

## Dietary n-3 Fatty Acid Deficiency in Mice Enhances Anxiety Induced by Chronic Mild Stress

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**Abstract** Docosahexaenoic acid (DHA), is the major polyunsaturated fatty acid in the brain and is important for both the structure and the function of the nervous system. Mice were fed either an n-3 fatty acid deficient (n-3 Def) or adequate (n-3 Adq) diet for two generations. The mice were housed under two conditions, as a group or in isolation and the major point of the study was to determine whether n-3 fatty acid deficiency would enhance isolation-induced anxiety. Isolation stress was assessed using the novelty suppressed feeding paradigm (NSF) after a 3-week period and the test lasted a maximal duration of 10 min. The number of successful mice consuming food pellets within 5 min in the n-3 Def diet group was low in both housing conditions (group housing, 33% and isolated, 30%), but was 92% in the group housed and 50% in the isolated group when fed the n-3 Adq diet. In the subsequent 5 min period, the isolated housing group consuming the n-3 Adq diet increased up to 79% and the group housed animals fed the n-3 Def diet increased to 67%. However, those that consumed the n-3 deficient diet combined with isolation stress exhibited no increase. These results suggested that the n-3 deficient mice had increased anxiety that was enhanced by the chronic mild stress of social isolation.

**Keywords** n-3 Fatty acid · Anxiety · Stress · Novelty suppressed feeding paradigm · Mice

### Abbreviations

DHA Docosahexaenoic acid, 22:6n-3  
PUFA Polyunsaturated fatty acid(s)

### Introduction

The n-3 fatty acids,  $\alpha$ -linolenic acid (ALA, 18:3n-3), eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) are nutritionally essential polyunsaturated fatty acids and cannot be synthesized de novo in mammals. These fatty acids, particularly DHA, exist mainly in the form of membrane phospholipids which are known to be crucial for maintaining normal brain structure and function [1]. It has been well documented that animal model of dietary n-3 fatty acids deprivation produces a loss of brain function. Several rodent studies have reported that lower DHA levels in the brain led to poorer performance on a variety of cognitive and learning tasks [2–5]. There have recently been several reports concerning various mental functions including mood variations and underlying mechanisms. For example, epidemiological studies of healthy people and postpartum women indicate a negative correlation between dietary n-3 PUFA intake and mental illness including serious mood disorders, aggression, depression, and bipolar disorder [6–9]. These findings suggest that mental illness may be associated with reduced dietary intake of n-3 fatty acids. In animal studies, Delion et al. [10] indicated that an n-3 fatty acid deficient diet specifically affects the monoaminergic system in brain.

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Kodas et al. [11] showed that dietary n-3 deficiency induced changes in the synaptic levels of 5-HT. On the other hand, it has been known that monoamine level in the brain are altered by chronic mild stress which may be induced by such animal housing conditions as lack of bedding, wet bedding, a tilted cage etc. [12, 13]. These lines of inquiry together suggest that n-3 fatty acid deficiency and stress are both factors that can modulate aspects of mental illness such as depression and anxiety.

In this study, the effects of n-3 fatty acid deficiency and social isolation stress were tested in mice using the novelty suppressed feeding paradigm (NSF) with the hypothesis that the combination of these factors would lead to the highest level of anxiety [14]. The NSF test has been considered useful for assessment of anxiety as it has lower sensitivity to changes in cognitive factors and thus has been used to evaluate anti-anxiety and anti-depressant drugs [15–17].

## Materials and Methods

### Animals and Study Design

Female CD-1 (ICR) mice were obtained at 3 weeks of age from Charles River Japan, Inc. and fed an n-3 deficient (n-3 Def) or n-3 adequate (n-3 Adq) diet (see experimental diet, Table 1). They were maintained within our animal facility under conventional conditions with controlled temperature ( $23 \pm 3$  °C), humidity ( $55 \pm 10\%$ ), and illumination (12 h; 0700–1900). At 7 weeks of age, they were mated with 8-week-old males of the same strain (experimental scheme presented in Fig. 1). Their litters were culled to ten pups and the pups were weaned onto the same diet as their mothers. Each individual in a cage of five mice was from a different litter. When the male offspring (second generation) were 8 weeks old, half of them in the each diet group were maintained in a cage containing five mice (group housed), the other half were housed individually for 3 weeks (isolation group). Behavioral experiments were conducted when the male mice were 11 weeks of age. Spontaneous motor activity was first measured for each mouse and after following a 15-h fast, each animal was given the NSF test [15–17].

