Specific Brain Regions of Female Rats Are Differentially Depleted of Docosahexaenoic Acid by Reproductive Activity and an (n-3) Fatty Acid-Deficient Diet¹,²

Beth Levant,³,⁶* Marlies K. Ozias,³ and Susan E. Carlson⁴–⁶

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

Abstract

Low tissue levels of (n-3) PUFA, particularly docosahexaenoic acid [DHA, 22:6(n-3)], are implicated in postpartum depression. Brain DHA content is depleted in female rats undergoing pregnancy and lactation when the diet supplies inadequate (n-3) PUFA. In this study, the effects of DHA depletion as a result of reproductive activity and an (n-3) PUFA-deficient diet were examined in 8 specific brain regions of female rats after undergoing 2 sequential reproductive cycles. Virgin females, fed the α-linolenic acid (ALA)-containing or deficient (low-ALA) diets for a commensurate duration (13 wk) served as a control for reproduction. Total phospholipid composition of each brain region was determined at weaning (postnatal d 21) by TLC/GC. The regional PUFA composition of ALA virgins was similar to that previously measured in male rats. All brain regions examined were affected by reproductive activity and/or the low-ALA diet; however, the magnitude of the loss of DHA and compensatory incorporation of docosapentaenoic acid [(n-6) DPA, 22:5(n-6)] varied among brain regions. In low-ALA parous dams, frontal cortex (77% of ALA virgin) and temporal lobe (83% of ALA virgin), regions involved in cognition and affect, were among those exhibiting the greatest depletion of DHA. Caudate-putamen also exhibited significant depletion of DHA (82% of ALA virgin), whereas only (n-6) DPA levels were altered in ventral striatum, hypothalamus, hippocampus, and cerebellum. This pattern of changes in regional DHA and (n-6) DPA content suggests that specific neuronal systems may be differentially affected by depletion of brain DHA in the postpartum organism.


Introduction

PUFA are a major component of the phospholipids that comprise the membranes of all cells including neurons. The fatty acid composition of these phospholipids affects the physicochemical properties of the membrane and thus influences conformation and function of receptors, ion channels, and other signaling proteins (1). PUFA also influence neuronal function by serving as precursors for prostaglandins and other signaling molecules and modulating gene expression through the activation of transcription factors (2).

Docosahexaenoic acid [DHA, 22:6(n-3)]⁷ which is derived from the essential fatty acid α-linolenic acid [ALA, 18:3(n-3)] and is the predominant species of PUFA in the brain accumulates primarily during the late prenatal and early postnatal periods, depending on species. Low availability of DHA during development results in decreased brain phospholipid DHA content and a compensatory incorporation of docosapentaenoic acid [(n-6) DPA, 22:5(n-6)] (3). In addition, the DHA content of the maternal brain can be reduced after only 1 reproductive cycle under dietary conditions with reduced availability of (n-3) PUFA, presumably due to the demands of supplying DHA to the developing fetus/neonate (4,5).

A growing body of clinical findings, such as tissue levels and trials with (n-3) PUFA supplements, implicate decreased levels of (n-3) long chain-PUFA (LC-PUFA), including DHA, in both nonpuerperal and postpartum depression [for review, see (6,7)]. Decreases in (n-3) PUFA are also associated with psychotic illness [for review see (8,9)]. Accordingly, depletion of brain DHA could, in conjunction with the hormonal and other changes associated with pregnancy and childbirth (10), affect maternal neurobiology. In humans, this could increase sensitivity to stress and thus susceptibility to postpartum depression and/or psychosis, consistent with the diathesis-stress model (11). This risk may be even greater after multiple pregnancies, which increases the risk of postpartum depression (12,13) and results in greater alterations in brain PUFA composition than a single reproductive cycle in rats (4).

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² Supplemental Tables 1–3 are available with the online posting of this paper at jn.nutrition.org.
⁷ Abbreviations used: ALA, α-linolenic acid; CRF, corticotrophin releasing factor; DHA, docosahexaenoic acid; (n-6) DPA, docosapentaenoic acid; 5-HT, serotonin; LC-PUFA, long-chain PUFA.
* To whom correspondence should be addressed. E-mail: blevant@kumc.edu.
To further address how reproductive activity and low dietary (n-3) PUFA content affect maternal brain phospholipid PUFA composition, this study determined the effects of the resulting DHA depletion in specific brain regions of postpartum dams that underwent 2 sequential reproductive cycles, a treatment that produces a 15% decrease in brain DHA content compared with virgin controls and maximal alterations in overall brain PUFA composition (4). We showed that brain regions are differentially affected and that regions associated with cognition and affect are among those most affected.

