Diet (n-3) Polyunsaturated Fatty Acid Content and Parity Interact to Alter Maternal Rat Brain Phospholipid Fatty Acid Composition\textsuperscript{1,2}

Beth Levant,\textsuperscript{3,6,*} Marlies K. Ozias,\textsuperscript{1} and Susan E. Carlson\textsuperscript{4,5,6}

\textsuperscript{3}Departments of Pharmacology, Toxicology, and Therapeutics; \textsuperscript{4}Dietetics and Nutrition; \textsuperscript{5}Pediatrics; and \textsuperscript{6}The Smith Mental Retardation Research Center, University of Kansas Medical Center, Kansas City, KS 66160

Abstract

Low tissue levels of (n-3) polyunsaturated fatty acids (PUFAs), particularly docosahexaenoic acid [DHA, 22:6(n-3)], are implicated in postpartum depression. The effects of 1–4 sequential reproductive cycles on maternal brain phospholipid fatty acid composition were determined in female rats fed diets containing \(\alpha\)-linolenic acid (ALA), containing ALA and pre-formed DHA (ALA+DHA), or lacking ALA (low-ALA). Virgin females, fed the diets for commensurate durations served as a control for reproduction. Whole-brain total phospholipid composition was determined at weaning by TLC/GC. A single reproductive cycle on the low-ALA diet decreased brain DHA content by 18% compared to ALA primiparas (\(P < 0.05\)), accompanied by incorporation of docosapentaenoic acid (n-6) DPA, 22:5(n-6) to 280% of ALA primiparas (\(P < 0.05\)). DHA was not further decreased after subsequent cycles; however, there was an additional increase in (n-6) DPA after the second cycle (\(P < 0.05\)). Brain DHA of virgin females fed the low-ALA diet for 27 wk decreased 15% (\(P < 0.05\)), but was accompanied by a more modest increase in (n-6) DPA than in parous low-ALA dams (\(P < 0.05\)). Virgin females and parous dams fed the diet containing ALA+DHA exhibited only minor changes in brain fatty acid composition. These observations demonstrate that brain DHA content of adult animals is vulnerable to depletion under dietary conditions that supply inadequate (n-3) PUFAs, that this effect is augmented by the physiological demands of pregnancy and lactation, and that maternal diet and parity interact to affect maternal brain PUFAs status. J. Nutr. 136: 2236–2242, 2006.

Introduction

Relative to other tissues, the brain has a particularly high lipid content (1). Of the lipids found in the brain, polyunsaturated fatty acids (PUFAs)\textsuperscript{7} play an important role, serving as a major component of the phospholipids that form cell membranes, being precursors of signaling molecules such as prostaglandins and leukotrienes, modulating gene expression through the activation of transcription factors, and forming the microenvironment around membrane-bound proteins (2–4). A predominant species of PUFA in brain is the long-chain PUFA (LC-PUFA) docosahexaenoic acid [DHA, 22:6(n-3)], which represents \(-15%\) of the fatty acids in brain and is derived from the essential fatty acid \(\alpha\)-linolenic acid [ALA, 18:3(n-3)]. Another biologically important LC-PUFA, arachidonic acid [AA, 20:4(n-6)], is synthesized from the essential fatty acid linoleic acid [18:2(n-6)]. Other major species of fatty acids in brain include vaccenic acid [18:1(n-7)], oleic acid [18:1(n-9)], and gadoleic acid [20:1(n-9)] (5).

Decreased levels of (n-3) LC-PUFAs, including DHA, are associated with depressive illnesses. Although brain fatty acid composition has not been examined in depression, the disease is associated with low (n-3) LC-PUFA consumption and/or altered content in peripheral tissues. Of note, erythrocyte and adipose DHA contents of depressed patients were decreased compared with controls (6–8) and were correlated with the severity of symptoms (6,8,9) or attempted suicide (10). The prevalence of depression is inversely related to fish consumption, a major dietary source of (n-3) LC-PUFAs (11,12). Furthermore, dietary supplementation with (n-3) LC-PUFAs appears to improve depressive symptoms in controlled clinical trials (13–16). Similar to nonpuerperal depression, several studies associate postpartum depression with decreased DHA levels in plasma or breast milk (17,18) or decreased ratio of DHA to (n-6) DPA in plasma (19), and n-3 LC-PUFA supplements were effective in reducing depressive symptoms in a pilot study (20).