After the behavioral experiments, they were killed by decapitation and the brain and blood were collected. Blood was spun in a refrigerated centrifuge at  $2,300 \times g$  for 15 min at 4 °C. An aliquot of the upper plasma phase was transferred to another tube. The plasma and brain samples were frozen at  $-80$  °C prior to lipid extraction and measurement of fatty acid composition. This experimental protocol was approved by the Animal Care and Use Committee of the Wakunaga Pharmaceutical Co. in Japan.

**Table 1** Composition of experimental diets

Ingredient	Amount (g/100 g diet)	
	n-3 Def.	n-3 Adq.
Casein, vitamin free	20	20
Carbohydrate	60	60
Cornstarch	15	15
Sucrose	10	10
Dextrose	19.9	19.9
Maltose-dextrin	15	15
Cellulose	5	5
Mineral-salt mix	3.5	3.5
Vitamin mix	1	1
L-cystine	0.3	0.3
Choline bitartrate	0.25	0.25
TBHQ	0.002	0.002
Fat	10	10
Hydrogenated coconut oil	8.1	7.75
Safflower oil	1.9	1.77
Flaxseed oil	None	0.48
Fatty acid composition <sup>a</sup>		
Saturates	80.3	76.7
Monounsaturates	3.8	5.2
18:2n-6	15.7	15.0
18:3n-3	0.14	2.5
n-6/n-3	112	5.9

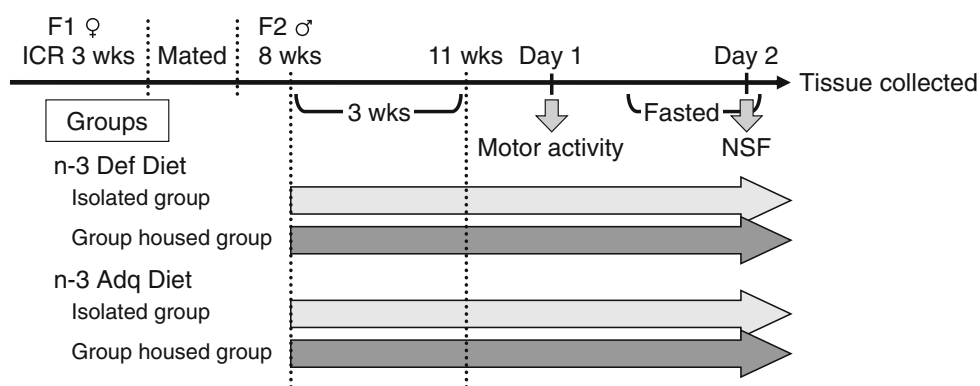
The two experimental diets, an n-3 fatty acid adequate diet (n-3 Adq) and an n-3 fatty acid deficient diet (n-3 Def), were based on the AIN-93 formulation with several modifications to obtain the extremely low basal level of n-3 fatty acid required in this study

<sup>a</sup> The 20:4n-6, 20:5n-3 and 22:6n-3 fatty acids were less than 0.01%

### Experimental Diet

The two experimental (n-3 Def and n-3 Adq) diets used were based on the AIN-93G diet recommendations for rodents (Table 1). The fat sources were altered to provide for the low n-3 fatty acid content required. The only difference between the n-3 Def and the n-3 Adq diets was the amount of n-3 fatty acids. This was achieved by adding a small amount of flaxseed oil to the n-3 Adq diet. The basal fat ingredients used were hydrogenated coconut and safflower oils for the n-3 Def diet. The fat content in both diets was 10/100 g diet and the amount of n-3 fatty acids as ALA in the n-3 Def and the n-3 Adq diets were 0.14 and 2.5% of total fatty acids, respectively. There was no difference in the total n-6 fatty acids between the two diets (n-3 Def, 15.7%; n-3 Adq, 15.0%). This diet was custom prepared and pelleted using low heat conditions (about 65 °C), then stored at 4 °C to prevent lipid oxidation (Oriental Yeast, Chiba, Japan) and the fatty acid distributions of the entire diet was quality assured within our own laboratory.

