Available online at www.sciencedirect.com

SciVerse ScienceDirect

Journal of Nutritional Biochemistry 24 (2013) 70–80

Journal of Nutritional Biochemistry

Exposure to a maternal n-3 fatty acid-deficient diet during brain development provokes excessive hypothalamic–pituitary–adrenal axis responses to stress and behavioral indices of depression and anxiety in male rat offspring later in life☆

Hui-Feng Chen, Hui-Min Su*

Department of Physiology, National Taiwan University College of Medicine, No. 1, Sec. 1, Jen-Ai Rd, Taipei, 100, Taiwan

Received 5 August 2011; received in revised form 13 February 2012; accepted 14 February 2012

Abstract

Brain docosahexaenoic acid (DHA, 22:6n-3) accumulates rapidly during brain development and is essential for normal neurological function. The aim of this study was to evaluate whether brain development was the critical period in which DHA deficiency leads to dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis in response to stress later in life. Rats were exposed to an n-3 fatty acid-deficient diet or the same diet supplemented with fish oil as an n-3 fatty acid-adequate diet either throughout the preweaning period from embryo to weaning at 3 weeks old or during the postweaning period from 3 to 10 weeks old. Exposure to the n-3 fatty acid-deficient diet during the preweaning period resulted, at weaning, in a significant decrease in hypothalamic DHA levels and a reduced male offspring body weight. DHA deficiency during the preweaning period significantly increased and prolonged restraint stress-induced changes in colonic temperature and serum corticosterone levels, caused a significant increase in $GABA_A$ antagonist-induced heart rate changes and enhanced depressive-like behavior in the forced swimming test and anxiety-like behavior in the plus-maze test in later life. These effects were not seen in male rats fed the n-3 fatty acid-deficient diet during the postweaning period. These results suggest that brain development is the critical period in which DHA deficiency leads to excessive HPA responses to stress and elevated behavioral indices of depression and anxiety in adulthood. We propose that these effects of hypothalamic DHA deficiency during brain development may involve a $GABA_A$ receptor-mediated mechanism.

Keywords: Hypothalamus; Stress; Brain development; n-3 fatty acids; Docosahexaenoic acid (DHA); HPA axis; Corticosterone; Gamma-aminobutyric $acid_A$ ($GABA_A$); GAD67; Depression; Anxiety

1. Introduction

Docosahexaenoic acid (DHA, 22:6n-3), which is specifically enriched in the brain and is essential for normal neurological function [1,2], accumulates rapidly during brain development from prenatal day 7 to beyond postnatal day 16 in the whole rat brain [3] and from the third trimester of gestation to 2 years after birth in the human forebrain [4,5]. It has been proposed that exposure to maternal stress, including nutritional insult, is associated with an increased risk of chronic diseases, such as hypertension, coronary heart disease, type 2 diabetes and cancer, and of neuropsychiatric disorders in the adult offspring in rats and humans [6–8]. In addition, in rats, sheep and humans, maternal stress has a profound impact on stress-induced hypothalamic–pituitary–adrenal (HPA) axis activity in later life in the offspring [9–11].

The magnitude of the HPA axis response to stress is limited by both the gamma-aminobutyric acid (GABA) inhibitory circuit and the glucocorticoid negative feedback system [10,12]. GABA, the inhibitory neurotransmitter that acts at inhibitory synapses, is the dominant neurotransmitter in the hypothalamus, especially in the paraventricular nucleus, a site rich in corticotrophin-releasing factor-secreting neurons, and thus provides the stimulus for secretion of adrenocorticotrophic hormone (ACTH), which controls HPA axis activity [13–15]. Lack of GABA has long been known to be associated with depression and anxiety, and positive modulators of $GABA_A$ receptors have antidepression effects [12,16]. Administration of a $GABA_A$ antagonist increases the corticosterone response [17], heart rate and blood pressure [18], and anxiety behavior [19,20], while administration of an agonist decreases these responses [17–20]. GABA is produced from glutamate by glutamate decarboxylase (GAD65 and GAD67), with GAD67 being the main isoenzyme responsible for GABA production under acute stress-inducing conditions [21].

This study was designed to evaluate whether brain development was the critical period in which a maternal n-3 fatty acid-deficient diet regulated the HPA axis response to stress in the male adult offspring. We hypothesized that hypothalamic DHA deficiency during brain development might modulate stress-induced GAD67

☆ This work was supported by grants from the National Science Council of Taiwan (grant NSC-99-2320-B-002-025) and the National Taiwan University (grant 99R71431).

* Corresponding author. 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 © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jnutbio.2012.02.006

expression and alter GABAergic regulation of the HPA axis activity, leading to anxiety- and depressive-like behaviors later in life. To test this, a high linoleic acid sunflower oil-based n-3 fatty acid-deficient diet was used to induce DHA deficiency during either the preweaning period (E0 to 3 weeks old) or the postweaning period (from 3 to 10 weeks old) and to examine the effects on the HPA axis response to stress and emotional behaviors in the male adult offspring.

2. Materials and methods

2.1. Animals and study design

Sprague–Dawley rats (7 weeks old) and pregnant rats at 2 days of gestation (8 weeks old) obtained from BioLasco Taiwan, a technology licensee of Charles River Laboratories in Taiwan, were housed in a humidity-controlled room at 24°C±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.

The study design is shown in Fig. 1. Chow diet-fed 8-week-old female rats were mated, and conception was confirmed by the presence of vaginal plugs.

To study the effect of exposure to an n-3 fatty acid-deficient diet during the preweaning period, two groups, preweaning deficient (prew-Def) and preweaning adequate (prew-Adq), were used (Fig. 1a). In group prew-Def, the dams were fed a high linoleic acid sunflower oil-based n-3 fatty acid-deficient diet and were given 0.1 ml of water per day by oral gavage throughout pregnancy and lactation; then, after weaning at 3 weeks old, the male offspring were fed chow diet (5001, LabDiet; Table 1) until sacrifice at 10 weeks old. In group prew-Adq, the dams were fed the same n-3 fatty acid-deficient diet supplemented by daily oral gavage with 0.2 ml of fish oil during pregnancy and 0.4 ml of fish oil during the 3 weeks of lactation. After weaning at 3 weeks old, the male offspring were fed chow diet until 10 weeks old.

To study the effect of exposure to an n-3 fatty acid-deficient diet during the postweaning period, groups postweaning deficient (postw-Def) and postweaning adequate (postw-Adq) were used (Fig. 1b). During pregnancy and lactation, the dams were fed the same n-3 fatty acid-deficient diet supplemented by daily oral gavage with fish oil as the dams in group prew-Adq; then, after weaning at 3 weeks old, the male pups were fed the n-3 fatty acid-deficient diet supplemented by daily oral gavage with either 0.1 ml of water (group postw-Def) or 0.1 ml of fish oil (group postw-Adq) until 10 weeks old.

In both studies, water was given by oral gavage to the control group to avoid bias due to the additional interaction between the rats and the experimenter. Since HPA axis responses may be gender specific [22–24] and to avoid the hormonal effect of the estrus cycle on depressive-like and anxiety-like behaviors, only male offspring were used. The male offspring used in each test was taken from four to five separate litters.

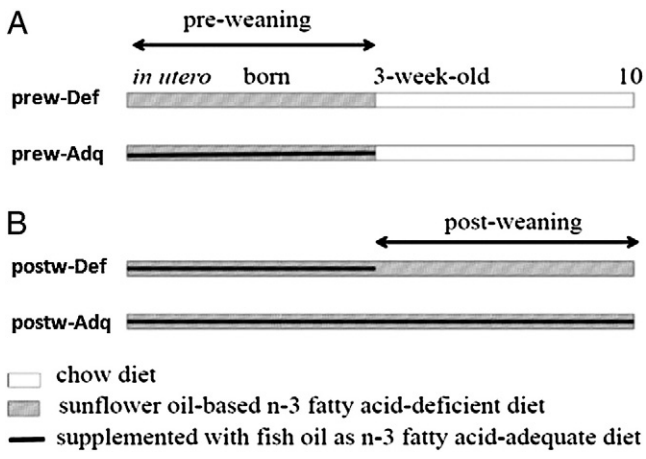

Fig. 1. Study design for rats exposed to a high linoleic acid sunflower oil-based n-3 fatty acid-deficient diet or the same diet supplemented with fish oil as an n-3 fatty acid-adequate diet during either the preweaning (prew) period (a) or the postweaning (postw) period (b). Group prew-Def: n-3 fatty acid-deficient diet for the dam during pregnancy and lactation; male offspring fed chow diet after weaning. Group prew-Adq: n-3 fatty acid-deficient diet supplemented with fish oil as an n-3 fatty acid-adequate diet for the dam during pregnancy and lactation; male offspring fed chow diet. Group postw-Def: n-3 fatty acid-deficient diet supplemented with fish oil as an n-3 fatty acid-adequate diet for the dam during pregnancy and lactation; male offspring fed the n-3 fatty acid-deficient diet. Group postw-Adq: n-3 fatty acid-deficient diet supplemented with fish oil as an n-3 fatty acid-adequate diet for the dam and male offspring.

Table 1
Composition of the n-3 fatty acid-deficient diet and chow diet

Ingredient (g/kg diet)	n-3 Fatty acid-deficient diet	Chow diet
Fat		50 (ether extract)
Sunflower oil	200	57 (acid hydrolysis)
Protein		
Casein	238	239
Methionine	3.5	
Carbohydrate		
Corn starch	150	487
Sucrose	294.3	
Fiber		
Alphacel	58.8	51
Vitamin mix		
AIN 76 vitamin mix	11.8	2.5
Mineral mix		
AIN 76 mineral mix	41.2	70
Choline chloride	2.4	
Energy density, kcal/g	4.5	3.4
Fat, % of energy	40	13.5(ether extract)
Protein, % of energy	21	28.5
Carbohydrate, % of energy	39	58

The details of the number of dams and the assignment of the male offspring to each test are given in Fig. 2.