Although the fatty acid composition of various tissues can vary over time depending on the availability of specific fatty acids and/or reproductive status (21–25), brain DHA content of nonreproducing adult rats appeared to be unaffected by a lack of dietary (n-3) PUFAs (2,21). Recently, we showed that the DHA

\textsuperscript{1} Supported by NIMH MH071599 and NIH Center Grant HD02528.
\textsuperscript{7} Abbreviations used: ALA, \(\alpha\)-linolenic acid; DHA, docosahexaenoic acid; (n-6) DPA, docosapentaenoic acid; FAME, fatty acid methyl ester; LC-PUFA, long-chain polyunsaturated fatty acid; MUFA, monounsaturated fatty acid; P#, postnatal day.
\textsuperscript{*} To whom correspondence should be addressed. E-mail: blevant@kumc.edu.

content of the maternal brain can be reduced after a single reproductive cycle under dietary conditions with reduced availability of (n-3) PUFAs (26), presumably due to the demands of supplying DHA to the developing fetus. This demonstration of the vulnerability of maternal brain DHA to reproduction-related depletion renders viable the hypothesis that altered brain LC-PUFA content, particularly decreased DHA, affects maternal neurobiology. In humans, this could increase sensitivity to stress, and thus susceptibility to postpartum depression, consistent with the diathesis-stress model (27). Despite the growing body of biological findings in nonpuerperal depression (28,29), the pathogenesis of postpartum depression remains unclear (30). Hormonal changes associated with pregnancy and childbirth appear to contribute (31,32); however, the etiology is complex and probably involves the interaction of environmental factors and genetic predispositions. Diets consumed by Western societies are noted for containing low levels of (n-3) PUFAs, particularly relative to (n-6) PUFAs, which compete for elongation and desaturation into LC-PUFAs (33). Moreover, although women appear to have higher rates of conversion of α-linolenic acid, the essential (n-3) PUFA, into DHA than men, humans are relatively inefficient in synthesizing DHA (≈8% conversion), and there is considerable variation among individuals (34–36). The low rate of conversion and high individual variability suggest the possible value of preformed DHA in the diet. It is recommended that pregnant and lactating women consume at least 300 mg DHA/d although average consumption is <100 mg (37,38). Accordingly, depletion of brain DHA, as a result of diet or metabolic capacity, identifies a potential risk factor for postpartum depression. This risk factor may be of particular importance for multiparous women, who are reported to have greater decreases in plasma DHA levels than primiparous women (23,39) and who appear to be at increased risk for postpartum depression (40,41).

In view of the potential importance of brain fatty acid composition for maternal health and the likelihood that women will consume suboptimal levels of (n-3) PUFAs, particularly LC-PUFAs, this study sought to further examine the effects of reproductive activity on the phospholipid fatty acid composition of the maternal brain after sequential reproductive cycles under dietary conditions varying in (n-3) PUFA content.

**Materials and Methods**

**Animals.** All experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the University of Kansas Medical Center Institutional Animal Care and Use Committee.

Rats were housed in a temperature- and humidity-controlled animal facility with a 12-h dark-light cycle (on at 0600) and consumed food and water ad libitum. Female, Long-Evans rats (70–78 g; Harlan) were obtained at least 5 d before the commencement of any treatments and were handled regularly.

**Experimental diets.** The control diet (ALA) was prepared by adding 7% by weight of pure soybean oil (without partial hydrogenation) as a source of α-linolenic acid to a purified baseline diet (Harlan Teklad Basal Diet TD00235). The ALA diet was identical to Harlan Teklad AIN 93G, which meets all current nutrient standards for rat pregnancy and growth (42). The ALA+DHA diet contained both α-linolenic acid and preformed DHA and was formulated with DHASCO (42.57% DHA by weight; Martek Biosciences) substituted on an equal weight basis for soybean oil such that DHA accounted for 0.7% of the total fat in the diet by weight. The low-ALA diet was formulated with sunflower oil (7% by weight), which contains negligible α-linolenic acid compared with soybean oil. The nutritional composition of the experimental diets is presented in Table 1. The fatty acid compositions of all diets are presented in Table 2.

