Dietary Echium Oil Increases Tissue (n-3) Long-Chain Polyunsaturated Fatty Acids without Elevating Hepatic Lipid Concentrations in Premature Neonatal Rats1,2

Qing Yang* and T. Michael O'Shea

Department of Pediatrics, Division of Neonatology, Wake Forest University Health Science, Winston-Salem, NC 27157

Abstract

Echium oil (EO) contains notable quantities of both (n-6) and (n-3) PUFA and has not, to our knowledge, been studied in neonates. We compared growth, tissue PUFA concentrations, and liver lipid profiles in premature neonatal Sprague-Dawley rats that were fed an EO diet with those that were dam-fed (DF) or fed rat milk substitute (RMS) or a fish oil (FO) diet. EO or FO comprised 10% of dietary fat. Rats were delivered prematurely at d 21 of gestation by caesarean section and then DF or fed one of the diets for 6 d. Rats were killed and the fatty acid (FA) concentrations in brain, liver, ileum, and serum and liver lipid profiles were analyzed. All diet-fed rats had similar weight gain and tissue protein concentrations. Compared with DF rats, EO-fed rats had similar brain docosahexaenoic acid (DHA) levels, similar brain and liver arachidonic acid (ARA) levels, higher liver and ileal eicosapentaenoic acid (EPA) levels (P < 0.05), and similar ARA:(EPA+DHA) ratios in brain, liver, and serum. Compared with RMS-fed rats, EO-fed rats had lower liver triglyceride FA and cholesterol ester concentrations (P < 0.05), higher EPA and DHA levels in liver, ileum, and serum, a higher DHA level in brain, and lower tissue and serum ratios of total (n-6):(n-3) PUFA and ARA:(EPA+DHA) (P < 0.05). Compared with FO-fed rats, EO-fed rats had higher ARA levels in brain, liver, ileum, and serum. In conclusion, dietary EO increases tissue EPA and DHA without reducing ARA in brain and liver and without elevating hepatic lipid concentrations of premature neonatal rats. J. Nutr. 139: 1353–1359, 2009.

Introduction

The intrauterine accretion rates of the long-chain PUFA, arachidonic acid (ARA),3 an (n-6) fatty acid (FA), and docosahexaenoic acid (DHA), an (n-3) FA, are the highest during the 3rd trimester of gestation (1). The accretion of ARA exceeds that of DHA until ~32 wk of gestation, but thereafter, the accretion of DHA exceeds that of ARA, which continues during the postnatal period (2–5). Humans have less effective endogenous synthesis of DHA than of ARA (6) and DHA concentrations in human milk vary widely with diet of lactating women worldwide (7,8). The concentration of DHA in preterm infant formula is similar to that in the average term milk of mothers in Western countries (7,8) and does not support the rate of fetal accretion of DHA (1). Thus, very low birth weight (VLBW) preterm infants (birth weight < 1500 g) tend to have DHA deficiency at birth, which often persists during the postnatal period.

Interventions to enhance VLBW infants’ accretion of (n-3) long-chain PUFA include supplementing lactating mothers with fish oil (FO) (9), a main source for DHA and eicosapentaenoic acid (EPA), and supplementing expressed human milk with algal DHA (10). However, the effectiveness of supplementing mothers’ diet with FO depends on compliance. FO is a poor source of ARA and supplementing formula with FO has been associated with poor growth in preterm infants (11,12). Algal oil, which is the only source of long-chain PUFA currently used in infant formula, lacks EPA, an important (n-3) long-chain PUFA in human milk. The endogenous synthesis of (n-3) long-chain PUFA is limited by poor conversion of the precursor α-linolenic acid to EPA and DHA due to the initial rate-limiting Δ6-desaturase (13,14). Stearidonic acid, the product of the Δ6-desaturation of α-linolenic acid and an intermediate in the biosynthesis of EPA and DHA, increases tissue EPA more efficiently than α-linolenic acid in rats (14) and humans (15).

Echium oil (EO), derived from the seeds of Echium plantagineum, is a natural source of stearidonic acid. In adult humans, dietary EO increases plasma and neutrophil EPA (but not DHA) and lowers serum triglycerides (16). In adult mice,

---

1 Supported by a pilot grant from the Center for Botanical Lipids (P50AT002782) at Wake Forest University Health Sciences.
2 Author disclosures: Q. Yang and M. O’Shea, no conflicts of interest.
3 Abbreviations used: ARA, arachidonic acid; DF, dam-fed; DHA, docosahexaenoic acid; EO, echium oil; EO-10, diet containing 10% echium oil as dietary fat; EPA, eicosapentaenoic acid; FA, fatty acid; FO, fish oil; FO-10, diet containing 10% fish oil as dietary fat; RMS, rat milk substitute; VLBW, very low birth weight.
* To whom correspondence should be addressed. E-mail: qyang@wfubmc.edu.