**Fig. 1** Schematic diagram illustrates study design. Half of the mice in the each diet group were switched to the isolated housing condition from 8 weeks of age for 3 weeks. NSF was conducted the day following measurement of motor activity at 11 weeks of age. After NSF, the mice were killed by decapitation and plasma and brain collected and the tissues analyzed for fatty acid composition



## Behavioral Experiment

The behavioral experiments including motor activity and NSF were performed in the morning (0900–1200 hours). Motor activity was measured using an activity sensor (Model NS-AS01, Neuroscience, Inc., Tokyo Japan). Each mouse was individually placed into a cage (22 × 34 × 14 cm), and the ambulatory time was measured during spontaneous motor activity for 30 min. After the measurement of motor activity, each mouse was returned to its home cage. The NSF was conducted the day following measurement of motor activity. All diet in the home cage was removed for 15 h prior to NSF testing. The NSF procedure was modified from that previously reported [15–17]. The test chamber was composed of an acrylic box (50 × 50 × 20 cm), and the floor was covered with approximately 2 cm of wooden bedding. At the time of testing, a single pellet of diet (n-3 Def or n-3 Adq diet) was placed on a platform positioned in the center of the box. A mouse was placed in a corner of the chamber and the session was recorded by video camera (HDR-SR1, SONY, Tokyo, Japan) until the mouse began to eat the pellet. The measurement time was set for 5 min and was extended to 10 min if eating behavior did not occur within the first 5 min. The parameters measured were the time to initially touch the food pellet, the latency of beginning to eat the pellet (defined as the mouse sitting on its haunches and biting the pellet with the use of forepaws) and the number of mice that were successful in eating (they ate within the measurement period). When the mouse failed to eat the pellet within 5 or 10 min, the latency was calculated as, 300 or 600 s, respectively. After starting to eat the pellet, the mouse was immediately transferred to a new cage in order to control for the time spent in the testing cage. The amount of food consumed over the 5 min period was measured. The feeding drive of each mouse was assessed by returning it to the familiar environment of its home cage

immediately after the NSF test and measuring the amount of food subsequently consumed over a 5 min period.

## Lipid Extraction, Transmethylation and Gas Chromatography

Tissue samples were thawed, weighed, and homogenized in methanol–hexane and methylated in acetyl chloride according to the modified method of Lepage and Roy [18]. Varying amounts of the internal standards methyl docosatrienoate (22:3n-3 for brain) or methyl tricosanoic (23:0 for plasma) were added to each sample to compensate for differences in tissue weight and lipid concentration (70 µg/250 mg brain, 20 µg/100 µl plasma). As an aid in preventing lipid oxidation during the procedures, 50 µg/ml of butylated hydroxytoluene was added in the methanol. The hexane extracts were concentrated into micro vials for gas chromatographic (GC) injection.

Fatty acid methyl esters were analyzed with a GC-2010 Network Gas Chromatograph (Shimadzu CO.LTD, Kyoto, Japan) equipped with a split injector, an AOC-20i automatic liquid sampler and an FID detector. The instrument was controlled and data was collected with GC solution (Shimadzu Co. Ltd, Kyoto, Japan). The GC column was a DB-FFAP 15 m × 0.10 mm i.d. × 0.10 µm film thickness (J&W Scientific from Agilent Technologies, California, US). The detector temperature was set at 260 °C and the injector at 250 °C. The oven temperature program began at 150 °C with a 0.25 min hold, and then ramped at 35 °C/min to 200 °C, then 3 °C/min to 225 °C with a 2.0 min hold, and finally ramped at 80 °C/min to 245 °C with a 25 min hold. Helium was used as carrier gas at a linear velocity of 56 cm/s [19]. A custom-mixed, 28-component, quantitative methyl ester standard containing 10–24 carbons and 0–6 double bonds was used for assignment of retention times and to ensure accurate quantification (Nu Chek Prep 462, Elysian, MN). The amount of each fatty acid was

expressed as the mole percentage (mol%) of total fatty acid content.

### Statistical Analysis

All data are expressed as the mean  $\pm$  the standard error of the mean (SEM). First touch time, food consumption and lipid composition in the plasma and brain were analyzed by Tukey test after one-way ANOVA. Latencies until eating the diet were evaluated using the Mann–Whitney *U* test, the number of successful mice were evaluated by Fisher's exact probability test (Statistica, Statsoft Japan, Tokyo, Japan).

