellefonte, PA).
Bellefonte, PA), which enabled separation of saturated and cis unsaturated fatty acids, as well as conjugated linoleic acid (20).

Molecular and biochemical analyses. Rat plasma triglycerides were analyzed in 5 μl plasma using the Triglyceride-SL reagent (Genzyme Diagnostics, www.biopacific.net), insulin was analyzed in 10 μl plasma using a rat specific insulin ELISA (Alpco, www.alpco.com), and β-hydroxybutyrate was analyzed by enzymatic assay (Cayman chemical, www.caymanchem.com). Neonatal liver fatty acids were analyzed by GC following extraction of total liver lipids (18). For gene expression analysis, RNA was extracted using the RNeasy Mini Kit (Qiagen, www.qiagen.com) and 1 μg of RNA reverse transcribed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, www.appliedbiosystems.com). Gene expression by real-time PCR was done using the comparative Ct method (ΔΔCt) of relative quantification, with commercially available primers and TaqMan probes specific for mitochondrial citrate transporter (solute carrier family 25, Slc25a1, Rn00820906_g1), ATP citrate lyase (Acly, Rn00566411_m1), fatty acid synthase (Fasn, Rn00594894_g1), pyruvate kinase, liver type (Pklr, Rn00561764_m1), stearoyl-CoA desaturase (Scd1, Rn00566411_m1), cytosolic serine hydroxymethyltransferase (Shmt1, Rn01751636_m1), and 3-hydroxy-3-methylglutaryl-CoA synthase 2 (Hmgcs2, Rn00597339_m1), with actin as the endogenous control. Figure 1 provides metabolic pathways to show the site of action of the enzymes for which gene expression was assessed.

Statistical analysis. For the milk fatty acids and gene expression, results are provided as means ± SE. Differences in milk or liver fatty acids, plasma metabolites and insulin, and gene expression due to the maternal diet in rats were analyzed by ANOVA with Fisher’s least significant difference (LSD) for comparisons among the high-fat diet groups and by unpaired t-tests for comparison between the low-fat and high-fat 18:1n-9 groups. Pearson correlations were used to determine relationships between plasma triglycerides or insulin and milk MCFA and between fatty acids in human milk. To assess growth, the mean pup weight for each litter was calculated, and then the mean of the mean pup weight for each diet was calculated for each diet group (n = 6 litters/diet group). Differences in litter weights at each age were assessed by ANOVA with LSD for post hoc analysis. Differences were considered significant at P < 0.05. All analyses were done with SPSS for WINDOWS (version 15; SPSS, Chicago, IL).

RESULTS

Maternal dietary fat and MCFA levels in rat milk. To address whether the proportion of dietary energy from fat

---

Fig. 1. Metabolic schematic illustrating enzyme functions of gene expression targets. Acly, ATP citrate lyase; Cpt1a, carnitine palmitoyl transferase; Fasn, fatty acid synthase; Hmgcs2, 3-hydroxy-3-methylglutaryl-coenzyme A synthase 2; Pklr, pyruvate kinase, liver type; Scd1, stearoyl-CoA desaturase; Shmt1, serine hydroxymethyltransferase; Slc25a1, solute carrier family 25 (mitochondrial citrate transporter).
Table 4. Fatty acid composition of milk from rats consuming a low- or high-fat maternal diet

