Dietary DHA during development affects depression-like behaviors and biomarkers that emerge after puberty in adolescent rats

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Abstract  DHA is an important omega-3 PUFA that confers neurodevelopmental benefits. Sufficient omega-3 PUFA intake has been associated with improved mood-associated measures in adult humans and rodents, but it is unknown whether DHA specifically influences these benefits. Furthermore, the extent to which development and puberty interact with the maternal diet and the offspring diet to affect mood-related behaviors in adolescence is poorly understood. We sought to address these questions by 1) feeding pregnant rats with diets sufficient or deficient in DHA during gestation and lactation; 2) weaning their male offspring to diets that were sufficient or deficient in DHA; and 3) assessing depression-related behaviors (forced swim test), plasma biomarkers [brain-derived neurotrophic factor (BDNF), serotonin, and melatonin], and brain biomarkers (BDNF) in the offspring before and after puberty. No dietary effects were detected when the offspring were evaluated before puberty. In contrast, after puberty depressive-like behavior and its associated biomarkers were worse in DHA-deficient offspring compared with animals with sufficient levels of DHA. The findings reported here suggest that maintaining sufficient DHA levels throughout development (both pre- and postweaning) may increase resiliency to emotional stressors and decrease susceptibility to mood disorders that commonly arise during adolescence. —Weiser, M. J., K. Wynalda, N. Salem, Jr., and C. M. Butt. Dietary DHA during development affects depression-like behaviors and biomarkers that emerge after puberty in adolescent rats. J. Lipid Res. 2015. 56: 151–166.

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The prevalence of depressive disorders increases dramatically after the rapid hormonal, physical, and neural changes of puberty (1, 2) from 1% of the population under 12 years old to ~25% of the population by the end of adolescence (3), and is more commonly diagnosed in girls than boys (2). Nearly half of depressed adolescents are treatment resistant, and selective serotonin reuptake inhibitors (SSRIs) that are typically effective for treating depressive disorders in adults have been shown to exacerbate suicidal behavior in some teens (4). These issues highlight the need for alternative approaches to juvenile depression, of which diet may be one (5, 6).

Mood is likely regulated by interactions between limbic and cortical brain regions (7). The volume of gray matter first increases during early development and then declines after puberty, possibly due to synaptic pruning (8). Thus, as the brain matures, these regions may be impacted by environmental factors such as stress, diet, and exercise. One particularly important dietary factor for optimal brain development is the omega-3 PUFA DHA (9). Interestingly, dietary consumption of DHA in the United States has dropped significantly during the 20th century coinciding with a significant rise in the prevalence of depressive disorders (10, 11), and it has been suggested that these two phenomena are linked (12).

DHA is the principal omega-3 PUFA in mammalian and human tissues (13, 14). It is particularly abundant in the central nervous system where it is enriched in synaptic membranes, astrocytes, growth cones, and mitochondria (15). Brain tissue is ~60% lipid (dry wt.), and DHA represents 5% to 10% of those lipids, including nearly half of the FAs comprising membrane aminophospholipids (16, 17). Unfortunately, conversion to DHA from dietary precursors is very limited in humans [<0.1% (18, 19)], and thus preformed DHA consumption is needed for optimal brain content (20). Sources of preformed DHA include foods such as fatty fish, organ meats, and eggs, or supplementation with oils derived from fish or microalgae.

Abbreviations: BDNF, brain-derived neurotrophic factor; FST, forced swim test; P, postnatal day; RBC, red blood cell; SSRI, selective serotonin reuptake inhibitor.

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DHA is acquired during gestation via maternal stores by way of placental transfer, and it is acquired after birth from mother’s milk or formula. As DHA is acquired primarily through the diet after birth, a decline in preformed DHA intake typically occurs immediately following weaning. This circumstance creates some developmental risk because weaning is a critical period for synaptogenesis and myelination. Thus, suboptimal DHA intake may influence synapse pruning, myelination, inhibitory synaptogenesis, and so forth, during this time. Indeed, DHA deficiency throughout development leads to decreased DHA levels in neural tissue and is associated with deficits in psychomotor development (21), problem solving (22), reading skills (23), visual acuity (24), and attention (25). Furthermore, emerging clinical data support a link between sufficient DHA intake with a reduction in the incidence and/or symptomatic relief of adolescent depression (5, 26–29), and there exists an abundance of clinical evidence supporting a positive role for the omega-3 PUFA EPA in the treatment of adult depression (30). However, the studies examining the importance of DHA, in particular, are limited in scope and interpretation, and no longitudinal studies have been reported to date. It is therefore unknown whether DHA sufficiency during development can help to establish optimal emotional resiliency to depressive mood states.

The work reported here sought to examine whether DHA sufficiency throughout development (gestation, preweaning, prepubescent, and adolescence) in rats could positively affect behavioral measures (forced swim test [FST]) and biomarkers (serotonin, melatonin, and brain-derived neurotrophic factor [BDNF]) associated with mood before or after puberty. The periods of dietary DHA supplementation were designed to include the majority of neural development as well as the transition from adolescence to adulthood in the rat (8–9 weeks). The study design also included the investigation of potential effects of DHA removal at weaning to model the postweaning drop in DHA intake that occurs in some infants. Furthermore, we wanted to determine whether feeding a postweaning diet rich in DHA to offspring weaned from DHA-deficient dams could affect these measures. Overall, the results described here suggest that DHA supplementation is required throughout development (pre- and postweaning) to positively affect mood-related behavioral measures and biomarkers after puberty in adolescent rats.

MATERIALS AND METHODS

Animals

Sprague-Dawley rats were housed individually (dams during gestation and during lactation with offspring) or in pairs (males after weaning) in polycarbonate cages in a temperature and humidity controlled environment, on a 12 h:12 h light:dark cycle, with chow and water ad libitum. All animal protocols were approved by the Institutional Animal Care and Use Committee at the University of Colorado (Boulder, CO) and were performed according to the Guide for the Care and Use of Laboratory Animals (8th edition, National Research Council).

Diet composition

The DHA-deficient and DHA-sufficient diet were prepared by Dyets Inc. (Bethlehem, PA) and were based on the AIN-93G formulation (31), with 7% total fat derived from a custom fat blend containing olive, hydrogenated coconut, safflower, soybean, and docosahexaenoic acid-rich single-cell oils. Composition of the two diets is shown in Table 1. Each diet contained, as a percentage of total FAs, ~11% linoleic acid (18:2n6) and 0.5% α-linolenic acid (18:3n3) but differed in amount of DHA (22:6n3; 0% for deficient vs. 0.89% for sufficient) and therefore total n3 FAs (0.61% vs. 1.50%) and n6:n3 ratio (17.89% vs. 7.55%).

Experimental design

The experimental design of the study is illustrated in Fig. 1. Timed-pregnant Sprague-Dawley rats were obtained from Harlan Laboratories (Indianapolis, IN) at embryonic day 4 and fed either a DHA-deficient or a DHA-sufficient diet. Shortly after parturition, the University of Colorado (Boulder, CO) and were performed

| Ingredient | DHA Deficient | DHA Sufficient |
|------------|---------------|----------------|
| Casein     | 20.00         | 20.00          |
| t-Cystine  | 0.30          | 0.90           |
| Sucrose    | 10.00         | 10.00          |
| Cornstarch | 39.75         | 39.75          |
| Dyetrosea  | 13.20         | 13.20          |
| Cellulose  | 5.00          | 5.00           |
| Mineral Mix 210025b | 3.50 | 3.50 |
| Vitamin Mix 310025b | 1.00 | 1.00 |
| Choline    | 0.25          | 0.25           |
| Fat composition: | 7.00 | 7.00 |
| Olive oil  | 1.68          | 1.53           |
| Coconut oil| 2.24          | 2.14           |
| Safflower oil | 2.81 | 2.87 |
| Soybean oil| 0.27          | 0.28           |
| DHA-S oil  | 0.19          |                |
| FA composition: | (% Total FAs) |     |
| 18:1n9     | 45.48         | 45.11          |
| 18:2n6     | 10.88         | 10.96          |
| 18:3n3     | 0.32          | 0.30           |
| 20:4n6     | 0.01          | 0.02           |
| 22:6n3     | —             | 0.89           |
| ΣSat       | 41.60         | 40.75          |
| ΣMono      | 46.88         | 46.42          |
| ΣPUFA      | 11.52         | 12.83          |
| Σn3        | 0.61          | 1.50           |
| Σn6        | 10.91         | 11.33          |
| n6:n3      | 17.89         | 7.55           |

