Available online at www.sciencedirect.com

SciVerse ScienceDirect

Journal of Nutritional Biochemistry 23 (2012) 299–305

Journal of Nutritional Biochemistry

Fish oil supplementation of maternal rats on an n-3 fatty acid-deficient diet prevents depletion of maternal brain regional docosahexaenoic acid levels and has a postpartum anxiolytic effect☆

Hui-Feng Chen, Hui-Min Su*

Department of Physiology, National Taiwan University College of Medicine, Taipei, Taiwan

Received 29 September 2010; accepted 20 December 2010

Abstract

Docosahexaenoic acid (DHA) and arachidonic acid (AA) are the major polyunsaturated fatty acids (PUFA) in the neuronal membrane. Most DHA and AA accumulation in the brain occurs during the perinatal period via placenta and milk. This study examined whether maternal brain levels of DHA and AA are depleted during pregnancy and lactation due to meeting the high demand of the developing nervous system in the offspring and evaluated the effects of the reproductive cycle on serotonin metabolism and of fish oil (FO) on postpartum anxiety. Pregnant rats were fed during pregnancy and lactation with a sunflower oil-based n-3 PUFA-deficient diet without or with FO supplementation, which provided 0.37% of the energy source as n-3 PUFA, and the age-matched virgin rats were fed the same diets for 41 days. In both sets of postpartum rats, decreased DHA levels compared to those in virgin females were seen in the hypothalamus, hippocampus, frontal cortex, cerebellum, olfactory bulb and retina, while AA depletion was seen only in the hypothalamus, hippocampus and frontal cortex. Serotonin levels were decreased and turnover increased in the brainstem and frontal cortex in postpartum rats compared to virgin rats. FO supplementation during pregnancy and lactation prevented the decrease in maternal brain regional DHA levels, inhibited monoamine oxidase-A activity in the brainstem and decreased anxiety-like behavior. We propose that the reproductive cycle depletes maternal brain DHA levels and modulates maternal brain serotonin metabolism to cause postpartum anxiety and suggest that FO supplementation may be beneficial for postpartum anxiety in women on an n-3 PUFA-deficient diet.
© 2012 Elsevier Inc. All rights reserved.

Keywords: Docosahexaenoic acid; Arachidonic acid; Serotonin; Fish oil; Reproductive cycle; Anxiety

1. Introduction

In the mammalian brain, lipids make up 10% of the fresh weight and 50% of the dry weight, and docosahexaenoic acid (DHA, 22:6n-3) and arachidonic acid (AA, 20:4n-6) are the major polyunsaturated fatty acids (PUFAs) in the neuronal membrane [1]. Most DHA and AA accumulation in the brain occurs during brain development from the beginning of the third trimester of gestation to 2 years after birth in humans and from Prenatal Day 7 to Postnatal Day 21 in rats, the PUFAs being supplied via the placenta to the fetus and in the milk to the pup [2-4]. DHA is essential for normal neurological function [5,6] and is enriched in the brain, retina and sperm, but not in other tissues [7]. It is important to know whether maternal brain and retinal DHA and AA levels are depleted during pregnancy and lactation due to meeting the high demand of the developing nervous system in the offspring.

In rats fed a low n-3 PUFA diet, maternal whole brain DHA levels are significantly reduced after one reproductive cycle compared to age-matched virgin females, but no change is seen in rats fed a control AIN-93G diet or an n-3 PUFA-rich diet [8,9]. In addition, in rats fed the same low n-3 PUFA diet, DHA levels were significantly reduced in three of the eight examined maternal brain regions after two consecutive reproductive cycles compared to age-matched virgins [10]; however, this low n-3 PUFA diet contained 0.32 g/kg diet of α-linolenic acid (18:3n-3), a DHA precursor, which might have supplied DHA to the offspring and prevented depletion of maternal DHA levels.

Many postpartum women are vulnerable to mood disturbances, often involving depression and anxiety [11-13]. Anxiety and depression are co-occurrent and both are modulated by serotonin

Abbreviations: AA, arachidonic acid, (20:4n-6); DHA, docosahexaenoic acid, (22:6n-3); FO, fish oil; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, 5-hydroxytryptamine or serotonin; HPA, hypothalamic-pituitary-adrenal; MAO-A, monoamine oxidase A; PUFA, polyunsaturated fatty acids; P, pregnant rats fed the n-3 PUFA-deficient diet; P+FO, pregnant rats fed the n-3 PUFA-deficient diet supplemented with fish oil; V, virgin female rats fed the n-3 PUFA-deficient diet; V+FO, virgin female rats fed the n-3 PUFA-deficient diet supplemented with fish oil.

☆ This work was supported by grants from the National Science Council of Taiwan (grant no. NSC-98-2320-B-002-058) and the Department of Health (grant no. DOH 96-TD-F-113-002).

* Corresponding author. Department of Physiology, National Taiwan University College of Medicine, Taipei, 100, Taiwan. Tel.: +886 2 2312 3456x88248; fax: +886 2 2396 4350.

E-mail address: hmsu1203@ntu.edu.tw (H.-M. Su).

0955-2863/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2010.12.010

(5-hydroxytryptamine, 5-HT), a target of medication for psychiatric disorders [14]. Given the side-effects of antidepressant medications on the developing offspring [15], it is important to find an alternative therapy for the prevention and treatment of postpartum depression and anxiety. It has been suggested that n-3 PUFAs are potentially beneficial in mood disorders [16,17].

The present study was therefore designed to examine whether maternal regional brain and retinal DHA levels in rats on an n-3 PUFA-deficient diet were reduced by supplying the demand of the developing brain in the offspring, whether reproductive activity altered serotonin metabolism and whether supplementation with n-3 PUFA-enriched fish oil (FO) of maternal rats during pregnancy and lactation prevented depletion of maternal brain regional DHA levels and had a postpartum anxiolytic effect.

2. Materials and methods

2.1. Animals and study design

Pregnant Sprague-Dawley rats at 2 days of gestation and virgin female rats (all 8 weeks old) were obtained from BioLasco Taiwan, a technology licensee of Charles River Laboratories in Taiwan, and immediately randomly assigned to a sunflower oil-based n-3 PUFA-deficient diet and 0.1 ml of water per day by oral gavage (P group, n=11) or the same diet supplemented with 0.2 ml of FO/day [180 mg of eicosapentaenoic acid (20:5n-3) and 120 mg of DHA/ml of FO, Leiner Health Products, LLC, California, USA] during the 20 days of gestation and 0.4 ml of FO/day during the 21 days of lactation (P+FO group, n=12). Age-matched virgin female rats were maintained for 41 days on the same diet with water (V group, n=6) or 0.1 ml of FO (V+FO group, n=6). The amounts of FO used were based on how much diet was eaten and to meet the n-3 PUFA dietary recommendation of about 0.4% of the energy source [18]. Postpartum rats were sacrificed at the time of weaning of the pups (Postnatal Day 22), and the age-matched virgin rats were killed after 41 days on the n-3 PUFA-deficient diet. All rats were housed in a humidity-controlled room at 24±1°C on a 12-h light–dark cycle with free access to tap water and diet. The protocols and animal treatments used in this study were approved by the Animal Care and Use Committee of the National Taiwan University College of Medicine.