<table>
<thead>
<tr>
<th>Dietary variables</th>
<th>Milk Fatty Acid</th>
<th>Low Fat</th>
<th>18:1n-9</th>
<th>16:0</th>
<th>n-6</th>
<th>n-3</th>
<th>Low LC n-3</th>
<th>High LC n-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>21.4 ± 1.08a</td>
<td>15.7 ± 0.52a</td>
<td>26.2 ± 0.54b</td>
<td>14.7 ± 0.30c</td>
<td>15.7 ± 0.51a</td>
<td>15.6 ± 0.87a</td>
<td>15.1 ± 0.38a</td>
<td></td>
</tr>
<tr>
<td>18:1n-9</td>
<td>24.5 ± 2.10a</td>
<td>48.1 ± 1.42a</td>
<td>32.9 ± 1.09a</td>
<td>28.7 ± 0.50a</td>
<td>43.3 ± 1.04a</td>
<td>47.1 ± 1.14a</td>
<td>14.3 ± 0.75a</td>
<td></td>
</tr>
<tr>
<td>18:2n-6</td>
<td>5.48 ± 0.41</td>
<td>5.59 ± 0.23b</td>
<td>4.54 ± 0.29a</td>
<td>20.1 ± 0.32a</td>
<td>6.40 ± 0.37c</td>
<td>6.11 ± 0.18b</td>
<td>6.56 ± 0.29c</td>
<td></td>
</tr>
<tr>
<td>18:3n-3</td>
<td>0.08 ± 0.01a</td>
<td>0.05 ± &lt;0.01a</td>
<td>0.08 ± 0.01ab</td>
<td>0.18 ± 0.01b</td>
<td>2.61 ± 0.10a</td>
<td>0.08 ± 0.01ab</td>
<td>0.49 ± 0.02c</td>
<td></td>
</tr>
<tr>
<td>Mammary derived</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΣMCFA</td>
<td>33.5 ± 4.33a</td>
<td>16.8 ± 2.60a</td>
<td>22.1 ± 1.72a</td>
<td>20.4 ± 1.22a</td>
<td>17.3 ± 1.99a</td>
<td>19.1 ± 2.03a</td>
<td>36.5 ± 2.25b</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE for n = 6-7 animals/diet group. Units are g/100 g fatty acid. MCFA, medium-chain fatty acid. *Different from the same fatty acid in the high-fat 18:1n-9 milk by unpaired t-test, P < 0.05. For each fatty acid, values in the high-fat columns with different superscripts are significantly different by ANOVA with least significant difference for post hoc analysis, P < 0.05.

Influences MCFA synthesis and secretion in milk, we compared the levels of MCFA in milk from lactating rats fed diets that differed in the proportion of energy from fat and carbohydrate but not protein or n-6 and n-3 fatty acids (low-fat compared with high-fat 18:1n-9 diet). These diets had no effect on the milk total fatty acid concentrations with 21.1 ± 2.38 and 21.5 ± 0.99 g fatty acid/dl for dams fed the low-fat and high-fat 18:1n-9 diets, respectively. These results show that uptake of high amounts of 18:2n-6 by the mammary gland, with subsequent suppression of milk MCFA seen in as well as the increased in 18:2n-6 to 20.1 g/100 g of the milk fatty acids in dams fed the high-fat 18:1n-9 diet (Fig. 2 and Table 4). Despite the marked increase in 18:2n-6 to 20.1 g/100 g of the milk fatty acids in dams fed the high-fat n-6 diet, the milk MCFA levels remained similar to milk from dams fed the high-fat 18:1n-9 diet (Table 4). These results show that uptake of high amounts of 18:2n-6 by the mammary gland, with subsequent secretion in milk, does not suppress secretion of MCFA in milk triglycerides. Rather, suppression of milk MCFA seen in association with a diet rich in 18:2n-6 is best explained by the high-fat content rather than the 18:2n-6 content of the maternal diet. The offspring body weight and growth was not different between pups from dams fed the low-fat and high-fat 18:1n-9 diets (P > 0.05, Fig. 3).

Maternal dietary fatty acid composition and milk MCFA and saturated and unsaturated fatty acids. Next, to address whether the maternal dietary fatty acid composition at constant fat intake influences milk MCFA, we fed a constant 20% kcal from fat but with different amounts of 16:0, 18:1n-9, 18:2n-6, and 18:3n-3 (Table 1). Again, the milk total fat concentration remained constant with 21.5 ± 0.99, 20.1 ± 1.18, 19.9 ± 1.38, and 19.8 ± 1.79 g fatty acid/dl in milk for the high-fat 18:1n-9, -16:0, -n-6, and -n-3 diets, respectively, P > 0.05. The dietary fatty acid composition of the lactating rat did, however, have a marked effect on the fatty acid composition of the milk. Thus, 16:0 was highest in milk from rats fed the high-fat 16:0 diet. Levels of 18:2n-6 and its metabolites, 20:4n-6, 22:4n-6, and 22:6n-6, were highest in milk from rats fed the high-fat n-6 diet. Levels of 18:3n-3 and its metabolites, 20:5n-3 and 22:6n-3, were highest in milk from dams fed the high-fat n-3 diet. Levels of 18:1n-9 were highest in milk from rats fed the high-fat 18:1n-9 diet (Fig. 2 and Table 4). Despite the marked increase in 18:2n-6 to 20.1 g/100 g of the milk fatty acids in dams fed the high-fat n-6 diet, the milk MCFA levels remained similar to milk from dams fed the high-fat 18:1n-9, -16:0, and -n-3 diets with only 4.5–6.4 g 18:2n-6/100 g milk fatty acid (Fig. 2 and Table 4). These results show that uptake of high amounts of 18:2n-6 by the mammary gland, with subsequent secretion in milk, does not suppress secretion of MCFA in milk triglycerides. Rather, suppression of milk MCFA seen in association with a diet rich in 18:2n-6 is best explained by the high-fat content rather than the 18:2n-6 content of the maternal diet. The offspring weight and growth was not different from postnatal day 1 to 15 among pups nursed by dams fed the high-fat 16:0, 18:1n-9, n-6, and n-3 diets (P > 0.05, Fig. 3).

