Maternal Parity and Diet (n-3) Polyunsaturated Fatty Acid Concentration Influence Accretion of Brain Phospholipid Docosahexaenoic Acid in Developing Rats\textsuperscript{1,2}

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Abstract

The long-chain PUFA, docosahexaenoic acid [22:6(n-3), DHA], a major component of neuronal membrane phospholipids, accumulates in brain during late prenatal and early neonatal development and is essential for optimal attentional and cognitive function. Because all nutrition is supplied to the developing fetus/neonate by the mother and maternal DHA status is affected by parity, this study examined the effects of maternal diet and parity on DHA accretion in the developing brain. Whole brain total phospholipid fatty acid composition was determined by TLC and GC in weanling male Long-Evans rats (n = 5) from the 1st, 2nd, 3rd, or 4th litters of dams fed diets containing \(\alpha\)-linolenic acid (ALA), containing ALA and preformed DHA (ALA + DHA), or lacking ALA (low-ALA). First-litter low-ALA offspring exhibited a decrease in phospholipid fatty acid DHA content to 68% of 1st-litter ALA pups. DHA in 2nd-litter low-ALA pups was further decreased to 55% of 1st-litter ALA pups, but further decreases were not observed in subsequent litters. DHA levels increased 15–20% in 2nd to 4th-litter ALA + DHA pups and 11% in 4th-litter ALA pups compared with 1st-litter ALA pups. These findings demonstrate that maternal diet and parity interact to affect offspring brain DHA status and suggest that maternal multiparity may place offspring at greater risk of decreased accretion of brain DHA if the maternal diet contains insufficient (n-3) PUFA. J. Nutr. 137: 125–129, 2007.

Introduction

Long-chain polyunsaturated fatty acids (LC-PUFA)\textsuperscript{7} are a major component of the phospholipids that form the cell membranes of all cells and are of particular importance in the central nervous system, which has a particularly high lipid content (1). \(\alpha\)-Linolenic acid [ALA, 18:3(n-3)] and linoleic acid [18:2(n-6)] are essential PUFA and can be elongated through desaturases and elongases to produce LC-PUFA such as docosahexaenoic acid [DHA, 22:6(n-3)] and arachidonic acid [20:4(n-6)] and docosapentaenoic acid [(n-6) DPA, 22:5(n-6)], respectively. Phospholipids form the microenvironment around membrane-bound proteins, which can affect receptor activity, membrane transport, hormonal and other signal transduction processes. In addition to their structural role, LC-PUFA serve as precursors for inter- and intracellular signaling molecules, such as prostaglandins, thromboxanes, and neuroprotectin D1; they also modulate gene expression through the activation of transcription factors [for review, see (2–5)].

DHA is the predominant species of LC-PUFA in the brain, which represents \(\sim 15\%\) of total fatty acids in that tissue (6). Most of the brain DHA accumulates during late prenatal and early postnatal development. In humans, rapid accretion of DHA occurs during the 3rd trimester of pregnancy (7,8), whereas, in rats, which are more immature at birth, DHA accumulates with a pronounced spike during the last 3 d of gestation and continues through weaning (9,10). DHA delivered to the fetus/neonate is primarily supplied by the mother, emphasizing the importance of the maternal diet (11). Under dietary conditions with insufficient availability of DHA, a compensatory substitution of (n-6) DPA ensues (12). This results in an alteration of the (n-6)/(n-3) ratio and changes the fatty acid composition of both neuronal and glial phospholipids (13), thus altering the physicochemical properties of the cell membranes (3,5).

Adequate availability of DHA during the prenatal/neonatal period is essential for optimal central nervous system development and function. In humans, low DHA availability is associated with decreased visual acuity in infants and suboptimal cognitive, attentional, and motor skills [for review, see (14–17)]. Likewise, rats fed diets with inadequate (n-3) PUFA displayed increased escape latency and had a defect in spatial retention.
(18). Moreover, decreased accretion of brain DHA during development resulted in altered dopaminergic neurotransmission in the mesolimbic and mesocortical dopamine systems (19) and behavior at adulthood indicative of modified dopaminergic function (20,21). Taken together with the growing body of clinical data [for review, see (22,23)], these observations suggest that low DHA levels may contribute to the etiology of neuropsychiatric disorders such as attention deficit hyperactivity disorder and schizophrenia.