18:1n9, oleic acid; 18:2n6, linoleic acid; 18:3n3, α-linolenic acid; 20:4n6, arachidonic acid (ARA); 22:6n3, DHA; ΣMono, sum of monounsaturated fatty acids; Σn3, sum of omega-3 fatty acids; Σn6, sum of omega-6 fatty acids; ΣPUFA, sum of polyunsaturated fatty acids; ΣSat, sum of saturated fatty acids; n6:n3, ratio of omega-6 to omega-3 fatty acids.

a Trademark Dyets, Inc. Carbohydrate composition (%): mono-saccharides, 1; disaccharides, 4; trisaccharides and higher, 0.9.

b AIN-93G mineral mix (mg/100 g diet): calcium, 500; phosphorus, 156.1; potassium, 360; sodium, 101.9; chloride, 157.1; sulfur, 30; magnesium, 50.7; iron, 3.5; copper, 0.6; manganese, 1; chromium, 0.1; iodine, 0.02; selenium, 0.02; fluoride, 0.1; boron, 0.05; molybdenum, 0.02; silicon, 0.5; nickel, 0.05; lithium, 0.01; vanadium, 0.01.

18:1n9, oleic acid; 18:2n6, linoleic acid; 18:3n3, α-linolenic acid; 20:4n6, arachidonic acid (ARA); 22:6n3, DHA; ΣMono, sum of monounsaturated fatty acids; Σn3, sum of omega-3 fatty acids; Σn6, sum of omega-6 fatty acids; ΣPUFA, sum of polyunsaturated fatty acids; ΣSat, sum of saturated fatty acids; n6:n3, ratio of omega-6 to omega-3 fatty acids.

TABLE 1. Compositions of diets used (g/100 g diet)
DHA Deficient Diet
DHA Pre-Weaning Diet
DHA Post-Weaning Diet
DHA Adequate Diet

Maternal Period
Prenatal
Weaning
Pubescent
Late-adolescence

Post-Weaning Period

Group 1
Group 2
Group 3
Group 4

Behavior & Tissue Analysis

1. Experimental design of the study. Timed pregnant females (embryonic day 4) were provided either a base diet containing no DHA or an equivalent diet containing DHA as ~1% of the total FAs throughout the gestation and lactation periods. At weaning, male offspring were either provided the base diet or the DHA-containing diet. Behavior was assessed in the FST before and after puberty (P40 and P60, respectively) in separate cohorts (each animal was only tested once). Tissue was taken at both time points for analysis of biomarkers for mood.

2. Behavior in the FST in two separate cohorts at either P39–P40 or P59–P60, (DHA-sufficient maternal diet and DHA-deficient postweaning diet), and DHA-deficient maternal and postweaning diets), and 4) sufficient (DHA-sufficient maternal and postweaning diets). Offspring were tested in the FST in two separate cohorts at either P39–P40 or P59–P60, but not at both time points. Thirty minutes following the final swim test, each animal was euthanized via decapitation. Whole brain was extracted and frozen in cold isopentane (approximately –1°C) and stored at –8°C (brains were not perfused with saline or buffer, but rather frozen directly after decapitation and extraction). Trunk blood was collected into K2-EDTA-coated vacutainers (BD Biosciences), inverted to mix, and centrifuged at 1,000 g for 15 min, and the resulting plasma aliquots were stored at –8°C until assayed. The cell pellet was then mixed with a 5-fold volume of saline, centrifuged at 1,000 g for 5 min (wash repeated twice), and the resulting pellet containing the red blood cell (RBC) fraction was stored at –8°C until assayed. After necropsy, brains were thawed on ice and bisected sagittally down the midline. One hemissection was used for FA analysis, and one hemisection was regionally dissected and stored at –80°C until further processing.

3. FA analysis

Tissues (RBCs, plasma, and brain) from the current study were analyzed for FA composition by gas chromatography. Sample preparation was optimized for each tissue matrix. Briefly, the plasma was aliquoted and dried under evaporative nitrogen; brain tissues were lyophilized, homogenized, and weighed; and

RBCs were vortexed, then aliquoted directly for assays. Internal standard (trinonadecanoic acid or pentadecanoic acid in toluene) was then added to each sample, and direct transesterification was accomplished by the addition of 1.5 N methanolic hydrochloric acid. Samples were heated to 100°C for 2 h. Following methylation, saturated sodium chloride was added, and the lipids were extracted into toluene for direct injection. Calibration curves were generated using GLC-502B (Nu-Chek Prep, Elysian, MN) for FA reference standards, with trinonadecanoic acid or pentadecanoic acid for the internal standard. Samples were analyzed on an Agilent 6890 gas chromatograph (split injection) equipped with a flame ionization detector. A 30 m × 0.32 mm × 0.2 µm SP-2580 fused silica capillary column (Supelco, Bellefonte, PA) was used with hydrogen as the carrier gas. The oven was temperature programmed from 140°C to 190°C at 5°C/min and held for 1 min at 190°C, then increased to 260°C at a rate of 17°C/min and held for 3 min for a total run time of 18.12 min. The flame ionization detector was set at 285°C. FA data are expressed as a wt percentage of total FAs.

4. FST

Animals were acclimated to the test room overnight prior to testing. The test was performed over two consecutive days. On day 1, the animals were acclimated to the test (0800 h to 1300 h) by placing them in a Plexiglas cylindrical container (45 cm × 20 cm; Stoelting Co., Wood Dale, IL) filled with 30 cm of fresh water (25°C) for 5 min, after which they were toweled dried and returned to their home cage. On day 2 (24 h later), the test was performed for a total swim time of 5 min, after which the rats were toweled dried and returned to their home cage. Both trials were recorded by a digital video camera secured to the ceiling above the cylinders. Total time swimming, immobile, and climbing, and number of dives were measured post hoc by an experimenter blind to the group assignments. Total time immobile was measured in real-time by behavioral software (ANY-maze, Stoelting Co.), and confirmed by the post hoc analysis. Swimming was defined as movement of the forelimbs and hind limbs that did not break the surface of the water. Immobility was defined as absence of any movement except for slight movements necessary for the animal to keep its head above water. Climbing was defined as rapid movement of the forelimbs that did not break the surface of the water. Dives were counted when the animal submerged its head in an effort to find an escape below the surface of the water.

5. Biomarker analysis

Testosterone. Plasma testosterone concentration was determined via a competitive ELISA (catalog number EIA-1559; DRG International Inc., Mountainside, NJ) according to the manufacturer’s specifications. Plasma samples were run neat in triplicate. All samples were analyzed in one assay. The intra-assay variance was 7.5%. The limit of detection for this assay is 0.083 ng/ml.

Serotonin. Plasma serotonin concentration was assayed via a competitive ELISA (catalog number RE59121; IBL International Inc., Hamburg, Germany). Plasma samples were centrifuged for 2 min at 10,000 g to ensure a platelet-free sample, and a 100 µl aliquot of the supernatant was taken for the assay. The manufacturer’s “Sample B” protocol (for platelet-free plasma) was followed as specified. All samples were analyzed in one assay. The intra-assay variance was 9.6%. The limit of detection for this assay is 0.014 ng/ml.

Melatonin. Plasma melatonin concentration was analyzed via a competitive ELISA (catalog number RE54021; IBL International Inc., Hamburg, Germany). Plasma samples were centrifuged for 2 min at 10,000 g to ensure a platelet-free sample, and a 100 µl aliquot of the supernatant was taken for the assay. The manufacturer’s “Sample B” protocol (for platelet-free plasma) was followed as specified. All samples were analyzed in one assay. The intra-assay variance was 9.6%. The limit of detection for this assay is 0.014 ng/ml.
RESULTS

Plasma testosterone

Mean plasma testosterone concentration was lower in animals at P40 (0.67 ± 0.06 ng/ml) than those at P60 (2.66 ± 0.16 ng/ml) as determined by an unpaired one-tailed $t$-test with Welch’s correction ($t_{(83)} = 11.34, P < 0.0001$). There were no significant group differences at either P40 or P60 (data not shown). Therefore, P40 and P60 were likely within the pre-/peripubertal and postpubertal time periods, respectively.