Maternal dietary LC n-3 fatty acids and milk MCFA. Previous studies have shown that feeding the LC n-3 fatty acids in fish oils increases plasma and tissue 20:5n-3 and 22:6n-3 and decreases plasma triglycerides (32, 39). Milk from dams fed the high-fat 18:1n-9, -low LC n-3, and -high LC n-3 diets,
providing 0, 0.29, or 4.3% kcal from 20:5n-3/22:6n-3 had
21.6/0.99, 18.4/2.29, and 18.7/1.35 g fatty acid/dl milk,
respectively, P < 0.05. Thus there were no triglyceride-lower-
ing effects of the LC n-3 fatty acids. However, 20:5n-3 in-
creased from 0.02/0.01 to 0.16/0.01 to 3.48/0.12,
and 22:6n-3 increased from 0.26 ± 0.01 to 0.68 ± 0.01 to 5.96 ±
0.26 g/100 g milk fat in milk from dams fed 0, 0.29, or 4.3%
energy from 20:5n-3 and 22:6n-3, respectively, *Different from high-fat
18:1n-9 and low LC n-3 fatty acid groups, P < 0.05.

Maternal plasma triglycerides are associated with differ-
ces in milk MCFA. The mammary gland must coordinate de novo fatty acid synthesis in the mammary epithelial cells with the uptake of fatty acids from plasma to maintain a constant milk fat supply. Feeding LC n-3 fatty acids increased milk MCFA, and the LC n-3 fatty acids are known to decrease plasma triglycerides (32, 39); therefore, we next hypothesized that lower maternal plasma triglyceride would be associated with higher MCFA secretion in milk. Lactating rats fed the low-fat and high-fat high-LC n-3 diets had higher milk MCFA than rats fed the high-fat 18:1n-9 and high-fat low-LC n-3 diets (Figs. 2 and 4 and Table 4). As hypothesized, the plasma triglyceride concentrations were significantly lower in rats fed the low-fat and high-fat high-LC n-3 diets, 89.4 ± 18.0 and 69.7 ± 15.0 mg triglyceride/dl, respectively, compared with rats fed the high-fat 18:1n-9 and high-fat low-LC n-3 diets, 195 ± 21.1 and 165 ± 18.0 mg triglyceride/dl, respectively, P < 0.05. There was also a significant inverse relationship between the maternal plasma triglycerides and milk MCFA, r = −0.580, P < 0.01 (Fig. 5). The maternal plasma insulin, on the other hand, was not different among the groups and showed no relationship to the milk MCFA concentration (Fig. 5). Overall, these results demonstrate that the availability of plasma-derived fatty acids, not their composition, is a primary determinant of MCFA secretion in milk.

Marginal dietary fatty acids influence liver fatty acid com-
position of the milk-fed neonate. To begin to address the
potential implications of milk fatty acid composition for the
neonate, we next analyzed the neonatal liver fatty acids at 3
days postnatal. Pups from mothers fed the low-fat diet had
higher liver 16:0 and lower 18:1n-9 compared with pups from

2.25 compared with 16.8 ± 2.60 g/100 g milk fatty acid from
rats fed 4.3% kcal compared with no 20:5n-3+22:6n-3 (Fig. 4
and Table 4). The increase in milk MCFA in rats fed 4.3% kcal
from 20:5n-3+22:6n-3 compared with no 20:5n-3+22:6n-3
was accompanied by a decrease in milk levels of 18:1n-9.
These results provide an unambiguous demonstration that
20:5n-3+22:6n-3 do not suppress mammary gland fatty acid
synthesis, even when fed in high amounts. However, differ-
ces in pup growth were also evident, with pups from rats fed
the high-fat high-LC n-3 diet having a higher body weight on
postnatal day 1 and maintaining a higher weight trajectory over
the first 10 days postnatal than pups from the high-fat 18:1n-9
or low LC n-3 fatty acid groups (P < 0.05, Fig. 3).