0022-3166/08 $8.00 © 2009 American Society for Nutrition.
dietary EO increases plasma and liver EPA more than DHA and decreases hepatic lipogenesis (17). However, the effect of EO on neonatal animals is unknown. We hypothesized that dietary EO would increase tissue EPA and DHA in neonatal rats. The aims of this study were to examine the safety or tolerance of supplementing diets with EO and to compare the effect of dietary EO with dam feeding (DF), rat milk substitute (RMS), and dietary FO on tissue long-chain PUFA and liver lipid profiles in premature neonatal rats.

Materials and Methods

Animals and diets

The animal protocol was approved by the Institutional Animal Care and Use Committee at Wake Forest University Health Sciences. Neonatal Sprague-Dawley rats were delivered prematurely at d 21 of gestation by cesarean section from timed pregnant female rats (Harlan Sprague Dawley) (18). The newborn pups were mixed and then divided into 4 groups by matching birth weight: the DF group was fed by a foster dam rat; the control diet group was fed RMS; the EO-10 and FO-10 groups were fed RMS containing 10% EO or 10% FO as dietary fat, respectively, n = 5 pups in each group. The dams were fed nonpurified diet ProLab RMH 3000 (LabDiet).

The RMS, a base diet, was prepared as described by Kanno et al. (19) with modifications. Briefly, the milk-base mixture was composed of sodium caseinate (American Casein Co.), whey protein (VitaNet) with casein:whey = 1:5, lactose (Harlan Teklad), mineral and micronutrient mixtures (all reagents from Fisher Scientific and Sigma-Aldrich), and vitamin mixture (Harlan Teklad). The additional water-soluble vitamins were mixed according to Auestad et al. (20) with modification (Table 1). Five edible oils were used as dietary fat in the RMS (12 g/L fat) to obtain a FA composition pattern similar to that of rat’s milk (19,20). The oil mixture contained 30% palm, 15% medium-chain triglyceride (Novatis), 25% coconut, 20% soybean, and 10% corn oils. The milk-base mixture and oil mixture were prepared separately. The pH of the milk-base was adjusted to 6.5. The oil mixture was slowly added to the milk-base with intermittent homogenization using a Polytron-like mixer and then the product was poured into 50-mL sterilized tubes and stored at −20°C. The EO-10 and FO-10 diets were made by replacing 10% of palm oil with 10% of EO or FO, respectively. The nutrient composition of the 3 diets is listed in Table 1. The FA compositions of rat’s milk, diets, and nonpurified rat diet were analyzed and listed in Table 2.

The diet group pups were housed in an incubator with a temperature of 35°C and 70% humidity and fed via intermittent orogastric silicon tubing placement. The feeding volume was 0.1 mL every 4 h for the first 24 h; thereafter, it was increased by 0.05 mL every 12 h. Before each feeding, urination and defecation were induced by gentle stimulation of the anogenital region. Daily weights were recorded. After feeding for 6 d, rats were food deprived for 4–6 h and then killed by decapitation. Samples of serum, forebrain (hereafter referred to simply as brain), liver, and terminal ileum (1 cm from distal end of the small bowel) were collected and stored at −80°C for analyzing FA compositions and protein.

Lipid analyses

FA. FA were isolated from the tissues, serum, rat’s milk, and diets using a chloroform-methanol (2:1) extraction procedure (21). FA of the phospholipid, triglyceride, and cholesterol ester fractions from brain, liver, and ileum were extracted in glass tubes containing measured 1,2-dihexadecanoyl-sn-glycerol-3-phosphatidylcholine, triheptadecanoyl, and cholesteryl heptadecanoate as internal standards and then isolated by TLC (Silica Gel 60, EM Sciences). The phospholipid, triglyceride, and cholesterol ester FA bands were scraped and transferred to 3 glass tubes. The FAME from 3 FA fractions of tissues and from total FA of serum, rat’s milk, and diets were prepared and extracted into isooctane (22). The FAME were separated and quantified by GC (6890N Gas Chromatograph, Agilent). The tissue FA was normalized to tissue protein and expressed as μmol/g protein. The FA in serum, rat’s milk, and diets were expressed as percent of total FA.