2.2. Diet composition

The compositions of the n-3 fatty acid-deficient diet, a modification of the AIN 76 purified diet, and of the chow are presented in Table 1. The fatty acid compositions of the n-3 fatty acid-deficient diet, chow diet and fish oil are presented in Table 2. The high linoleic acid sunflower oil-based n-3 fatty acid-deficient diet contained 61% of the total fatty acids as linoleic acid (18:2n-6) (Table 2). The n-3 fatty acid-adequate diets consisted of the above n-3 fatty acid-deficient diet supplemented with different amounts of fish oil [0.1 ml of fish oil contained 0.5 mg of α-linolenic acid (18:3n-3), 13.7 mg of eicosapentaenoic acid (EPA, 20:5n-3), 1.1 mg of docosapentaenoic acid (22:5n-3) and 7.1 mg of DHA, and a total of 22.4 mg of n-3 fatty acids; Leiner Health Products, L.L.C., CA, USA] in order to meet the 18:3n-3 dietary recommendation of about 0.4% of the energy source [25] and to provide a source of preformed DHA since it has been suggested by ourselves [2] and others [26] that preformed DHA is the best source of DHA for maintaining brain DHA levels in the adult. Except for the methionine and choline, which were from Sigma-Aldrich Inc. (MO, USA), and the sunflower oil, corn starch and sucrose, which were purchased from a local supermarket, all diet ingredients were obtained from MP Biomedicals, L.L.C. (OH, USA).

2.3. Anxiety-like behavior test

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

The rats were habituated to the dark room illuminated with dim red light used for testing for at least 30 min before testing, which was performed during the period of 13:00–15:00 h. 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.

2.4. Depressive-like behavior test

A forced swim test was used to assess depressive-like behavior in the rats at 10 weeks old. The rats were placed in a cylinder (25 cm wide×46 cm high) filled to a depth of 30 cm with water at room temperature for 15 min on day 1 and again for a 5-min test session on day 2, and the time spent climbing, swimming or immobile was measured during the test session. Climbing was defined as upward struggling movements of the forepaws at the side of the cylinder, swimming as movement around the cylinder, and immobility as no additional activity other than that required keeping the head above water. A video camera was mounted above the center of the apparatus. The behavior test was performed between 13:00 and 15:00 h.

2.5. Restraint stress and sacrifice

Restraint stress was applied in an acrylic cylindrical rat restrainer (Model STM-6, Shineteh Instruments Co., Ltd.) for 60 min between 13:00 and 16:00 h at 10 weeks old. The rat was able to move its limbs and head, but not its trunk. The colonic core body temperature was measured before restraint and every 15 min during restraint using a

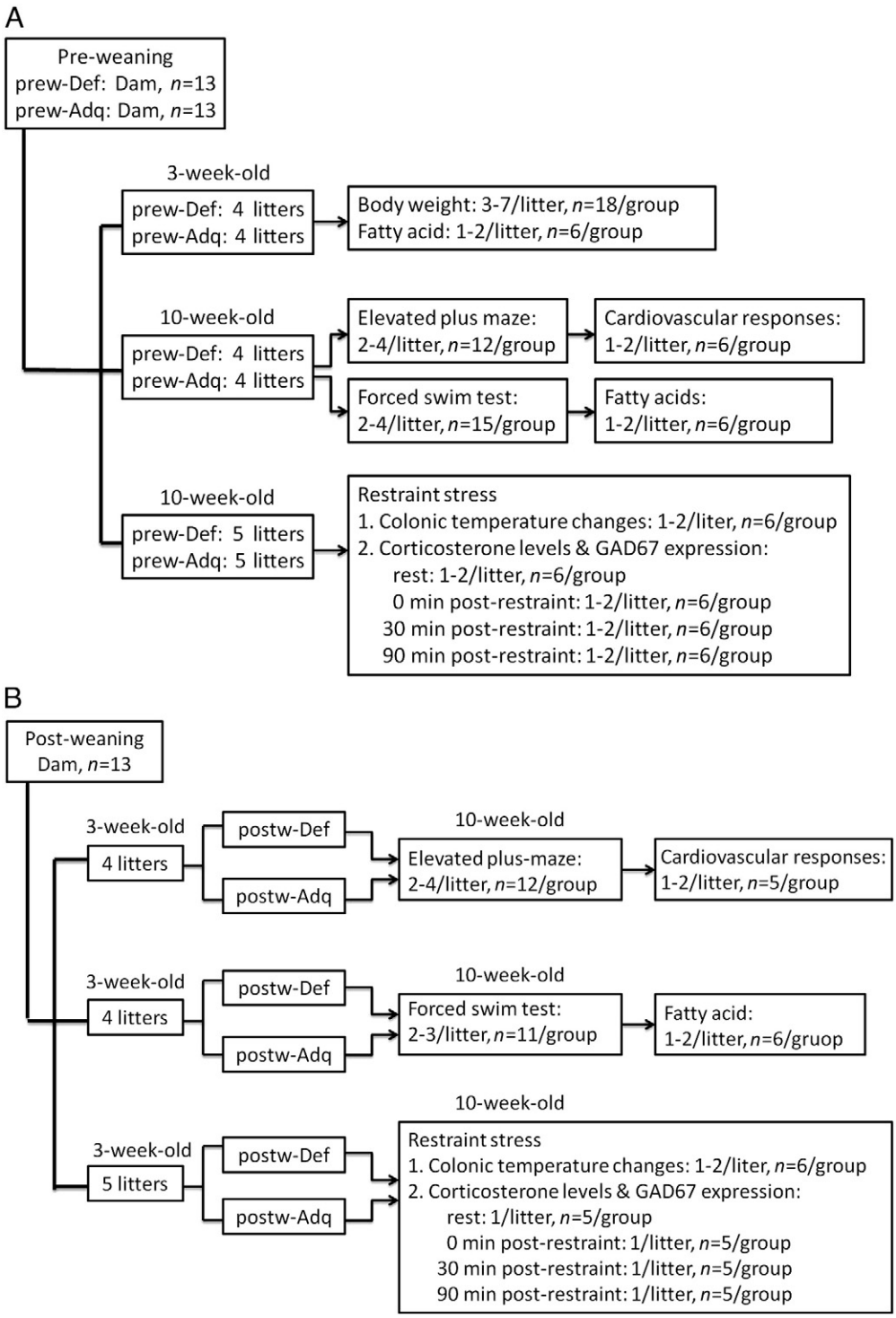

Fig. 2. Number of dams used and assignment of male offspring exposed to an n-3 fatty acid-deficient alone or the same diet supplemented with fish oil as an n-3 fatty acid-adequate diet during either the preweaning period (a) or the postweaning period (b) to each test.

digital thermometer. Rats were sacrificed without having been subjected to restraint stress (rest condition) or at 0, 30 or 90 min after the 60 min of restraint stress. The rats were anesthetized with CO_2, blood was collected by cardiac puncture, serum was immediately separated by centrifugation, the hypothalamus was rapidly removed, and then all samples were frozen in liquid nitrogen and stored at a −80°C freezer until analysis.

2.6. Corticosterone

Corticosterone levels were measured in the serum samples taken without restraint and at 0, 30 or 90 min after the above restraint using an enzyme-linked immunosorbent assay kit (Cayman Chemical, MI, USA) according to the manufacturer's instructions.

2.7. Hypothalamic GAD67 protein expression

The hypothalamus was homogenized at 4°C in lysis buffer [20 mM HEPES, pH 7.5, 250 mM sucrose, 10 mM KCl, 1.5 mM MgCl2, 1 mM EDTA, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), 1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, and protease inhibitor cocktail mix (Sigma, MO, USA)], the homogenate was centrifuged at 15,000*g* for 20 min at 4°C, and the supernatant was collected. The protein concentration was determined using a Bio-Rad Bradford protein assay kit

Table 2
Fatty acid compositions of the n-3 fatty acid-deficient diet, chow diet and fish oil[1,2]

	n-3-Deficient diet	Chow diet	Fish oil
% of total fatty acids			
14:0	0.2±0.0	2.4±0.2	15.7±0.5
16:0	8.0±0.1	24.8±0.8	24.8±0.4
18:0	3.5±0.0	7.5±0.3	3.3±0.1
20:0	0.1±0.0	–	0.1±0.0
16:1n-9	0.3±0.0	3.0±0.1	14.7±0.2
18:1n-9	26.5±0.6	27.7±0.6	9.3±0.2
18:1n-7	0.7±0.2	2.0±0.2	3.4±0.2
20:1n-9	0.1±0.0	0.1±0.0	0.5±0.1
18:2n-6	61.0±0.6	30.5±0.8	1.6±0.3
20:2n-6	–	–	0.3±0.1
20:3n-6	–	0.3±0.0	0.5±0.1
20:4n-6	–	–	0.3±0.1
22:4n-6	–	–	0.1±0.0
22:5n-6	–	–	0.1±0.1
18:3n-3	–	1.6±0.1	0.6±0.1
20:5n-3	–	0.8±0.1	15.2±0.4
22:5n-3	–	–	1.2±0.0
22:6n-3	–	0.6±0.1	7.9±0.1

[1] The data are presented as the mean±S.E.M. ($n=6$).
[2] Fatty acids accounting for less than 0.05% of the total fatty acids are not shown.