Body and brain weight

There were no differences in body weight between dams on either the DHA-sufficient or DHA-deficient diet after parturition or after weaning, and no differences were detected in whole brain weight at the time of euthanization following weaning.

Among the offspring, there were no group differences in body weight at either P40 or P60, or brain mass at P40. However, analysis by one-way ANOVA detected a significant main effect of group on brain weight at P60 [$F_{(3,61)} = 6.16, P = <0.001$]. Tukey’s post hoc test indicated that preweaning sufficient animals had significantly lower whole brain weight (1.982 ± 0.012 g) than either sufficient (2.088 ± 0.025 g, $P = 0.021$) or deficient (2.114 ± 0.024 g, $P < 0.0001$) animals, but not postweaning sufficient animals (2.072 ± 0.026 g, $P = 0.080$).

FA analysis

Diet affected the DHA status of peripheral and central tissues in the dam at the time of euthanization (P21; 39 days on diet). Sufficient dams had higher DHA content in RBCs (4.39 ± 0.09% of total FAs, $N = 8$) than deficient dams (1.96 ± 0.11%, $N = 9$; $t_{(15)} = 16.80, P < 0.0001$), and higher DHA content in brain (13.50 ± 0.20%, $N = 8$) than deficient dams (12.59 ± 0.17%, $N = 9$; $t_{(15)} = 3.50, P = 0.0016$) as determined by an unpaired one-tailed $t$-test. In the offspring, supplementation of the maternal or postweaning diets with DHA had profound effects on the FA composition of central and peripheral tissues both before and after puberty. The FA composition of brain, RBCs, plasma, and their statistical analyses are detailed in Tables 2, 3, and 4, respectively. In addition, the data for the primary PUFAs of interest within brain tissue [DHA, 22:6n3; arachidonic acid (ARA, 20:4n6); and docosapentaenoic acid (DPAn6; 22:5n6)] are graphed in Fig. 2. As found in previous studies, dietary supplementation of DHA during any developmental time period (maternal, postweaning, or throughout) significantly increased brain DHA content both before and after puberty relative to deficient controls. Furthermore, at P40 sufficient animals had significantly higher brain DHA content (14.80% of total FAs) than preweaning (12.96%) and postweaning (13.56%) sufficient cohorts, both of which had higher brain DHA content than the deficient animals (9.50%). At P60, brain DHA content was similar in the sufficient and postweaning sufficient groups (14.01% and 13.51%, respectively), and both groups had significantly greater brain DHA content than both the preweaning sufficient and deficient groups (11.89% and 9.71%, respectively).

With the other brain PUFAs of interest, again similar to previous studies, there were differences in ARA and DPAn6 brain content that tracked inversely to the change in brain DHA levels (Table 2). For example, ARA and DPAn6 were...
highest in the brains from animals that were not supplemented with DHA during development, and lowest in those supplemented throughout development. DPA:n6 brain content was quicker to change in response to post-weaning than in brain. For example, the 24 day period from weaning (P16) to prepuberty (P40) was long enough to allow DHA reductions (P < 0.001) and performed more dives (P = 0.045) in the animals deficient in DHA throughout development. At P60, one-way ANOVA detected a main effect of group on time spent immobile (F(3,61) = 6.93, P = 0.023). Tukey’s post hoc pairwise analysis indicated that completely sufficient animals spent significantly less time immobile (P = 0.026) and more time climbing (P < 0.001) and performed more dives (P = 0.045) than the animals deficient in dietary DHA throughout development. In addition, postweaning sufficient animals spent significantly more time climbing (P = 0.007) than deficient cohorts, yet were no different from deficient animals in measures of immobility or dives. Postweaning sufficient (postweaning deficient) offspring

### TABLE 2. Dietary DHA affects brain FA profiles

| FA      | Age (P) | Deficient | Prewean Sufficient | Postwean Sufficient | Sufficient | F     | P    | Power |
|---------|---------|-----------|--------------------|--------------------|------------|-------|------|-------|
| 16:0    | 40      | 18.32 ± 0.07<sup>a</sup> | 18.16 ± 0.10<sup>b</sup> | 18.20 ± 0.08<sup>c</sup> | 17.87 ± 0.07<sup>d</sup> | 5.68   | 0.002 | 0.94  |
| 16:0    | 60      | 18.60 ± 0.14 | 18.60 ± 0.17 | 18.39 ± 0.15 | 18.57 ± 0.15 | NS     |      |      |
| 18:1n9  | 40      | 16.87 ± 0.09<sup>a</sup> | 17.42 ± 0.14<sup>b</sup> | 17.13 ± 0.12<sup>c</sup> | 17.75 ± 0.09<sup>d</sup> | 11.02  | <0.001 | 1.00  |
| 18:1n9  | 60      | 17.37 ± 0.13<sup>a</sup> | 17.63 ± 0.14<sup>b</sup> | 17.84 ± 0.15<sup>c</sup> | 17.87 ± 0.13<sup>d</sup> | 2.97   | 0.038 | 0.68  |
| 18:2n6  | 40      | 0.46 ± 0.01<sup>a</sup> | 0.65 ± 0.01<sup>b</sup> | 0.68 ± 0.01<sup>c</sup> | 0.71 ± 0.01<sup>d</sup> | 7.02   | <0.001 | 0.97  |
| 18:2n6  | 60      | 0.10 ± 0.00 | 0.09 ± 0.00 | 0.09 ± 0.00 | 0.09 ± 0.00 | NS     |      |      |
| 20:4n6  | 40      | 10.57 ± 0.05<sup>a</sup> | 10.30 ± 0.04<sup>b</sup> | 9.98 ± 0.07<sup>c</sup> | 9.81 ± 0.05<sup>d</sup> | 42.69  | <0.001 | 1.00  |
| 20:5n3  | 40      | 6.92 ± 0.06<sup>a</sup> | 9.45 ± 0.08<sup>b</sup> | 8.99 ± 0.07<sup>c</sup> | 9.12 ± 0.08<sup>d</sup> | 16.72  | <0.001 | 1.00  |
| 22:5n3  | 60      | 0.46 ± 0.01<sup>a</sup> | 0.44 ± 0.01<sup>b</sup> | 0.52 ± 0.01<sup>c</sup> | 0.49 ± 0.01<sup>d</sup> | 19.67  | <0.001 | 1.00  |
| 22:5n3  | 60      | 0.09 ± 0.00 | 0.09 ± 0.00 | 0.08 ± 0.00 | 0.08 ± 0.00 | NS     |      |      |
| 20:4n6  | 40      | 10.57 ± 0.05<sup>a</sup> | 10.30 ± 0.04<sup>b</sup> | 9.98 ± 0.07<sup>c</sup> | 9.81 ± 0.05<sup>d</sup> | 42.69  | <0.001 | 1.00  |
| 20:5n3  | 40      | 6.92 ± 0.06<sup>a</sup> | 9.45 ± 0.08<sup>b</sup> | 8.99 ± 0.07<sup>c</sup> | 9.12 ± 0.08<sup>d</sup> | 16.72  | <0.001 | 1.00  |
| ARA:DHA | 40      | 1.12 ± 0.02<sup>a</sup> | 0.80 ± 0.02<sup>b</sup> | 0.74 ± 0.02<sup>c</sup> | 0.66 ± 0.02<sup>d</sup> | 152.72 | <0.001 | 1.00  |

16:0, palmitic acid; 18:1n9, oleic acid; 20:5n3, EPA; 22:5n3, docosapentaenoic acid (DPA:n6 and DPA:n6); n.d., not detectible; NS, not significant in multifactorial ANOVA. Data are presented as the mean percent of total FAs ± the SEM. The degrees of freedom and their errors for the analysis of each FA at age P40 were 3 and 68, and at age P60 were 3 and 67, respectively. Significant differences (P < 0.05) between groups were determined with Tukey’s post hoc test and are designated by a lack of common superscripts across each row.