Free and total cholesterol. Tissue free cholesterol was obtained from the chloroform-methanol extract, which contained measured amounts of 5α-cholestanol as the internal standard. A small portion of chloroform-methanol lipid extract was transferred to a glass tube and the solvent was evaporated under a stream of nitrogen at 60°C. The dried lipids were dissolved in a small volume of hexane. The free cholesterol concentration was determined by GC. Cholesterol ester was calculated from the cholesterol ester FA (described above) as cholesterol ester-FA/0.7. Total cholesterol was calculated as the sum of free cholesterol and cholesterol ester. Both tissue free cholesterol and cholesterol ester were normalized to tissue protein and expressed as μmol/g protein.

Protein assay

Following chloroform-methanol extraction, the tissue that remained in the water-methanol mixture was allowed to air-dry for 24 h. The dry delipidated tissues were digested in 1 mol/L sodium hydroxide at 60°C.
for 3–4 h. Tissue protein was measured by the Lowry protein assay (23) and reported as μg/mg wet tissue.

**Statistical analysis**

All results are expressed as means ± SEM. To compare the 4 groups with respect to body weight, we used 2-way (age, group) ANOVA for repeated measures. We then used Dunnett’s post hoc test to compare the body weight of the EO-10 group with those of the DF, RMS, or FO-10 groups. To compare the 4 groups’ concentrations of tissue protein and FA and liver lipid, we used 1-way ANOVA. We then used Dunnett’s post hoc test to compare the EO-10 group with the DF, RMS, or FO-10 groups. A P-value of <0.05 was considered significant.

**Results**

**FA compositions of diets**

In all 3 diets, the concentrations of linoleic acid [18:2(n-6)] and α-linolenic acid [18:3(n-3)] were higher than those in rat’s milk. The EO-10 diet contained the highest concentration of α-linolenic acid and stearidonic acid [18:4(n-3)]. Rat’s milk contained the highest concentration of EPA and DHA. The RMS and EO-10 diets lacked detectable concentrations of both the (n-6) and (n-3) long-chain PUFA, ARA, EPA, and DHA. The order of total (n-6):(n-3) PUFA ratios was: RMS > DF > FO-10 > EO-10.

**Daily weight and tissue protein concentrations**

Birth weight did not differ among the 4 groups, with an overall mean of 5.41 ± 0.05 g. The EO-10 group had similar weight gain to the RMS and FO-10 groups but gained less than the DF group (11.70 ± 0.44 μg/mg) and FO-10 (17.40 ± 0.82 μg/mg) groups, but was less than that of the DF group (195.00 ± 5.29) (P < 0.05). Similarly, the protein concentration of ileum in the EO-10 group (96.61 ± 5.19 μg/mg) did not differ from that in the RMS (95.81 ± 3.77 μg/mg) and FO-10 (99.24 ± 3.37 μg/mg) groups but was less than that in the DF group (117.40 ± 2.92 μg/mg) (P < 0.05).

**Tissue phospholipids and serum PUFA concentrations and liver lipid profile**

**Comparison of EO-10 and DF groups.** Compared with the DF group, the EO-10-fed rats had higher concentrations of 18:2(n-6), 18:3(n-6), and 20:3(n-6) in brain, liver, ileum, and serum, lower concentrations of ARA in ileum and serum (P < 0.05), and similar concentrations of ARA in brain and liver. The EO-10-fed rats had no detectable EPA. Compared with the DF group, they had similar 22:5(n-3) and DHA concentrations in brain and higher EPA but lower DHA concentrations in liver and ileum. In serum, LCPUFA, EPA, ARA, and DHA were all lower in EO-10-fed rats than DF rats (P < 0.05) (Table 3). EO-10-fed rats had a higher total (n-6):(n-3) PUFA ratio in brain, liver, and ileum (P < 0.05) (Fig. 1A) and a lower ARA:(EPA + DHA) ratio in ileum (P < 0.05) (Fig. 1B). The EO-10 and DF groups had similar ARA:(EPA + DHA) ratios in brain, liver, and serum (Fig. 1B). The liver phospholipid-FA of EO-10-fed rats was higher than in DF rats, but triglyceride-FA (Fig. 2A), free cholesterol, and cholesterol ester concentrations (Fig. 2B) did not differ between the EO-10-fed and DF rats.