Fig. 5. Association between maternal plasma tri-
glyceride and insulin and MCFA in milk. Maternal
plasma triglyceride concentrations were inversely
associated with milk MCFA, P < 0.01, but there was
no significant association between maternal insulin
concentrations and milk MCFA, P > 0.05.
mothers fed the high-fat 18:1n-9 diet (Fig. 6). Neonatal livers of pups fed by dams in the high-fat groups showed similar relative amounts of fatty acids to those in the milk group (Fig. 6). The retention of high levels of 20:4n-6 and 22:6n-3 in neonatal liver relative to the small amounts of these fatty acids in milk is particularly notable. Specifically, 20:4n-6 never exceeded 3 g/100 g milk fatty acid, but reached 10–18 g/100 g of neonatal liver fatty acid, and 22:6n-3 represented <1 g/100 g milk fatty acid in the low-fat and high-fat 18:1n-9, 16:0, n-6, n-3 and low LC n-3 groups, with retention in the neonatal liver to levels ranging from 7 to 12 g/100 g liver fatty acid. An increase in the milk 22:6n-3 to 6 g/100 g fatty acid in rats fed the high-fat high-LC n-3 diet led to a mean of 28 g/100 g fatty acid in neonatal liver.

Maternal dietary fatty acids influence offspring hepatic expression of genes of fatty acid and glucose metabolism. We next sought to understand whether the differences in milk MCFA, n-6, or n-3 fatty acids are relevant to metabolic regulation in the neonatal liver through analysis of gene expression for enzymes relevant to the expected effects of high MCFA, n-6, or n-3 fatty acids provided in milk. In this regard, MCFA absorbed from the intestine are preferentially trans-

Fig. 6. Rat milk and neonatal offspring liver fatty acids at 3 days postnatal. Bars represent, from left to right, low-fat and high-fat 18:1, -16:0, -n-6, -n-3, -low LC n-3, and -high LC n-3. *Different from high-fat 18:1n-9. For each fatty acid and sample type, milk or liver, bars with different superscripts are significantly different, P < 0.05.
ported directly to the liver and may undergo rapid β-oxidation to acetyl-CoA (2), which is a key intermediate for biosynthesis of other fatty acids and ketones. Oxidation of MCFA may also spare glucose and oxidation of other fatty acids. The n-6 and n-3 fatty acids are known to increase fatty acid β-oxidation and decrease fatty acid synthesis in adult liver through mechanisms that involve regulation of several transcription factors (24, 27, 40, 41). Therefore, to elucidate potential effects of altered milk fatty acid composition on liver metabolism, we examined the expression of key genes involved in fatty acid and glucose metabolism in 3-day-old rat liver.

Fig. 7. Gene expression of enzymes involved in fatty acid and glucose metabolism in 3-day-old rat liver. *Different from high-fat 18:1n-9 and high-fat n-6, \( P < 0.05 \).
MCFA, n-6, or n-3 fatty acids, we probed gene expression of enzymes of fatty acid, glucose, and acetyl-CoA metabolism in neonatal liver. In addition, to address potential effects extending to amino acid metabolism, we also determined expression of \( \text{Shmt1} \). \( \text{Shmt1} \) encodes the enzyme that catalyzes the reversible conversion of serine to glycine. Our results show no effect of higher MCFA in milk on expression of any of the target genes in 3-day-old pup liver (Fig. 7). In contrast, the higher LC n-3 fatty acids in neonatal liver of rats born to dams fed the high-fat high-LC n-3 diet was associated with lower \( \text{PkIr} \) expression, suggesting lower glycolysis, although no effect on \( \text{Cpt1a} \), a rate-limiting enzyme of fatty acid \( \beta \)-oxidation, was found. Neonates with higher LC n-3 fatty acids also had lower \( \text{Acly} \), the enzyme that cleaves cytosolic citrate to generate acetyl-CoA, as well as lower \( \text{Fasn} \) and \( \text{Scd1} \), which encode enzymes involved in fatty acid synthesis, and higher \( \text{Hmgcs2} \), which is involved in ketogenesis. Together these results suggest that n-3 fatty acids directly acetyl-CoA away from fatty acid synthesis and toward increased ketone synthesis. We found no difference in \( \text{Sle25a1} \), which encodes the citrate transporter or \( \text{Shmt1} \). Because differences in gene expression were apparent in offspring of rats fed the high LC n-3 diet, but not those born to mothers fed the high-fat 18:1n-9 or 18:2n-6 diets, the results suggest that the n-3 fatty acids have uniquely different effects on 18:2n-6 on hepatic gene expression in the milk-fed neonate.