### Depression-like behavior

Before puberty (P40), there were no differences among the four groups in measures of passive behaviors (time immobile; Fig. 3A) or active behaviors (time climbing and dives; Fig. 3C and Fig. 3E). Interestingly, after puberty there was a shift toward more average time spent immobile (Fig. 3B; 175.5 min vs. 144.2 min prepuberty), less average time spent climbing (Fig. 3D; 12.4 min vs. 46.8 min prepuberty), and a lower mean number of dives (Fig. 3F; 0.44 vs. 1.25 prepuberty) in the animals deficient in DHA.
exhibited behavior similar to the completely deficient controls. At P60, brain DHA level was negatively correlated to immobility time (0.293, \(P = 0.016\)) and positively correlated to climbing time (0.376, \(P = 0.002\)) and number of dives (0.224, \(P = 0.036\)). No correlations were seen at P40.

**Plasma biomarkers**

For plasma BDNF, one-way ANOVA did not identify a main effect of group at P40 (Fig. 4A) but did at P60 (Fig. 4B; \(F_{(3,56)} = 2.93, P = 0.041\)). Tukey’s post hoc analysis determined that only offspring that received DHA throughout development had significantly higher plasma BDNF concentrations after puberty than the deficient animals (\(P = 0.039\)). Both pre- and postweaning DHA supplementation was required to positively affect plasma BDNF concentration. Additionally, plasma BDNF concentration was positively correlated to plasma serotonin concentration. Therefore, dietary DHA in these animals as there was only a trend with DHA after weaning was necessary to enhance plasma BDNF concentrations after puberty. DHA supplementation during only gestation and lactation was ineffective in modulating plasma serotonin after puberty. Plasma serotonin was positively correlated to brain DHA levels at P40 (0.346, \(P = 0.002\)), but not at P60.

For plasma melatonin, one-way ANOVA identified a main effect of group before puberty at P40 (Fig. 4E; \(F_{(3,56)} = 4.54, P = 0.006\)) and after puberty at P60 (Fig. 4F; \(F_{(3,56)} = 11.77, P < 0.0001\)). Before puberty, Tukey’s post hoc analysis showed that only offspring that received DHA throughout development had significantly increased plasma concentrations of melatonin (\(P = 0.015\)). Preweaning sufficient animals had melatonin concentrations that were approaching those of sufficient animals, but it appeared that DHA deficiency during the 24 day window between weaning and P40 was long enough to impair the effect of preweaning dietary DHA in these animals as there was only a trend for significance as compared with deficient controls (\(P = 0.088\)). After puberty, both groups that received postweaning dietary DHA, regardless of maternal diet, exhibited significantly increased plasma melatonin concentrations as compared with deficient controls (sufficient, \(P < 0.0001\); postweaning sufficient, \(P = 0.001\)). Dietary supplementation with DHA after weaning was necessary to enhance plasma melatonin concentrations after puberty, and DHA supplementation during only gestation and lactation was ineffective in modulating plasma melatonin after puberty.

![Table 3. Dietary DHA affects RBC FA profiles](https://example.com/table3.png)

| FA        | Age (d) | Deficient | Prewean Sufficient | Postwean Sufficient | Sufficient | F | P | Power |
|-----------|---------|-----------|--------------------|---------------------|------------|---|---|-------|
| 16:0      | 40      | 27.16 ± 0.33 | 26.16 ± 0.76       | 27.74 ± 0.52        | 27.92 ± 0.75 | NS |   |       |
| 20:4n6    | 40      | 25.77 ± 0.22 | 26.52 ± 0.12       | 26.03 ± 0.14        |            | NS |   |       |
| 16:0      | 60      | 10.47 ± 0.26 | 10.22 ± 0.16       | 10.11 ± 0.21        | 10.20 ± 0.24 | NS |   |       |
| 20:4n6    | 60      | 5.12 ± 0.08  | 5.08 ± 0.10        | 5.36 ± 0.10         | 5.29 ± 0.12  | NS |   |       |
| 18:3n3    | 40      | 4.96 ± 0.10  | 4.83 ± 0.10        | 5.54 ± 0.10         | 5.04 ± 0.09  | 8.97 <0.001 | 0.999 |
| 18:3n3    | 60      | 0.05 ± 0.01  | 0.04 ± 0.01        | 0.04 ± 0.01         | 0.04 ± 0.01  | NS |   |       |

Data are presented as the mean percent of total FAs ± the SEM. The degrees of freedom and their errors for the analysis of each FA at age P40 and P60 are designated by a lack of common superscripts across each row.
Brain BDNF

One-way ANOVA identified a significant main effect of group on BDNF content in hippocampus (Fig. 5A; $F_{3(45)} = 4.23$, $P = 0.015$) and hypothalamus (Fig. 5B; $F_{3(61)} = 3.99$, $P = 0.012$) at P60. Tukey’s post hoc analysis indicated that sufficient animals had significantly higher BDNF content in both the hippocampus ($P = 0.015$) and hypothalamus ($P = 0.017$) as compared with deficient controls. These effects were absent in animals from DHA-sufficient mothers that lacked DHA supplementation after weaning (preweaning sufficient), although there was a slight trend toward increased hippocampal BDNF in this cohort ($P = 0.194$). DHA supplementation after weaning in offspring from DHA-deficient mothers (postweaning sufficient) was able to increase hippocampal BDNF levels ($P = 0.030$), but not hypothalamic BDNF content. Brain DHA levels were positively correlated to both hippocampal BDNF content (0.454, $P < 0.001$) and BDNF content in the hypothalamus (0.259, $P = 0.019$).

DISCUSSION

This study demonstrated that DHA supplementation provided to rats during early and late development increased brain DHA levels considerably and affected depressive-like behaviors and mood-associated biomarkers that emerged following puberty. Removal of DHA supplementation after weaning (late development) negated nearly all of these behavioral and biochemical effects, whereas starting dietary DHA supplementation after weaning was too late to affect most measures. The circulating biomarkers chosen in this study are clinically reported to be affected by at least one or more successful therapies aimed at treating depression (antidepressant drugs, cognitive behavioral therapy, electroconvulsive treatment, etc.). Overall, we observed an interesting pubertal shift in all of the peripheral biomarkers to lower plasma concentrations postpuberty as compared with prepuberty. This decrease coincided with the overall increase in depressive-like behaviors seen postpuberty in the DHA-deficient offspring.

Maternal and postweaning dietary supplementation with DHA had an effect on overall FA composition of tissues that depended greatly on the timing and duration of supplementation, the location of the tissue, and the specific FA of interest. Addition of DHA to the maternal diet successfully increased brain DHA content in the offspring that was further enhanced or diminished depending on whether DHA was provided in the postweaning diet. Deficient offspring had ~30% less brain DHA than sufficient offspring both before and after puberty, or a difference of about 4% to 5% of total FAs. This spread is similar to the differences (−22% on average) that have been noted in postmortem tissue from depressed patients as compared...
Dietary DHA supplementation during development affected the concentration of FAs in brain tissue. A: Before puberty, dietary DHA at any time point resulted in higher brain DHA concentration relative to controls on a DHA-deficient diet, and DHA throughout development increased brain DHA content to a greater extent than DHA during only the preweaning or postweaning period. B: After puberty, dietary DHA at any time point resulted in higher brain DHA concentration relative to deficient controls, and DHA throughout development or after weaning increased brain DHA content higher than DHA during only the preweaning period. C: Before puberty, dietary DHA at any time point resulted in lower brain ARA concentration relative to deficient controls, and DHA throughout development or after weaning decreased brain ARA content to a greater extent than DHA during only the preweaning period. D: After puberty, dietary DHA postweaning, regardless of maternal diet, resulted in lower brain ARA concentration relative to deficient controls, yet DHA supplementation just during the preweaning period did not affect brain ARA content. E: Before puberty, dietary DHA at any time point resulted in lower brain DPA(n6) concentration relative to deficient controls, and DHA throughout development decreased brain DPA(n6) content to a greater extent than DHA during only the preweaning or postweaning period. F: After puberty, dietary DHA at any time point resulted in lower brain DPA(n6) content relative to deficient controls, DHA throughout development decreased brain DPA(n6) content lower than DHA during only the preweaning or postweaning period, and DHA supplementation only after weaning decreased brain DPA(n6) concentration lower than DHA during just the preweaning period. Data are presented as averages ± the SEM. Significant differences (*P < 0.05) between groups were determined by one-way ANOVA followed by Tukey’s post hoc tests and are indicated by an absence of shared superscripts.