(Bio-Rad, CA, USA), samples containing 20 μg of protein were denatured at 95°C for 5 min and separated on a 10% SDS polyacrylamide gel by electrophoresis, and the proteins were transferred to a polyvinylidene fluoride membrane (Bio-Rad). The membrane was blocked at room temperature for 1 h in blocking buffer [5% nonfat milk in Tris-HCl-buffered saline containing 0.1% Tween 20 (TBS-T)], washed several times with TBS-T and incubated overnight at 4°C with a 1:5000 dilution in blocking buffer mouse monoclonal anti-GAD67 antibody (MAB5406, Millipore, MA, USA) or mouse monoclonal anti-GAPDH antibody (Cell Signaling, MA, USA) used as the loading control. After washing with TBS-T, the blots were incubated at room temperature for 1 h with a horseradish peroxidase-conjugated secondary antibody (Santa Cruz, CA, USA) diluted in blocking buffer, and bound antibodies were visualized using enhanced chemiluminescence (Amersham Biosciences, Buckinghamshire, England), with the optical density measured using a Dolphin-Chemi mini Image System (WEALTEC, NV, USA).

2.8. $GABA_A$ antagonist microinjection

The rat was anesthetized with urethane (800 mg/kg intraperitoneally, supplemented as needed) (Sigma, St. Louis, MO, USA), and the right common carotid artery was cannulated with PE-50 polyethylene tubing. The arterial line was connected to an NL108T2 disposable pressure transducer to record the arterial blood pressure and heart rate on a CP122 AC/DC strain gage amplifier (Grass Instruments, West Warwick, RI, USA). The rat was then placed prone, and the head was mounted in a stereotaxic apparatus so that the bregma and lambda were positioned on the same horizontal plane. A thermistor probe was inserted into the rectum to measure core body temperature.

The overlying skin and connective tissue were removed from the skull, and a small hole was drilled to allow access to the brain. After surgery was complete, the core body temperature and cardiovascular parameters were monitored for at least 30 min to assure a stable baseline. Using a fine glass micropipette with a tip diameter of 20–30 μm, bicuculline methiodide (10 pmol/50 nl, Sigma) was microinjected unilaterally into the hypothalamic paraventricular nucleus (stereotaxic coordinates: 1.8 mm caudal to the bregma, 0.4 mm lateral to the midline and 7.8 mm ventral to the dura), and the same parameters were continuously monitored for 60 min.

2.9. Fatty acid analyses

Total lipids were extracted according to the method of Bligh and Dyer [27], dried down under nitrogen gas and converted to their methyl esters, which were analyzed on a Hewlett-Packard 5890 gas chromatograph using flame ionization detection [28]. The fatty acid peaks were identified from external standard runs 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).

Table 3
Fatty acid composition of the hypothalamus at weaning or at 10 weeks old in male rats exposed to an n-3 fatty acid-deficient diet or the same diet supplemented with fish oil as an n-3 fatty acid-adequate diet during either the preweaning or postweaning period[1,2]

	3 Weeks old (at weaning)		10 Weeks old		10 Weeks old	
Fatty acid	prew-Adq	prew-Def	prew-Adq	prew-Def	postw-Adq	postw-Def
% Of total fatty acids						
14:0	0.2±0.1	0.3±0.1	–	0.1±0.0	0.2±0.2	0.1±0.0
16:0	26.4±1.0	28.2±1.0	22.0±0.6	22.3±0.8	21.1±1.3	21.1±1.7
18:0	22.6±1.7	23.0±2.6	21.9±1.2	22.1±1.6	21.7±2.0	24.8±1.8
20:0	0.4±0.1	0.4±0.1	0.3±0.1	0.5±0.1	0.5±0.1	0.6±0.1
22:0	0.2±0.1	0.1±0.0	0.3±0.1	0.3±0.1	0.3±0.1	0.4±0.1
24:0	0.4±0.1	0.2±0.1	0.4±0.1	0.3±0.2	0.6±0.1	0.6±0.1
Total saturated	50.1±1.9	52.1±3.3	45.0±1.0	45.6±1.1	44.5±1.4	47.4±3.1
14:1n-5	0.1±0.0	0.3±0.2	0.2±0.2	0.4±0.2	0.8 ±0.8	0.1±0.1
16:1n-7	0.4±0.0	0.3±0.1	0.1±0.0	0.1±0.0	0.2±0.1	0.1±0.0
16:1n-9	0.4±0.0	0.4±0.0	0.4±0.0	0.4±0.0	0.4±0.0	0.3±0.0
18:1n-9	14.1±0.5	13.1±1.1	20.2±0.7	19.7±0.5	18.3±0.2	17.7±1.1
18:1n-7	3.2±0.2	2.9±0.3	4.0±0.3	4.2±0.1	4.1±0.1	4.0±0.2
20:1n-9	0.6±0.1	0.7±0.2	1.3±0.1	1.3±0.1	1.1±0.1	1.1±0.1
20:3n-9	0.1±0.0	0.1±0.1	0.1±0.0	–	0.1±0.0	0.1±0.0
22:1n-9	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
24:1n-9	0.2±0.1	0.1±0.0	0.5±0.1	0.6±0.1	0.6±0.1	0.5±0.1
Total monounsaturated	19.1±0.8	17.9±1.4	26.8±0.8	26.8±0.8	25.7±1.1	24.0±1.5
18:2n-6	1.4±0.1	1.5±0.1	0.7±0.1	0.7±0.1	1.4±0.4	1.0±0.1
18:3n-6	0.1±0.1	0.1±0.0	–	–	–	–
20:2n-6	0.2±0.1	0.3±0.1	0.2±0.0	0.2±0.0	0.3±0.0	0.2±0.1
20:3n-6	0.7±0.1	0.5±0.1	0.3±0.1	0.3±0.1	0.3±0.1	0.2±0.1
20:4n-6	10.9±0.5	11.3±0.7	9.2±0.2	9.2±0.3	9.6±0.2	9.6±0.6
22:4n-6	4.2±0.2	4.6±0.3	4.2±0.2	4.1±0.2	4.4±0.1	4.6±0.3
22:5n-6	**1.1±0.1**	**4.3±0.2*****	**0.4±0.0**	**0.8±0.1***	**0.6±0.1**	**3.0±0.2*****
Total n-6 PUFA	18.6±0.8	22.5±1.3*	14.8±0.4	15.2±0.4	16.6±0.4	18.5±1.2
18:3n-3	0.2±0.1	0.1±0.1	0.3±0.1	0.4±0.0	0.3±0.0	0.3±0.0
18:4n-3	0.2±0.0	0.1±0.0	0.1±0.0	0.0±0.0	0.2±0.2	0.1±0.0
20:5n-3	–	–	0.1±0.1	0.4±0.1	–	0.2±0.1
22:5n-3	0.2±0.1	0.1±0.1	0.1±0.0	0.1±0.0	0.2±0.0	0.1±0.0
22:6n-3	**11.7 ±0.5**	**7.1±0.8*****	**12.8±0.5**	**11.5±0.7**	**12.6±0.4**	**9.5±0.6****
Total n-3 PUFA	12.3±0.5	7.5±0.7***	13.4±0.6	12.5±0.7	13.3±0.3	10.1±0.6***

Bold text indicates the 2 groups with significant difference between groups.
[1] The data are presented as the mean±S.E.M. ($n=4$ litters, 1–2 male rats/litter for a total of $n=6$ per group). $^{*}P<.05$; $^{**}P<.01$; $^{***}P<.001$ in a two-tailed Student's t test.
[2] Fatty acids accounting for less than 0.05% of the total fatty acids are not shown.

Table 4
Body weight at weaning and at 10 weeks old of the male offspring of rats exposed to an n-3 fatty acid-deficient diet or the same diet supplemented with fish oil as an n-3 fatty acid-adequate diet during either the preweaning or the postweaning period

	prew-Adq	prew-Def	postw-Adq	postw-Def
		Body weight (g)		
3 Weeks old (weaning)	52.3±1.7	43.5±2.9*	53.2±1.6	50.3±1.7
10 Weeks old	341.4±7.7	344.0±8.1	338.1±6.4	317.0±9.9

The data are presented as the mean±S.E.M. (n=4 litters, 4–5 male rats/litter for a total of n=18 per group for groups prew-Adq and prew-Def or n=5 litters, 3–5/litter for a total of n=20 per group for groups postw-Adq and postw-Def), *P value<.05 in a two-tailed Student's t test.

The fatty acid composition of each lipid was expressed as the weight of that lipid as a percentage of the total weight of the carbon 14 to carbon 22 fatty acids (wt %).

2.10. Statistical analysis

The data are presented as the mean±S.E.M. A two-tailed Student's t test was used to compare differences between two groups. Two-way analysis of variance (ANOVA) and two-way repeated-measures ANOVA followed by a Bonferroni *post hoc* analysis were used to determine group and time effects. Statistics GraphPad Prism 5.0 (Graph Pad Software, Inc., San Diego, CA, USA) was used to perform graphical and statistical analysis. A P value<.05 was considered statistically significant.

3. Results

3.1. DHA levels in the hypothalamus

In the experiment in which the rats were exposed preweaning to the n-3 fatty acid-deficient diet, hypothalamic DHA levels in the male pups at weaning (3 weeks old) were significantly reduced in group prew-Def compared to group prew-Adq, and this reduction in DHA levels was compensated by a significant increase in n-6 decosapentaenoic acid (22:5n-6) levels in group prew-Def compared to group prew-Adq (Table 3). After weaning, the pups were fed a chow diet until 10 weeks old, when hypothalamic DHA levels were found to be restored, with no difference between the prew-Def and prew-Adq groups, although 22:5n-6 levels were still statistically higher in group prew-Def (Table 3). In contrast, in the experiment in which the rats were postweaning exposed to the n-3 fatty acid-deficient diet, hypothalamic DHA levels in 10-week-old male offspring were significantly decreased in group postw-Def compared to group postw-Adq, and 22:5n-6 levels increased (Table 3).