**Study design.** Individually housed dams (n = 6) were fed the respective experimental diets at the time of initial mating with male proven breeders and consumed that diet for the duration of the study. Litters were culled to 8 pups on postnatal day 1 (P1) and weaned on P21. For sequential reproductive cycles, dams were remated 8–10 d after weaning. Age-matched, virgin females served as a control for reproduction and were fed the respective diets (n = 4/time point for the ALA diet, 6/time point for the ALA+DHA and low-ALA diets) for 6, 13, 20, or 27 wk, reflecting the time required to complete 1–4 reproductive cycles. Before purchase, rats were fed Harlan Teklad Global 18% Protein Rodent Diet 2018S, Rats received Teklad Rodent Diet (W) 8604 for 5–7 d during acclimatization.

At the time of weaning of the last litter, or treatment for the commensurate duration for virgins, rats were killed by decapitation and the brains rapidly removed, frozen on dry ice, and stored at −70°C until used for analysis of fatty acids. Fatty acid composition was determined in one hemisphere of each brain by TLC and GC as previously described (43) and expressed as area percentage.

**Data analysis.** Results are presented as the mean ± SEM. Data were analyzed for statistically significant main effects by 3-way ANOVA with factors of diet, number of cycles/duration of treatment, and reproductive status. ANOVA and Tukey’s test were then used to determine the effects of number of cycles/duration of treatment within each diet group and between virgins and parous dams. Differences were considered significant at P < 0.05. After initial analysis indicating no effect of duration of treatment of the ALA diet in virgin females, data for these rats were pooled across the 4 time points and used as a single “virgin control” group for all subsequent analyses.

**Results**

Long-Evans dams underwent 1–4 sequential reproductive cycles while consuming diets varying in (n-3) PUFA content. Age-matched virgin females fed the diets for commensurate periods of time served as a control for reproduction.

**TABLE 1** Composition of the experimental diets

<table>
<thead>
<tr>
<th></th>
<th>ALA</th>
<th>ALA+DHA</th>
<th>Low ALA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal mix</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Casein</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>i-Cystine</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>398</td>
<td>398</td>
<td>398</td>
</tr>
<tr>
<td>Maltdextrin</td>
<td>132</td>
<td>132</td>
<td>132</td>
</tr>
<tr>
<td>Sucrose</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Cellulose</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Mineral mix</td>
<td>35</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Vitamin mix</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>TerButylhydroquinone</td>
<td>0.014</td>
<td>0.014</td>
<td>0.014</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>—</td>
<td>70</td>
<td>—</td>
</tr>
<tr>
<td>Sunflower oil</td>
<td>—</td>
<td>—</td>
<td>70</td>
</tr>
<tr>
<td>DHASCO</td>
<td>1.85</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Harlan Teklad TD00235. The individual basal mix components are presented as supplied by the manufacturer. The basal mix (TD 94045) is modified from AIN 93G (42) for purchase by adding 70 g/kg of the selected oil(s) to the basal mix (930 g/kg).
2 Harlan Teklad TDI94046 (AIN 93G-MX).
3 Harlan Teklad TDI94047 (AIN 93-VX).
4 42.57% DHA by weight (Martek Biosciences).
TABLE 2  Fatty acid composition of diets

