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Omega-3 Fatty Acid Plasma Levels Before and After Supplementation: Correlations with Mood and Clinical Outcomes in the Omega-3 and Therapy Studies

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Abstract

Objective: To examine fatty acid profiles, their response to omega-3 fatty acid (Ω3) supplementation, and associations with clinical status and treatment response in youth with mood disorders.

Methods: In a placebo-controlled 2X2 design, 7–14 year-olds ($N = 95$) in parallel pilot trials (depression $N = 72$; bipolar $N = 23$) were randomly assigned to 12 weeks of Ω3 supplementation (1.4 g eicosapentaenoic acid [EPA], 0.2 g docosahexaenoic acid [DHA], and 0.27 g other Ω3 per day); psychoeducational psychotherapy (PEP); their combination; or placebo (mainly oleic and linoleic acid) alone. Blood was drawn at baseline ($N = 90$) and endpoint ($n = 65$). Fatty acid levels were expressed as percent of total plasma fatty acids. Correlational and moderator/mediator analyses were done with SPSS Statistics 23.

Results: *At baseline:* (1) DHA correlated negatively with alpha-linolenic acid (ALA) ($r = -0.23$, $p = 0.029$); (2) Arachidonic acid (AA, Ω6) correlated negatively with global functioning ($r = -0.24$, $p = 0.022$); (3) Total Ω3 correlated negatively with age ($r = -0.22$, $p = 0.036$) and diastolic blood pressure ($r = -0.31$, $p = 0.006$). *Moderation:* Baseline ALA moderated response to Ω3 supplementation: ALA levels above the sample mean (lower DHA) predicted significantly better placebo-controlled response ($p = 0.04$). *Supplementation effects:* Compared to placebo, 2 g Ω3 per day increased EPA blood levels sevenfold and DHA levels by half (both $p < 0.001$). Body weight correlated inversely with increased EPA ($r = -0.52$, $p = 0.004$) and DHA ($r = -0.54$, $p = 0.003$) and positively with clinical mood response. *Mediation:* EPA increase baseline-to-endpoint mediated placebo-controlled global function and depression improvement: the greater the EPA increase, the less the placebo-controlled Ω3 improvement.

Conclusion: Ω3 supplementation at 2 g/day increases blood levels substantially, more so in smaller children. A possible U-shaped response curve should be explored.

Keywords: mood disorders, omega-3 fatty acids, supplementation as treatment, plasma levels, moderation, mediation

Introduction

There is considerable interest in the affective, behavioral, and cognitive benefits of omega-3 (Ω3) fatty acids, both in adults and children. However, some fundamental questions, including the optimal dose for treating various conditions, ratio of the two main long chain Ω3 fatty acids (eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA]), ratio of Ω3 to Ω6 fatty acids, expectable effect sizes, relationship of blood fatty acid levels to clinical symptom levels, and relationship of baseline blood levels to treatment response, remain unanswered. Addressing such questions requires an understanding of fatty acid biochemistry and physiology.

Essential fatty acids (EFAs) are required for growth, maintenance, and repair of tissues and cannot be manufactured *de novo* by humans (Lawrence 2010; Arnold et al. 2013). A saturated bond is a single bond between adjacent carbon atoms, with two hydrogen atoms on each carbon (i.e., saturated with hydrogen) (Lawrence 2010). Desaturation substitutes a double bond between carbons at the expense of hydrogen atoms (Lawrence 2010). Fatty acids with two or more double bonds are polyunsaturated fatty acids (PUFAs).

Humans cannot desaturate fatty acids at carbons 3 and 6 from the methyl (or omega) end of the hydrocarbon. Thus, the two EFAs that humans cannot synthesize are alpha-linolenic acid (ALA, 18:3 Ω3), an 18-carbon chain with three unsaturated bonds and the first

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unsaturated bond three carbons from the noncarboxyl "tail" (Ω3), and linoleic acid (LA, 18:2 Ω6), an 18-carbon chain with two unsaturated bonds, the first unsaturated bond six carbons from the tail, Ω6 (Lawrence 2010). Once the sixth carbon is unsaturated (by plants), additional unsaturated bonds can, to a modest extent, be added at every three carbons (at 9, 12, 15, etc.), with addition of two carbons at the carboxyl head between desaturations (Sinclair 1990).