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.

Long-Evans rats (70- to 78-d-old females, and male proven breeders; Harlan) 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. Rats were obtained at least 5 d before the commencement of any treatments and were handled regularly.

Experimental diets. The formulation of the ALA and low-ALA diets and the fatty acid composition of all diets were detailed in a previous publication (4) (Supplemental Tables 1 and 2). The ALA diet was prepared by adding 7% by weight of pure soybean oil (without partial hydrogenation) to Teklad Basal diet TD00235. This resulted in an ALA concentration of 5.09 g/kg diet and was identical to Teklad Carin-93G (14). The low-ALA diet was formulated with linoleic sunflower oil (7% by weight) and contained 0.32 g/kg ALA. Before purchase, rats were fed Teklad Global 18% protein rodent diet number 2018S and Teklad Rodent diet (W) number 8604 for 5–7 d during acclimatization. The ALA and low-ALA diets were prepared by adding 7% by weight of pure soybean oil (without partial hydrogenation) to Teklad Basal diet TD00235. This resulted in an ALA concentration of 5.09 g/kg diet and was identical to Teklad AIN-93G (14). The low-ALA diet was formulated with linoleic sunflower oil (7% by weight) and contained 0.32 g/kg ALA. Before purchase, rats were fed Teklad Global 18% protein rodent diet number 2018S and Teklad Rodent diet (W) number 8604 for 5–7 d during acclimatization.

Study design. Individually housed dams (n = 6 per group) were taken through 2 sequential reproductive cycles. Dams were randomly assigned to the ALA or low-ALA diet groups at the time of initial mating and were fed that diet for the rest of the study. Litters were culled to 8 pups on postnatal d 1 and weaned on postnatal d 21. Dams were remated 8–10 d after weaning. Age-matched, virgin females served as a control for repeated reproductive activity did not affect the percentage of phospholipid DHA in or (n-6) DPA of any brain region in parous dams fed the ALA diet compared with ALA virgins.

Results

In ALA virgins, regional phospholipid DHA levels ranged from 11.3% of total fatty acids in the substantia nigra/ventral tegmental area to 17.7% in the frontal cortex (Table 1). The level of (n-6) DPA ranged from 0.14% in the cerebellum to 0.50% in the temporal lobe (Table 2). Levels of other fatty acids were similar to previous reports (16) (Table 3). The percentages of DHA of each (n-6) DPA (Fig. 1A) and (n-6) DPA (Fig. 1B) in specific brain regions were correlated with those of adult male rats (16) (r² = 0.884, P < 0.001 for DHA and r² = 0.610, P < 0.05 for (n-6) DPA).

Because DHA and (n-6) DPA were the primary fatty acids affected by reproduction and an (n-3) PUFA-deficient diet in whole brain (4), data for specific brain regions are presented only for DHA and (n-6) DPA. For DHA, significant main effects of diet (P < 0.001), reproductive status (P < 0.001), and brain region (P < 0.001) and interaction of reproductive status and diet (P = 0.010) were detected by 3-way ANOVA. For DHA, significant main effects of diet (P < 0.001), reproductive status (P < 0.001), and brain region (P < 0.001) were detected, as well as interactions of reproductive status and diet (P < 0.001), diet and brain region (P < 0.001), and reproductive status and brain region (P = 0.018).

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Reproductive activity did not affect the percentage of phospholipid DHA or (n-6) DPA of any brain region in parous dams fed the ALA diet compared with ALA virgins.