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Teklad 2018S¹</th>
<th>Teklad 8604¹</th>
<th>ALA²</th>
<th>ALA+DHA²</th>
<th>Low ALA²</th>
</tr>
</thead>
<tbody>
<tr>
<td>g/kg diet</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:0</td>
<td>0.06</td>
<td>0.59</td>
<td>0.06</td>
<td>0.25</td>
<td>0.19</td>
</tr>
<tr>
<td>16:0</td>
<td>7.64</td>
<td>5.80</td>
<td>6.94</td>
<td>7.11</td>
<td>4.00</td>
</tr>
<tr>
<td>18:0</td>
<td>1.50</td>
<td>1.15</td>
<td>2.80</td>
<td>2.73</td>
<td>2.80</td>
</tr>
<tr>
<td>20:0</td>
<td>0.1</td>
<td>0.08</td>
<td>0.19</td>
<td>0.19</td>
<td>0.13</td>
</tr>
<tr>
<td>22:0</td>
<td>0.03</td>
<td>0.02</td>
<td>0.25</td>
<td>0.19</td>
<td>0.14</td>
</tr>
<tr>
<td>24:0</td>
<td>0.00</td>
<td>0.05</td>
<td>0.06</td>
<td>0.05</td>
<td>0.06</td>
</tr>
<tr>
<td>16:1</td>
<td>0.07</td>
<td>0.80</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>18:1</td>
<td>12.59</td>
<td>8.68</td>
<td>14.32</td>
<td>14.32</td>
<td>12.72</td>
</tr>
<tr>
<td>20:1(n-9)</td>
<td>0.17</td>
<td>0.22</td>
<td>0.13</td>
<td>0.13</td>
<td>0.06</td>
</tr>
<tr>
<td>18:2(n-6)</td>
<td>31.25</td>
<td>19.67</td>
<td>33.73</td>
<td>33.09</td>
<td>42.89</td>
</tr>
<tr>
<td>18:3(n-3)</td>
<td>2.76</td>
<td>1.75</td>
<td>5.09</td>
<td>4.90</td>
<td>0.32</td>
</tr>
<tr>
<td>20:2(n-6)</td>
<td>0.00</td>
<td>0.01</td>
<td>0.02</td>
<td>0.02</td>
<td>0.13</td>
</tr>
<tr>
<td>20:4(n-6)</td>
<td>0.00</td>
<td>0.16</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>20:5(n-3)</td>
<td>0.00</td>
<td>1.08</td>
<td>ND</td>
<td>0.05</td>
<td>ND</td>
</tr>
<tr>
<td>22:5(n-3)</td>
<td>0.00</td>
<td>0.22</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>22:6(n-3)</td>
<td>0.00</td>
<td>0.79</td>
<td>ND</td>
<td>0.44</td>
<td>ND</td>
</tr>
</tbody>
</table>

¹ Fatty acid composition supplied by Harlan Teklad. Trace amounts of other fatty acids reported in these formulations are not presented.
² Data are the total fatty acid composition of the extracted whole diet determined quantitatively using Supelco 37 Component FAME Mix. Trace amounts of other fatty acids detected in these formulations are not presented.
³ ND, not detected.

Adult, virgin, female rats (n = 4/time point) were fed the ALA diet for 6, 13, 20, or 27 wk. Brain phospholipid composition was similar to that previously reported, with palmitic (16:0), stearic (18:0), oleic [18:1(n-9)], and DHA [22:6(n-3)] representing the predominant fatty acid species (44) (Fig. 1). No main effect of duration of treatment on brain fatty acid composition, or interaction of duration of treatment and fatty acid, was detected by 2-way ANOVA (data not shown).

Undergoing sequential reproductive cycles while consuming the ALA diet produced only minor alterations in maternal brain fatty acid composition with 20:1(n-9), “other (n-3)” fatty acids [18:3(n-3), 20:5(n-3), and 22:5(n-3) combined] and “other (n-6)” fatty acids [18:2(n-6), 20:2(n-6), and 20:3(n-6) combined] exhibiting transient changes of 15–18% at some time points (P < 0.05) (Fig. 1). Similarly, the ratio of DHA to (n-6) DPA was not altered after multiple reproductive cycles (Fig. 2).

In virgin females fed the low-ALA diet, brain DHA content decreased gradually over time, reaching 85% of virgin controls (P < 0.05) after the time equivalent to 4 reproductive cycles (27 wk) (Fig. 3). (n-6) DPA increased to 175% of that of virgin controls after the time equivalent to 1 reproductive cycle (6 wk) (P < 0.05) and continued to increase with duration of treatment, reaching 325% of virgin controls at the time equivalent to 4 cycles (27 wk) (P < 0.05 v. 1 cycle). Consequently, the DHA:(n-6) DPA ratio was decreased to 47% of that of virgin controls after the time equivalent to 1 reproductive cycle (P < 0.05) and gradually decreased further to 23% of virgin controls after the time equivalent to 4 reproductive cycles (Fig. 2). Transient changes over the course of treatment were also observed for 20:1(n-9) and other (n-3) fatty acids. Levels of saturated fatty acids (16:0, 18:0, and other), other MUFAs (16:1 and 24:1), 18:1(n-9), 20:4(n-6), 22:4(n-6), and other (n-6) acids did not differ from those of virgin controls at any time point (data not shown).