2.2. Diet composition

The ingredients of the n-3 PUFA-deficient diet and its fatty acid composition are shown in Table 1. The oil used was sunflower oil. The n-3 PUFA-deficient diet contained 60% of the total fatty acids as linoleic acid (18:2n-6) and 0.02% as 18:3n-3. All diet ingredients were obtained from MP Biomedicals (Ohio, USA), except the methionine and choline, which were from Sigma-Aldrich (Missouri, USA), and the sunflower oil, corn starch and sucrose, which were purchased from a local supermarket.

2.3. Postpartum anxiety behavior test

An elevated plus-maze test was used to assess anxiety-like behavior in postpartum rats at Day 15 of lactation. The plus-maze consisted of two open arms (50×10 cm) and two closed arms with walls (50×10×30 cm) connected by a central platform (10×10 cm) in the shape of a plus sign and was painted in black and elevated 50 cm above the floor. A video camera was mounted above the center of the apparatus.

The rats were habituated to the test dark room with dim red light for at least 30 min before testing during the nighttime. The rat was then placed on the central platform with its head facing an open arm and allowed to explore the maze for 5 min while being videotaped. The time spent in the open arms, the number of times the rat entered the open arms and the total number of times the rat entered any of the arms were recorded. The time spent in the open arms and the number of times the rat entered the open arms were taken as indexes of anxiety levels (the shorter the time or the smaller the number, the greater the anxiety), while the total number of times the rat entered any of the arms was taken as an index of motor activity.

2.4. Fatty acid analyses

The rats were anesthetized with CO_2 and blood collected by cardiac puncture and the serum and erythrocytes immediately separated by centrifugation, frozen in liquid nitrogen and stored in a −80°C freezer until analysis. The liver and brain were rapidly removed and the liver frozen as above, as were the retina, olfactory bulb, cerebellum, hypothalamus, hippocampus, frontal cortex and brainstem after dissection on ice. Total lipids were extracted from aliquots of tissue homogenate according to the method of Bligh and Dyer [19] and were dried down under nitrogen gas, then, as described previously [20], were converted to their methyl esters and analyzed on a Hewlett-Packard 5890 gas chromatograph using flame ionization detection [21]. The fatty acid peaks were identified by comparison of the retention times with those of an authentic standard mixture of 68A (Nu-Chek Prep, Elysian, MN, USA), 37 FAME, PUFA2 and PUFA3 (all from SUPELCO, Bellefonte, PA, USA). The fatty acid composition was expressed as the weight as a percentage of the total weight of carbon 14 to carbon 22 fatty acids (wt.%).

Table 1
Ingredients and fatty acid composition of the n-3 PUFA-deficient diet and FO[a,b,c]

Ingredient (g/kg diet)	n-3 PUFA-deficient diet	
Sunflower oil	200	
Casein	238	
DL-Methionine	3.5	
Corn starch	150	
Sucrose	294.3	
Alphacel	58.8	
AIN 76 vitamin mix	11.8	
AIN 76 mineral mix	41.2	
Choline chloride	2.4	
Fatty acid composition (%)		**FO**
14:0	0.11±0.04	15.62±0.57
16:0	7.85±0.30	25.15±0.62
18:0	3.56±0.19	3.44±0.13
20:0	0.06±0.04	0.12±0.04
16:1n-7	0.04±0.01	14.99±0.26
18:1n-9	28.31±0.53	9.32±0.24
18:1n-7	0.42±0.18	3.32±0.19
20:1n-9	0.02±0.02	0.52±0.14
18:2n-6	59.58±0.63	1.48±0.33
20:2n-6	–	0.37±0.17
20:3n-6	–	0.44±0.11
20:4n-6	–	0.17±0.17
22:4n-6	–	0.06±0.02
22:5n-6	–	0.05±0.04
18:3n-3	0.02±0.00	0.54±0.14
20:5n-3	–	15.92±0.82
22:5n-3	–	1.18±0.06
22:6n-3	–	7.96±0.14

[a] The composition of the n-3 PUFA-deficient diet was modified from that of the AIN 76 purified diet. The amounts of casein, methionine, fiber, vitamin mixture, mineral mixture and choline in the n-3 PUFA-deficient diet were adjusted to maintain the same nutrient/energy ratio as the AIN-76 purified diet.
[b] The data are presented as the mean±S.E.M. (n=6).
[c] Fatty acids accounting for less than 0.05% of the total fatty acids are not shown.

2.5. Measurement of serotonin and its main metabolite

The brainstem, frontal cortex and hippocampus were homogenized in 5% acetic acid (Sigma, Missouri, USA) and the homogenate centrifuged at 25,000×g for 30 min at 4°C. The supernatants were filtered on a 0.22-μm centrifugal filter (Millipore, Bedford, MA, USA) by centrifugation at 10,000×g for 10 min at 4°C, then 5-HT and its main metabolite, 5-hydroxyindoleacetic acid (5-HIAA), in the filtrates were separated by reversed-phase high-performance liquid chromatography. The filtrates were applied to a 4.6×150-mm LUNA C-18(2) 5 μm C18 column (Phenomenex, Torrance, CA, USA) and eluted at 1 ml/min with a mobile phase of 80 mM monobasic phosphate sodium, 2 mM sodium octylsulfate and 10% acetonitrile (pH 2.8), and components were detected using an eight-channel coulometric electrochemical detector arranged in series and set at increasing potentials from −50 to +300 mV (CoulArray Model 5600 HPLC system, ESA, Inc., Chelmsford, MA, USA). The data was acquired, processed and analyzed using CoulArray software. 5-HIAA and 5-HT were identified by comparison of the retention time and potential of the main peak with those of authentic standards (Sigma). The amounts of 5-HT and 5-HIAA were calculated from the peak heights using linear regression analysis of the peak heights obtained using the known amounts of the standards. The 5-HIAA/5-HT ratio was taken as an estimate of 5-HT turnover.