Table 1 Effects of diet (n-3) fatty acid concentration and reproductive status on phospholipid fatty acid DHA in specific brain regions of female rats

<table>
<thead>
<tr>
<th>Brain region</th>
<th>ALA virgin</th>
<th>ALA parous</th>
<th>Low-ALA virgin</th>
<th>Low-ALA parous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal cortex</td>
<td>17.72 ± 0.67</td>
<td>16.99 ± 0.43</td>
<td>16.76 ± 0.52</td>
<td>13.42 ± 0.59*</td>
</tr>
<tr>
<td>Caudate-putamen</td>
<td>13.31 ± 0.39</td>
<td>12.76 ± 0.39</td>
<td>12.51 ± 0.60</td>
<td>10.92 ± 0.30†</td>
</tr>
<tr>
<td>Ventral striatum</td>
<td>12.91 ± 0.67</td>
<td>13.70 ± 0.58</td>
<td>13.47 ± 0.41</td>
<td>12.18 ± 0.51</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>12.73 ± 0.50</td>
<td>11.67 ± 0.42</td>
<td>11.94 ± 0.21</td>
<td>11.10 ± 0.51</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>12.16 ± 0.39</td>
<td>11.58 ± 0.75</td>
<td>11.36 ± 0.68</td>
<td>10.50 ± 0.28</td>
</tr>
<tr>
<td>Temporal lobe</td>
<td>15.63 ± 0.58</td>
<td>16.05 ± 0.32</td>
<td>14.98 ± 0.20</td>
<td>13.05 ± 0.74*</td>
</tr>
<tr>
<td>Substantia nigra/Ventral tegmental area</td>
<td>11.27 ± 0.68</td>
<td>11.10 ± 0.41</td>
<td>9.87 ± 0.82</td>
<td>9.92 ± 0.63</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>15.62 ± 0.47</td>
<td>14.93 ± 0.36</td>
<td>15.57 ± 0.46</td>
<td>14.66 ± 0.65</td>
</tr>
</tbody>
</table>

* Different than ALA virgin, ALA parous, and low-ALA virgin, P < 0.05. † Different than ALA virgin and ALA parous, P < 0.05.
Low-ALA virgins exhibited no alterations in regional DHA; however, (n-6) DPA was increased 1-to-3-fold in the frontal cortex, caudate-putamen, ventral striatum, hippocampus, and temporal lobe and 5.4-fold in the cerebellum compared with ALA virgins and ALA parous dams.

Low-ALA parous dams had 14–23% lower levels of DHA in the frontal cortex, caudate-putamen, and temporal cortex than all other treatment groups (P < 0.05) and tended to have lower levels in the hypothalamus (13%, P = 0.06) and hippocampus (14%, P = 0.15) than the ALA virgin group. The level of (n-6) DPA was increased 3-to-5-fold compared with ALA virgins in all brain regions except cerebellum where it was increased 10.6-fold. In all brain regions, the percentages of (n-6) DPA were significantly higher in low-ALA parous dams than in low-ALA virgins (P < 0.05).

### Discussion

In view of the potential importance of (n-3) PUFA for maternal mental health, the effects of reproductive activity and a diet low in (n-3) PUFA on the phospholipid brain fatty acid composition of specific regions of the female rat were assessed.

Phospholipid fatty acid compositions of specific brain regions of female rats were similar to those previously reported in mice and rats (16–18). Furthermore, regional PUFA composition was highly correlated with that observed in adult male rats (16). This observation supports previous reports that brain PUFA composition is similar in male and female rats under similar dietary conditions (19).

Brain regions of parous female rats fed a diet containing inadequate (n-3) PUFA exhibited regionally specific changes in phospholipid PUFA composition. Three of the 8 brain regions examined (frontal cortex, caudate-putamen, and temporal lobe) exhibited significant decreases in DHA content. DHA in these brain regions was not affected by either reproductive status or the ALA-deficient diet alone, indicating that this effect was due to the interaction of the diet and physiological status.