In reproducing females fed the low-ALA diet, brain DHA content decreased after a single reproductive cycle to 82% of ALA primiparas (P < 0.05) and to 84% of virgin controls (Fig. 3). No further decrease in brain DHA occurred after reproductive cycles 2–4. (n-6) DPA levels were increased after a single reproductive cycle to 280% of primiparous ALA dams (P < 0.05) and continued to increase over subsequent cycles, reaching 480% of ALA primiparas after 4 cycles (P < 0.05 v. 1 cycle). As a result, the DHA:(n-6) DPA ratio decreased to 28% of ALA primiparas after a single reproductive cycle (P < 0.05) (Fig. 2). The DHA:(n-6) DPA ratio was not further decreased by subsequent reproductive cycles but was lower than that of low-ALA virgin females after cycles 1–3 (P < 0.05). Additional alterations in brain fatty acid composition in parous females consuming the low-ALA diet included an increase in 20:1(n-9) to 120% of ALA primiparas (P < 0.05) after two cycles of consuming the low (n-3) diet. This increase in 20:1(n-9) became maximal after 3 cycles when 20:1(n-9) levels were significantly higher than in low-ALA virgin females (P < 0.05). Levels of saturated fatty acids, other MUFAs, 18:1(n-9), 20:4(n-6),

Figure 1  Effects of sequential reproductive cycles on brain phospholipid fatty acid composition of female rats fed the control, α-linolenic acid-containing, diet (ALA). Data are presented as means ± SEM, n = 6 (parous dams) or 16 (virgin controls). All fatty acids measured are shown. The break in the y-axis indicates a change in scale. *Different from the virgin control, P < 0.05.
22:4(n-6), other (n-3), and other (n-6) did not differ from ALA primiparas at any time point (data not shown).

In virgin rats fed the ALA+DHA diet, brain DHA did not differ from that of virgin controls at any time point (Fig. 4). However, (n-6) DPA gradually decreased with duration of treatment, reaching 50% of that of virgin controls at the time equivalent to 3 reproductive cycles (20 wk) \( (P < 0.05) \). Accordingly, the DHA:(n-6) DPA ratio gradually increased, reaching a maximum of 197% of that of virgin controls at the time equivalent to 3 reproductive cycles \( (P < 0.05) \) (Fig. 2).

Other (n-3) fatty acids also decreased to 73% of that of virgin controls after the time equivalent to 1 cycle (6 wk) \( (P < 0.05) \), but did not decrease further with additional duration of treatment. Transient decreases in 18:1(n-7) and 20:1(n-9) also occurred over the course of treatment. Levels of saturated fatty acids, other MUFAs, 20:4(n-6), 22:4(n-6), and other (n-6)s did not differ from those of virgin controls at any time point (data not shown).

In reproducing dams fed the ALA+DHA diet, the brain content of 20:1(n-9) increased to 124% of ALA primiparas after 2 reproductive cycles \( (P < 0.05) \) (Fig. 4). Levels of 20:1(n-9) were not further increased by subsequent reproductive cycles and did not differ from those of virgin females fed the ALA+DHA diet at any time point. Levels of DHA, (n-6) DPA, or any other fatty acid measured did not differ from those of ALA primiparas nor was the DHA:(n-6) DPA ratio altered (Fig. 2).

Levels of saturated fatty acids, other MUFAs, 20:4(n-6), 22:4(n-6), and other (n-6)s did not differ from those of ALA primiparas any time point (data not shown).

Discussion

The demonstration of the depletion of maternal brain DHA content under dietary conditions supplying inadequate (n-3) PUFAs, combined with preclinical, clinical, and epidemiologic findings, suggest that such a depletion could affect maternal neurobiology and thus increase susceptibility to postpartum depression in humans. In view of the potential importance of (n-3) PUFAs for maternal health, the effects of diets varying in PUFAs composition on brain fatty acid status were examined in female rats undergoing sequential reproductive cycles and in virgin controls.

The present findings demonstrate that brain phospholipid fatty acid composition in both nonreproducing and reproducing female rats can be modulated by a diet low in α-linolenic acid even when the diet preceding this experimental manipulation should have resulted in adequate (n-3) PUFA stores. Although some changes in other fatty acids occurred \[ e.g., 18:1(n-7), 20:1(n-9) \], these alterations were either transitory, small in magnitude, or both. The major effects of the diet treatments in parous and virgin female rats were on DHA with a compensatory alteration in (n-6) DPA, a product derived from linoleic acid. Accordingly, the ensuing discussion will focus on the effects on DHA and (n-6) DPA.