2.6. Measurement of monoamine oxidase A activity

Monoamine oxidase A (MAO-A) is the major enzyme metabolizing 5-HT in the brain [22]. As described previously [23], brain tissues were homogenized in 0.32 M ice-cold sucrose using a Potter-Elvehjem homogenizer and the homogenate was centrifuged at 12,000×g for 10 min at 4°C. The pellets were suspended in 0.32 M ice-cold sucrose and centrifuged at 21,000×g for 20 min at 4°C, then the resultant pellets were resuspended in 0.1 M phosphate-buffered saline (pH 7.4) and centrifuged at 12,000×g for 10 min at 4°C, and the final pellets resuspended in the same buffer. MAO-A activity was measured using an Amplex Red Monoamine Oxidase Assay kit (Molecular Probes, Oregon, USA) according to the manufacturer's instructions using p-tyramine as substrate

and pargyline as a specific MAO-B inhibitor and measuring the fluorescence at 544/590 nm. The protein concentration was measured using a Bradford protein assay kit (Bio-Rad, Hercules, CA, USA).

2.7. Statistical analyses

The data are presented as the mean±S.E.M. The significance of differences between the four groups and the main effects was tested by two-way ANOVA (postpartum status×FO supplementation). When the interaction was significant, comparisons among groups were performed using Duncan's multiple range tests. Student's unpaired *t* test was used to compare differences between two groups. All statistical analyses were performed using the SAS program (version 9.1.3, SAS Institute, Cary, NC, USA). A two-sided *P* value ≤.05 was considered statistically significant.

3. Results

3.1. Postpartum anxiety behavior

To examine the effect of FO supplementation on anxiety-like behavior in rats fed an n-3 PUFA-deficient diet during pregnancy and lactation, an elevated plus maze test was performed at Day 15 of lactation on postpartum rats fed the n-3 PUFA-deficient diet without (P group) or with (P+FO group) FO supplementation. The time spent in the open arms (Fig. 1A; *P*=.03) and the number of times the rat entered the open arms (Fig. 1B; *P*=.01) were significantly greater in the P+FO group than in the P group, showing that FO supplementation of the maternal rats during pregnancy and lactation had a postpartum anxiolytic effect. The total number of times P or P+FO rats entered any of the arms was not significantly different (Fig. 1C), showing that FO supplementation during the reproductive cycle had no effect on motor activity.

3.2. DHA levels in different brain regions and the retina

As shown by the major PUFA composition analysis (Table 2) (levels of other fatty acids are presented in Supplemental Tables 1–6), all of the five examined maternal brain regions showed reduced DHA levels, in the postpartum rats (P and P+FO groups) compared to the age-matched virgin female rats (V and V+FO groups) fed the n-3 PUFA-deficient diet. Two-way ANOVA revealed a main effect of postpartum status in the hypothalamus, hippocampus, frontal cortex, cerebellum and olfactory bulb, but not in the retina, while a main effect of FO supplementation was seen in all five examined brain regions and the retina; no postpartum×FO interaction was seen in the hippocampus, frontal cortex, cerebellum and olfactory bulb, but a significant interaction was seen in the hypothalamus and retina.

The greatest DHA depletion in the P group compared to the V group was seen in the hypothalamus (37% loss), followed by the frontal cortex (29% loss), hippocampus (24% loss), cerebellum (22% loss), olfactory bulb (22% loss) and retina (20% loss). DHA levels in these brain regions in the P group were 8.0–10.2% of total fatty acids compared to 11.7–14.3% in the V group. Comparing DHA levels in the P+FO and V+FO groups, the greatest DHA depletion in the P+FO group was in the frontal cortex (21% decrease), followed by the hippocampus (15%), hypothalamus (13%), olfactory bulb (11%) and cerebellum (7%). DHA levels in the depleted brain regions in the P+FO group were 10.7–13.5% of total fatty acids compared to 12.3–16.8% in the V+FO group. In contrast, there was no significant difference in DHA levels in the retina between the P+FO and V+FO groups.

In the P+FO group, the depletion of brain DHA levels was significantly less than that in the P group. The highest recovered DHA levels were seen in the retina (104% of the V group level), cerebellum (103%), olfactory bulb (102%), hippocampus (93%) and frontal cortex (92%), followed by the hypothalamus (85%). In contrast, virgin rats supplemented with FO for 41 days (V+FO) showed some increase in brain regional DHA levels compared to the V group, but the difference was not statistically significant except in the frontal cortex.

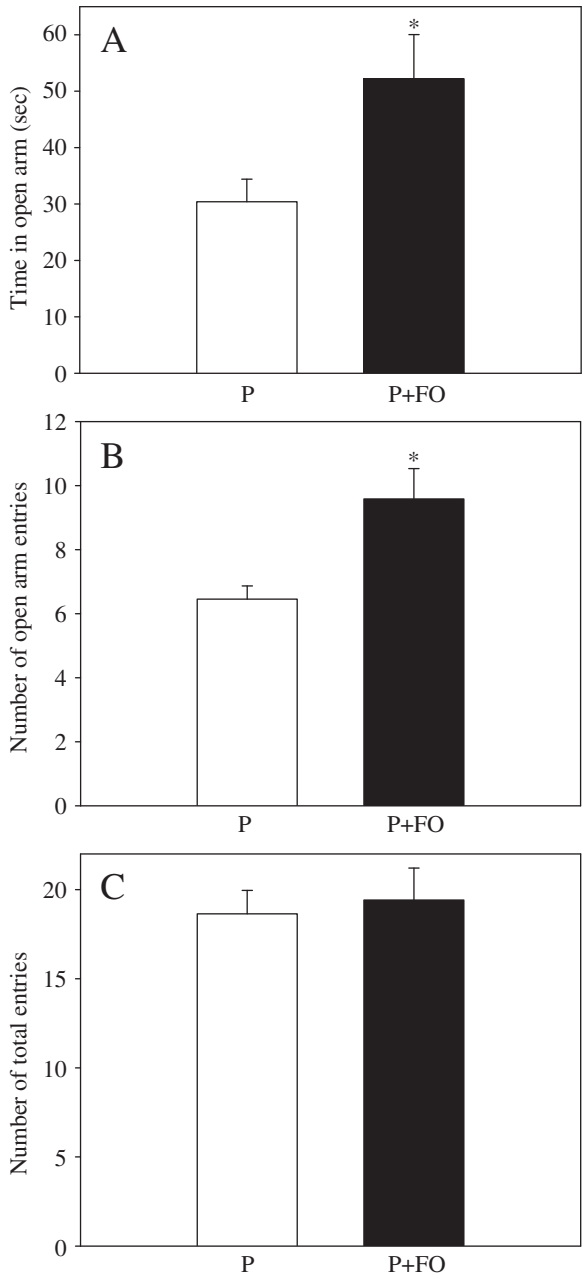

Fig. 1. Effect of FO supplementation on anxiety-like behavior in the elevated plus maze test in postpartum rats on Day 15 of lactation. The time spent in the open arms (A), the number of times the rat entered the open arms (B) and the total number of time the rat entered any of the arms (C) are shown as the mean±S.E.M. (*n*=11–12/group). * indicates a significant difference between the P and P+FO groups by the *t* test.