In contrast to DHA, regional (n-6) DPA levels, which represent a much smaller percentage of phospholipid fatty acids relative to DHA, were affected by both reproductive status and diet. Increased percentages of (n-6) DPA were observed in all brain regions of parous dams fed the ALA-deficient diet. At least some of the incorporation of (n-6) DPA can be attributed to the effects of diet alone, because (n-6) DPA levels were significantly increased in all regions except the substantia nigra/ventral tegmental area in virgin rats fed the ALA-deficient diet for 13 wk, the time required for 2 reproductive cycles, and may reflect the slightly higher level of linoleic acid in sunflower oil compared with soy oil as well as the relative lack of ALA. Although reproductive activity with adequate dietary ALA alone produced no effect on (n-6) DPA levels, regional content of the fatty acid was further increased by the combined effects of reproduction and the ALA-deficient diet in all brain regions except the hypothalamus, where (n-6) DPA was not different from in low-ALA virgins and the increase can thus be attributed entirely to the effect of the ALA-deficient diet.
Although there was no clear delineation of systems exhibiting altered PUFA status in the parous dams with DHA depletion, frontal cortex and temporal lobe, which had the greatest decreases in DHA, are brain regions involved in cognition and affect and with known neurochemical alterations in depression. For example, increased densities of serotonin (5-HT) 5-HT₁A, 5-HT₂A, and β adrenergic receptors and decreased density of corticotrophin releasing factor (CRF) receptors are noted in prefrontal or frontal cortex of postmortem depressive or suicide victims (20). Likewise, alteration in DHA status was not observed in the cerebellum, which is primarily involved in motor function, or the substantia nigra/ventral tegmental area, which contains the dopaminergic perikarya. However, other limbic brain regions, such as the ventral striatum, hypothalamus, and hippocampus exhibited little, if any, change in PUFA composition, suggesting that reward, endocrine, and memory processes might be less affected. In contrast, the caudate-putamen, a component of the extrapyramidal motor system, was among the regions exhibiting larger alterations. Thus, the combined actions of reproductive activity and inadequate dietary (n-3) PUFA affected, but were not specific for, brain regions thought to be associated with depression and/or psychosis.

It is particularly noteworthy that the changes in regional PUFA composition observed in this study of postpartum depletion differ from those reported in models of decreased DHA accretion during development. In both models, frontal cortex exhibited decreases in DHA of the greatest magnitude (16–18). However, in comparison with our previous study of reduced DHA accretion during development, which measured regional phospholipid fatty acid composition at adulthood using the same rat strain, diet, and methods for dissection and analysis as those used in this study (16), fewer brain regions exhibited large decreases in DHA in the postpartum model. The substantia nigra/ventral tegmental area was noteworthy, however, in exhibiting relatively little decrease in DHA content in either model. Although the magnitude of the decrease in whole brain DHA content varies somewhat between these models, thus limiting the ability to make a definitive comparison, these data strongly suggest that regional DHA depletion/decreased accretion associated with different physiological states is not the same. Accordingly, the consequent neurobiological changes are likely different as well.

Future studies must determine how the regionally selective modifications in LC-PUFA composition observed in this study actually contribute to altered neurobiology in the postpartum organism. However, consistent with a role for depletion of brain DHA in depression, rats placed on an (n-3) PUFA-deficient diet at weaning exhibited increased immobility in the Porsolt forced swim test, an animal model of depression, at adulthood (21). Alterations involving neurotransmitters of particular relevance in depression, such as serotonin, CRF, and dopamine (20,22), are also associated with reduced DHA. Of note, low plasma DHA levels were correlated with increased cerebrospinal fluid concentrations of CRF and the serotonin metabolite 5-hydroxyindoleacetic acid in the cerebrospinal fluid of alcoholics and perpetrators of domestic violence, respectively (23,24). In addition, developmental models of decreased DHA accretion are associated with altered serotonergic neurotransmission (25), increased density of cortical 5-HT₂A receptors (26), altered dopaminergic neurotransmission, decreased cortical D₂ receptor mRNA expression, and increased D₂ receptor density in the nucleus accumbens (27).

In conclusion, these data demonstrate that although all brain regions examined were affected by the combined effects of reproductive activity and/or an (n-3)-deficient diet, regional LC-PUFA composition was differentially affected. The frontal cortex and the temporal lobe, regions involved in cognition and affect, were among those most affected, although brain regions associated with motor systems also exhibited significant depletion of DHA. This pattern of regional variation in change in DHA and (n-6) DPA, which differs from that produced by decreased accretion of DHA during development, suggests that specific neuronal systems may be differentially affected in the postpartum organism. The functional consequences of these alterations in brain PUFA composition must be determined in future studies; however, these changes may contribute to changes in neurobiology that could increase vulnerability of the maternal organism to stress.

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