**Comparison of EO-10 and RMS groups.** The EO-10 and RMS groups had similar (n-6) PUFA profiles in brain, liver, ileum, and serum, except that the EO-10 group had higher 18:3(n-6) and 20:3(n-6) levels in ileum and higher 18:3(n-6) in serum (P < 0.05). The EO-10-fed rats had higher EPA and DHA

<table>
<thead>
<tr>
<th>FA</th>
<th>Rat’s milk</th>
<th>RMS</th>
<th>EO-10</th>
<th>FO-10</th>
<th>Nonpurified rat diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>12:0</td>
<td>10.26</td>
<td>12.47</td>
<td>12.81</td>
<td>13.15</td>
<td>0.12</td>
</tr>
<tr>
<td>14:0</td>
<td>12.10</td>
<td>5.89</td>
<td>5.28</td>
<td>6.37</td>
<td>1.12</td>
</tr>
<tr>
<td>15:0</td>
<td>0.14</td>
<td>0.04</td>
<td>0.11</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>16:0</td>
<td>26.40</td>
<td>21.89</td>
<td>17.21</td>
<td>18.86</td>
<td>18.13</td>
</tr>
<tr>
<td>16:1</td>
<td>1.07</td>
<td>0.17</td>
<td>0.10</td>
<td>1.31</td>
<td>1.51</td>
</tr>
<tr>
<td>16:0</td>
<td>17.80</td>
<td>23.18</td>
<td>20.19</td>
<td>19.90</td>
<td>1.84</td>
</tr>
<tr>
<td>18:0</td>
<td>4.44</td>
<td>4.11</td>
<td>3.70</td>
<td>3.78</td>
<td>3.78</td>
</tr>
<tr>
<td>18:1 Δ 9</td>
<td>17.80</td>
<td>23.18</td>
<td>20.19</td>
<td>19.90</td>
<td>1.84</td>
</tr>
<tr>
<td>18:1 Δ 11</td>
<td>1.27</td>
<td>0.39</td>
<td>0.60</td>
<td>0.90</td>
<td>1.84</td>
</tr>
<tr>
<td>18:2(n-6)</td>
<td>16.12</td>
<td>21.46</td>
<td>21.39</td>
<td>20.34</td>
<td>40.86</td>
</tr>
<tr>
<td>18:3(n-6)</td>
<td>0.42</td>
<td>n.d.</td>
<td>1.13</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>20:2(n-6)</td>
<td>0.71</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.23</td>
</tr>
<tr>
<td>20:3(n-6)</td>
<td>0.73</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>20:4(n-6)</td>
<td>1.57</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.12</td>
</tr>
<tr>
<td>20:5(n-6)</td>
<td>0.56</td>
<td>2.21</td>
<td>5.75</td>
<td>2.20</td>
<td>1.10</td>
</tr>
<tr>
<td>22:5(n-6)</td>
<td>0.28</td>
<td>0.08</td>
<td>1.40</td>
<td>0.29</td>
<td>0.20</td>
</tr>
<tr>
<td>22:6(n-3)</td>
<td>0.51</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.18</td>
</tr>
<tr>
<td>Total (n-6):(n-3) PUFA</td>
<td>7.84</td>
<td>9.37</td>
<td>3.15</td>
<td>4.56</td>
<td>1.44</td>
</tr>
</tbody>
</table>

1 Prolab RMH 3000 (LabDiet) contains 22.5% protein, 10.9% fat, and 31.5% carbohydrate.

2 n.d., Not detectable for a peak of GC with a signal:noise ratio < 10, and/or <1000 area units, and/or no peak within a retention time window for a component FA.
In ileum, the DHA concentration was lower in the EO-10 rats in the EO-10 group than in the RMS group (Fig. 1A) in all tissues and serum were lower in the EO-10 group than in FO-10 (Table 3) compared with RMS-fed rats. The total (n-6):(n-3) PUFA ratio (Fig. 1A) and ARA:EPA + DHA ratio (Fig. 1B) in all tissues and serum were lower in the EO-10 group than in the RMS group (P < 0.05). The liver triglyceride-FA (Fig. 2A) and cholesterol ester concentrations (Fig. 2B) were lower in the EO-10-fed than in RMS rats (P < 0.05).

**Comparison of EO-10 and FO-10 groups.** Compared with FO-10-fed rats, EO-10-fed rats had higher ARA and lower EPA and DHA concentrations in brain, liver, and serum (P < 0.05). In ileum, the DHA concentration was lower in the EO-10 rats than in the FO-10 group. EO-10-fed rats had higher 18:2(n-6) and 18:3(n-6) levels in brain and ileum and higher 18:3(n-6) in liver and serum (P < 0.05) (Table 3). The total (n-6):(n-3) PUFA ratio (Fig. 1A) and ARA:EPA + DHA ratio (Fig. 1B) in all tissues and serum were higher in the EO-10 group than in FO-10 group (P < 0.05). Liver triglyceride-FA (Fig. 2A) and cholesterol ester (Fig. 2B) concentrations did not differ between the EO-10 and FO-10 groups.