3.3. AA levels in brain regions and the retina

AA is the other major PUFA in the neuronal membrane, but whether its levels in maternal brain regions and the retina are depleted during the reproductive cycle has received less attention. As shown in Table 2, AA levels in the hypothalamus, hippocampus and frontal cortex were reduced after pregnancy and lactation in rats fed the n-3 PUFA-deficient diet compared to age-matched virgin female rats fed the same diet. Two-way ANOVA revealed a main effect of postpartum status in the hypothalamus, hippocampus and frontal cortex, but not in the cerebellum, olfactory bulb or retina, while no

Table 2
Major PUFA composition of different brain regions in postpartum or virgin rats fed the n-3 PUFA-deficient diet with or without FO supplementation for 41 days[a,b,c]

	V (n=6)[4]	V+FO (n=6)	P (n=7)	P+FO (n=6)	Postpartum	FO	P×FO
22:6n-3, DHA							
Hypothalamus	12.60±0.65[a]	12.25±0.61[a]	7.96±0.16[c]	10.73±0.42[b]	<0.0001	0.0174	0.0032
Hippocampus	11.67±0.58	12.76±0.40	8.92±0.40	10.85±0.49	<0.0001	0.0038	0.3748
Frontal cortex	14.34±0.81	16.78±0.91	10.09±0.60	13.23±0.27	<0.0001	0.0006	0.6213
Cerebellum	13.13±0.94	14.54±0.32	10.24±0.60	13.51±0.70	0.0063	0.0016	0.1668
Olfactory bulb	12.27±0.86	13.95±0.82	9.64±0.32	12.52±0.61	0.0069	0.0003	0.3912
Retina	17.13±0.71[a]	17.10±0.88[a]	13.68±1.13[b]	17.71±1.29[a]	0.1557	0.0498	0.0467
20:4n-6, AA							
Hypothalamus	10.06±0.44[a]	9.20±0.32[a]	7.86±0.19[b]	8.25±0.14[b]	<0.0001	0.4272	0.0414
Hippocampus	12.76±0.54	13.65±0.63	10.81±0.51	11.49±0.62	0.0019	0.1900	0.8626
Frontal cortex	10.21±0.69	10.45±0.29	9.00±0.25	9.24±0.19	0.0062	0.5536	0.9926
Cerebellum	6.94±0.39	7.53±0.31	6.86±0.12	6.87±0.34	0.2235	0.3274	0.3340
Olfactory bulb	9.07±0.76	9.17±0.61	8.58±0.38	9.40±0.36	0.8159	0.4140	0.5239
Retina	9.20±0.79	9.43±0.47	8.59±0.44	8.03±0.67	0.1192	0.7935	f0.5317
22:4n-6							
Hypothalamus	4.36±0.43	3.92±0.18	2.76±0.13	3.32±0.15	0.0010	0.8126	0.0505
Hippocampus	3.03±0.30	2.96±0.15	2.31±0.15	2.30±0.19	0.0023	0.8436	0.8939
Frontal cortex	3.00±0.28	2.90±0.20	1.88±0.13	2.15±0.10	<0.0001	0.6682	0.3394
Cerebellum	1.73±0.13	1.82±0.08	1.64±0.06	1.23±0.27	<0.0001	0.6682	0.3394
Olfactory bulb	2.37±0.29[a]	1.97±0.21[ab]	1.62±0.11[b]	2.16±0.11[ab]	0.1576	0.7010	0.0236
Retina	1.23±0.11	1.15±0.09	1.32±0.25	0.98±0.15	0.7831	0.1398	0.3595
22:5n-6							
Hypothalamus	0.45±0.19	0.10±0.07	0.35±0.08	0.04±0.02	0.3350	0.0030	0.9910
Hippocampus	0.89±0.23	0.14±0.07	1.11±0.27	0.42±0.07	0.2016	0.0009	0.8956
Frontal cortex	0.87±0.19	0.10±0.08	0.77±0.14	0.19±0.02	0.9884	<0.0001	0.4886
Cerebellum	0.50±0.05[b]	0.17±0.08[c]	0.88±0.04[a]	0.12±0.10[a]	0.0247	<0.0001	0.0051
Olfactory bulb	2.32±0.81	0.65±0.27	0.91±0.34	0.35±0.16	0.0698	0.0210	0.2276
Retina	1.11±0.14	0.13±0.02	1.32±0.16	0.50±0.10	0.0115	<0.0001	0.4716
22:5n-6+22:6n-3							
Hypothalamus	12.90±0.61[a]	12.35±0.58[a]	8.31±0.21[c]	10.73±0.42[b]	<0.0001	0.0545	0.0040
Hippocampus	12.46±0.54	12.86±0.42	10.02±0.30	11.27±0.52	0.0003	0.0835	0.3562
Frontal cortex	15.21±0.86	18.92±2.26	10.87±0.60	13.41±0.26	0.0006	0.0175	0.6346
Cerebellum	13.63±0.97	14.72±0.38	11.11±0.63	13.63±0.74	0.0160	0.0158	0.3098
Olfactory bulb	14.59±0.83	14.60±1.05	10.55±0.41	12.87±0.76	0.0015	0.1595	0.1624
Retina	18.24±0.81[a]	17.23±0.89[ab]	15.00±1.25[b]	18.21±1.31[a]	0.2793	0.2918	0.0498
n-3+n-6							
Hypothalamus	29.54±2.03[a]	26.74±0.81[a]	20.06±0.46[c]	23.67±0.74[b]	<0.0001	0.0906	0.0040
Hippocampus	29.16±1.37	31.13±0.94	24.98±0.58	26.87±1.31	0.0007	0.0798	0.9715
Frontal cortex	29.88±1.61	31.62±1.19	24.12±0.98	27.01±0.51	0.0002	0.0538	0.6159
Cerebellum	24.65±1.47	26.61±0.68	22.32±0.72	24.59±1.12	0.0411	0.0469	0.8754
Olfactory bulb	27.62±0.74	27.36±1.06	23.62±0.83	26.97±0.96	0.0264	0.1083	0.0628
Retina	30.33±1.18	29.70±1.16	30.03±1.63	29.15±1.37	0.7420	0.5620	0.9235

[a] The data are presented as the mean±SEM.
[b] Levels of other fatty acids are presented in Supplemental Tables 1–6.
[c] Statistical differences between the four groups were determined by two-way ANOVA. When the interaction was significant, comparisons among groups were performed using Duncan's multiple range tests. Means without a common superscript letter differ, $P \leq .05$.

main effect of FO supplementation was seen in any of the five examined brain regions and the retina.