### Results

In this study, two types of diets were used including an n-3 Adq diet containing both n-6 (about 15% linoleic acid) and n-3 fatty acids (about 2.5% ALA) and an n-3 Def diet with the same level of linoleic acid but nearly devoid in n-3 fatty acids (0.14%). These diets produced no significant differences in body weight gain between dietary groups during the testing period (Table 2). Prior to starting the NSF measurements, mice were subjected to a single 30 min motor activity trial in which their moving time was recorded. There were no significant differences between any of the dietary groups in spontaneous motor activity (data not shown).

In the time of first touch of the food pellet in the NSF experiment, there was no significant difference between the n-3 Def and n-3 Adq groups in either housing condition (Table 2). The latency to eat diet in the n-3 Adq-group housed group in the first 5 min was  $179.9 \pm 20.7$  (sec) and was significantly shorter than that of the other three groups (the n-3 Def-isolated group,  $264.0 \pm 24.0$ ,  $P < 0.05$ ; the n-3 Def-group housed,  $248.8 \pm 22.1$ ,  $P < 0.05$ ; the n-3 Adq-isolated group,  $250.3 \pm 19.9$ ,  $P < 0.05$ ). Also, these differences continued to be evident when measured after 5 min (Table 2).

The proportion of successful mice, those eating a food pellet within the first 5 min, was 30% (3/10 animal) in the n-3 Def-isolated housing group, 33% (5/15) in the n-3 Def-group housed, 50% (7/14) in the n-3 Adq-isolated group and 92% (12/13) in the n-3 Adq-group housed group, respectively (Fig. 2a). Most mice in the n-3 Def-isolated group were not able to perform the task, but the n-3 Adq-group housed animals were very successful. These proportions indicated a significant difference ( $P < 0.01$ ) between the n-3 Def and n-3 Adq diet groups in the control breeding condition, i.e., group housed animals, but there was no significant difference between dietary groups in the isolated housing groups. Also, there was a significant difference between the isolated and group housed animals who consumed the n-3 Adq diet ( $P < 0.05$ ), but this difference was not detected between the groups consuming the n-3 Def diet.

When the analysis of the measurement time was extended to its maximum of 10 min, the proportion of successful mice in each group increased to 67% (10/15) in the n-3 Def-group housed and to 79% (11/14) in the n-3 Adq-isolated, thus increasing to a level similar to that of the n-3 Adq-group housed animals (Fig. 2b). It was of importance that only the n-3 Def-isolated group remained at the same low level of success (30%) even when the trial was extended to 10 min. There was a significant difference between the n-3 Def and n-3 Adq diet groups in the isolated housing groups ( $P < 0.01$ ). The significant difference between dietary groups in the group housed condition which was detected in the 5 min evaluation disappeared during the extended period. Also, there was the tendency for a difference between housing conditions in the n-3 Def diet group, although this was not observed in the 5 min evaluation. There was no appreciable difference between any of the groups in food consumption at the 5 min time point during NSF testing (data not shown).

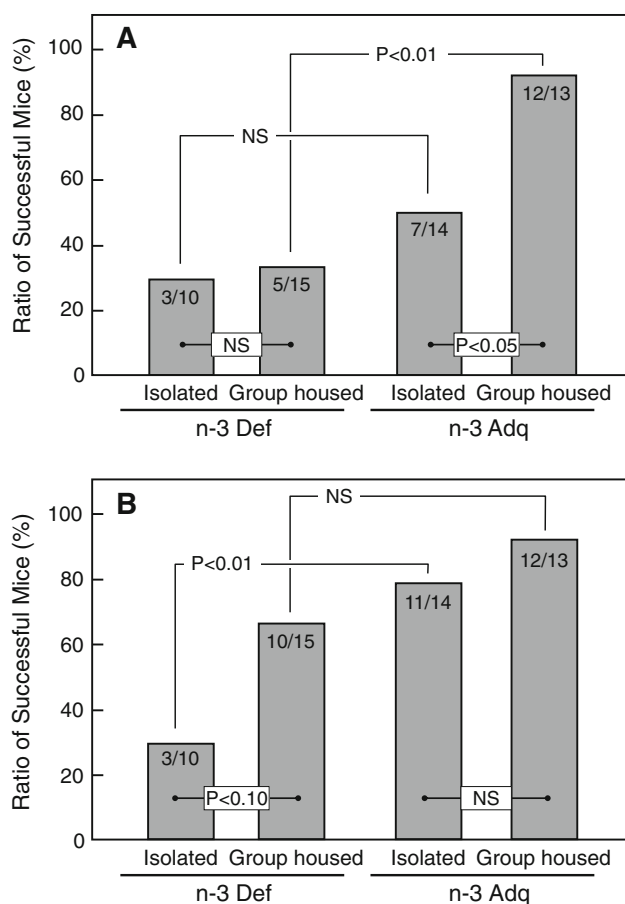
The mice were killed and plasma and brain collected after the behavioral testing was completed. The lipid composition in these tissues was analyzed in order to