Previous studies indicate that maternal diets containing inadequate (n-3) PUFA decrease the DHA content of the offspring and that this effect increases when animals are maintained on (n-3)-deficient diets for multiple generations (24). Clinical and animal studies also demonstrate that pregnancy and lactation can deplete maternal tissues of DHA and that this effect increases with multiparity (25–29). Accordingly, in this study, the effects of maternal dietary (n-3) PUFA content and maternal parity (i.e., number of litters produced) on offspring brain DHA accretion were assessed.

Materials and Methods

Animals. All experiments were conducted in accordance with the NIH guidelines 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 (Harlan) were obtained at least 5 d prior to the beginning of experimentation and were handled regularly. They were housed in a temperature- and humidity-controlled facility with a 12-h light-dark cycle and consumed food and water ad libitum.

Experimental diets. The formulation and fatty acid composition of the experimental diets are detailed in a previous publication (28, Supplement Tables 1 and 2). The ALA diet was prepared by adding 7% by weight of pure soybean oil (without partial hydrogenation) to a baseline diet [Teklad Basal Diet (TD00235)]. This resulted in α-linolenic acid and linoleic acid concentrations of 5.09 g/kg and 33.73 g/kg, respectively, and is identical in composition to Teklad AIN-93G (30). The low-ALA diet was prepared by adding pure linoleic sunflower oil to the same baseline diet and contained 0.32 g/kg α-linolenic acid and 42.89 g/kg linoleic acid. The ALA + DHA diet contained α-linolenic acid (4.90 g/kg) and preformed DHA and was prepared by substituting DHASCO (42.57% DHA by weight, Martek Biosciences) on an equal weight basis for soybean oil such that DHA accounted for 0.44 g/kg in the diet. The linoleic acid concentration of the ALA + DHA diet was 33.09 g/kg.

Study design. In a between-groups design, weaning (postnatal d 21) males were randomly collected from the 1st, 2nd, 3rd, or 4th litters produced by dams fed 1 of 3 experimental diets (n = 5, each from a different litter). Individually housed dams (80–89 d old) were randomly assigned a diet group at the time of initial mating as part of a larger ongoing breeding program. Litters were culled to 8 pups on postnatal d 1 and weaned on postnatal d 21. Dams were remated 8–10 d after weaning. Litters of >15 pups were excluded and those dams were not used for further breeding. Likewise, dams that failed to achieve a pregnancy upon remating were excluded from further use. Pups were weighed regularly throughout the experiment. Breeder males were maintained on standard laboratory chow [Teklad Rodent Diet (W) 8604] when not mated. Pups were killed by decapitation. Brains were rapidly removed, frozen on dry ice, and stored at −70°C. Total phospholipid fatty acid composition was determined in 1 hemisphere of each brain by TLC and GC as previously described (31) and expressed as area percentage.

Data analysis. Data are presented as the mean ± SEM. Data were analyzed by 2-way ANOVA, with factors of diet and maternal parity (Sysstat, version 10.2). Post hoc analysis was performed by 1-way ANOVA with all groups, followed by Tukey’s test. Differences were considered significant at P < 0.05.

Results

Pup weight. Two-way ANOVA indicated an effect of maternal parity (P = 0.003) but no effect of diet or an interaction of maternal parity and diet on the weights of the resulting offspring at weaning (Fig. 1). Post hoc analysis indicated no significant effects of maternal parity within diet groups or any difference between diet groups for any particular maternal parity.

First-litter brain phospholipid fatty acid composition. Whole brain phospholipid fatty acid composition of weanling males produced by primiparas fed the ALA diet was similar to that previously reported with DHA representing 13.9% of total fatty acids (20) (Table 1). One-way ANOVA indicated effects of diet in DHA (P < 0.0001) and (n-6) DPA (P < 0.0001). Post hoc analysis with Tukey’s test indicated that the low-ALA diet resulted in 32% less DHA compared with pups of dams fed the ALA diet (P < 0.001). This decrease in DHA was accompanied by an increase in (n-6) DPA to 530% of the ALA group (P < 0.001). Pups produced by primiparas fed the ALA + DHA diet exhibited no differences in the content of any individual fatty acids compared with the ALA group.