The greatest AA depletion in the P group compared to the V group was seen in the hypothalamus (22% decrease), followed by the hippocampus (16%) and frontal cortex (12%). AA levels in the depleted regions in the P group were 7.9–10.8% of total fatty acids compared to 10.1–12.8% in the V group. AA levels were not altered in the cerebellum, olfactory bulb or retina in the P group compared to the V group. Comparison of AA levels in the P+FO and V+FO groups showed that the greatest AA depletion in the P+FO group was seen in the hippocampus (15% decrease), followed by the frontal cortex (12%) and hypothalamus (11%). AA levels in the depleted regions in the P+FO group were 8.2–11.5% of total fatty acids compared to 9.2–13.6% in the V+FO group. Again, AA levels were not altered in the cerebellum, olfactory bulb or retina.

3.4. DHA and AA levels in the liver, erythrocytes and serum

DHA levels in the liver, erythrocytes and serum in the P group were significantly lower than in the other three groups (Table 3) (levels of other fatty acids are presented in Supplemental Tables 7–9). Two-way ANOVA revealed a main effect of postpartum and FO and a Postpartum×FO interaction.

AA levels in the liver, erythrocytes and serum in the postpartum rats (P and P+FO groups) were significantly lower than those in the age-matched virgin groups (V and V+FO groups). Two-way ANOVA revealed a main effect of postpartum, but no main effect of FO. In contrast, levels of 18:2n-6 (source of AA) in the postpartum rats were significantly increased in the erythrocytes and serum, but not in the liver, compared to the virgin rats. It is interesting to note that total n-3 and n-6 PUFA levels in the five examined brain regions and liver, but not in the erythrocytes and serum, in the postpartum rats (P and P+FO groups) were significantly reduced compared to the age-matched virgin groups (V and V+FO groups).

3.5. 5-HT levels and metabolism

As shown in Table 4, two-way ANOVA revealed there was no FO effect or Postpartum×FO interaction effect on 5-HT levels, but there was a main effect of postpartum in the brainstem, hippocampus and frontal cortex, indicating that 5-HT levels were significantly reduced in the postpartum groups compared to the age-matched virgin groups. When the 5-HIAA/5-HT ratio was used as an estimate of the

Table 3
Major PUFA composition of the liver, erythrocytes and serum in postpartum or virgin rats fed the n-3 PUFA-deficient diet with or without FO supplementation for 41 days[a,b,c]

	V (n=6)	V+FO (n=6)	P (n=7)	P+FO (n=6)	Postpartum	FO	P×FO
22:6n-3, DHA							
Liver	3.65±0.53[a]	3.93±0.35[a]	1.26±0.31[b]	4.16±0.54[a]	0.0372	0.0013	0.0014
Erythrocytes	1.71±0.15[a]	2.15±0.21[a]	0.54±0.12[b]	1.92±0.17[a]	0.0003	<0.0001	0.0101
Serum	1.66±0.13[b]	2.56±0.29[a]	0.50±0.11[c]	2.73±0.24[a]	0.0225	<0.0001	0.0034
18:2n-6							
Liver	19.94±1.25	24.50±1.72	21.74±2.52	20.66±2.50	0.2803	0.8331	0.0967
Erythrocytes	14.52±0.79	15.19±0.65	24.76±2.47	23.64±1.63	<0.0001	0.8871	0.5702
Serum	21.08±2.37	22.71±1.14	36.03±2.25	28.83±1.42	<0.0001	0.1740	0.0372
20:4n-6, AA							
Liver	20.27±1.76[a]	14.61±0.74[b]	11.82±1.91[b]	13.86±1.71[b]	0.0038	0.1655	0.0016
Erythrocytes	27.62±1.19	24.91±0.88	18.56±1.01	19.83±1.63	<0.0001	0.5474	0.1046
Serum	32.85±2.12[a]	30.41±1.34[a]	16.06±1.72[c]	22.56±1.03[b]	<0.0001	0.1740	0.0372
22:4n-6							
Liver	0.93±0.17	0.56±0.13	0.71±0.23	0.14±0.06	0.0537	0.0105	0.8278
Erythrocytes	0.20±0.20	0.20±0.13	0.71±0.14	0.40±0.08	0.0265	0.3319	0.3119
Serum	0.50±0.04	0.05±0.03	0.59±0.21	0.11±0.05	0.6139	0.0014	0.9788
22:5n-6							
Liver	3.16±0.34	0.30±0.11	2.08±0.72	0.30±0.20	0.2321	0.0001	0.2263
Erythrocytes	1.39±0.22	0.24±0.06	1.07±0.10	0.25±0.06	0.2272	<0.0001	0.2123
Serum	1.45±0.22	0.15±0.05	1.33±0.16	0.25±0.15	0.9650	<0.0001	0.5017
22:6n-3+22:5n-6							
Liver	6.81±0.81[a]	4.23±0.34[b]	3.35±0.96[b]	4.46±0.65[ab]	0.0640	0.3861	0.0117
Erythrocytes	3.10±0.31	2.40±0.26	1.61±0.20	2.17±0.20	0.0022	0.7727	0.0184
Serum	3.11±0.21[a]	2.71±0.31[a]	1.83±0.18[b]	2.98±0.26[a]	0.0491	0.1340	0.0034
n-3+n-6							
Liver	48.49±2.25	44.48±1.81	39.13±3.74	40.01±2.21	0.0068	0.2635	0.1931
Erythrocytes	46.07±1.05	43.09±1.67	46.43±2.18	46.63±2.78	0.3301	0.4858	0.4275
Serum	58.47±1.45	56.40±0.70	55.99±1.03	56.19±0.50	0.2090	0.3767	0.2855

[a] The data are presented as the mean±S.E.M.
[b] Levels of other fatty acids are presented in Supplemental Tables 7–9.
[c] Statistical differences between the four groups were determined by two-way ANOVA. When the interaction was significant, comparisons among groups were performed using Duncan's multiple range tests. Means without a common superscript letter differ, $P \leq .05$.

serotonin turnover rate, two-way ANOVA revealed that there was no FO effect or Postpartum×FO interaction, but there was a main effect of postpartum in the brainstem and frontal cortex and the 5-HIAA/5-HT ratios in these two areas were higher in the postpartum groups than in the virgin groups. There was no significant difference in the 5-HIAA/5-HT ratio in the hippocampus between the four groups.

3.6. MAO-A activity

In the brainstem, two-way ANOVA revealed a main effect of both postpartum and FO with a Postpartum×FO interaction. The *post hoc* Duncan's multiple range test showed that MAO-A activity in the brainstem was significantly lower in the P+FO group than in the other three groups (Fig. 2). There was no postpartum, FO or Postpartum×FO effect on MAO-A activity in the hippocampus and frontal cortex.