**Table 2** Enhancement of the level of anxiety by n-3 fatty acid deficiency and mild stress on the novelty suppressed feeding paradigm

Group	No. of mice	Body weight (g)	First touch (s)	First 5 min latency 1 (s)	Max. 10 min latency 2 (s)
n-3 Def					
Isolated	10	$47.5 \pm 2.0$	$99.7 \pm 23.0$	$264.0 \pm 24.0^{\#}$	$474.0 \pm 66.0^{\#}$
Group housed	15	$45.5 \pm 1.3$	$61.6 \pm 10.9$	$248.8 \pm 22.1^{\#}$	$403.1 \pm 53.0^{\#}$
n-3 Adq					
Isolated	14	$47.6 \pm 1.0$	$63.7 \pm 11.1$	$250.3 \pm 19.9^{\#}$	$360.0 \pm 49.1^{\#}$
Group housed	13	$48.5 \pm 1.0$	$76.7 \pm 12.8$	$179.9 \pm 20.7$	$203.0 \pm 37.7$

Each parameter is presented as the mean  $\pm$  SEM

$^{\#} P < 0.05$ , vs. n-3 Adq group housed (Mann–Whitney *U*-test)



**Fig. 2** The influence of the n-3 fatty acid deficiency and isolated housing on the anxiety level of mice. The measurement time was set initially for 5 min (a) and was extended to 10 min (b), if mice could not eat the food pellet within the first 5 min. The ratio of successful mice (mice who found the food and began to eat it) was calculated for each group. Significant differences between groups were analyzed by Fisher's exact probability test ( $n = 10$ – $15$  animals per group)

confirm the expected effects of the dietary fatty acid (Tables 3, 4). In both plasma and brain, there were no striking differences within the families of saturated or monounsaturated fatty acids nor the amount of total fatty acids between the n-3 Def and n-3 Adq groups in either housing condition. However, the n-3 Def group showed a marked increase in three n-6 fatty acids, 20:4n-6, 22:4n-6, 22:5n-6, and a decrease in two n-3 fatty acids, 22:5n-3 and 22:6n-3, in both tissues when compared to the n-3 Adq group and in both housing conditions. As expected, the ratio of n-6/n-3 fatty acids in the n-3 Def group markedly increased with a concomitant change in the level of n-6 and n-3 fatty acids. When animals in the two different housing conditions were compared, there were no significant differences in brain, although there were rather small differences in some plasma saturated, monounsaturated and n-6 fatty acids.

## Discussion

In this study, effects of n-3 fatty acid deficiency and isolation stress on anxiety were studied. In all four groups, there was no significant difference in motor activity and the time of first touch on the NSF task, important controls for motor function, general level of arousal and motivation. However, the latency of eating the diet in the n-3 Adq group housed animals was significantly shorter than that of the other three groups in both evaluation periods ( $P < 0.05$ ). In the first 5 min, the ratio of successful mice in the n-3 Def group was clearly lower than that of the n-3 Adq group in the group housed case ( $P < 0.01$ ). Also, in the 10 min evaluation, the ratio of successful mice in the n-3 Adq group was greater than that of the n-3 Def group in the isolation stressed groups ( $P < 0.01$ ). These results suggested that the n-3 Def mice had a higher anxiety level than the n-3 Adq mice. The proportion of successful mice was a better indicator than the latency to consume diet when comparing experimental diets or housing conditions.