Maternal parity and diet on brain phospholipid polyunsaturated fatty acid composition. Two-way ANOVA indicated main effects of diet (P < 0.0001) and maternal parity (P < 0.0001) and an interaction of these factors (P < 0.0001) for both DHA and (n-6) DPA. Significant main effects of diet (P < 0.0001), maternal parity (P < 0.0001), and an interaction of these factors (P < 0.05) were also detected for “other (n-3)” fatty acids [18:3(n-3), 20:5(n-3), and 22:5(n-3) combined].

In pups produced by dams fed the ALA diet, no significant alterations in DHA or (n-6) DPA across litters were detected (Fig. 2). One-way ANOVA indicated differences in other (n-3) fatty acids (P < 0.0001), which were 72% higher in 3rd-litter ALA pups than in 2nd-litter ALA pups (P < 0.05; data not shown). No other alterations in LC-PUFA across litters were detected (data not shown).

Figure 1 Effects of maternal parity and diet (n-3) PUFA concentration on offspring weight at weaning (postnatal d 21) in rats. Data are presented as means ± SEM, n = 5. *Different from ALA + DHA 2nd litter, P < 0.05.
TABLE 1  Effects of maternal dietary (n-3) PUFA concentration on whole brain phospholipid fatty acid composition at weaning of 1st-litter offspring in rats

<table>
<thead>
<tr>
<th>Diet/fatty acid</th>
<th>ALA</th>
<th>Low-ALA</th>
<th>ALA + DHA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Area %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16:0</td>
<td>22.3 ± 0.6</td>
<td>23.5 ± 0.8</td>
<td>22.5 ± 0.9</td>
</tr>
<tr>
<td>18:0</td>
<td>18.7 ± 0.1</td>
<td>18.4 ± 0.2</td>
<td>18.7 ± 0.2</td>
</tr>
<tr>
<td>Other SFA</td>
<td>3.9 ± 0.1</td>
<td>3.9 ± 0.1</td>
<td>4.2 ± 0.1</td>
</tr>
<tr>
<td>18:1(n-9)</td>
<td>0.5 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>18:1(n-7)</td>
<td>0.5 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>20:1(n-9)</td>
<td>0.5 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>Other MUFA1</td>
<td>0.5 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>22:6(n-3)</td>
<td>13.0 ± 0.3</td>
<td>9.4 ± 0.4</td>
<td>15.1 ± 0.6</td>
</tr>
<tr>
<td>Other (n-3)</td>
<td>0.5 ± 0.1</td>
<td>0.3 ± 0.2</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>20:4(n-6)</td>
<td>12.2 ± 0.3</td>
<td>12.5 ± 0.4</td>
<td>12.2 ± 0.4</td>
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<tr>
<td>22:4(n-6)</td>
<td>4.0 ± 0.5</td>
<td>4.5 ± 0.2</td>
<td>3.7 ± 0.2</td>
</tr>
<tr>
<td>22:5(n-6)</td>
<td>1.0 ± 0.04</td>
<td>5.3 ± 0.4</td>
<td>0.8 ± 0.03</td>
</tr>
<tr>
<td>Other (n-6)</td>
<td>0.5 ± 0.1</td>
<td>1.7 ± 0.1</td>
<td>2.0 ± 0.1</td>
</tr>
</tbody>
</table>

1  Data are presented as the means ± SEM, n = 5 (each from a different litter). Means in a row with superscripts without a common letter differ, P < 0.05.
2  MUFA, monounsaturated fatty acid.

Pups produced by dams fed the low-ALA diet had lower brain phospholipid fatty acid DHA contents than ALA pups, regardless of maternal parity (P < 0.0001). The DHA level of 1st-litter low-ALA pups decreased to 68% of 1st-litter ALA pups (P < 0.001). A further decrease in DHA to 55% of 1st-litter ALA occurred in 2nd-litter pups on the low-ALA diet (P < 0.001 vs. 1st-litter low-ALA). These decreases in DHA were accompanied by increases in (n-6) DPA of 430% (P < 0.001 vs. 1st-litter ALA) and 780% (P < 0.001 vs. 1st-litter ALA and 1st-litter low-ALA) of 1st litter ALA pups, respectively (P < 0.0001). No further decrease in DHA or increase (n-6) DPA content occurred in the 3rd or 4th litters. No other alterations in LC-PUFA were detected in any litter borne by dams fed the low-ALA diet (data not shown).