**Differences in neonatal liver gene expression are not associated with differences in plasma triglyceride or ketones.** We next determined whether the changes in neonatal liver \( \text{Acly} \), \( \text{Fasn} \), and \( \text{Hmgcs2} \) expression were associated with differences in plasma triglycerides or ketones. Plasma triglyceride concentrations were lower in pups in the low-fat diet compared with the high-fat 18:1n-9 diet group (Fig. 8). However, despite differences in liver \( \text{Hmgcs2} \) expression, there was no significant difference in plasma \( \beta \)-hydroxybutyrate concentrations among the 3-day-old pups.

**Human milk unsaturated fatty acids are inversely associated with MCFA.** The total MCFA content of human milk was not different at 1 and 2 mo postpartum, with 11.8 ± 0.27 (range 4.34–26.7, \( n = 175 \)) and 11.2 ± 0.25 (range 4.25–25.2, \( n = 149 \)) \( \text{g/100 g milk fatty acid} \), respectively (\( P > 0.05 \)). Lauric acid (12:0) and 14:0 were the most abundant and together represented 90% of the total milk MCFA, with a significant correlation between 12:0 and 14:0, \( r = 0.562, P < 0.001, n = 324 \). No differences were found in the milk MCFA between women assigned to 22:6n-3 and the placebo during pregnancy (\( P > 0.05 \)); thus, results for all women were combined for further analysis. The relationship between MCFA and other fatty acids in milk was assessed by grouping the milks by quartile of total MCFA, with results for 1 mo postpartum shown in Table 5 and for 2 mo postpartum in Table 6. As the milk MCFA increased, the levels of 18:1n-9, 18:2n-6, and 18:3n-3 decreased. The sum of milk MCFA, 18:1n-9, 18:2n-6, and 18:3n-3 was remarkably constant, representing ~60% of total fatty acids, further showing the reciprocal changes of MCFA derived from mammary gland synthesis with the 18-carbon chain unsaturated fatty acids (Fig. 9). We found no evidence of any significant relationship between milk MCFA and 20:5n-3 (\( r = 0.088, P = 0.248 \)) or 22:6n-3 (\( r = -0.022, P = 0.703 \)).