**Discussion**

There is considerable interest in interventions that increase the intake of (n-3) long-chain PUFA by preterm infants (9,10), concentrations in all tissues and serum and higher 18:3(n-3) and 18:4(n-3) in serum (P < 0.05) (Table 3) compared with RMS-fed rats. The total (n-6):(n-3) PUFA ratio (Fig. 1A) and ARA:EPA + DHA ratio (Fig. 1B) in all tissues and serum were lower in the EO-10 group than in the RMS group (P < 0.05). The liver triglyceride-FA (Fig. 2A) and cholesterol ester concentrations (Fig. 2B) were lower in the EO-10-fed than in RMS rats (P < 0.05).
because the amount of DHA in preterm infant formula is not sufficient to support the rate of fetal accretion of DHA (1). As a basis for future studies in humans, we investigated the possibility that dietary EO, a new edible oil, could increase tissue levels of EPA and DHA in premature neonatal rats. We found that premature neonatal rats fed an EO-10 diet had similar growth and tissue protein concentrations to those fed RMS and FO-10 diets, suggesting that dietary EO has no adverse effects on the growth of neonatal rats, as has been reported in adult mice (17) and chickens (24). In the current study, rats fed experimental diets grew more slowly than DF rats. It is possible that the better weight gain of DF rats resulted from a higher intake and/or better digestion and absorption of rat milk than experimental diets.

The most important findings in the current study were that dietary EO increased serum and tissue phospholipid (n-3) long-chain PUFA, EPA, and DHA concentrations compared with RMS, did not reduce the ARA concentration in brain and liver compared with the FO-10 diet, and resulted in the same ratio of ARA:(EPA + DHA) in brain, liver, and serum as in DF rats. In contrast to the reports in adult mice (17) and chickens (24), the tissue concentrations of both EPA and DHA in neonatal rats were higher in the EO-10 group than in the RMS group, except EPA was undetectable in the brains of both groups. Recently, Zhang et al. (17) reported that in adult mice, the EO diet, compared with a palm oil diet, increased liver DHA concentrations in the triglyceride and cholesterol ester fractions but not in the phospholipid fraction. In contrast, in premature neonatal rats, we found that the EO-10 diet, compared with RMS, increased liver DHA concentrations in the triglyceride fraction (primarily in the phospholipid fraction) but not in the cholesterol ester fraction (Q. Yang, unpublished observations). The mechanism for the difference in endogenous DHA synthesis and cellular location between adults and neonates is unknown. The difference in tissue concentrations of EPA and DHA between the EO-10 and DF groups was tissue dependent. For example, in the brain, EO-10-fed rats had no detectable phospholipid EPA but had the same level of DHA as in DF rats, whereas in the liver and ileum, EO-10-fed rats had higher concentrations of phospholipid EPA but lower concentrations of phospholipid DHA than in DF rats. In both brain and liver, the levels of 22:5(n-3), a final precursor to DHA, were similar in the EO-10 and DF rats. It appears that the developing brain of neonatal rats has a high demand for DHA that is met by incorporating DHA synthesized in the liver and brain (25). Dietary EO increased serum EPA and DHA concentrations in neonatal rats (Table 3) and adult mice (17) but increased only serum EPA in humans (16), suggesting a difference in long-chain PUFA metabolism between rodents and humans.

Nevertheless, our data suggest that premature neonatal rats can actively synthesize EPA and DHA from their 18-carbon precursors, as can human VLBW preterm infants (26–28). In neonatal rats, enrichment of tissue (n-3) long-chain PUFA by dietary EO, compared with RMS, appears to result from either increasing the amount of α-linolenic acid [18:3(n-3)] and stearidonic acid [18:4(n-3)] or decreasing the ratio of α-linolenic acid:stearidonic acid, or both, in the diet. In fact, the EO-10 diet had much more α-linolenic acid and stearidonic acid than rats’ milk and RMS, with a ratio of α-linolenic acid:stearidonic acid (4:1) close to that of rat’s milk (2:1) but much lower than that of

![FIGURE 1](Echium oil affects tissue fatty acid in rat pups)
RMS (28:1). Because the endogenous synthesis rate of (n-3) long-chain PUFA is negatively correlated with the dietary ratio of linoleic acid-linolenic acid (29,30), the fact that this ratio is lower in the EO-10 diet (3.72) than in RMS diet (9.71) may also account for the increased tissue and serum EPA and DHA in EO-10-fed rats. Our results are consistent with the finding in human term infants fed formula that lowering the ratio of linoleic acid-linolenic acid in formula increases DHA levels in erythrocytes (31) and plasma (32).