The α-linolenic acid–containing control diet is identical to AIN 93G, a diet that meets all current nutrient standards for reproducing and developing rats \( (42) \). However, this diet has not been investigated with regard to adequacy for maternal brain fatty acid status. Because there were no alterations in brain DHA or (n-6) DPA content in female rats fed the α-linolenic

Figure 2
Effects of dietary PUFAs and reproductive status on the ratio of docosahexaenoic acid to (n-6) docosapentaenoic acid of female rats. Data are presented as means ± SEM, \( n = 6 \) (all groups except virgin controls) or 16 (virgin controls). \( \alpha \)Different from virgin control, \( P < 0.05 \); \( \beta \)Different from ALA primiparas, \( P < 0.05 \); \( \gamma \)Different from 1 cycle of respective diet, \( P < 0.05 \); \( \delta \)Virgin different from parous respective time point within diet group, \( P < 0.05 \).

Figure 3
Effects of low dietary α-linolenic acid content (low ALA) and reproductive status on brain phospholipid fatty acid composition of female rats. Data are presented as means ± SEM, \( n = 6 \), and are shown only for fatty acids exhibiting significant effects of diet and/or reproductive status. Control groups are virgin controls, \( n = 16 \), and ALA primiparas, \( n = 6 \). The break in the y-axis indicates a change in scale. \( \alpha \)Different from virgin control, \( P < 0.05 \); \( \beta \)Different from ALA primiparas; \( \gamma \)Different from 1 cycle of respective diet, \( P < 0.05 \); \( \delta \)Different from 2 cycles of respective diet, \( P < 0.05 \); \( \epsilon \)Virgin different from parous at respective time-point within diet group, \( P < 0.05 \).
Figure 4  Effects of a diet containing α-linolenic acid and docosahexaenoic acid (ALA+DHA) and reproductive status on brain phospholipid fatty acid composition of female rats. Data are presented as means ± SEM, n = 6, and are shown only for DHA and fatty acids exhibiting significant effects of diet and/or reproductive status. Control groups are virgin controls, n = 16, and ALA primiparas, n = 6. The break in the y-axis indicates a change in scale. *Different from virgin control, P < 0.05; †Different from ALA primiparas, P < 0.05; "Virgin different from parous at respective time-point within diet group, P < 0.05.

acid-containing diet undergoing as many as 4 sequential reproductive cycles, nor was there any alteration in the ratio of DHA to (n-6) DPA, the data suggest that the α-linolenic acid in this diet is adequate for reproducing female rats. This observation also indicates that the metabolic capacity and/or the tissue DHA stores of the dam are sufficient to maintain maternal brain DHA content after multiple sequential reproductive cycles without the need for preformed DHA in the diet.

In this study, the inclusion of preformed DHA in the diet containing α-linolenic acid and DHA (ALA+DHA) did not increase brain DHA content in the virgin females, even after 27 wk of treatment. However, brain (n-6) DPA content was reduced after 20 wk of treatment, resulting in an increase in the ratio of DHA to (n-6) DPA, suggesting substitution of DHA for (n-6) DPA in cell membranes (45). Other studies showed that the brain DHA content of adult rats can be increased after treatment with diets containing DHA (46–48). The small effect on brain LC-PUFA composition in this study is most likely due to the use of a lower concentration of DHA in the diet, and perhaps also differences in strain and sex. A similar lack of effect of feeding preformed DHA on brain DHA content occurred in reproducing females. However, no gradual decrease in brain (n-6) DPA content occurred, suggesting that what little substitution of DHA for (n-6) DPA that occurred in virgin females did not take place under the demands of pregnancy and lactation.

In contrast to previous reports indicating that brain DHA content of nonreproducing adult rats appeared to be unaffected by a lack of dietary (n-3) PUFA (2,21), our findings demonstrate that brain DHA is vulnerable to depletion when rats are fed a diet containing inadequate (n-3) PUFA for a sufficient period of time. Differences between the present results and previous studies may reflect strain and/or sex differences. However, the formulation of the diets is likely not a factor because the sunflower oil used to prepare the low-ALA diet in this study does contain somewhat higher trace levels of α-linolenic acid than those found in the peanut, safflower, and other oils used in other studies.