4. Discussion

In this study, we compared postpartum rats fed an n-3 PUFA-deficient diet during pregnancy and lactation and age-matched virgin female rats fed the same diet (41 days for both groups) and found that DHA levels in the five examined maternal brain regions and AA levels in three brain regions were depleted and that FO supplementation of the maternal rats maintained brain DHA levels, but not AA levels. 5-HT levels were reduced, and 5-HT turnover increased, in the selected

Table 4
Effect of postpartum status and FO supplementation on 5-HT metabolism in the brainstem, frontal cortex and hippocampus of postpartum or virgin rats fed the n-3 PUFA-deficient diet with or without FO supplementation[a]

	V	V+FO	P	P+FO	Postpartum	FO	P×FO
Brainstem							
5-HT[b]	3.89±0.31	4.05±0.21	2.57±0.54	3.25±0.07	0.0067	0.2432	0.4566
5-HIAA[b]	1.64±0.10	1.71±0.06	1.25±0.26	1.91±0.14	0.5598	0.0369	0.1858
5-HIAA/5-HT[c]	0.43±0.03	0.43±0.01	0.51±0.03	0.59±0.03	0.0003	0.1858	0.1586
Frontal cortex							
5-HT	2.13±0.28	2.69±0.56	1.70±0.31	0.94±0.16	0.0511	0.4695	0.0512
5-HIAA	1.57±0.17	1.46±0.22	1.72±0.13	1.55±0.24	0.5432	0.4904	0.8946
5-HIAA/5-HT	0.77±0.09	0.58±0.07	1.03±0.25	1.78±0.33	0.0018	0.1673	0.0276
Hippocampus							
5-HT	1.71±0.27	2.02±0.32	1.23±0.37	1.03±0.19	0.0251	0.8505	0.4157
5-HIAA	2.24±0.25	2.26±0.18	1.18±0.26	1.91±0.36	0.0149	0.1649	0.1933
5-HIAA/5-HT	1.47±0.29	1.33±028	1.16±0.20	1.85±0.15	0.6535	0.2726	0.1060

[a] The data are presented as the mean±S.E.M. (n=5–6/group).
[b] Units: nmol/g wet tissue.
[c] 5-HIAA/5-HT ratio as an index of 5-HT turnover.

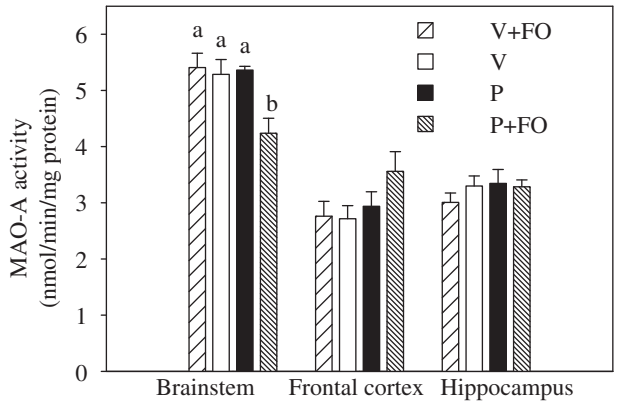

Fig. 2. MAO-A activity in the brainstem, frontal cortex and hippocampus of postpartum or virgin rats fed the n-3 PUFA-deficient diet with or without FO supplementation for 41 days. The data are presented as the mean±S.E.M. (n=5–6/group). Means without a common letter differ, $P \leq .05$.

serotoninergic brain regions (brainstem, frontal cortex and hippocampus) in the postpartum rats compared to virgin rats. In addition, FO supplementation of maternal rats resulted in a decrease in MAO-A activity in the brainstem and in postpartum anxiety behavior. We conclude that maternal brain regional DHA levels in rats fed an n-3 PUFA-deficient diet during pregnancy and lactation were depleted by supplying DHA to the offspring and that this was avoided by FO supplementation. We propose that reproduction modulates maternal brain 5-HT levels and 5-HT turnover and causes postpartum anxiety and suggest that FO supplementation of women on an n-3 PUFA-deficient diet during pregnancy and lactation may have a postpartum anxiolytic effect.

In rats fed a low n-3 PUFA diet containing 0.32 g/kg of 18:3n-3 estimated as about 0.08% of the energy source, maternal whole brain DHA levels were reduced by 20% after a single reproductive cycle compared to age-matched virgins [8]. In addition, in rats fed the same low n-3 PUFA diet, maternal DHA levels were significantly reduced by 20% in the frontal cortex, 13% in the caudate-putamen and 13% in the temporal lobe after two consecutive reproductive cycles compared to age-matched virgins rats, but were not altered in the hypothalamus, hippocampus, cerebellum and ventral striatum [10]. We suspected that this amount of 18:3n-3 in the diet might supply DHA to prevent the loss of maternal brain DHA. In the present study on rats fed an n-3 PUFA-deficient diet containing 0.04 g/kg of 18:3n-3 estimated as about 0.008% of the energy source, we found that maternal rat DHA levels were significantly reduced by 37% in the hypothalamus, 29% in the frontal cortex, 24% in the hippocampus, 22% in the cerebellum, 22% in the olfactory bulb and 20% in the retina after a single reproductive cycle compared to age-matched virgins. These three studies showed that maternal DHA levels in the brain in rats fed an n-3 PUFA-deficient diet are depleted by one reproduction cycle. Thus, brain DHA levels in young adult rats are not affected by feeding an n-3 PUFA-deficient diet for up to 7 months [24], but can be depleted in rats on an n-3 PUFA-deficient diet by pregnancy and lactation.

In rats fed a low n-3 PUFA diet, maternal whole brain AA levels were reduced by 15% in postpartum rats (8.3% of total fatty acids) compared to age-matched virgin rats (9.8% of total fatty acids) [8]. We found that maternal rat AA levels were significantly reduced by 12–22% in the hypothalamus, hippocampus and frontal cortex, but were unchanged in the olfactory bulb, cerebellum and retina after a single reproductive cycle in postpartum rats compared to virgin rats. In addition, maternal DHA and AA levels in the liver, erythrocytes and serum were significantly reduced in postpartum rats compared to age-matched virgin rats. This result is supported by previous findings that, in rats fed a low n-3 or normal n-3 PUFA diets, DHA levels in the maternal liver and erythrocytes and AA levels in the maternal liver are significantly reduced in postpartum rats compared to age-matched virgin rats [25].