with their healthy counterparts (33). In fact, DHA was the only FA found to be significantly altered in these postmortem brains. Another interesting phenomenon observed in this study was the effect of diet change at the time of weaning on brain DHA content before and after puberty. Switching preweaning sufficient animals to a DHA-deficient diet at weaning caused a slow decline in brain DHA content (~15% relative to sufficient animals) that did not reach the levels observed in deficient controls by the end of puberty (44 days). This rate was clearly slower than the rate of increase seen the preweaning deficient animals (+39% relative to deficient animals) indicating that the developing brain was able to retain DHA to some extent in the face of dietary deficiency. Indeed, previous studies show that accretion is relatively faster than depletion of DHA in neural tissue (34). However, infants are typically weaned to rather DHA-poor foods and children’s diets are usually no better than the usual Western adult diet, providing plenty of time throughout childhood and adolescence for depletion of DHA from neural tissue. Unfortunately, longitudinal data on DHA status in children from birth to adolescence are lacking, but in one particular study, healthy term infants who were weaned at 4–6 months of age to formula without DHA had significant losses in RBC DHA content at 1 year of age (~50%) as compared with levels measured at the time of weaning. Conversely, infants fed formula containing DHA not only maintained tissue levels of DHA but had increases in RBC DHA...
DHA reduces depression-like behavior in adolescent rats

Dietary DHA inhibited postpuberty increases in depressive-like behaviors in the FST. Diet did not affect immobility (a passive behavior) before puberty at P40 (A), and only dietary DHA throughout development reduced immobility after puberty at P60 (B). Similarly, diet did not affect climbing or diving (active behaviors) before puberty (C and E, respectively). After puberty, dietary DHA during the gestation and lactation (preweaning) period, regardless of postweaning diet, increased climbing (D). However, only dietary DHA throughout development increased diving (F). Data are presented as averages ± the SEM. Significant differences ($P < 0.05$) between groups were determined by one-way ANOVA followed by Tukey’s post hoc tests and are indicated by an absence of shared superscripts.

**Fig. 3.** Dietary DHA inhibited postpuberty increases in depressive-like behaviors in the FST. Diet did not affect immobility (a passive behavior) before puberty at P40 (A), and only dietary DHA throughout development reduced immobility after puberty at P60 (B). Similarly, diet did not affect climbing or diving (active behaviors) before puberty (C and E, respectively). After puberty, dietary DHA during the gestation and lactation (preweaning) period, regardless of postweaning diet, increased climbing (D). However, only dietary DHA throughout development increased diving (F). Data are presented as averages ± the SEM. Significant differences ($P < 0.05$) between groups were determined by one-way ANOVA followed by Tukey’s post hoc tests and are indicated by an absence of shared superscripts.

In this study, maternal and postweaning dietary DHA supplementation reduced postpubertal measures of depression-like behaviors that increased in deficient animals after puberty. We used the modified FST to measure depression-like behaviors before and after puberty (36). Typical antidepressants limit the amount of passive coping (immobility) and promote the amount of active coping (swimming/climbing) in adult animals (37, 38). In juvenile animals, immobility is particularly sensitive to SSRIs and not tricyclic antidepressants and thereby effectively models the clinical evidence observed in cases of adolescent depression (39). The data from this study indicated that subsequent to puberty there was a shift in deficient animals toward a depression-like behavioral phenotype and biomarker profile. This shift may be akin to the increased incidence of depression following puberty in humans. Because the FST requires two tests separated by 24 h, it is entirely possible that the stress of the first test differentially sensitized the animals to the second test, subsequently influencing the behavioral responses seen. Accordingly, prior stressors have been shown to affect behavior in a synergistic fashion with omega-3 deficiency (40, 41). Animals provided preweaning dietary supplementation with DHA displayed a more active postpubertal coping style than deficient controls. However, improvements in all active behaviors (climbing and diving) and in passive behavior (immobility) were only observed in sufficient animals.

In this study, maternal and postweaning dietary DHA supplementation reduced postpubertal measures of depression-like behaviors that increased in deficient animals after puberty. We used the modified FST to measure depression-like behaviors before and after puberty (36). Typical antidepressants limit the amount of passive coping (immobility) and promote the amount of active coping (swimming/climbing) in adult animals (37, 38). In juvenile animals, immobility is particularly sensitive to SSRIs and not tricyclic antidepressants and thereby effectively models the clinical evidence observed in cases of adolescent depression (39). The data from this study indicated that subsequent to puberty there was a shift in deficient animals toward a depression-like behavioral phenotype and biomarker profile. This shift may be akin to the increased incidence of depression following puberty in humans. Because the FST requires two tests separated by 24 h, it is entirely possible that the stress of the first test differentially sensitized the animals to the second test, subsequently influencing the behavioral responses seen. Accordingly, prior stressors have been shown to affect behavior in a synergistic fashion with omega-3 deficiency (40, 41). Animals provided preweaning dietary supplementation with DHA displayed a more active postpubertal coping style than deficient controls. However, improvements in all active behaviors (climbing and diving) and in passive behavior (immobility) were only observed in sufficient animals. This indicated that these effects were dependent on early and late organizational (developmental) effects of DHA supplementation, as switching to a DHA-rich postweaning diet...
diet in preweaning deficient offspring was unable to affect these measures. However, climbing behaviors were exclusively dependent on an early organizational effect of DHA, as increased time climbing was seen only in preweaning sufficient and entirely sufficient offspring, but not postweaning sufficient or deficient offspring. These data show that both maternal and postweaning dietary DHA supplementation were required to effectively buffer the postpubertal rise in passive behaviors and fall in active behaviors seen in the deficient controls. Conversely, postweaning dietary DHA was too late, and maternal-only DHA supplementation was insufficient, to overcome all aspects of this shift in behavior.

In line with the decrease in time immobile, there was a higher plasma concentration of serotonin detected in sufficient animals from this study relative to their deficient counterparts. This was only observed in the sufficient group, and not maintained in preweaning sufficient or rescued in postweaning sufficient animals, suggesting that the effect of dietary DHA supplementation on this measure was dependent on its actions in early and late development.

Previous reports suggest that early, but not late, omega-3 PUFA supplementation can restore brain serotonin and dopamine levels in omega-3 PUFA-deficient rats. Taken with our results, it may be that peripheral serotonin levels are more dependent upon current omega-3 PUFA status, whereas brain serotonin levels are more sensitive to early developmental omega-3 PUFA intake (42). Interestingly, in our study there was an apparent drop in plasma serotonin levels after puberty in all animals, a phenomenon that has been reported previously in boys (43, 44). Importantly, concentrations of circulating serotonin and its precursor tryptophan have been reported to be lower in depressed adults relative to healthy controls (45, 46). Brain serotonin content is well known to be dependent on plasma tryptophan concentration (47), and the circulating serotonin level may also be an indicator of brain serotonin concentration (48). In a recent study, rats supplemented with high-DHA fish oil from gestation to adulthood exhibited decreased immobility in the FST and had increased levels of serotonin in the hippocampus and cortex, effects that were inhibited by acute treatment with a
DHA reduces depression-like behavior in adolescent rats

Fig. 5. Dietary DHA throughout development increased BDNF in the hippocampus (A) and hypothalamus (B) when measured at P60, an effect that was absent in animals where dietary DHA was removed at weaning. Dietary DHA supplementation in deficient offspring increased hippocampal, but not hypothalamic, BDNF at P60. Data are presented as averages ± the SEM. Significant differences \((P < 0.05)\) between groups were determined by one-way ANOVA followed by Tukey’s post hoc tests and are indicated by an absence of shared superscripts.

Addition of DHA to the postweaning diet, regardless of maternal diet, also increased plasma levels of melatonin relative to deficient offspring, an effect absent in preweaning sufficient animals. This indicates that plasma melatonin levels may be more acutely dependent on DHA tissue content than on organizational effects of DHA. Melatonin has an important role in the entrainment of many biological systems (including sleep/wake cycles) to the central circadian clockmaker, the suprachiasmatic nucleus. Mood disorders have been associated with disruptions in circadian rhythms that govern essential function such as sleep, eating, and neuroendocrine function. Depressive symptoms are typically highest in morning, suggesting a phase advance of circadian rhythm, and depressed adolescents have been shown to have altered rest-activity rhythms (52).