### Table 5. Major fatty acids in human milk at 1 mo postpartum separated by quartiles of milk MCFA

<table>
<thead>
<tr>
<th>Quartile</th>
<th>4.31–9.99</th>
<th>10.0–12.5</th>
<th>12.6–14.9</th>
<th>15.0–26.7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatty acid, g/100g fatty acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCFA</td>
<td>8.24 ± 0.23</td>
<td>11.2 ± 0.09</td>
<td>13.7 ± 0.13</td>
<td>18.2 ± 0.64</td>
</tr>
<tr>
<td>18:1n-7*</td>
<td>2.19 ± 0.05</td>
<td>1.91 ± 0.04</td>
<td>1.88 ± 0.05</td>
<td>1.79 ± 0.06</td>
</tr>
<tr>
<td>18:1n-9*</td>
<td>37.4 ± 0.39</td>
<td>35.0 ± 0.43</td>
<td>32.9 ± 0.49</td>
<td>30.4 ± 0.50</td>
</tr>
<tr>
<td>18:2n-6*</td>
<td>13.8 ± 0.36</td>
<td>13.9 ± 0.41</td>
<td>12.6 ± 0.50</td>
<td>11.4 ± 0.51</td>
</tr>
<tr>
<td>18:3n-3*</td>
<td>1.63 ± 0.07</td>
<td>1.64 ± 0.08</td>
<td>1.29 ± 0.09</td>
<td>1.24 ± 0.07</td>
</tr>
<tr>
<td>Σ18 unsat*</td>
<td>55.0 ± 0.66</td>
<td>52.5 ± 0.58</td>
<td>48.7 ± 0.69</td>
<td>44.9 ± 0.83</td>
</tr>
<tr>
<td>16:0</td>
<td>20.0 ± 0.37</td>
<td>20.0 ± 0.34</td>
<td>20.7 ± 0.47</td>
<td>20.4 ± 0.53</td>
</tr>
<tr>
<td>18:0</td>
<td>6.10 ± 0.17</td>
<td>6.20 ± 0.16</td>
<td>6.42 ± 0.18</td>
<td>6.23 ± 0.18</td>
</tr>
<tr>
<td>20:3a-6</td>
<td>0.34 ± 0.01</td>
<td>0.39 ± 0.01</td>
<td>0.40 ± 0.02</td>
<td>0.39 ± 0.01</td>
</tr>
<tr>
<td>20:4a-6</td>
<td>0.41 ± 0.01</td>
<td>0.41 ± 0.01</td>
<td>0.40 ± 0.02</td>
<td>0.39 ± 0.01</td>
</tr>
<tr>
<td>22:4n-6</td>
<td>0.07 ± 0.01</td>
<td>0.07 ± 0.01</td>
<td>0.07 ± 0.01</td>
<td>0.07 ± 0.01</td>
</tr>
<tr>
<td>22:5a-6</td>
<td>0.02 ± 0.01</td>
<td>0.02 ± 0.01</td>
<td>0.02 ± 0.01</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>22:5n-3</td>
<td>0.07 ± 0.01</td>
<td>0.09 ± 0.01</td>
<td>0.08 ± 0.01</td>
<td>0.08 ± 0.01</td>
</tr>
<tr>
<td>22:6n-3</td>
<td>0.27 ± 0.01</td>
<td>0.28 ± 0.02</td>
<td>0.27 ± 0.03</td>
<td>0.24 ± 0.02</td>
</tr>
<tr>
<td>CLA</td>
<td>0.27 ± 0.01</td>
<td>0.27 ± 0.01</td>
<td>0.31 ± 0.02</td>
<td>0.30 ± 0.02</td>
</tr>
<tr>
<td>Other</td>
<td>9.21 ± 0.32</td>
<td>8.58 ± 0.27</td>
<td>8.72 ± 0.29</td>
<td>8.78 ± 0.43</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n \), no. of animals/diet group. The 5th to 95th percentile of milk MCFA was divided into 4 equal quartiles. *Highest and lowest quartiles are significantly different by unpaired \( t \)-test, \( P < 0.05 \). CLA, conjugated linoleic acid. \(^{a}\)Other includes 16:1, 18:3n-6, 18:4n-3, 20:0, 20:1, 20:3n-9, 20:2n-6, 22:0, 22:1n-11, 22:1n-9, 22:5n-3, 24:0, 24:1, and trans fatty acids.

---

Fig. 8. Plasma triglyceride and \( \beta \)-hydroxybutyrate in 3-day-old rats. *Different from high-fat 18:1n-9, \( P < 0.05 \).
and 0.48 g/100 g fatty acid, respectively, at 2 mo postpartum. The percentile content of 20:5n-3 and 22:6n-3 was relatively low, at 18% energy from fat as soybean oil (34). However, the specific energy as corn oil compared with standard rodent chow with acetyl-CoA carboxylase, and fatty acid synthase protein abundance in the mammary gland epithelial cell, not the composition of the fatty acids, is a pivotal factor contributing to regulation of MCFA secretion in milk.