The evaluations using two different periods (5 and 10 min) in the NSF test produce a change in the level of difficulty or what may be considered the sensitivity of the test since 10 min is long enough to allow for eating behavior in most mice. In the longer evaluation period only, the number of successful mice in the n-3 Def-group housing condition was increased and there was a tendency for a difference according to housing condition for the proportions of successful mice ( $P < 0.10$ ). These results indicated that the anxiety level of the n-3 Def mice was increased in the isolated housing case.

Fedorova and Salem studied the anxiety level of n-3 fatty acid deficient mice exposed to sound stress using elevated plus maze performance [5]. In both n-3 fatty acid adequate and deficient dietary groups, the number of entries into the open arms and the time spent in the open arm decreased in the high-stress condition (a loud noise of 120 dB) but there was no difference between dietary groups under low-stress conditions. However, these parameters were markedly decreased in the n-3 deficient group relative to the n-3 adequate group [5, 20]. This result suggests that the n-3 fatty acid deficient animals exhibit an enhanced vulnerability to acute stressors. Nakashima et al. [21] demonstrated that n-3 fatty acid deficient mice (supplemented with safflower oil) tended to spend more time in the open arm relative to an n-3 adequate group (perilla oil) in the elevated plus maze task. However, in this task, a decline in cognitive function in the n-3 deficient animals may be an important factor rather than solely an increase in anxiety. Also the safflower oil group exhibited no change in drug sensitivities to scopolamine and pentobarbital, drugs known to alter behavior. From these considerations, it may be surmised that n-3 Def animals have a decrease

**Table 3** Effects of n-3 fatty acid deficiency on fatty acid composition of mouse plasma (mol% of total fatty acids)

Fatty acid	n-3 Def		n-3 Adq	
	Isolated (n = 10)	Group housed (n = 15)	Isolated (n = 14)	Group housed (n = 13)
10:0	0.15 ± 0.04	0.17 ± 0.04	0.18 ± 0.03	0.21 ± 0.04
12:0	3.03 ± 0.43	3.50 ± 0.32	3.14 ± 0.39	4.08 ± 0.41
14:0	2.29 ± 0.18	2.60 ± 0.15	2.45 ± 0.15	2.82 ± 0.19
16:0 DMA	0.40 ± 0.03	0.34 ± 0.02	0.38 ± 0.03	0.37 ± 0.03
16:0	20.61 ± 0.25	22.30 ± 0.35**	21.90 ± 0.32	23.03 ± 0.22
18:0 DMA	0.12 ± 0.02	0.12 ± 0.01	0.13 ± 0.01	0.13 ± 0.02
18:0	11.48 ± 0.24	10.58 ± 0.31	11.37 ± 0.30	10.36 ± 0.14
20:0	0.20 ± 0.02	0.26 ± 0.02	0.21 ± 0.01	0.33 ± 0.02*##
22:0	0.34 ± 0.03	0.37 ± 0.03	0.30 ± 0.02	0.41 ± 0.02#
24:0	0.10 ± 0.01	0.13 ± 0.01	0.12 ± 0.01	0.16 ± 0.01#
Total sat.	38.73 ± 0.69	40.37 ± 0.47	40.16 ± 0.52	41.90 ± 0.46
14:1	0.02 ± 0.02	0.08 ± 0.02	0.09 ± 0.02	0.04 ± 0.02
16:1n-7	3.29 ± 0.14	3.57 ± 0.15	3.35 ± 0.14	3.56 ± 0.17
18:1 DMA	0.25 ± 0.03	0.24 ± 0.02	0.24 ± 0.03	0.23 ± 0.02
18:1n-9	14.72 ± 0.51	14.89 ± 0.69	14.92 ± 0.55	13.32 ± 0.47
20:1n-9	0.28 ± 0.02	0.34 ± 0.02	0.29 ± 0.02	0.29 ± 0.01
24:1n-9	0.32 ± 0.02	0.29 ± 0.02	0.30 ± 0.02	0.23 ± 0.01#
Total mono.	18.88 ± 0.54	19.40 ± 0.81	19.19 ± 0.59	17.66 ± 0.60
18:2n-6	21.83 ± 0.69	22.84 ± 0.46	23.94 ± 0.49	25.78 ± 0.44##
18:3n-6	0.20 ± 0.03	0.22 ± 0.02	0.17 ± 0.01	0.24 ± 0.02
20:2n-6	0.19 ± 0.01	0.19 ± 0.01	0.21 ± 0.01	0.16 ± 0.01###
20:3n-6	1.62 ± 0.07	1.50 ± 0.04	1.32 ± 0.07**	1.39 ± 0.04
20:4n-6	15.90 ± 0.92	13.11 ± 0.80	9.52 ± 0.58**	7.97 ± 0.52**
22:4n-6	0.21 ± 0.04	0.18 ± 0.01	0.09 ± 0.01**	0.04 ± 0.01**
22:5n-6	1.81 ± 0.12	1.64 ± 0.08	0.31 ± 0.03**	0.19 ± 0.02**
Total n-6 PUFA	41.77 ± 0.81	39.68 ± 0.98	35.56 ± 0.71**	35.78 ± 0.68**
18:3n-3	nd	nd	0.53 ± 0.04**	0.63 ± 0.04**
22:5n-3	0.04 ± 0.02	0.02 ± 0.01	0.33 ± 0.03**	0.39 ± 0.02**
22:6n-3	0.58 ± 0.04	0.53 ± 0.04	4.23 ± 0.25**	3.64 ± 0.25**
Total n-3 PUFA	0.62 ± 0.04	0.54 ± 0.04	5.09 ± 0.22**	4.66 ± 0.23**
n-6/n-3	69.10 ± 4.38	75.83 ± 3.49	7.10 ± 0.22**	7.85 ± 0.32**
Total fatty acids (μg/μl)	5.92 ± 0.20	5.78 ± 0.20	6.23 ± 0.27	5.60 ± 0.25