Our finding that dietary EO did not affect the level of phospholipid ARA, especially compared with dietary FO, in brain and liver could be of clinical importance. Like DHA, ARA is a major structural FA, particularly in the early developing brain (3,4). Low ARA levels in infants given formula supplemented with FO appear to impair preterm infants’ growth (11,12). Our study suggests that supplementing the diet with EO might have an advantage over FO. The similar concentrations of phospholipid ARA in the brain and liver of premature rats fed long-chain PUFA-depleted diets (RMS and EO-10) and of DF rats is probably attributable to enhanced endogenous conversion of linoleic acid to ARA to compensate for the dietary deficiency of ARA, even though the ratio of linoleic acid-linolenic acid in the EO-10 diet was lower than that in RMS. Enhanced synthesis of ARA has also been observed in human VLBW infants (26–28), probably because ARA synthesis, compared with that of DHA (27), involves a shorter and more direct enzyme-catalyzed pathway. Possible explanations for the lower ARA levels in the ileum phospholipids and serum of all diet-fed rats include: 1) the liver might be an important organ for endogenous synthesis of ARA that supplies the brain; 2) the amount of ARA in small intestine might be more dependent on dietary ARA than on endogenous synthesis; and 3) the low circulating ARA in serum could be due to a high uptake by the premature brain.

EO contains substantial quantities (>10% of total FA) of 2 (n-3) 18-carbon PUFA (28% α-linolenic acid and 13% stearidonic acid) and 2 (n-6) 18-carbon PUFA (19% linoleic acid and 11% γ-linolenic acid) (16). Both stearidonic acid and γ-linolenic acid are downstream from the rate-limiting Δ6-desaturase and thereby enhance synthesis of EPA and ARA, respectively. On the other hand, because dietary EO provides more (n-3) 18-carbon PUFA, it lowered the ratios of total (n-6):(n-3) PUFA and ARA:(EPA + DHA) in the tissue phospholipids and serum compared with RMS, but kept the ratio of ARA:(EPA + DHA) in the brain, liver, and serum similar to those in DF rats. Preterm infants have a high capability to synthesize endogenous long-chain PUFA (26–28), so an optimal dose of 18-carbon PUFA and an optimal ratio of (n-6):(n-3) PUFA in formula may improve tissue (n-3) long-chain PUFA status in the preterm infants.

Finally, both EO-10 and FO-10 diets reduced the levels of liver triglyceride-FA and cholesterol ester concentrations compared with RMS, as has been observed in adult mice fed EO and FO diets (17). This result suggests that the association of hepatic long-chain PUFA depletion with the development of fatty liver (33) is due primarily to (n-3) long-chain PUFA depletion rather than (n-6) long-chain PUFA depletion, because rats fed an EO-10 or FO-10 diet had significantly higher liver phospholipid EPA and DHA concentrations but lower ARA than rats fed RMS, which enhanced neither (n-3) nor (n-6) long-chain PUFA. Others have shown that (n-3) PUFA reduced the expression of genes controlling hepatic triglyceride and FA synthesis (17).

In summary, the EO-10 diet, compared with RMS, increased EPA and DHA concentrations and decreased the total (n-6):(n-3) PUFA ratio in brain, liver, and ileal phospholipid and in serum, while keeping the ARA:(EPA + DHA) ratio comparable to those in DF rats in brain, liver, and serum. The EO-10 diet also lowered hepatic triglyceride-FA and cholesterol ester concentrations to the levels in DF- and FO-fed premature neonatal rats. EO provides more α-linolenic acid and stearidonic acid and lowers the ratio of linoleic acid-linolenic acid, enhancing endogenous synthesis of (n-3) long-chain PUFA. Thus, botanical EO could serve as an alternative fat source in preterm infant formula to improve tissue (n-3) long-chain PUFA status in VLBW infants.

Acknowledgments
We thank Lingyao Yang for helping with animal feeding and Drs. John Parks and Lawrence Rudel for kind provision of EO and FO.

Literature Cited