Interestingly, we observed a substantial drop in plasma melatonin concentration after puberty, an effect that has been previously reported in humans (53, 54). Circulating melatonin has been shown to be low in depressed patients, and positively affected by SSRI treatment (55, 56). Corroborating our effects reported here, a diet low in omega-3 PUFAs has been shown to decrease plasma melatonin in hamsters (57), and exogenous melatonin and melatonin receptor agonists administered to rats and mice exert antidepressant-like effects in the FST (58, 59) possibly through a central serotonin-dependent mechanism (60). Both serotonin and melatonin receptor systems have been linked to the expression of BDNF in the brain (61, 62), an important neural growth factor implicated in the neurotrophin theory of depression (63).

In accordance with the increases in plasma melatonin and serotonin, there was a higher plasma concentration of BDNF detected in sufficient animals from this study relative to their deficient counterparts. As with serotonin, but not melatonin, this increase was only observed in the sufficient group, and not maintained in preweaning sufficient or rescued in postweaning sufficient animals. This suggests that the effect of dietary DHA supplementation on plasma BDNF was dependent on its actions in early and late development. Furthermore, in alignment with melatonin and serotonin, plasma BDNF concentrations were lower after puberty, a phenomenon also reported in humans (64). Circulating levels of BDNF correlate well with the expression of BDNF in brain, at least in rodents (65), thus providing a convenient indicator of central BDNF status. It also appears that there is evidence, albeit controversial, that BDNF can cross the blood-brain barrier (66, 67). Interestingly, peripheral administration of BDNF in mice reportedly decreased immobility in the FST after 2 weeks of treatment (68) suggesting that circulating BDNF may have a functional effect on behavioral measures of mood. BDNF has a prominent role in shaping neurotransmission and plasticity in the brain by aiding in the growth, maintenance, and survival of neurons (69, 70), including the normal development and function of serotonin neurons (71). Deficits of neural plasticity appear to be associated with depression (72). Accordingly, peripheral BDNF has been shown to be lower in depressed patients as compared with healthy counterparts, and these levels rise with antidepressant

5-HT₁A serotonin receptor antagonist (49). This suggests that optimal DHA status may enhance serotonergic neurotransmission, thereby improving depressive-like behaviors. Our data extend this previous study to suggest that optimal DHA status during both early and late development is important for measures of juvenile mood and peripheral indicators of serotonin function following puberty. Interestingly, 5-HT₁A receptors are highly expressed at birth in humans but decline rapidly in expression through adolescence (50, 51). Thus, it is intriguing to speculate that this decline is somehow buffered by enhanced levels of DHA in brain, perhaps by facilitating 5-HT₁A receptor activity.
BNF immunoreactivity was detected in postmortem samples from patients who were treated with antidepressants (75). BDNF protein levels are highest in the hippocampus and hypothalamus, two brain regions that exhibit a high degree of plasticity and are essential elements of the neuroendocrine response to stress (76, 77). The hypothalamus in particular also plays a critical role in the onset, duration, and completion of puberty (78, 79). Thus, increases in the levels of hypothalamic and hippocampal BDNF content may provide an adolescent enhanced resiliency to stressors and negative mood states during a time of great physical, mental, and hormonal change.

In this study, dietary DHA supplementation throughout early and late development increased postpubertal hippocampal and hypothalamic BDNF protein levels relative to deficient offspring. Loss of dietary DHA after weaning resulted in BDNF protein levels no different than deficient animals; however, preweaning deficient animals placed on a postweaning DHA-supplemented diet had increased BDNF protein levels in the hippocampus but not the hypothalamus. The effects seen in hypothalamus are similar to the observations of plasma BDNF concentrations in this study. This suggests that BDNF protein expression in the hypothalamus is dependent upon effects of DHA during both early and late development, but that hippocampal BDNF is sensitive to current DHA tissue status and/or late developmental effects of DHA. Other studies have also reported an effect of an omega-3 sufficient diet during development on brain levels of BDNF protein. For example, rats fed DHA-adequate diets from gestation to 18 weeks old had elevated BDNF protein in the hippocampus and hypothalamus (80), and those fed an ω-6-linolenic acid sufficient diet from weaning to 18 weeks old had elevated BDNF protein expression in the frontal cortex (81) compared with animals on deficient diets. In addition, fish oil supplementation during gestation and lactation increased hippocampal and cortical BDNF protein levels in 21-day-old and 90-day-old rats (49). Our data are in line with these reports but suggest that the effects of dietary DHA during development on brain BDNF protein levels are likely dependent on the timing of DHA supplementation in a brain region-specific manner. These changes in BDNF protein expression may have contributed to the behavioral phenotype observed in the FST because acute infusion of BDNF into the midbrain or hippocampus has been shown to decrease depression-like behavior in the FST (82, 83).

Our study expands upon those described previously that have examined the effect of dietary omega-3 PUFA consumption, or lack thereof, during development on depression-like behaviors in rats. Both Naliwaiko et al. (84) and Vines et al. (49) reported that fish oil supplementation throughout gestation, lactation, and after weaning resulted in male offspring that exhibited reduced immobility in the FST compared with controls when tested as adults. In addition, studies by Ferraz et al. (85) and DeMar et al. (86) indicated that feeding male rats diets supplemented with fish oil or ω-6-linolenic acid, respectively, after weaning (P21) into adulthood for 6 months decreased FST immobility compared with unsupplemented controls. On the other hand, McNamara et al. (87) reported that feeding omega-3 PUFA-sufficient diets from weaning to adulthood (P21–P90) in female rats had no effect on FST behavior compared with omega-3 PUFA-insufficient controls. This suggests that there may be a sex difference in the effect of postweaning omega-3 PUFA supplementation on depressive-like behaviors. However, similar to the females in McMamara et al. (87), we did not observe a benefit of postweaning DHA on time spent immobile in male offspring when examined at P60. Taken together with the previous studies, this suggests that perhaps postweaning omega-3 PUFA supplementation is most beneficial in males when provided past adolescence into adulthood or when behaviors are assessed in adulthood rather than late adolescence. What is unknown is whether pre- and postweaning omega-3 PUFA supplementation in female offspring when examined at P60. Taken together with the previous studies, this suggests that perhaps postweaning omega-3 PUFA supplementation is most beneficial in males when provided past adolescence into adulthood or when behaviors are assessed in adulthood rather than late adolescence. What is unknown is whether pre- and postweaning omega-3 PUFA supplementation in female offspring when examined at P60. Taken together with the previous studies, this suggests that perhaps postweaning omega-3 PUFA supplementation is most beneficial in males when provided past adolescence into adulthood or when behaviors are assessed in adulthood rather than late adolescence. What is unknown is whether pre- and postweaning omega-3 PUFA supplementation in female offspring when examined at P60. Taken together with the previous studies, this suggests that perhaps postweaning omega-3 PUFA supplementation is most beneficial in males when provided past adolescence into adulthood or when behaviors are assessed in adulthood rather than late adolescence. It is common to identify patterns in the data that are not related to the effects of the intervention, such as differences in baseline characteristics or other confounding factors. Additionally, the study may have been underpowered to detect smaller effects or differences in behavior between groups. The limitations of the study include the relatively small sample size, which may have limited the power to detect subtle differences. Furthermore, the study only examined male offspring, and it is unclear whether the results would be generalizable to female offspring or other populations. Finally, the study did not control for potential confounding factors, such as maternal diet, which may have influenced the results. Despite these limitations, the study provides valuable insights into the effects of DHA supplementation during development on adolescent behavior.
161 healthy adolescents, there was a higher percentage of DHA, but not EPA (20:5n3), present in the RBCs from healthy controls as compared with their depressed counterparts (28). Interestingly, there was an increase in RBC α-linolenic acid content in depressed adolescents suggesting that the conversion of α-linolenic acid to EPA, which is already inefficient in humans (~0.1% conversion), may be impaired in these individuals. In a cross-sectional study, DHA content was lower (−16%) in the RBCs of depressed teenagers with eating disorders than those with eating disorders and no depressive symptoms (91). In a cross-sectional study of Japanese teenagers ages 12 to 15, there was an inverse correlation of EPA plus DHA intake with symptoms of depression in boys, but not girls (5). The lifetime major depressive disorder prevalence rates in Japan are some of the lowest in the world (92). However, to achieve time major depressive disorder prevalence rates in Japan teenagers with eating disorders than those with eating disorders is emerging yet limited, but given the intervention role of DHA and omega-3 PUFAs on adolescent depression, a supposition in alignment with the results of our study.