For example, desaturation and elongation of the 18-carbon Ω3 alpha-linolenic acid (ALA, with three unsaturated bonds), can theoretically produce, through numerous steps, the 20-carbon five-unsaturated-bond EPA and then the 22-carbon six-unsaturated-bond DHA (Brookes et al. 2006). The longer-chain, more unsaturated acids thus produced are considered in the Ω3 or Ω6 series depending on the location of the first unsaturated bond from the tail. Although this anabolic route contributes relatively little, with most of the longer-chain acids coming directly from the diet, it may become important in a dietary context deficient in the longer-chain acids. The balance between Ω3 and Ω6 may be important, because the two series compete for the fatty acid desaturase (FADS) enzymes (Simopoulos 2010, 2016).

Humans evolved in a food environment with a ratio of ~1 Ω3 to 1 Ω6 molecules; but the modern Western diet has a ratio of 1 to 5–30, indicating that current diets are unbalanced, relatively deficient in Ω3 (Singh 2005; Bradbury 2011; Gomez Candela et al. 2011; Simopoulos 2011), possibly neutralizing the value of ingested Ω3 ALA (Simopoulos 2016). It is also possible that some individuals have a genetic deficiency of FADS, leading to the same EPA/DHA deficiency or complicating the dietary imbalance (Glaser et al. 2010). In the context of cardiovascular health, Harris (2006) argues convincingly that the ratio of total Ω6 to total Ω3 is not relevant since many things affect desaturase and elongase activities and hence the formation of arachidonic acid (AA), docosapentaenoic acid (DPA), EPA, and DHA. Instead, the ratio of AA to EPA/DHA may be more relevant since these molecules are precursors for eicosanoids involved with inflammation and resolution of inflammation.

The possible imbalance of AA to EPA/DHA, either in the diet or through altered metabolism, has several implications for brain function, because (1) DHA ideally constitutes ~33% of the fatty acids in the cerebral cortex (the dry weight of the brain being 60% phospholipids), optimizing membrane fluidity and receptor nesting sites (Chang et al. 2009; Bradbury 2011); and (2) EPA is also the precursor for the series 3 eicosanoids (prostaglandins, thromboxanes, and leukotrienes), which are anti-inflammatory and necessary for cell communication (Neuringer and Connor 1986; Singh 2005; Chang et al. 2009; Bradbury 2011). The Ω6 20-carbon four-unsaturated-bond AA is the precursor of the pro-inflammatory two series of eicosanoids (Harris et al. 2009; Lenihan-Geels et al. 2013). AA is important for brain health/growth in its own right (Henriksen et al. 2008), and extra may also be incorporated into neuronal membranes as a substitute for EPA and DHA when the latter are deficient; it will work, but not as well as the optimal proportion of each.

Another series of eicosanoids is series 1, which come from 20-carbon dihomo-gamma-linolenic acid, the next metabolite from gamma-linolenic acid, an 18-carbon three-unsaturated-bond ingredient in some EFA supplements (Sinclair 1990); series 1 eicosanoids have both anti-inflammatory and proinflammatory actions (Harris et al. 2009; Lenihan-Geels et al. 2013). Several psychiatric disorders are thought to involve excess inflammation and/or deficiency of Ω3 fatty acids (Kaplan et al. 2015; Rechenberg 2016). Laboratory animal and human data, including some meta-analyses, have suggested a benefit of Ω3 supplementation for cognition and mood (Richardson 2003; Martins 2009; Bloch and Qawasmi 2011; Sarris et al. 2012).

