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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☆

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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

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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.

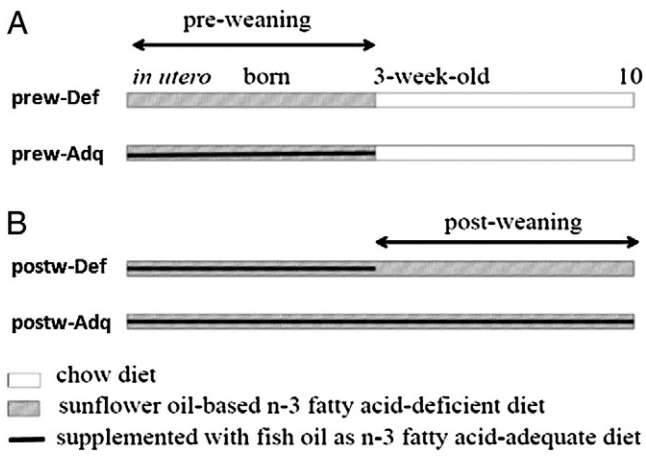

Fig. 1. Study design for rats exposed to a high linoleic acid sunflower oil-based n-3 fatty acid-deficient diet or the same diet supplemented with fish oil as an n-3 fatty acid-adequate diet during either the preweaning (prew) period (a) or the postweaning (postw) period (b). Group prew-Def: n-3 fatty 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