3.2. Offspring body weight

Male offspring body weight at weaning was significantly decreased in group prew-Def compared to group prew-Adq (Table 4), while there was no significant difference in body weight at 10 weeks old between any of the groups.

3.3. Anxiety-like behavior in the elevated plus-maze test

In the rats exposed preweaning to the n-3 fatty acid-deficient diet, the time spent in the open arms was significantly decreased in group prew-Def compared to group prew-Adq (Fig. 3a). The number of times the rat entered the open arms was reduced in group prew-Def compared to group prew-Adq, but the difference was not significant (Fig. 3b, P=.08). The total number of times the rats entered any of the arms was not significantly different between the two groups (Fig. 3c).

In the rats exposed postweaning to the n-3 fatty acid-deficient diet, the time spent in the open arms (Fig. 3 d), the number of times

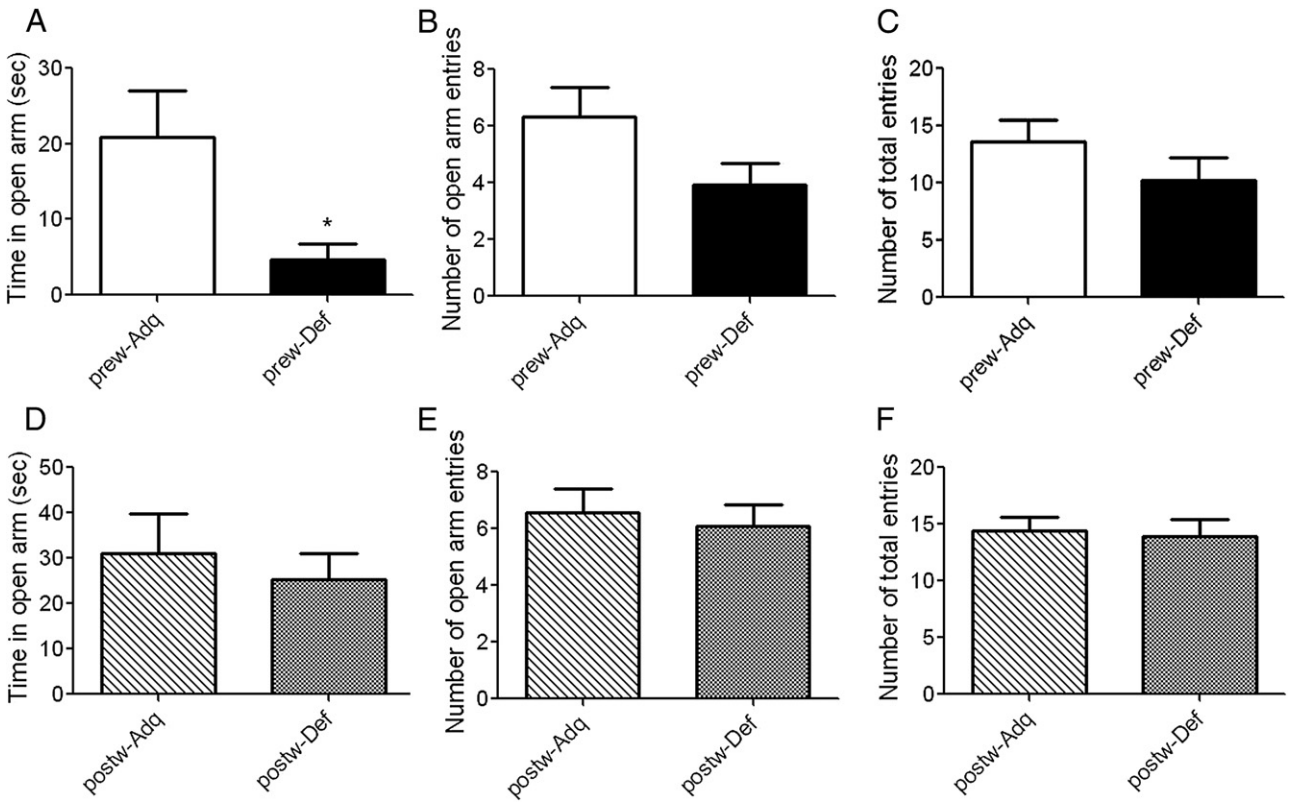

Fig. 3. Effect of exposure to an n-3 fatty acid-deficient diet or the same diet supplemented with fish oil as an n-3 fatty acid-adequate diet during either the preweaning period (a–c) or the postweaning period (d–f) on anxiety-like behavior in the elevated plus-maze test in male offspring at 10 weeks old. The time spent in the open arms (a, d), the number of times the rat entered the open arms (b, e) and the total number of times the rat entered any of the arms (c, f) are shown as the mean±S.E.M. (4 litters and 2–4 male rats/litter, making a total of n=12 per group). *P value<.05 in a two-tailed Student's t test.

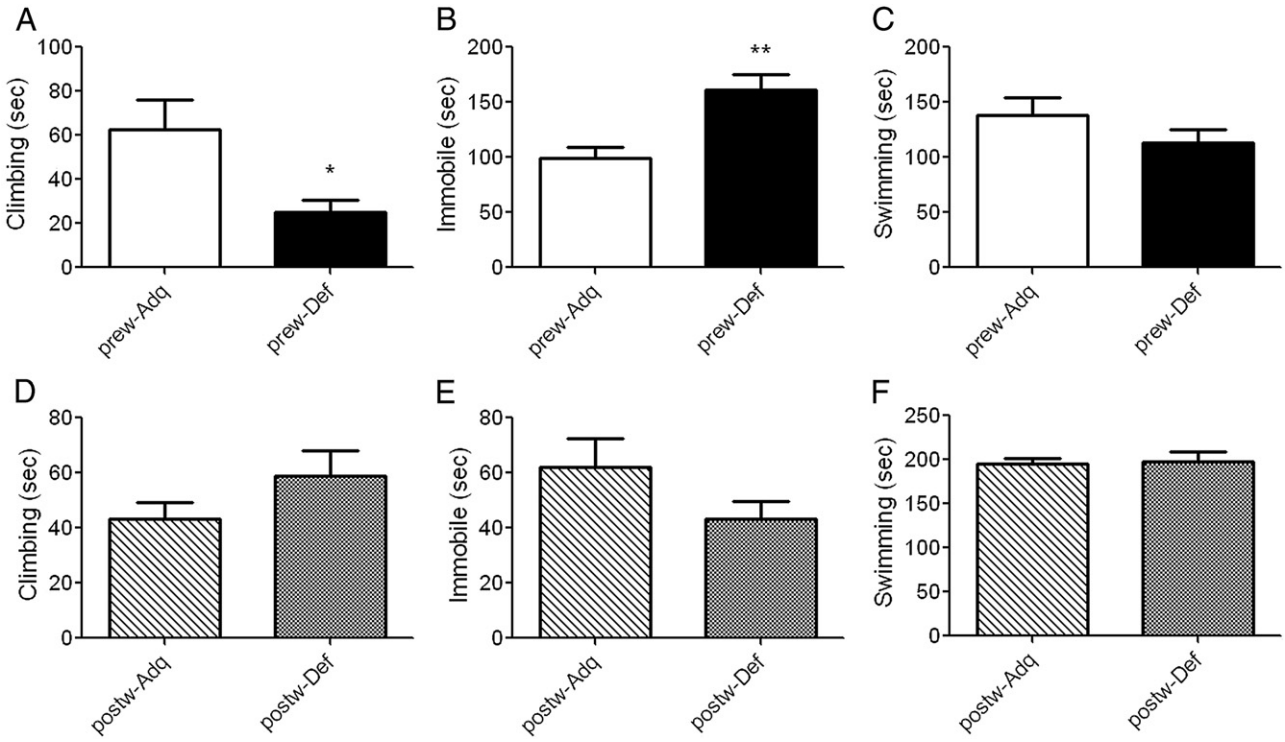

Fig. 4. Effect of exposure to an n-3 fatty acid-deficient diet or the same diet supplemented with fish oil as an n-3 fatty acid-adequate diet during either the preweaning period (a–c) or the postweaning period (d–f) on depressive-like behavior in the forced swim test in male offspring at 10 weeks old. The time spent in climbing (a, d), immobile (b, e) or swimming (c, f) is shown as the mean±S.E.M. (4 litters and 2–4 male rats/litter, making a total of 15 per group for groups prew-Adq and prew-Def; 4 litters and 2–3 male rats/litter, making a total of 11 per group for groups postw-Adq and postw-Def). *P<.05; **P<.01 in a two-tailed Student's t test.

the rat entered the open arms (Fig. 3e) and the total number of times the rat entered any of the arms (Fig. 3f) were not significantly different between groups postw-Adq and postw-Def.

3.4. Depressive-like behavior in the forced swim test

In the rats exposed preweaning to the n-3 fatty acid-deficient diet, group prew-Def exhibited a significantly shorter climbing time (Fig. 4a) and significantly longer immobility time (Fig. 4b) than group prew-Adq, with no significant difference in swimming time between the two groups (Fig. 4c). In contrast, in the rats exposed postweaning to the n-3 fatty acid-deficient diet, there were no significant differences between groups postw-Adq and postw-Def in climbing, immobility or swimming time.

3.5. Changes in colonic temperature during restraint stress

Colonic temperature changes during the 60 min of restraint stress are shown in Fig. 5.