The Omega-3 and Therapy Studies (OATS) were a pair of 2X2 pilot randomized clinical trials of omega-3 fatty acids (Ω3) and psychoeducational psychotherapy (PEP) for youth (age 7–14 years) with mood disorders. In addition, all subjects were provided a daily multivitamin. The bipolar study randomized 23 (target $N=60$); the depression study randomized 72 (target $N=60$). Primary and secondary clinical results are reported elsewhere. Briefly,

- In the bipolar study, depression symptoms improved more with the combination of Ω3 and PEP than with placebo (PBO) ($p=0.01$, $d=1.7$); effect of Ω3 alone on depression was medium ($d=0.48$) (Fristad et al. 2015).
- In the depression study, maternal history of depression and psychosocial stress moderated treatment response such that those with maternal depression or low levels of family psychosocial stress responded significantly better to both treatments (compared to PBO) than those with no maternal depression or high levels of family stress. Specifically, with high psychosocial stress or no maternal depression, all four treatment assignments, including PBO, showed significant improvement ($p=0.01$–0.001), while with maternal depression or low psychosocial stress, only the three active treatments (Ω3, PEP, and the combination) did (Fristad et al. 2016).
- In 48 depressed children whose parents completed an omnibus measure of externalizing behaviors at each assessment, the combined treatment group showed more improvement than the PBO group ($p=0.022$, $d=0.80$), with Ω3 and PEP monotherapy groups in between (Young et al. 2016).
- In 38 depressed children whose parents completed the Eyberg Child Behavior Inventory, combined treatment showed more improvement than PBO on the Intensity Scale ($p=0.012$, $d=1.07$) with Ω3 and PEP monotherapies intermediate (Young et al. 2016).

This study sought to examine the relationship of blood fatty acids before treatment and at the end of treatment in 95 children randomized to an Ω3 supplement or PBO. The following hypotheses were tested:

1. Participants who were supplemented with Ω3, but not those receiving PBO, would have a significant increase in EPA and DHA levels from baseline to endpoint.
2. Baseline EPA and DHA levels would correlate inversely with baseline clinical measures of depressive and manic symptoms. Because blood levels are quoted in percent of total blood fatty acids, exploratory analyses would show opposite effects for Ω6 fatty acids.
3. Baseline Ω3 fatty acid blood levels would moderate response to Ω3 supplementation. Exploratory analyses would show opposite effects for Ω6 fatty acids.
4. Increases in EPA and DHA would correlate with improvement in clinical mood measures.

Methods

Participants were recruited from community advertisements and clinician referrals in a large Midwestern city. Parents provided written informed consent and youth, written assent, with forms and procedures approved by the local Institutional Review Board.

Inclusion criteria: (1) age 7–14 years; (2) diagnosis of Diagnostic and Statistical Manual of Mental Disorders, 4th edition, Text

Revision (DSM-IV-TR) major depression, dysthymic disorder, depressive disorder not otherwise specified (NOS), bipolar disorder NOS, or cyclothymia; and (3) for those in the depression study, clinically significant symptom severity [≥40 on the Child Depression Rating Scale-Revised (CDRS-R) (Poznanski et al. 1984)].

Exclusion criteria: (1) inability to swallow capsules the size of the study supplement; (2) a major medical disorder; (3) DSM-IV-TR autistic disorder; (4) psychosis warranting antipsychotic medication; (5) active suicidal concern (i.e., suicidal plans or recent attempt); (6) ≥3 ''marked'' or ''severe'' mood symptoms on the Kiddie Schedule for Affective Disorders and Schizophrenia (KSADS) for youth with BP-NOS/cyclothymia; (7) mental health intervention (psychotherapy or pharmacotherapy other than stable medication for attention deficit/hyperactivity disorder or a sleep aid) or Ω3 in the month preceding randomization; (8) enrollment in the ninth grade or higher; or (9) intellectual disability (IQ <70 and impaired adaptive functioning).