Fatty acid methyl esters from 10:0 to 24:1n-9 were analyzed and 18:1n-7, 18:3n-3 and 20:5n-3 were not detected (nd, i.e. <0.01%). Each parameter is presented as the mean ± SEM

\*  $P < 0.05$ , \*\*  $P < 0.01$ , compared between n-3 Def and n-3 Adq diet group in each breeding condition. #  $P < 0.05$ , ##  $P < 0.01$  compared between in the each diet group (one-way ANOVA and Tukey test)

in the threshold or an enhancement of vulnerability to stressors, a factor independent of any acute social stress induced by isolation. It is important to note here though that the balance of these factors likely depends upon the details of the conditions of each behavioral experiment as they determine the intensity of the stress.

The n-6 fatty acids increased and the n-3 fatty acids markedly decreased in the n-3 Def group compared with the n-3 Adq group in both brain and plasma. There was a marked decline in brain DHA with a concomitant increase

in n-6 fatty acids including 22:5n-6, 22:4n-6 and 20:4n-6 as a result of the n-3 fatty acid dietary deficiency. However, there was no change in fatty acid composition between groups given different types of housing, even though we detected significant differences in the anxiety level between the isolated and group housed animals. Vancassel et al. [12] reported a change in monoamine content in the mouse brain when exposed to unpredictable chronic mild stress (UCMS) in various housing conditions including no-bedding, wet-bedding, a tilted cage, water in the home