In the rats exposed preweaning to the n-3 fatty acid-deficient diet, two-way ANOVA for repeated measures revealed a significant main effect of group and a group×time interaction between groups prew-Adq and prew-Def, but no time effect (Fig. 5a). The change in colonic temperature during the stress was significantly greater

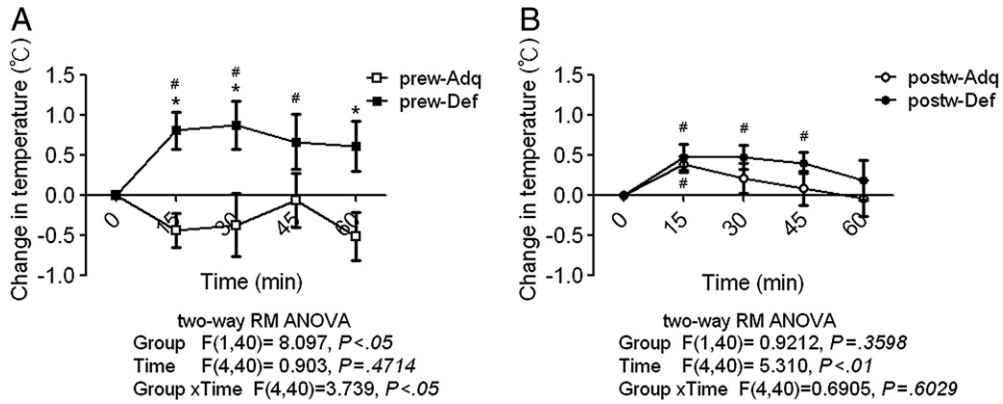

Fig. 5. Effect of exposure of rats to an n-3 fatty acid-deficient diet or the same diet supplemented with fish oil as an n-3 fatty acid-adequate diet during either the preweaning period (a) or the postweaning period (b) on changes in colonic temperature during 60-min restraint-induced stress in the 10-week-old male offspring. Colonic temperature was recorded before restraint (0 min) and at 15, 30, 45 or 60 min during restraint. The data are presented as the mean±S.E.M., (n=5 litters, 1–2 male rats/litter, making a total of 6 per group). Two-way ANOVA for repeated measures followed by a Bonferroni posttest was used to compare group and time effects. * or # indicates a significant difference (P<.05) between the two groups or between the indicated time and 0 min, respectively.

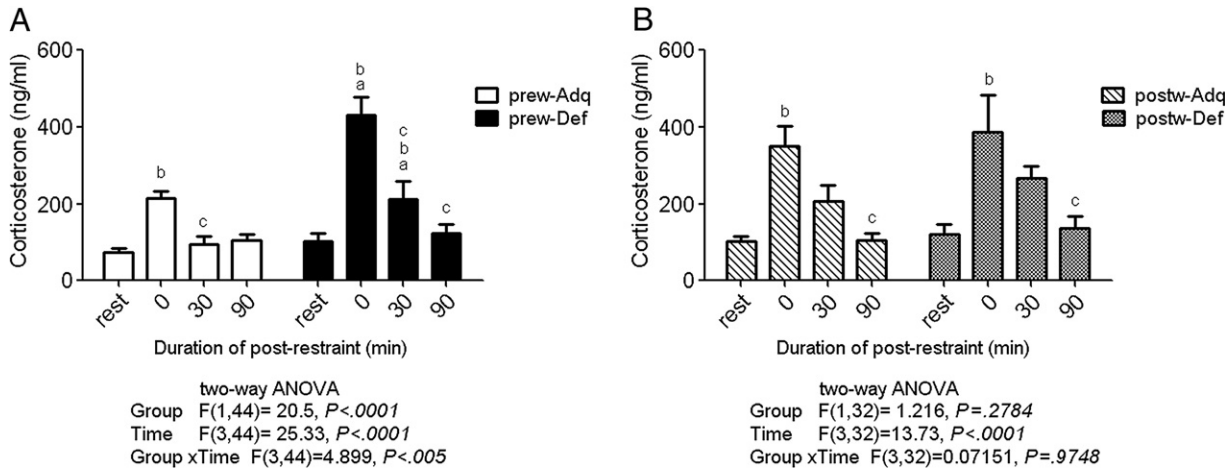

Fig. 6. Effect of exposure of rats to an n-3 fatty acid-deficient diet or the same diet supplemented with fish oil as an n-3 fatty acid-adequate diet during either the preweaning period (a) or the postweaning period (b) on serum corticosterone levels at different times after the end of 60-min restraint-induced stress in the 10-week-old male offspring. Serum corticosterone was measured without restraint (rest) and at 0, 30 and 90 min after the end of restraint. The data are presented as the mean±S.E.M., (5 litters and 1–2 male rats/litter, making a total of 6 per group for groups prew-Adq and prew-Def; 5 litters and 1 male rats/litter, making a total of 5 per group for groups postw-Adq and postw-Def for each time point). Two-way ANOVA followed by a Bonferroni posttest was used to compare group and time effects. a, b or c indicates a significant difference between two groups or compared to the rest condition or to 0 min postrestraint, respectively.

in group prew-Def than group prew-Adq. *Post hoc* analysis revealed that colonic temperature in group prew-Def increased significantly from 15 to 45 min of restraint compared to baseline (0 min), while no significant change in temperature was seen in group prew-Adq.

In the rats exposed postweaning to the n-3 fatty acid-deficient diet, two-way ANOVA for repeated measures revealed a significant main effect of time between groups postw-Adq and postw-Def, but no group effect or group×time interaction (Fig. 5b). There was no significant difference in the change in colonic temperature between

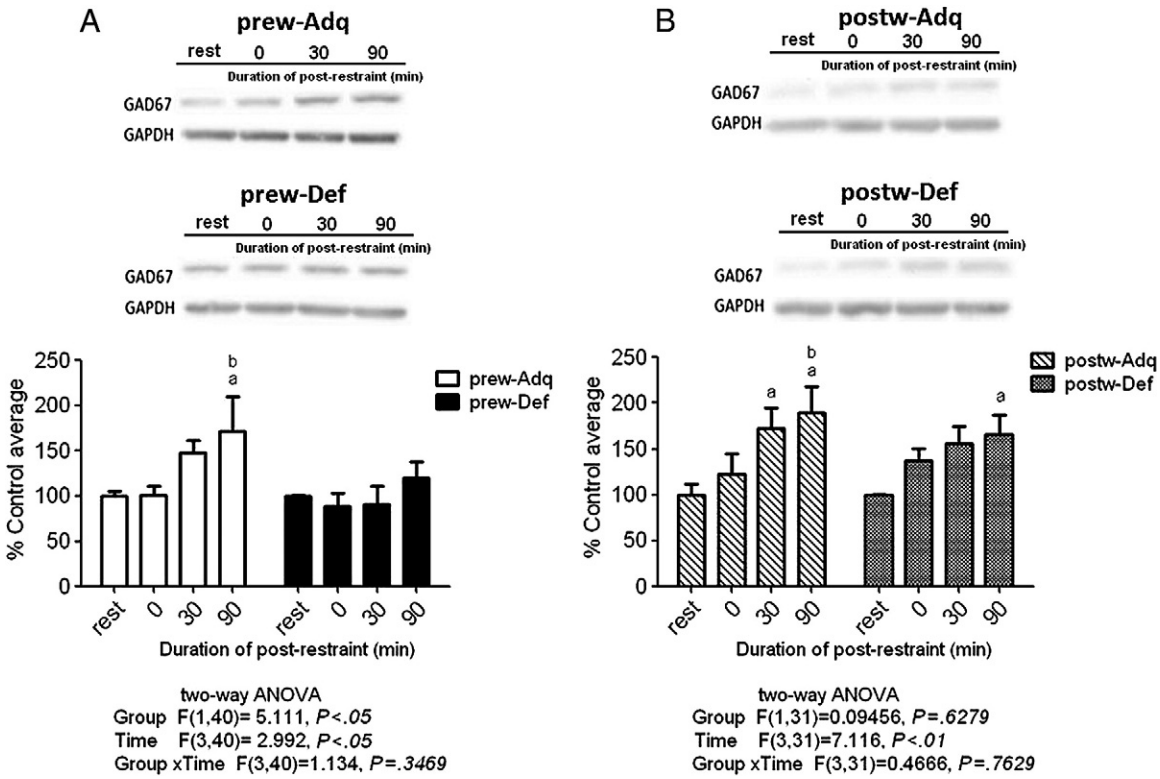

Fig. 7. Effect of exposure of rats to an n-3 fatty acid-deficient diet or the same diet supplemented with fish oil as an n-3 fatty acid-adequate diet during either the preweaning period (a) or the postweaning period (b) on hypothalamic GAD67 protein expression at different times after the end of 60-min restraint-induced stress in the 10-week-old male offspring. GAD67 protein expression without restraint (rest) and at 0, 30 and 90 min after the end of restraint was analyzed by Western blotting, with GAPDH as the loading control. The levels are expressed as a percentage of the respective basal level and are presented as the mean±S.E.M., (5 litters and 1–2 male rats/litter, making a total of 6 per group for groups prew-Adq and prew-Def; 5 litters and 1 male rat/litter, making a total of 5 per group for groups postw-Adq and postw-Def for each time point). Two-way ANOVA followed by a Bonferroni *post hoc* test was used to determine group and time effects. a or b indicates a significant difference compared to the rest condition or to 0 min postrestraint, respectively.

groups postw-Def and postw-Adq. *Post hoc* analysis revealed that colonic temperature changes in group postw-Def increased significantly from 15 to 45 min of restraint, while those in group postw-Adq were only significantly increased at 15 min compared to before restraint.