In agreement with our previous study (26), brain DHA content was significantly decreased after a single reproductive cycle with consumption of the low-α-linolenic acid diet, accompanied by an increase in (n-6) DPA. This observation concurs with previous reports of declines in plasma DHA levels during pregnancy in women (22,25,49) and suggests that reproduction-related depletion of brain DHA may also occur in humans. Interestingly, brain DHA content was not further decreased after subsequent reproductive cycles. This suggests that maternal brain DHA may be resistant to depletion once some minimum level has been reached, at least under the specific conditions of the low α-linolenic acid diet employed here. However, although no further decrease in brain DHA occurred after the first reproductive cycle, (n-6) DPA content continued to increase, reaching maximal levels after 2 cycles, suggesting that the effects of diet and reproductive activity on brain fatty acid composition were not maximal until 2 cycles had been completed. Again, these findings are similar to observations on plasma or erythrocyte DHA levels in humans, indicating greater depletion in multiparous than in null- or primiparous women (23,24,39), but no significant correlation of DHA level with parity (24). The mechanism underlying this apparent basement effect for maternal brain DHA must be determined in future studies but could involve mobilization of peripheral stores and/or increased synthetic capacity, which is stimulated by estradiol (50) and appears to increase during pregnancy (51–53). These mechanisms could be further augmented after multiple reproductive cycles.

The specific contributory role of brain fatty acid composition in depressive illnesses remains to be determined. However, LC-PUFAs influence neuronal function in several ways. Within the membranes of neuronal cells, phospholipids form the surrounding microenvironment, and thus influence the conformation and function of receptors, ion channels, and other membrane-bound proteins (2). A number of LC-PUFAs, most notably arachidonic acid, serve as precursors for intracellular signaling molecules such as prostaglandins. Recently, DHA was shown to be the precursor of the neurotrophic factor neuroprotectin D1 (54). In addition, LC-PUFAs can activate transcription factors and thus modulate gene expression (3). Accordingly, any alteration in brain LC-PUFA composition resulting from the interaction of diet and reproductive activity would likely alter maternal neurobiology. The neurobiological effects of altered LC-PUFA in the parous organism, as well as in virgin females fed an α-linolenic-deficient diet long-term, must be determined in future studies. However, alterations in serotonergic function, which is of particular relevance in depression (28,29), are associated with decreased brain DHA. Of note, low plasma DHA levels resulting from alcoholism are correlated with...
increased cerebrospinal fluid concentrations of the serotonin metabolite 5-hydroxyindoleacetic acid (55). Altered serotonergic function was also found in several animal models of decreased brain DHA content. For example, rats with decreased brain DHA content from conception exhibit altered serotonergic neurotransmission and increased density of 5-HT2A receptors (56,57), whereas diets that increased cortical DHA content increased serotonin levels in piglets (58). Other neurotransmitter systems that may play a role in depression, such as dopamine (59), are also affected in rats with low brain DHA content throughout development (60). Consistent with these neurochemical alterations, rats fed an (n-3) PUFA-deficient diet at weaning exhibited increased immobility in the Porsolt forced swim test, an animal model of depression, at adulthood (61).

In conclusion, the present findings indicate that brain DHA content of adult, female rats is vulnerable to depletion when they consume a diet supplying inadequate (n-3) PUFAs. Depletion of brain DHA, and the reciprocal incorporation into (n-6) DPA, is increased by the physiological demands of pregnancy and lactation, with a baseline effect occurring after 2 sequential reproductive cycles. Although brain DHA content was maintained in reproducing female rats fed a diet containing a-linolenic acid, but no DHA, the relatively inefficient synthesis of DHA in humans (36) opens the possibility that parous women could be vulnerable to depletion of brain DHA content without the inclusion of preformed DHA in the diet. Furthermore, these observations provide additional support for the hypothesis that reproduction-related depletion of brain DHA content could produce alterations in maternal neurobiology that might increase vulnerability to postpartum mental illness in women and suggest that appropriate (n-3) PUFA content and composition of the diet may be important for maternal health as well as optimal development of the offspring.

Acknowledgments
The authors thank Paul F. Davis and Mike L. Richards for technical assistance.

Literature Cited
18. De Vriese SR, Christophe AB, Maes M. Lowered serum n-3 polyunsaturated fatty acid (PUFA) levels predict the occurrence of postpartum depression: further evidence that lowered n-PUFAs are related to major depression. Life Sci. 2003;73:3181–7.