In human studies, it has been reported that plasma DHA and AA levels are reduced in pregnant women compared to nonpregnant women of child-bearing age [26]. Plasma and erythrocyte DHA levels are significantly lower in lactating women than in nonlactating women after parturition and decrease even further with a longer duration of breast-feeding [27]. Maternal plasma and erythrocyte DHA levels, but not AA levels, are significantly lower during the postpartum period than during gestation or at delivery [28]. Moreover, maternal plasma DHA levels are significantly lower in multigravidae than in primigravidae [29]. These studies indicate that human maternal DHA levels are reduced by a reproductive cycle. We found that, in rats fed an n-3 PUFA-deficient diet during pregnancy and lactation, maternal DHA levels were reduced not only in the serum and erythrocytes but also in the five examined brain regions, suggesting that brain DHA deprivation by the offspring may occur in women on an n-3 PUFA-deficient diet during pregnancy and lactation.

Maternal brain DHA depletion was prevented in rats on an n-3 PUFA-deficient diet by FO supplementation in this study or by feeding an n-3 PUFA-rich diet in previous studies [8-10,30]. According to our estimate of maternal energy intake during pregnancy and lactation and to our analysis of the FO in this study, the n-3 PUFA provided by the FO accounted for about 0.37% of the energy source (0.24% of the energy source as 20:5n-3 and 0.13% as DHA). This amount of FO supplementation of the n-3 PUFA-deficient diet during pregnancy and lactation maintained brain regional and retina DHA levels at 85–104% of those in age-matched virgin rats, while in maternal rats without FO supplementation the levels were reduced to 63–80% in the depleted brain regions and retina. This result is supported by previous findings that, in rats fed an n-3 PUFA-rich diet containing 4.2–5.1 g/kg of 18:3n-3 estimated for about 1–1.2% of energy source, maternal whole brain or regional DHA levels are not changed compared to age-matched virgin rats [8-10,30].

Many postpartum women are susceptible to mood disturbances, especially depression and anxiety [11-13], but the mechanism is not known. Anxiety or depression is modulated by 5-HT, which is synthesized in the raphe nuclei of the brainstem and projected to the cortex, hippocampus and other areas, and is metabolized mainly by MAO-A [14,22]. We therefore examined 5-HT levels and MAO-A activity in these serotoninergic regions and found that, in postpartum rats compared to virgin rats, 5-HT levels were decreased in the brainstem, frontal cortex and hippocampus, and 5-HT turnover increased in the brainstem and frontal cortex, indicating that reproduction modulates 5-HT metabolism and that this may contribute to postpartum anxiety. In addition, FO supplementation of rats fed an n-3 PUFA-deficient diet during pregnancy and lactation suppressed MAO-A activity in the brainstem, leading to an increase in available 5-HT and decreased anxiety-like behavior, indicating an anxiolytic-like action of FO. It has been suggested that postpartum depression is associated with lower DHA status [31,32]. A cross-national analysis showed that higher DHA levels in maternal milk and higher seafood consumption are correlated with a lower prevalence of postpartum depression [33]. Moreover, supplementation of the diet with n-3 PUFA for 8 weeks resulted in a decrease in the depression score in pregnant women with major depression or women with postpartum depression [16,34].

It is interesting to note that the hypothalamus was identified as one of the brain regions most susceptible to DHA loss after pregnancy and lactation. The hypothalamus is responsible for controlling the hypothalamic–pituitary–target organ axis endocrine system in order to meet the requirements of pregnancy and lactation [35]. Dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis and oxytocin

and prolactin secretion has been suggested to lead to postpartum depression or anxiety [36-39]. In addition, depression and anxiety disorders are associated with excessive stress responses, HPA hyperactivity, and increased secretion of hypothalamic corticotrophin releasing factor [40], a modulator of the HPA axis. In perpetrators of domestic violence, lower plasma DHA levels are associated with higher cerebrospinal fluid levels of corticotrophin-releasing hormone [41], indicating that DHA deficiency may facilitate hyperactivity of the HPA axis. Whether the reduced DHA levels in the hypothalamus affect the HPA axis or hypothalamic secretion of hormones, resulting in postpartum anxiety or depression, requires further study.

This study shows that DHA levels in the maternal hypothalamus, hippocampus, frontal cortex, cerebellum, olfactory bulb and retina in rats fed an n-3 PUFA-deficient diet are depleted by pregnancy and lactation, and that FO supplementation during the reproductive cycle maintains maternal brain regional and retina DHA levels and has an anxiolytic-like effect. In addition, 5-HT levels are reduced and its turnover increased in postpartum rats compared to virgin female rats and this may contribute to postpartum anxiety or depression. This suggests that depletion of maternal brain DHA levels by the offspring may occur in women on an n-3 PUFA-deficient diet during pregnancy and lactation. We propose that FO has potential as a natural supplement that can maintain maternal brain regional DHA levels and inhibit MAO-A activity in the maternal brainstem and may have a postpartum anxiolytic effect on women on an n-3 PUFA-deficient diet during pregnancy and lactation.

Supplementary materials related to this article can be found online at doi:10.1016/j.jnutbio.2010.12.010.