The data reported here present a totality of pubertal changes in depression-like behaviors and central and peripheral biomarkers of depression in male rats and strongly suggest that these changes can be positively modulated by DHA supplementation throughout development. These data highlight the particular importance of dietary supplementation with preformed DHA after weaning, a time when most infants are transitioned to foods containing low levels of omega-3 PUFAs, a trend that continues onward into adulthood. The clinical evidence regarding the role of DHA and omega-3 PUFAs on adolescent depression is emerging yet limited, but given the intervention and epidemiological data currently available and potential societal impact, further studies are certainly warranted.

REFERENCES

1. Petersen, A. C. 1988. Adolescent development. Annu. Rev. Psychol. 39: 583–607.
2. Merikangas, K. R., J. P. He, M. Burstein, S. A. Swanson, S. Avenevoli, L. Cui, C. Benjet, K. Georgiades, and J. Swendsen. 2010. Lifetime prevalence of mental disorders in US adolescents: results from the National Comorbidity Survey Replication–Adolescent Supplement (NCS-A). J. Am. Acad. Child Adolesc. Psychiatry. 49: 980–989.
3. Kessler, R. C., S. Avenevoli, and K. Ries Merikangas. 2001. Mood disorders in children and adolescents: an epidemiologic perspective. Biol. Psychiatry. 49: 1002–1014.
4. Bridge, J. A., S. Iyengar, C. B. Salary, R. P. Barbe, B. Birmaher, H. A. Pincus, L. Ren, and D. A. Brent. 2007. Clinical response and risk for reported suicidal ideation and suicide attempts in pediatric antidepressant treatment: a meta-analysis of randomized controlled trials. J. Am. Med. Assoc. 297: 1683–1696.
5. Murakami, K., Y. Miyake, S. Sasaki, K. Tanaka, and M. Arakawa. 2010. Fish and n-3 polysaturated fatty acid intake and depressive symptoms: Ryukyus Child Health Study. Pediatr. 126: e629–e630.
6. Jacka, F. N., P. J. Kremer, E. R. Leslie, M. Berk, G. C. Patton, J. W. Toubourour, and J. W. Williams. 2010. Associations between diet quality and depressed mood in adolescents: results from the Australian Healthy Neighbourhoods Study. Aust. N. Z. J. Psychiatry. 44: 435–442.
7. Mayberg, H. S., M. Iotti, S. K. Braman, S. McGinnis, R. K. Mahurin, P. A. Jerabek, J. A. Silva, J. L. Tekell, C. C. Martin, J. L. Lancaster, et al. 1999. Reciprocal limbic-cortical function and negative mood: converging PET findings in depression and normal sadness. Am. J. Psychiatry. 156: 675–682.
8. Giedd, J. N., J. Blumenthal, N. O. Jeffries, F. X. Castellanos, H. Liu, A. Zijdenbos, T. Paus, A. C. Evans, and J. L. Rapoport. 1999. Brain development during childhood and adolescence: a longitudinal MRI study. Nat. Neurosci. 2: 861–863.
9. Innis, S. M. 2007. Dietary (n-3) fatty acids and brain development. J. Nutr. 137: 855–859.
10. Blasbalg, T. L., J. R. Hibbelen, C. E. Ramsden, S. F. Majchrzak, and R. R. Rawlings. 2011. Changes in consumption of omega-3 and omega-6 fatty acids in the United States during the 20th century. Am. J. Clin. Nutr. 93: 950–962.
11. Klerman, G. L., and M. M. Weissman. 1989. Increasing rates of depression. J. Am. Med. Assoc. 261: 2229–2235.
12. Hibbeln, J. R. 1998. Fish consumption and major depression. *Lancet* 351: 1213.
13. Arterburn, L. M., E. B. Hall, and H. Oken. 2006. Distribution, interconversion, and dose response of n-3 fatty acids in humans. *Am. J. Clin. Nutr.* 83 (6, Suppl.): 1467S–1476S.
14. Matzka, M. Z. 1992. Tissue levels of polyunsaturated fatty acids during early human development. *J. Pediat.* 120: S129–S138.
15. Grandgirard, A., J. M. Bourre, F. Julliard, P. Homayoun, O. Dumont, M. Piciotti, and J. L. Sebedio. 1994. Incorporation of trans-long chain n-3 polyunsaturated fatty acids in rat brain structures and retina. *Lipids* 29: 251–258.
16. Bourre, J. M., M. Bonneil, J. Chaudiere, M. Clement, O. Dumont, G. Durand, H. Lafont, G. Nalbone, G. Pascal, and M. Piciotti. 1992. Structural and functional importance of dietary polyunsaturated fatty acids in the nervous system. *Adv. Exp. Med. Biol.* 318: 211–229.
17. Salem, N., Jr., H-Y. Kim, and J. A. Yergey. 1986. Docosahexaenoic acid: membrane function and metabolism. In *Health Effects of Polyunsaturated Fatty Acids in Seafoods*. A. P. Simopoulos, R. R. Colombo, J. Anderson, O. M. Blaga, and S. E. Carlson. 2004. Visual acuity and the essentiality of docosahexaenoic acid and DHA in the human visual system of formula-fed term infants weaned to formula with or without long-chain polyunsaturates at 4 to 6 months: a randomized clinical trial. *J. Pediat.* 142: 660–677.

18. Lin, Y. H., A. Llanos, P. Mena, R. Uauy, N. Salem, Jr., and R. J. Pawlsky. 2010. Componental analyses of 24S-α-linolenic acid and α-linolenic acid supplementation and conversion to n-3 long-chain polyunsaturated fatty acids in humans. *Prostaglandins Leukot. Essent. Fatty Acids* 80: 85–91.
19. Brennan, J. T., N. Salem, Jr., A. J. Sinclair, and S. C. Cunnane. 2009. International Society for the Study of Fatty A. L. M. Bucolo, F. Drago, and F. Caraci. 2014. Role of omega-3 fatty acids in the rat forced-swim test model the human response to antidepressant treatment for pediatric depression. *Psychopharmacology (Berl.)* 187: 433–441.
20. Reed, A. L., H. K. Harpe, F. Petty, and D. B. Bryun. 2008. Juvenile rats in the forced-swim test. *Neurosci. Biobehav. Rev.* 29: 547–569.
21. Cryan, J. F., M. E. Page, and I. Lucki. 2005. Differential behavioral effects of the antidepressants reboxetine, fluoxetine, and moclobemide in a modified forced-swim test following chronic treatment. *Psychopharmacology (Berl.)* 182: 355–344.
22. Detke, M. J., M. Rickels, and I. Lucki. 1995. Active behaviors in the rat forced-swimming test differentially produced by serotoninergic and noradrenergic antidepressants. *Psychopharmacology (Berl.)* 121: 66–72.
51. Dillon, K. A., R. Gross-Isseroff, M. Israeli, and A. Biegon. 1991. Autoradiographic analysis of serotonin 5-HT1A receptor binding in the human brain postmortem: effects of age and alcohol. Brain Res. 554:50–64.

52. Teicher, M. H., C. A. Olof, D. Harper, E. Magnus, C. Brasher, F. Wen, and K. Palhaim. 1993. Locomotor activity in depressed children and adolescents. I. Circadian dysregulation. J. Am. Acad. Child Adolesc. Psychiatry. 32:760–769.

53. Silman, R. E., R. M. Leone, R. J. Hooper, and M. A. Preece. 1979. Melatonin, the pineal gland and human puberty. Nature. 282:301–303.

54. Waldhauser, F., G. Weissenbacher, H. Frisch, U. Zeitlhuber, M. Waldhauser, and R. J. Wurtman. 1984. Fall in nocturnal serum melatonin during prepuberty and pubescence. Lancet. 1:362–365.

55. Beck-Friis, J., D. von Rosen, B. F. Kjellman, J. G. Ljunggren, and G. Lu. 2003. BDNF and activity-dependent synaptic modulation. Melatonin, the pineal gland and human puberty. Nature. 282:301–303.

56. Carvalho, L. A., C. Gorenstein, R. Moreno, C. Pariente, and R. P. Markus. 2009. Effect of antidepressants on melatonin metabolite in depressed patients. J. Psychopharmacol. 23:475–489.