In pups produced by dams fed the ALA + DHA diet, brain phospholipid fatty acid DHA content were 15–20% higher in the 2nd, 3rd, and 4th litters compared with 1st-litter ALA pups (P < 0.01, P < 0.01, and P < 0.001, respectively); however, the ALA and ALA + DHA groups did not differ for the 3rd and 4th litters. No alterations in LC-PUFA were detected in any litter borne by dams fed the ALA + DHA diet (data not shown).

Discussion

Adequate availability of DHA is essential for optimal CNS development. Because all nutrition is supplied to the developing fetus/neonate by the mother and maternal DHA status is affected by parity, this study examined the interaction of maternal diet and parity on DHA accretion in the developing brain.

In this study, the diet-induced changes in accretion of brain DHA during development are similar to those previously reported. Of note, the decrease in brain phospholipid fatty acid DHA content in 1st-litter offspring raised on the diet lacking α-linolenic acid is similar to that observed in other studies using diets formulated with sunflower oil (20,21). This decrease in DHA is less than that produced by diets made with oils that contain even less α-linolenic acid, such as safflower or peanut (32), but is similar to the decreases in tissue DHA levels reported in clinical populations with schizophrenia or attention deficit hyperactivity disorder (32–38) and is thus likely to model clinical conditions. Similar to previous reports, the diet containing DHA and α-linolenic acid did not produce a significant increase in whole brain DHA levels (31,39); however, such treatment increases the phospholipid fatty acid DHA content in specific brain regions (31).

The diet deficient in α-linolenic acid resulted in decreased accretion of DHA in the developing brain accompanied by increased incorporation of (n-6) DPA, a well-established compensatory substitution (12). Brain phospholipid fatty acid DHA content was further decreased in the 2nd litter; however, no additional decrease in DHA or increase in (n-6) DPA was observed in subsequent litters. These findings are similar to observations in humans where the umbilical cord plasma DHA status of primigravidas newborns was higher than multigravidas newborns (25,26,40). Such observations in the offspring likely reflect depletion of maternal DHA stores, as plasma and erythrocyte DHA concentrations in humans are lower in multiparous than in null- or primiparous women (25,26,40). Likewise, in rats, depletion of maternal brain phospholipid fatty acid DHA content and compensatory incorporation of (n-6) DPA reaches a plateau after 2 reproductive cycles (28). Future studies must determine the mechanism underlying this apparent basement effect for offspring brain DHA; however, mobilization of maternal peripheral stores and/or increased maternal synthetic capability, which is stimulated by estrogen (41) and appears to increase during
pregnancy (42–44), could contribute and might be further augmented after multiple litters. Alternatively, equilibrium between α-linoleic intake and utilization could occur after several reproductive cycles in rats fed the low (n-3) diet.

Although brain phospholipid fatty acid DHA contents of 1st-litter offspring raised on the diet containing preformed DHA did not differ from 1st-litter ALA pups, DHA levels were increased in the 2nd, 3rd, and 4th litters produced by dams fed the ALA + DHA diet compared with 1st-litter ALA pups. This observation also suggests that maternal ability to mobilize or deliver DHA may be augmented after multiple reproductive cycles.

A large body of work indicates that adequate availability of DHA during early development is essential for optimal central nervous system development [for review, see (14–17)]. Moreover, a growing body of evidence suggests decreased availability of DHA may represent an environmental factor that contributes to the development of several neuropsychiatric disorders [for review, see (22)]. The present findings clearly demonstrate that maternal diet and parity interact to affect brain DHA accretion in developing offspring. Because humans are relatively inefficient in synthesizing DHA from α-linolenic acid (45–47), it is recommended that pregnant and lactating women consume 300 mg of DHA per day (48). However, the average pregnant or lactating woman in North America consumes only one-third of the recommended amount (49) and far less than the amount of DHA ingested by populations that regularly consume marine fish and mammals (50). The present findings further underscore the need for appropriate nutrition during pregnancy and lactation and suggest that maternal multiparity may place offspring at greater risk for a decreased accretion of brain DHA if the maternal diet contains insufficient (n-3) PUFA.

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