Early studies on MCFA synthesis and secretion in human milk reported an increase in MCFA with high dietary carbohydrate intakes (13, 21, 31) and implicated insulin and glucose stimulation of mammary gland fatty acid synthesis. More recent studies have revealed a potential role for 18:2n-6 in decreasing MCFA synthesis in the mammary epithelial cell, possibly through mechanisms involving SREBP, Srebp and fatty acid synthase gene expression were lower in the mammary glands of rats fed 23% compared with 4.8% energy from corn oil (33), which is rich in 18:2n-6 (10). Lower citrate lyase, acetyl-CoA carboxylase, and fatty acid synthase protein abundance was also reported in mammary glands of mice fed 46% energy as corn oil compared with standard rodent chow with 18% energy from fat as soybean oil (34). However, the specific effect of 18:2n-6 remained unclear, since altering the 18:2n-6 content of the diet by changing fat intake also involved inverse changes in the proportion of energy from carbohydrate. Therefore, we designed diets to dissociate the effects of n-6 and n-3 fatty acids from total fat and carbohydrate intake on mammary gland secretion of MCFA in milk. Our results show that, at constant fat and carbohydrate intakes, changing the dietary fatty acid, 16:0, 18:1n-9, 18:2n-6, or 18:3n-3, had no effect on the milk MCFA, even when 18:2n-6 was increased from 4.5 to 20% of the milk fatty acids by a maternal diet rich in this fatty acid. Based on this, we conclude that the apparent inhibition of MCFA synthesis by diets high in 18:2n-6 are explained by the higher fat, rather than 18:2n-6 in the diet. Similarly, the concomitant secretion of high 20:5n-3 and 22:6n-3 and high MCFA in milk of lactating dams fed the high LC n-3 diets shows that dietary LC n-3 fatty acids do not suppress MCFA secretion in milk. However, lower maternal plasma triglycerides, either due to a high-carbohydrate low-fat diet or a high-fat diet rich in 20:5n-3 and 22:6n-3, led to a remarkable almost twofold increase in milk MCFA (Figs. 4 and 5). Overall, the results provide evidence that MCFA secretion in milk is determined by the availability of plasma fatty acid for uptake by the mammary gland. Here we note that the inverse association of 18:2n-6 and MCFA in human milk could also be interpreted as evidence that unsaturated fatty acids influence mammary gland fatty acid synthesis in humans. However, because the levels of 18:1n-9, 18:2n-6, and 18:3n-3 in human milk increase with increasing maternal dietary intakes of these fatty acids (6, 15), higher levels of unsaturated fatty acids in human milk may simply reflect higher maternal fat intakes. No association was evident between 20:5n-3 or 22:6n-3 and MCFA levels in human milk; however, in our study, median (5th to 95th percentile) dietary intakes of 20:5n-3 and 22:6n-3 were 65 (10–228) and 105 (10–430) mg/day, respectively (12), which is below the amounts associated with significant triglyceride lowering in normolipemic individuals (39). Similarly, in lactating rats, the low LC n-3 diet provided 0.29% kcal as 20:5n-3 and 22:6n-3, equivalent to ~640 mg 20:5n-3 and 22:6n-3 in a 2,000 kcal diet, and this had no effect on the MCFA levels in rat milk (Fig. 4).

The implications of variability in milk fatty acids for metabolism in the neonatal liver have received little attention, although several studies have reported that n-3 fatty acids

Table 6. Major fatty acids in human milk at 2 mo postpartum separated by quartiles of milk MCFA

<table>
<thead>
<tr>
<th>Quartile</th>
<th>4.25-9.99</th>
<th>10.0-12.5</th>
<th>12.6-14.9</th>
<th>15.0-25.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>52</td>
<td>54</td>
<td>26</td>
<td>17</td>
</tr>
<tr>
<td>Fatty acid, g/100 g fatty acid</td>
<td>MCFA</td>
<td>8.26 ± 0.20</td>
<td>11.1 ± 0.11</td>
<td>13.5 ± 0.13</td>
</tr>
<tr>
<td>18:1n-7*</td>
<td>2.06 ± 0.05</td>
<td>1.94 ± 0.04</td>
<td>1.82 ± 0.04</td>
<td>1.76 ± 0.06</td>
</tr>
<tr>
<td>18:1n-9*</td>
<td>37.9 ± 0.47</td>
<td>35.6 ± 0.36</td>
<td>32.6 ± 0.37</td>
<td>31.1 ± 0.74</td>
</tr>
<tr>
<td>18:2n-6*</td>
<td>15.2 ± 0.44</td>
<td>13.7 ± 0.38</td>
<td>12.0 ± 0.41</td>
<td>11.1 ± 0.51</td>
</tr>
<tr>
<td>18:3n-3*</td>
<td>1.77 ± 0.09</td>
<td>1.60 ± 0.07</td>
<td>1.39 ± 0.07</td>
<td>1.38 ± 0.10</td>
</tr>
<tr>
<td>18:4n-6*</td>
<td>0.01 ± 0.01</td>
<td>0.01 ± 0.01</td>
<td>0.01 ± 0.01</td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td>20:4n-6*</td>
<td>0.01 ± 0.01</td>
<td>0.01 ± 0.01</td>
<td>0.01 ± 0.01</td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td>22:4n-6*</td>
<td>0.01 ± 0.01</td>
<td>0.01 ± 0.01</td>
<td>0.01 ± 0.01</td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td>20:5n-3*</td>
<td>0.08 ± 0.01</td>
<td>0.08 ± 0.01</td>
<td>0.07 ± 0.01</td>
<td>0.07 ± 0.01</td>
</tr>
<tr>
<td>22:5n-6*</td>
<td>0.02 ± 0.01</td>
<td>0.02 ± 0.01</td>
<td>0.02 ± 0.01</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>22:6n-3*</td>
<td>0.02 ± 0.01</td>
<td>0.02 ± 0.01</td>
<td>0.02 ± 0.01</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>CLA*</td>
<td>0.02 ± 0.01</td>
<td>0.02 ± 0.01</td>
<td>0.02 ± 0.01</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>Other*</td>
<td>8.23 ± 0.26</td>
<td>8.24 ± 0.26</td>
<td>8.40 ± 0.22</td>
<td>9.12 ± 0.34</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of animals/diet group. The 5th to 95th percentile of milk MCFA was divided into 4 equal quartiles. *Highest and lowest quartiles are significantly different by unpaired t-test, P < 0.05. †Other includes 16:1, 18:3n-6, 18:4n-3, 20:3, 20:1, 20:3n-9, 20:2n-6, 22:0, 22:1n-11, 22:1n-9, 22:5n-3, 24:0, 24:1, and trans fatty acids.