There were no differences in colonic temperature before restraint between any groups (group prew-Adq, 36.8°C±0.2°C; group prew-Def, 36.5°C±0.3°C; group postw-Adq, 37.5°C±0.2°C; group postw-Def, 37.2°C±0.2°C).

3.6. Changes in serum corticosterone levels after restraint stress

Serum corticosterone levels after the 60 min of restraint stress are shown in Fig. 6.

In the rats exposed preweaning to the n-3 fatty acid-deficient diet, two-way ANOVA revealed a significant main effect of group and time between prew-Adq and prew-Def groups and a group×time interaction (Fig. 6a). The changes in serum corticosterone levels after stress were significantly greater in group prew-Def than in group prew-Adq. *Post hoc* analysis revealed that there was no difference between groups prew-Adq and prew-Def in basal corticosterone levels without restraint (rest). Immediately after the 60 min of restraint (0 min), corticosterone levels were significantly increased in both groups, but were significantly higher in group prew-Def. At 30 min postrestraint, levels in group prew-Adq were indistinguishable from basal levels, but were significantly higher in group prew-Def. At 90 min postrestraint, levels in both groups were at basal levels.

In the rats exposed postweaning to the n-3 fatty acid-deficient diet, two-way ANOVA revealed a significant main effect of time between groups postw-Adq and postw-Def, but no group effect or group×time interaction (Fig. 6b). *Post hoc* analysis revealed that corticosterone levels were significantly increased in both groups after

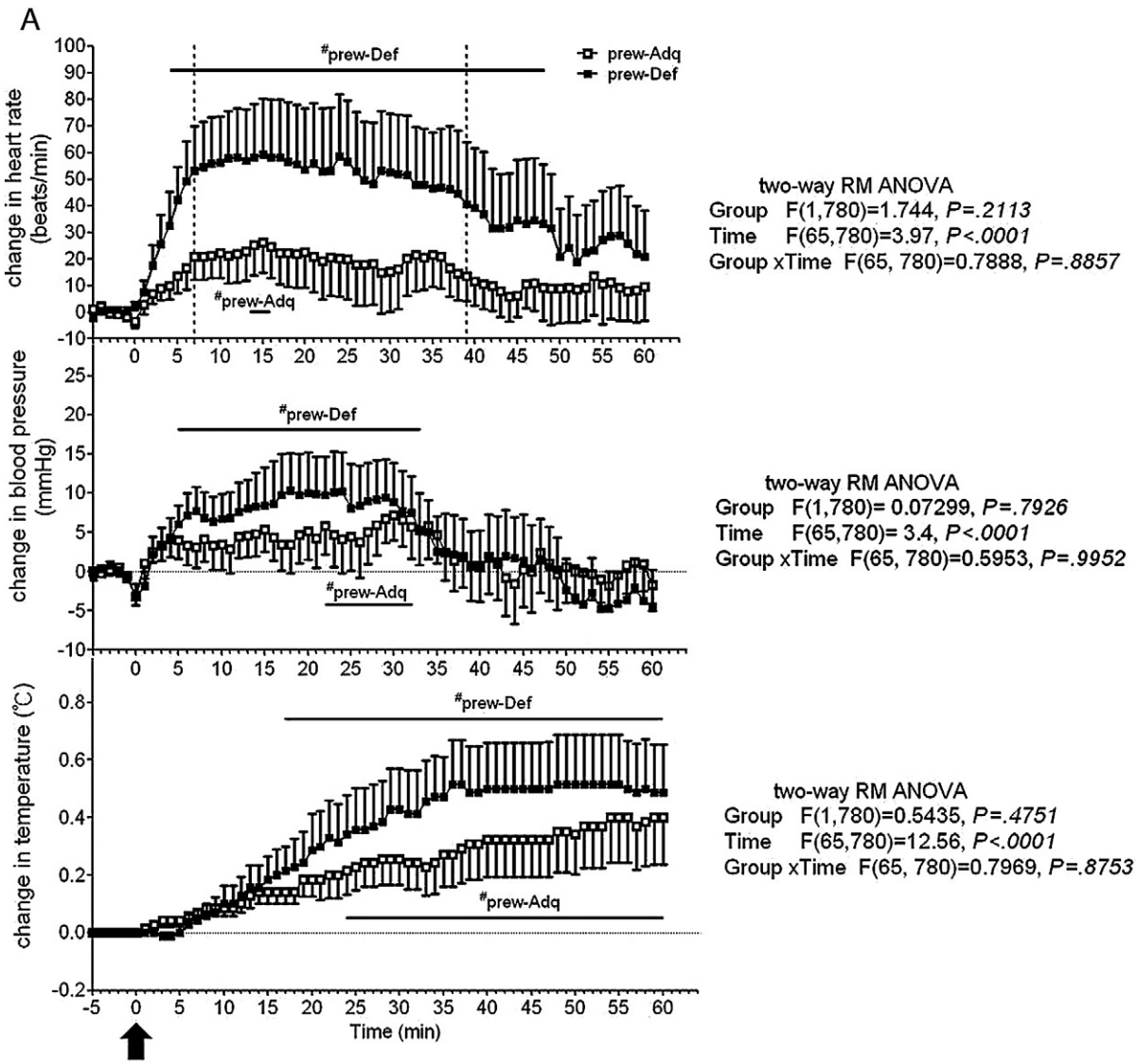

Fig. 8. Effect of exposure of rats to an n-3 fatty acid-deficient diet or the same diet supplemented with fish oil as an n-3 fatty acid-adequate diet during either the preweaning period (a) or the postweaning period (b) on $GABA_A$ receptor antagonist-stimulated stress-like cardiovascular responses. Heart rate (top panel), blood pressure (center panel) and core body temperature (bottom panel) were continuously recorded after microinjection of bicuculline methiodide (0 min, arrow) into the hypothalamic paraventricular nucleus of the 10-week-old male offspring. The data are presented as the mean±S.E.M., (4 litters and 1–2 male rats/litter, making a total of 6 per group for groups prew-Adq and prew-Def; 4 litters and 1–2 male rats/litter, making a total of 5 per group for groups postw-Adq and postw-Def). Two-way ANOVA for repeated measures followed by a Bonferroni *post hoc* test was used to determine group and time effects. # indicates a significant difference compared to before microinjection.

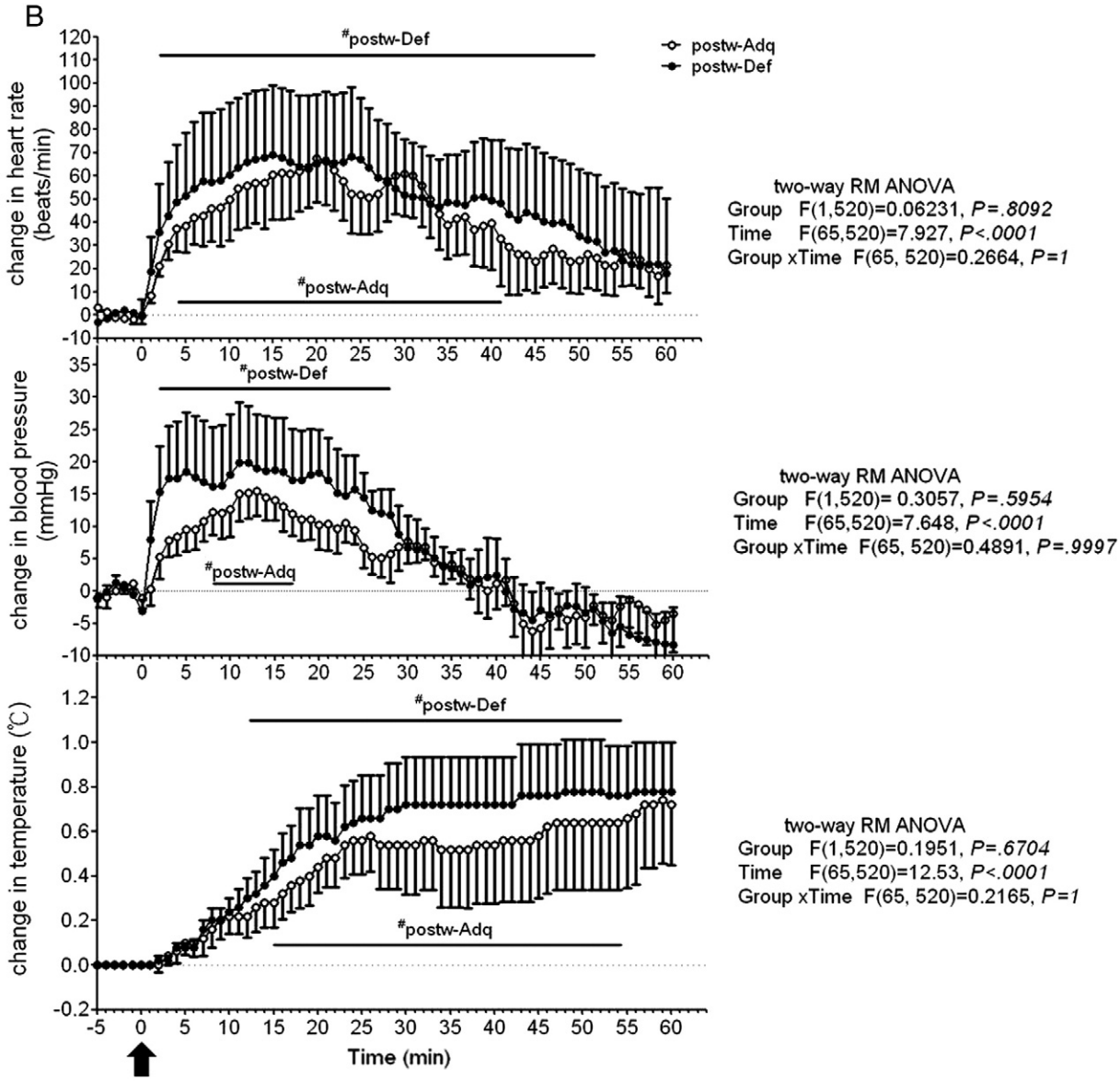

Fig. 8. continued.

the 60 min of restraint (0 min postrestraint) and returned to basal levels at 30 min postrestraint. There were no differences in serum corticosterone levels without restraint between any of the groups.