**Table 4** Effects of n-3 fatty acid deficiency on mouse brain fatty acid composition (mol% of total fatty acids)

Fatty acid	n-3Def		n-3 Adq	
	Isolated (n = 10)	Group housed (n = 15)	Isolated (n = 14)	Group housed (n = 13)
14:0	0.21 ± 0.01	0.22 ± 0.004	0.19 ± 0.01	0.20 ± 0.01
16:0 DMA	2.76 ± 0.02	2.65 ± 0.02	2.56 ± 0.02**	2.63 ± 0.02
16:0	21.15 ± 0.13	21.32 ± 0.10	20.23 ± 0.09**	20.16 ± 0.14**
18:0 DMA	4.00 ± 0.02	3.92 ± 0.03	4.16 ± 0.04*	4.18 ± 0.03**
18:0	20.03 ± 0.06	20.03 ± 0.08	20.24 ± 0.04	20.23 ± 0.05
20:0	0.48 ± 0.01	0.47 ± 0.01	0.48 ± 0.01	0.48 ± 0.01
22:0	0.67 ± 0.01	0.67 ± 0.01	0.69 ± 0.01	0.69 ± 0.01
23:0	0.16 ± 0.004	0.16 ± 0.004	0.16 ± 0.003	0.16 ± 0.004
24:0	0.81 ± 0.02	0.79 ± 0.02	0.81 ± 0.02	0.82 ± 0.03
Total sat.	50.27 ± 0.10	50.23 ± 0.12	49.52 ± 0.09**	49.55 ± 0.11**
16:1n-7	0.72 ± 0.07	0.82 ± 0.01	0.80 ± 0.02	0.77 ± 0.02
18:1 DMA	1.80 ± 0.03	1.78 ± 0.02	1.69 ± 0.02*	1.75 ± 0.02
18:1n-9	14.65 ± 0.10	14.71 ± 0.11	15.28 ± 0.07**	15.35 ± 0.12**
18:1n-7	4.39 ± 0.05	4.58 ± 0.05	4.27 ± 0.05	4.33 ± 0.09*
20:1n-9	1.81 ± 0.04	1.80 ± 0.03	1.93 ± 0.04	1.92 ± 0.04
22:1n-9	0.20 ± 0.01	0.20 ± 0.01	0.22 ± 0.01	0.22 ± 0.01
24:1	2.16 ± 0.04	2.09 ± 0.04	2.43 ± 0.05**	2.45 ± 0.04**
Total mono.	25.73 ± 0.19	25.98 ± 0.15	26.62 ± 0.16*	26.78 ± 0.22*
18:2n-6	0.42 ± 0.02	0.45 ± 0.02	0.49 ± 0.01	0.52 ± 0.02*
18:3n-6	0.06 ± 0.001	0.06 ± 0.001	0.06 ± 0.002	0.06 ± 0.002
20:2n-6	0.11 ± 0.003	0.11 ± 0.003	0.12 ± 0.004	0.13 ± 0.003**
20:3n-6	0.29 ± 0.01	0.27 ± 0.01	0.43 ± 0.02**	0.44 ± 0.02**
20:4n-6	8.31 ± 0.08	8.33 ± 0.07	7.35 ± 0.04**	7.38 ± 0.06**
22:4n-6	2.80 ± 0.04	2.75 ± 0.02	2.08 ± 0.03**	2.12 ± 0.01**
22:5n-6	5.51 ± 0.15	5.39 ± 0.10	0.30 ± 0.02**	0.28 ± 0.01**
Total n-6 PUFA	17.50 ± 0.23	17.36 ± 0.10	10.83 ± 0.06**	10.93 ± 0.07**
22:5n-3	0.01 ± 0.01	0.01 ± 0.004	0.16 ± 0.005**	0.17 ± 0.01**
22:6n-3	6.48 ± 0.13	6.42 ± 0.10	12.87 ± 0.07**	12.57 ± 0.10**
Total n-3 PUFA	6.49 ± 0.13	6.43 ± 0.10	13.02 ± 0.07**	12.74 ± 0.11**
n-6/n-3	2.71 ± 0.07	2.71 ± 0.05	0.83 ± 0.01**	0.86 ± 0.01**
Total fatty acids (µg/mg wet wt)	40.51 ± 0.70	41.07 ± 0.45	37.53 ± 0.29**	38.12 ± 0.87**

Fatty acid methyl esters from 14:0 to 24:1n-9 were analyzed. Each parameter is presented as the mean ± SEM. \* $P < 0.05$ , \*\* $P < 0.01$  compared between n-3 Def and n-3 Adq diet groups in each housing condition. (one-way ANOVA and Tukey test)

cage, switching the light/dark cycle, etc. The UCMS induced a significant decrease of norepinephrine in the frontal cortex and striatum and tissue levels of serotonin. Moreover, Kudas et al. [11] demonstrated that chronic n-3 fatty acid dietary deficiency induced changes in the synaptic levels of 5-HT and subsequent provision of n-3 fatty acid was able to restore both biochemical and neurochemical factors altered by the n-3 deficient diet. Therefore, our results may be due in part to stress-induced alterations in brain monoamines that can alter emotional behavior. It is likely then that a decline in brain DHA and the level of stress are important factors modulating anxiety-related behavior.

In the present study, we demonstrated that the anxiety level of n-3 Def mice was greater than that of n-3 Adq mice and was enhanced by the social isolation stress of individual housing. These results suggest that n-3 fatty acid deficiency may decrease the threshold to stress and, conversely, that increased intake of dietary n-3 fatty acids may suppress the risk of emotional disorders. The relationship between various brain monoamines and behavioral performance in n-3 fatty acid deficient animals requires further investigation.

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