References

[1] Sastry PS. Lipids of nervous tissue: composition and metabolism. Prog Lipid Res 1985;24(2):69–176.
[2] Dobbing J, Sands J. Comparative aspects of the brain growth spurt. Early Hum Dev 1979;3(1):79–83.
[3] Green P, Glozman S, Kamensky B, Yavin E. Developmental changes in rat brain membrane lipids and fatty acids. The preferential prenatal accumulation of docosahexaenoic acid. J Lipid Res 1999;40(5):960–6.
[4] Martinez M. Tissue levels of polyunsaturated fatty acids during early human development. J Pediatr 1992 Apr;120(4 Pt 2):S129–138.
[5] Fedorova I, Salem Jr N. Omega-3 fatty acids and rodent behavior. Prostaglandins Leukot Essent Fatty Acids 2006;75(4-5):271–89.
[6] Innis SM. Essential fatty acids in growth and development. Prog Lipid Res 1991;30(1):39–103.
[7] Salem Jr N, Litman B, Kim HY, Gawrisch K. Mechanisms of action of docosahexaenoic acid in the nervous system. Lipids 2001;36(9):945–59.
[8] Levant B, Radel JD, Carlson SE. Reduced brain DHA content after a single reproductive cycle in female rats fed a diet deficient in N-3 polyunsaturated fatty acids. Biol Psychiatry 2006;60(9):987–90.
[9] Levant B, Ozias MK, Carlson SE. Diet (n-3) polyunsaturated fatty acid content and parity interact to alter maternal rat brain phospholipid fatty acid composition. J Nutr 2006;136(8):2236–42.
[10] Levant B, Ozias MK, Carlson SE. Specific brain regions of female rats are differentially depleted of docosahexaenoic acid by reproductive activity and an (n-3) fatty acid-deficient diet. J Nutr 2007;137(1):130–4.
[11] Wenzel A, Haugen EN, Jackson LC, Brendle JR. Anxiety symptoms and disorders at eight weeks postpartum. J Anxiety Disord 2005;19(3):295–311.
[12] Ross LE, McLean LM. Anxiety disorders during pregnancy and the postpartum period: a systematic review. J Clin Psychiatry 2006;67(8):1285–98.
[13] Stuart S, Couser G, Schilder K, O'Hara MW, Gorman L. Postpartum anxiety and depression: onset and comorbidity in a community sample. J Nerv Ment Dis 1998;186(7):420–4.
[14] Ressler KJ, Nemeroff CB. Role of serotonergic and noradrenergic systems in the pathophysiology of depression and anxiety disorders. Depress Anxiety 2000;12(Suppl 1):2–19.
[15] Moses-Kolko EL, Bogen D, Perel J, Bregar A, Uhl K, Levin B, et al. Neonatal signs after late in utero exposure to serotonin reuptake inhibitors: literature review and implications for clinical applications. JAMA 2005;293(19):2372–83.
[16] Freeman MP, Hibbeln JR, Wisner KL, Davis JM, Mischoulon D, Peet M, et al. Omega-3 fatty acids: evidence basis for treatment and future research in psychiatry. J Clin Psychiatry 2006;67(12):1954–67.
[17] Parker G, Gibson NA, Brotchie H, Heruc G, Rees AM, Hadzi-Pavlovic D. Omega-3 fatty acids and mood disorders. Am J Psychiatry 2006;163(6):969–78.
[18] Bourre JM, Francois M, Youyou A, Dumont O, Piciotti M, Pascal G, et al. The effects of dietary alpha-linolenic acid on the composition of nerve membranes, enzymatic activity, amplitude of electrophysiological parameters, resistance to poisons and performance of learning tasks in rats. J Nutr 1989;119(12):1880–92.
[19] Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 1959;37(8):911–7.
[20] Moser AB, Jones DS, Raymond GV, Moser HW. Plasma and red blood cell fatty acids in peroxisomal disorders. Neurochem Res 1999;24(2):187–97.
[21] Chung WL, Chen JJ, Su HM. Fish oil supplementation of control and (n-3) fatty acid-deficient male rats enhances reference and working memory performance and increases brain regional docosahexaenoic acid levels. J Nutr 2008;138(6):1165–71.
[22] Shih JC, Chen K, Ridd MJ. Role of MAO A and B in neurotransmitter metabolism and behavior. Pol J Pharmacol 1999;51(1):25–9.
[23] Mathews TA, Fedele DE, Coppelli FM, Avila AM, Murphy DL, Andrews AM. Gene dose-dependent alterations in extraneuronal serotonin but not dopamine in mice with reduced serotonin transporter expression. J Neurosci Methods 2004;140(1-2):169–81.
[24] Bourre JM, Dumont OS, Piciotti MJ, Pascal GA, Durand GA. Dietary alpha-linolenic acid deficiency in adult rats for 7 months does not alter brain docosahexaenoic acid content, in contrast to liver, heart and testes. Biochim Biophys Acta 1992;1124(2):119–22.
[25] Levant B, Ozias MK, Carlson SE. Diet (n-3) polyunsaturated fatty acid content and parity affect liver and erythrocyte phospholipid fatty acid composition in female rats. J Nutr 2007;137(11):2425–30.
[26] Holman RT, Johnson SB, Ogburn PL. Deficiency of essential fatty acids and membrane fluidity during pregnancy and lactation. Proc Natl Acad Sci U S A 1991;88(11):4835–9.
[27] Otto SJ, van Houwelingen AC, Badart-Smook A, Hornstra G. Comparison of the peripartum and postpartum phospholipid polyunsaturated fatty acid profiles of lactating and nonlactating women. Am J Clin Nutr 2001;73(6):1074–9.
[28] Stark KD, Beblo S, Murthy M, Buda-Abela M, Janisse J, Rockett H, et al. Comparison of bloodstream fatty acid composition from African-American women at gestation, delivery, and postpartum. J Lipid Res 2005;46(3):516–25.
[29] Al MD, van Houwelingen AC, Hornstra G. Relation between birth order and the maternal and neonatal docosahexaenoic acid status. Eur J Clin Nutr 1997;51(8):548–53.
[30] Levant B, Ozias MK, Davis PF, Winter M, Russell KL, Carlson SE, et al. Decreased brain docosahexaenoic acid content produces neurobiological effects associated with depression: Interactions with reproductive status in female rats. Psychoneuroendocrinology 2008;33(9):1279–92.
[31] Otto SJ, de Groot RH, Hornstra G. Increased risk of postpartum depressive symptoms is associated with slower normalization after pregnancy of the functional docosahexaenoic acid status. Prostaglandins Leukot Essent Fatty Acids 2003;69(4):237–43.
[32] 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(25):3181–7.
[33] Hibbeln JR. Seafood consumption, the DHA content of mothers' milk and prevalence rates of postpartum depression: a cross-national, ecological analysis. J Affect Disord 2002;69(1-3):15–29.
[34] Su KP, Huang SY, Chiu TH, Huang KC, Huang CL, Chang HC, et al. Omega-3 fatty acids for major depressive disorder during pregnancy: results from a randomized, double-blind, placebo-controlled trial. J Clin Psychiatry 2008;69(4):644–51.
[35] Brunton PJ, Russell JA. The expectant brain: adapting for motherhood. Nat Rev Neurosci 2008;9(1):11–25.
[36] Jolley SN, Elmore S, Barnard KE, Carr DB. Dysregulation of the hypothalamic-pituitary-adrenal axis in postpartum depression. Biol Res Nurs 2007;8(3):210–22.
[37] Mastorakos G, Ilias I. Maternal and fetal hypothalamic-pituitary-adrenal axes during pregnancy and postpartum. Ann N Y Acad Sci 2003;997:136–49.
[38] Asher I, Kaplan B, Modai I, Neri A, Valevski A, Weizman A. Mood and hormonal changes during late pregnancy and puerperium. Clin Exp Obstet Gynecol 1995;22(4):321–5.
[39] Nissen E, Gustavsson P, Widstrom AM, Uvnas-Moberg K. Oxytocin, prolactin, milk production and their relationship with personality traits in women after vaginal delivery or cesarean section. J Psychosom Obstet Gynaecol 1998;19(1):49–58.
[40] Arborelius L, Owens MJ, Plotsky PM, Nemeroff CB. The role of corticotropin-releasing factor in depression and anxiety disorders. J Endocrinol 1999;160(1):1–12.
[41] Hibbeln JR, Bissette G, Umhau JC, George DT. Omega-3 status and cerebrospinal fluid corticotrophin releasing hormone in perpetrators of domestic violence. Biol Psychiatry 2004;56(11):895–7.