3.7. Changes in hypothalamic GAD67 protein expression after restraint stress

Changes in hypothalamic GAD67 protein expression after the 60 min of restraint stress are shown in Fig. 7.

In the rats exposed preweaning to the n-3 fatty acid-deficient diet, two-way ANOVA revealed a significant main effect of group and time between groups prew-Adq and prew-Def, with no group×time interaction (Fig. 7a). GAD67 expression was significantly higher in group prew-Adq than group prew-Def after the stress. *Post hoc* analysis revealed that GAD67 expression was significantly increased at 90 min postrestraint in group prew-Adq, whereas no increased expression was observed in group prew-Def at any time poststress.

In the rats exposed postweaning to the n-3 fatty acid-deficient diet, two-way ANOVA revealed a significant main effect of time between groups postw-Adq and postw-Def, but no group effect or group×time interaction (Fig. 7b). There was no difference in GAD67 expression between groups postw-Adq and postw-Def after the stress. *Post hoc* analysis revealed that GAD67 expression was significantly increased at 90 min postrestraint in both groups.

There were no differences in GAD67 expression between any of the groups without stress (data not shown).

3.8. $GABA_A$ antagonist stimulation of stress-like cardiovascular responses

Microinjection into the paraventricular nucleus of bicuculline methiodide, a $GABA_A$ antagonist, elicited increases in heart rate, blood pressure and core body temperature, as shown in Fig. 8. There were no significant differences in any parameter prior to microinjection between any groups, the values for groups prew-Adq, prew-Def, postw-Adq and postw-Def for heart rate being 326±5.1, 332±0.0, 314±21.9 and 364±21.8 beats/min, those for blood pressure being 83.4±2.6, 85.1±3.1, 82.9±7.7 and 82.2±2.3 mmHg and those for core body temperature being 33.7°C±0.1°C, 34.3°C±0.3°C, 33.7°C±0.4°C and 34.6°C±0.1°C.

In the rats exposed preweaning to the n-3 fatty acid-deficient diet, two-way ANOVA for repeated measures revealed a significant main effect of time in these three parameters between groups prew-Def and prew-Adq, but no group effect or group×time interaction (Fig. 8a). Multiple marginal regression models for repeated measures using the generalized estimating equations method showed that heart rate was significantly increased in group prew-Def at 7–39 min after bicuculline methiodide injection compared to group prew-Adq. In group prew-Def, heart rate (top panel) was maximal (58.6±23.2 beats/min) from 3 to 24 min and returned to baseline levels at 49 min, whereas in group prew-Adq, it only showed a modest increase, which was maximal (26.1±1.3 beats/min) at 15 min. In group prew-Def, blood pressure (center panel) was maximal (10.3±4.8 mmHg) at 5–18 min and returned to baseline levels at 34 min, whereas in group prew-Adq, it was slightly increased at 22–32 min (maximal increase 7.2±3.1 mmHg at 30 min). The increase in core body temperature (bottom panel) developed gradually over 17–60 min in group prew-Def (maximal increase 0.51°C±0.16°C) or over 24–60 min in group prew-Adq (maximal increase 0.40°C±0.16°C).

In the rats exposed postweaning to the n-3 fatty acid-deficient diet, two-way ANOVA for repeated measures revealed a significant main effect of time in these three parameters between groups postw-Def and postw-Adq, but no group effect or group×time interaction (Fig. 8b). Heart rate, blood pressure and core body temperature were slightly increased in group postw-Def compared to group postw-Adq.

4. Discussion

This study demonstrated that DHA deficiency during the preweaning period enhances the stress-induced HPA axis responses, leading to anxiety-like and depressive-like behaviors in the male offspring adulthood. Male rats exposed to a maternal n-3 fatty acid-deficient diet during the preweaning period showed increased and prolonged stress-induced increases in colonic temperature, serum corticosterone levels and heart rate changes in adulthood, effects not seen in male rats fed the same n-3 fatty acid-deficient diet after weaning.

The maternal n-3 fatty acid-deficient diet caused a 39% reduction in pup hypothalamus DHA levels at weaning. Although hypothalamic DHA levels were restored by subsequent feeding of chow diet containing adequate n-3 fatty acid levels for 7 weeks after weaning, exposure to an n-3 fatty acid-deficient diet preweaning still resulted in increased and prolonged changes in colonic temperature and serum corticosterone levels in response to restraint-induced stress and in enhanced anxiety-like behavior in the elevated plus-maze test and depressive-like behavior in the forced swimming task during adulthood. These effects were not seen in adult male rats fed an n-3 fatty acid-deficient diet after weaning, in which hypothalamic DHA levels were normal at weaning but showed a significant 25% decrease in the adult. These results suggest that deficiency of hypothalamic DHA during the preweaning period may facilitate hyperactivity of the HPA axis resulting in anxiety and depression behaviors in the adult.

The adult male rats exposed to a sunflower oil-based n-3 fatty acid-deficient diet during the preweaning period, which had a reduced body weight at weaning, showed an increased and prolonged serum corticosterone response to restraint-induced stress in adulthood, as well as depressive-like in the forced swim test and anxiety-like behavior but no motor activity deficit in the elevated plus-maze test. In a previous study, pup body weight at weaning was not affected by a maternal low n-3 fatty acid diet prepared from a mixture of safflower and soybean oils containing 0.38 g/kg diet of 18:3n-3, a DHA precursor, which may have supplied DHA to the offspring [29]. In studies in humans, birth weight has been positively associated with maternal intake of fish or seafood during pregnancy [30,31]. Adult men who were born with a lower birth weight have higher plasma cortisol levels and show increased total urinary cortisol metabolite excretion in response to ACTH challenge [32], and young humans born with a lower birth weight show greater right frontal electroencephalogram activity at rest and more severe internalizing problems, including depression and anxiety [33,34]. These results indicate that a low body weight in early life may facilitate stress-induced HPA responses in the adult male. In addition, depression and anxiety disorders are associated with excessive stress responses and HPA axis hyperactivity in adult rats, monkeys and humans [35], and a low birth weight is positively correlated with HPA axis hyperactivity and depression in humans [8,36].

The developing brain is more vulnerable to DHA deficiency because of rapid neurologic processing, including synapse formation, neurite outgrowth, myelination, neurotransmitter secretion and neurological function [2,37–39]. Although low brain DHA levels during development can be restored to normal by subsequent n-3 fatty acid supplementation, physiological functions are still affected. In rats exposed to an n-3 fatty acid-deficient diet from the embryo to postnatal day 21 and then switched to an n-3 fatty acid-adequate diet for 6 weeks, prefrontal cortex and hippocampal DHA levels were restored to normal, but dopaminergic neurotransmission and serotonin release were not [40,41]. In rats exposed to an n-3 fatty acid-deficient diet from the embryo to 2 months old and then supplemented with n-3 fatty acid-enriched fish oil for about 3 months, hippocampal DHA levels were restored, and water-maze learning memory performance improved but was not fully restored [28]. Rats exposed to an n-3 fatty acid-deficient diet during early life from the embryo to postnatal day 64 are more vulnerable to hypertension in later life than those exposed to the same diet during adulthood [42]. In rats exposed to an n-3 fatty acid-deficient diet from the embryo to postnatal 7 weeks old and then supplemented with n-3 fatty acid-enriched egg yolk or pig brain phospholipids for 2 months, brain DHA levels were restored, but anxiety-like behavior in the plus-maze test was still significantly increased [43]. In the present study, in rats exposed to an n-3 fatty acid-deficient diet from the embryo to 3 weeks old and then switched to an n-3 fatty acid-adequate chow diet for 7 weeks, hypothalamic DHA levels were restored, but anxiety-like behavior in the plus-maze test and depressive-like behavior in the forced swimming task were still significantly increased. These studies suggest that DHA deficiency during brain development may lead to irreversible damage to certain brain functions. In contrast, DHA-deficient mice or rats fed an n-3 fatty acid-deficient diet from the embryo throughout their entire life, as opposed to only in early life, as in this study, showed either no difference in or lower anxiety-like behavior in the plus-maze test and depressive-like behavior in the forced swimming task compared to animals fed an n-3 fatty acid-adequate diet [44–48]. This may be partly due to the different experimental design regarding the period of DHA deficiency. Further studies are needed on the effect of n-3 fatty acids on anxiety and depression.

Lack of GABA has long been associated with depression and anxiety, and positive modulators of $GABA_A$ receptors can have antidepression effects [12]. In the present study, the $GABA_A$ antagonist bicuculline induced an increase in heart rate that was significantly higher in adult rats exposed to an n-3 fatty acid-deficient diet during the preweaning period than in rats with the same deficient diet supplemented with fish oil as an adequate n-3 fatty acid diet during the same period. Moreover, hypothalamic GAD67 expression after restraint stress was not changed in adult rats exposed to an n-3 fatty acid-deficient diet during the preweaning period, in contrast to the other groups fed an n-3 fatty acid-deficient diet postweaning or the same deficient diet supplemented with fish oil as an n-3-adequate diet throughout life, in which it was increased at 90 min after stress. This suggests that DHA deficiency during the preweaning period may have an impact on GABAergic regulation later

in life. In addition, DHA supplementation attenuates $GABA_A$ receptor antagonist-enhanced conditioned fear-induced freezing behavior in DHA-deficient rats [49]. Moreover, DHA and EPA supplementation or GABA agonist administration decreases the frequency, duration and severity of seizures in patients with epilepsy, who show impaired GABAergic regulation and exhibit anxiety disorders [19,20,50]. These studies suggest that n-3 fatty acid may have antistress effects involving GABAergic modulation.