DISCUSSION

These studies began with the knowledge of the large variability in human milk fatty acids, ranging from 4 to ~25% of milk fatty acids (15, 22). MCFA are synthesized in the mammary gland (7, 9, 13, 28, 29, 35, 37), whereas unsaturated fatty acids, particularly the n-6 and n-3 fatty acids, are taken up from plasma (15, 22). The mammary gland must integrate the supply and synthesis of fatty acids with the need for fatty acids for secretion of milk triglycerides. Our results convincingly demonstrate that the availability of plasma lipids for the mammary gland epithelial cell, not the composition of the fatty acids, is a pivotal factor contributing to regulation of MCFA secretion in milk.

P = 0.773 at 1 or 2 mo postpartum (r = 0.025, P = 0.766 and r = −0.111, P = 0.179, respectively). However, the 95th percentile content of 20:5n-3 and 22:6n-3 was relatively low, at 0.16 and 0.53 g/100 g fatty acid, respectively, at 1 mo and 0.18 and 0.48 g/100 g fatty acid, respectively, at 2 mo postpartum.

Fig. 9. Sum of MCFA and 18-carbon unsaturated fatty acids in milk at 1 mo postpartum separated by quartile of MCFA in milk.
regulate gene expression for enzymes of fatty acid synthesis and oxidation and glycolysis in adult rodent liver (24). Our results show that the fatty acid composition of the milk diet had a marked effect on the accumulation of fatty acids in the neonatal liver (Fig. 6), consistent with previous reports (1, 3, 30). In the present study, higher hepatic LC n-3 fatty acids, specifically 20:5n-3 and 22:6n-3, secondary to maternal feeding with the high LC n-3 diet resulted in altered hepatic gene expression in the neonate, with lower Pklr, Acly, Fasn, and Scd1 and higher Hmgcs2. The absence of a difference in Cpt1a expression may simply reflect that all neonates were receiving a milk diet, which is high in fat (22). The lower Fasn, Acly, and Scd1 and higher Hmgcs2 together suggest that higher LC n-3 fatty acids are associated with decreased export of acetyl-CoA as citrate from the mitochondria, decreased fatty acid synthesis, and increased use of acetyl-CoA for generation of ketones. Interestingly, ketones have been proposed to play an important role as a carbon source during early brain development (8). At 3 days of age, no significant effects of the milk fatty acid composition on neonatal plasma triglycerides or ketones were apparent, but whether these appear after longer milk feeding is unknown.

In summary, our results show that, within the range of usual diets, the quantity, not composition, of fatty acid available for uptake by the mammary gland determines MCF/secretion in milk. While the liver and mammary gland are both able to synthesize and secrete triglycerides, the liver integrates the energy supply to maintain normoglycemia and directs excess energy toward fatty acid synthesis for storage in adipose tissue, whereas the mammary gland must coordinate fatty acid synthesis and fatty acid uptake from plasma to maintain triglyceride secretion in milk, which, if altered, has implications for the dietary energy density and potential growth of the infant. In this context, although the n-6 and n-3 fatty acids regulate SREBP and lipogenesis in the liver (24, 27, 40, 41), it is reasonable to expect and consistent with our results that the dietary fatty acid composition does not impact fatty acid secretion in milk. The implications of the variability in fatty acid composition for the developing infant liver, however, merit further understanding.

ACKNOWLEDGMENTS

We acknowledge D. Janette King, Russell Friesen, Michael George, and Roger Dyer for providing laboratory assistance.

GRANTS

This work was supported by a grant from the Canadian Institute of Health Research. S. M. Innis was supported as a senior scientist at the Child and Family Research Institute. E. M. Novak was supported by a graduate student-ship from the Michael Smith Foundation for Health Research.

DISCLOSURES

No conflicts of interest are declared by the authors.

REFERENCES