57. Lavialle, M., G. Champeil-Potokar, J. M. Alessandri, L. Balasse, P. Guesnet, C. Papillon, P. Pevet, S. Vancassel, B. Vivien-Roels, and G. Lu. 2003. BDNF and activity-dependent synaptic modulation. Melatonin, the pineal gland and human puberty. Nature. 282:301–303.

58. Lu, B. 2003. BDNF and activity-dependent synaptic modulation. Brain Res. 995:99–106.

59. Lyons, W. E., L. A. Mamouns, G. A. Ricarte, V. Coppola, S. W. Redd, S. H. Boyer, C. Whiler, V. E. Koliniotis, and L. T. Lapid. 1999. Brain-derived neurotrophic factor-deficient mice develop aggressiveness and hyperaggression in conjunction with brain serotoninergic abnormalities. Proc. Natl. Acad. Sci. USA. 96:1529–1534.

60. Duman, R. S., J. Malberg, and J. Thome. 1999. Neural plasticity to stress and antidepressant treatment. Biol. Psychiatry. 46:1181–1191.

61. Karege, F., G. Perret, G. Bondolfi, M. Schwald, G. Bertschy, and J. M. Aubry. 2002. Decreased serum brain-derived neurotrophic factor levels in major depressed patients. Psychiatry Res. 109:143–148.

62. Sen, S., R. Duman, and G. Sanacora. 2008. Serum brain-derived neurotrophic factor, depression, and antidepressant medications: meta-analyses and implications. Biol. Psychiatry. 64:527–532.

63. Chen, B., D. Dowlatshahi, G. M. MacQueen, J. F. Wang, and L. T. Young. 2001. Increased hippocampal BDNF immunoreactivity in subjects treated with antidepressant medication. Biol. Psychiatry. 50:260–263.

64. Herman, J. P., C. M. Prevett, and W. E. Cullinan. 1996. Neuronal circuit regulation of the hypothalamic-pituitary-adrenocortical stress axis. Crit. Rev. Neurobiol. 10:371–394.

65. Yan, Q., R. D. Rosenfled, C. R. Matheson, N. Hawkins, O. T. Lopez, L. Bennett, and A. A. Welcher. 1997. Expression of brain-derived neurotrophic factor protein in the adult rat central nervous system. Neuroscience. 78:431–448.

66. Plant, T. M., V. L. Gay, G. R. Marshall, and M. Arslan. 1987. Puberty in monkeys is triggered by chemical stimulation of the hypothalamus. Proc. Natl. Acad. Sci. USA. 84:2506–2510.

67. Shahab, M., C. Mastronardi, S. B. Seminara, W. F. Crowley, S. R. Ojeda, and T. M. Plant. 2005. Increased hypothalamic GPR34 signaling: a potential mechanism for initiation of puberty in primates. Proc. Natl. Acad. Sci. USA. 102:2129–2134.

68. Bhatia, H. S., R. Agrawal, S. Sharma, Y. X. Huo, Z. Ying, and F. Gomez-Pinilla. 2011. Omega-3 fatty acid deficiency during brain maturation reduces neuronal and behavioral plasticity in adulthood. PLoS ONE. 6(12):e28978.

69. Rao, J. S., R. N. Ertley, H. J. Lee, J. C. DeMar, Jr., J. T. Arnold, S. Naliwaiko, K., R. L. Araujo, R. V. da Fonseca, J. C. Castilho, R. Ojeda, and T. M. Plant. 2005. Increased hypothalamic GPR34 signaling: a potential mechanism for initiation of puberty in primates. Proc. Natl. Acad. Sci. USA. 102:2129–2134.

70. Siuciak, J. A., D. R. Lewis, S. J. Wiegand, and R. M. Lindsay. 1997. Antidepressant-like effect of brain-derived neurotrophic factor (BDNF). Pharmacol. Biochem. Behav. 56:131–137.

71. Naliwaiko, K., R. L. Araujo, R. V. da Fonseca, J. C. Castilho, R. Ojeda, and T. M. Plant. 2005. Increased hypothalamic GPR34 signaling: a potential mechanism for initiation of puberty in primates. Proc. Natl. Acad. Sci. USA. 102:2129–2134.

72. DeMar, J. C., Jr., K. Ma, J. M. Bell, M. Igarashi, D. Greenstein, and J. W. Lipton. 2013. Omega-3 fatty acid deficiency does not affect the effects of chronic fluoxetine treatment on central serotonin tone or behavior in the forced swim test in female rats. Pharmacol. Biochem. Behav. 114–115:1–8.

73. Ferraz, A. C., A. M. Delattre, R. G. Almendra, M. Sonagli, C. Borges, P. Araujo, M. L. Andersen, S. Tufik, and M. M. Lima. 2011. Chronic omega-3 fatty acids supplementation promotes beneficial effects on anxiety, cognitive and depressive-like behaviors in rats subjected to a restraint stress protocol. Behav. Brain Res. 212:119–122.

74. Ferraz, A. C., M. Shahab, M. A. Kiss, F.绿色-Pinilla. 2011. Omega-3 fatty acid deficiency during brain maturation reduces neuronal and behavioral plasticity in adulthood. PLoS ONE. 6(12):e28978.

75. Shaw, A. V., and S. K. Kulkarni. 1998. Central nervous system depression: mechanisms of activity in mice and rats. Indian J. Exp. Biol. 36:257–263.

76. Vaidya, V. A., G. J. Marek, G. K. Aghajanian, and R. S. Duman. 1997. 5-HT2A receptor-mediated regulation of brain-derived neurotrophic factor mRNA in the hippocampus and the neocortex. J. Neurosci. 17:2785–2795.

77. Lu, B. 2003. BDNF and activity-dependent synaptic modulation. Brain Res. 995:99–106.

78. Lyons, W. E., L. A. Mamouns, G. A. Ricarte, V. Coppola, S. W. Redd, S. H. Boyer, C. Whiler, V. E. Koliniotis, and L. T. Lapid. 1999. Brain-derived neurotrophic factor-deficient mice develop aggressiveness and hyperaggression in conjunction with brain serotoninergic abnormalities. Proc. Natl. Acad. Sci. USA. 96:1529–1534.

79. Duman, R. S., J. Malberg, and J. Thome. 1999. Neural plasticity to stress and antidepressant treatment. Biol. Psychiatry. 46:1181–1191.

80. Karege, F., G. Perret, G. Bondolfi, M. Schwald, G. Bertschy, and J. M. Aubry. 2002. Decreased serum brain-derived neurotrophic factor levels in major depressed patients. Psychiatry Res. 109:143–148.
current status, future directions, and dietary recommendations. 
*Prostaglandins Leukot. Essent. Fatty Acids*. **81**: 223–231.

94. Martins, J. G. 2009. EPA but not DHA appears to be responsible for the efficacy of omega-3 long chain polyunsaturated fatty acid supplementation in depression: evidence from a meta-analysis of randomized controlled trials. *J. Am. Coll. Nutr.* **28**: 525–542.

95. Mozaffari-Khosravi, H., M. Yassini-Ardakani, M. Karamati, and S. E. Shariati-Bafghi. 2013. Eicosapentaenoic acid versus docosahexaenoic acid in mild-to-moderate depression: a randomized, double-blind, placebo-controlled trial. *Eur. Neuropsychopharmacol.* **23**: 636–644.

96. Zhao, Y., S. Joshi-Barve, S. Barve, and L. H. Chen. 2004. Eicosapentaenoic acid prevents LPS-induced TNF-alpha expression by preventing NF-kappaB activation. *J. Am. Coll. Nutr.* **23**: 71–78.

97. Chen, C. T., and R. P. Bazinet. β-oxidation and rapid metabolism, but not uptake regulate brain eicosapentaenoic acid levels. *Prostaglandins Leukot. Essent. Fatty Acids*. Epub ahead of print. June 5, 2014; doi:10.1016/j.plefa.2014.05.007.

98. Makrides, M., M. A. Neumann, R. W. Byard, K. Simmer, and R. A. Gibson. 1994. Fatty acid composition of brain, retina, and erythrocytes in breast- and formula-fed infants. *Am. J. Clin. Nutr.* **60**: 189–194.