In conclusion, this study shows that DHA deficiency during the preweaning period can lead to excessive HPA axis responses to stress and enhanced anxiety- and depressive-like behaviors in the male offspring in adulthood. We propose that the effect of brain DHA deficiency during brain development on HPA activity may involve a $GABA_A$ receptor-mediated mechanism.

References

[1] Fedorova I, Salem Jr N. Omega-3 fatty acids and rodent behavior. Prostaglandins Leukot Essent Fatty Acids 2006;75:271–89.
[2] Su HM. Mechanisms of n-3 fatty acid-mediated development and maintenance of learning memory performance. J Nutr Biochem 2010;21:364–73.
[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;40:960–6.
[4] Martinez M. Tissue levels of polyunsaturated fatty acids during early human development. J Pediatr 1992;120:S129–38.
[5] Lauritzen L, Hansen HS, Jorgensen MH, Michaelsen KF. The essentiality of long chain n-3 fatty acids in relation to development and function of the brain and retina. Prog Lipid Res 2001;40:1–94.
[6] Jackson AA. Integrating the ideas of life course across cellular, individual, and population levels in cancer causation. J Nutr 2005;135:2927S–33S.
[7] Barker DJ. In utero programming of chronic disease. Clin Sci (Lond) 1998;95: 115–28.
[8] Kajantie E. Fetal origins of stress-related adult disease. Ann N Y Acad Sci 2006;1083:11–27.
[9] Mastorakos G, Ilias I. Maternal and fetal hypothalamic-pituitary-adrenal axes during pregnancy and postpartum. Ann N Y Acad Sci 2003;997:136–49.
[10] Darnaudery M, Maccari S. Epigenetic programming of the stress response in male and female rats by prenatal restraint stress. Brain Res Rev 2008;57:571–85.
[11] Bale TL. Is mom too sensitive? Impact of maternal stress during gestation. Front Neuroendocrinol 2005;26:41–9.
[12] Kalueff AV, Nutt DJ. Role of GABA in anxiety and depression. Depress Anxiety 2007;24:495–517.
[13] Decavel C, Van den Pol AN. GABA: a dominant neurotransmitter in the hypothalamus. J Comp Neurol 1990;302:1019–37.
[14] Cullinan WE, Ziegler DR, Herman JP. Functional role of local GABAergic influences on the HPA axis. Brain Struct Funct 2008;213:63–72.
[15] Miklos IH, Kovacs KJ. GABAergic innervation of corticotropin-releasing hormone (CRH)-secreting parvocellular neurons and its plasticity as demonstrated by quantitative immunoelectron microscopy. Neuroscience 2002;113:581–92.
[16] Skilbeck KJ, Johnston GA, Hinton T. Stress and GABA receptors. J Neurochem 2010;112:1115–30.
[17] Roland BL, Sawchenko PE. Local origins of some GABAergic projections to the paraventricular and supraoptic nuclei of the hypothalamus in the rat. J Comp Neurol 1993;332:123–43.
[18] Chen F, Cham JL, Badoer E. High-fat feeding alters the cardiovascular role of the hypothalamic paraventricular nucleus. Am J Physiol Regul Integr Comp Physiol 2010;298:R799–807.
[19] Treiman DM. GABAergic mechanisms in epilepsy. Epilepsia 2001;42(Suppl. 3): 8–12.
[20] Vazquez B, Devinsky O. Epilepsy and anxiety. Epilepsy Behav 2003;4(Suppl. 4): S20–5.
[21] Bowers G, Cullinan WE, Herman JP. Region-specific regulation of glutamic acid decarboxylase (GAD) mRNA expression in central stress circuits. J Neurosci 1998;18:5938–47.
[22] Goel N, Bale TL. Examining the intersection of sex and stress in modelling neuropsychiatric disorders. J Neuroendocrinol 2009;21:415–20.
[23] Mueller BR, Bale TL. Sex-specific programming of offspring emotionality after stress early in pregnancy. J Neurosci 2008;28:9055–65.
[24] Kajantie E, Phillips DI. The effects of sex and hormonal status on the physiological response to acute psychosocial stress. Psychoneuroendocrinology 2006;31: 151–78.
[25] Bourre JM, Francois M, Youyou A, 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;119:1880–92.
[26] Brenna JT, Salem Jr N, Sinclair AJ, Cunnane SC. alpha-Linolenic acid supplementation and conversion to n-3 long-chain polyunsaturated fatty acids in humans. Prostaglandins Leukot Essent Fatty Acids 2009;80:85–91.
[27] Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 1959;37:911–7.
[28] 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: 1165–71.
[29] Levant B, Ozias MK, Davis PF, et al. Decreased brain docosahexaenoic acid content produces neurobiological effects associated with depression: interactions with reproductive status in female rats. Psychoneuroendocrinology 2008;33:1279–92.
[30] Olsen SF, Grandjean P, Weihe P, Videro T. Frequency of seafood intake in pregnancy as a determinant of birth weight: evidence for a dose dependent relationship. J Epidemiol Community Health 1993;47:436–40.
[31] Olsen SF, Olsen J, Frische G. Does fish consumption during pregnancy increase fetal growth? A study of the size of the newborn, placental weight and gestational age in relation to fish consumption during pregnancy. Int J Epidemiol 1990;19: 971–7.
[32] Reynolds RM, Walker BR, Syddall HE, et al. Altered control of cortisol secretion in adult men with low birth weight and cardiovascular risk factors. J Clin Endocrinol Metab 2001;86:245–50.
[33] Boyle MH, Miskovic V, Van Lieshout R, et al. Psychopathology in young adults born at extremely low birth weight. Psychol Med 2010:1–12.
[34] Schmidt LA, Miskovic V, Boyle M, Saigal S. Frontal electroencephalogram asymmetry, salivary cortisol, and internalizing behavior problems in young adults who were born at extremely low birth weight. Child Dev 2010;81:183–99.
[35] Arborelius L, Owens MJ, Plotsky PM, Nemeroff CB. The role of corticotropin-releasing factor in depression and anxiety disorders. J Endocrinol 1999;160:1–12.
[36] Kajantie E. Early-life events. Effects on aging. Hormones (Athens) 2008;7:101–13.
[37] Georgieff MK. Nutrition and the developing brain: nutrient priorities and measurement. Am J Clin Nutr 2007;85:614S–20S.
[38] Wurtman RJ. Synapse formation and cognitive brain development: effect of docosahexaenoic acid and other dietary constituents. Metabolism 2008;57(Suppl. 2): S6–10.
[39] Carlson SE. Docosahexaenoic acid supplementation in pregnancy and lactation. Am J Clin Nutr 2009;89:678S–84S.
[40] Kodas E, Vancassel S, Lejeune B, Guilloteau D, Chalon S. Reversibility of n-3 fatty acid deficiency-induced changes in dopaminergic neurotransmission in rats: critical role of developmental stage. J Lipid Res 2002;43:1209–19.
[41] Kodas E, Galineau L, Bodard S, et al. Serotoninergic neurotransmission is affected by n-3 polyunsaturated fatty acids in the rat. J Neurochem 2004;89:695–702.
[42] Weisinger HS, Armitage JA, Sinclair AJ, Vingrys AJ, Burns PL, Weisinger RS. Perinatal omega-3 fatty acid deficiency affects blood pressure later in life. Nat Med 2001;7:258–9.
[43] Carrie I, Clement M, de Javel D, Frances H, Bourre JM. Phospholipid supplementation reverses behavioral and biochemical alterations induced by n-3 polyunsaturated fatty acid deficiency in mice. J Lipid Res 2000;41:473–80.
[44] Nakashima Y, Yuasa S, Hukamizu Y, et al. Effect of a high linoleate and a high alpha-linolenate diet on general behavior and drug sensitivity in mice. J Lipid Res 1993;34:239–47.
[45] Belzung C, Leguisquet AM, Barreau S, Delion-Vancassel S, Chalon S, Durand G. Alpha-linolenic acid deficiency modifies distractibility but not anxiety and locomotion in rats during aging. J Nutr 1998;128:1537–42.
[46] Moriguchi T, Greiner RS, Salem Jr N. Behavioral deficits associated with dietary induction of decreased brain docosahexaenoic acid concentration. J Neurochem 2000;75:2563–73.
[47] Frances H, Monier C, Bourre JM. Effects of dietary alpha-linolenic acid deficiency on neuromuscular and cognitive functions in mice. Life Sci 1995;57:1935–47.
[48] Raygada M, Cho E, Hilakivi-Clarke L. High maternal intake of polyunsaturated fatty acids during pregnancy in mice alters offsprings' aggressive behavior, immobility in the swim test, locomotor activity and brain protein kinase C activity. J Nutr 1998;128:2505–11.
[49] Takeuchi T, Iwanaga M, Harada E. Possible regulatory mechanism of DHA-induced anti-stress reaction in rats. Brain Res 2003;964:136–43.
[50] Al Khayat HA, Awadalla MM, Wakad AA, Marzook ZA. Polyunsaturated fatty acids in children with idiopathic intractable epilepsy: serum levels and therapeutic response. J Pediatr Neurol 2010;8:175–85.