Of 178 youth screened, 72 (37 Major Depressive Disorder; 5 Dysthymic Disorder; 30 Depressive Disorder NOS) were randomized into the depression study (Clinicaltrials.gov Identifier: NCT01341925) and 23 (11 BP-NOS; 12 cyclothymic disorder) into the bipolar study (ClinicalTrials.gov identifier: NCT01341925). The CONSORT charts were published earlier (Fristad et al. 2015; Fristad et al. 2016).

Procedures

At the screening assessment, history and physical examinations by a licensed physician or nurse practitioner ruled out medical disorders. After baseline assessment, eligible youth were randomized into one of four treatment arms: PEP+Ω3; Ω3 monotherapy; PEP monotherapy (with pill PBO); or PBO (without any active intervention). Families, regardless of randomized condition, participated in five follow-up assessments across 12 weeks of intervention at 2, 4, 6, 9, and 12 weeks following baseline assessment. Those randomized into one of the two PEP conditions participated in two therapy sessions (one parent, one child) per week. Families were financially compensated for completing assessments.

Treatment

PEP incorporates psychoeducation, family systems concepts, and cognitive-behavioral therapy techniques delivered in weekly parent sessions and weekly youth individual sessions, each lasting 45–50 minutes. Content of sessions for children include symptom identification, awareness of strengths, emotion recognition and regulation, understanding treatment components (medication, identifying school-based resources, and psychotherapy), development of coping strategies, cognitive restructuring, problem-solving skills, and verbal and nonverbal communication. Parent sessions cover parallel content to the child sessions (at an adult level), and also include coverage of school advocacy, symptom management, and self-care. (See Fristad et al. 2011 for additional details on session content.)

Ω3 supplements or PBO were provided in a pill box at each assessment after baseline. Both Ω3 and PBO capsules were provided by OmegaBrite Corporation (www.omegabrite.com; Las Vegas, NV). Groups receiving Ω3 took two capsules of 500 mg each (350 mg EPA: 50 mg DHA; 65 mg other Ω3) twice daily for a total daily dose of 2000 mg, 1860 mg Ω3. PBO groups received two PBO capsules twice daily containing oleic acid (40%), linoleic acid (LA, 42%), and palmitic acid (13%) matched to the Ω3 capsules for size, odor, and appearance. See Supplementary Table S1 for complete analysis of both preparations (Supplementary Data are available online at www.liebertpub.com/cap). Straka et al. (2015) found a significant accumulation of EPA and DHA in plasma within 1 month in adults receiving similar doses (1680 mg/day EPA+DHA, ~26 mg Ω3/kg body weight) compared to doses used here for children (1600 mg/day EPA+DHA, ~32 mg/kg). Levels remain elevated in plasma for up to 6 months if participants continue consuming supplements. Straka et al.'s (2015) findings in adults (fourfold plasma increase) suggest the dose in the present study should result in at least a twofold increase of Ω3 levels, although we might expect more EPA increase than DHA given the 7:1 formulation used here.

All participants were given an over-the-counter daily multivitamin/mineral tablet (Centrum Jr.) to standardize micronutrition; no other nutritional supplements were permitted. Adherence to taking the oil supplement was monitored by pill counts from returned pill boxes at each assessment. In addition, plasma fatty acid composition provides another check on adherence because it reflects fatty acid intake of the past few days to week, both dietary and supplementation with Ω3. Of course, genetic metabolic aberrations, such as FADS deficiency, may also influence fatty acid composition.

Randomization

Block randomization was not contingent on any demographic or clinical variables. Lab personnel not directly involved in the study generated the random allocation sequence and assigned participants a number linked with a treatment condition. These staff provided the appropriate study capsules to the family and notified the family if they were randomized to participate in PEP.

Study masking

Participants, interviewers, therapists, coordinator, and other study staff who had contact with families were masked to Ω3/PBO assignment. Interviewers completing study assessments were additionally masked to which participants were assigned to PEP. Following their final assessment, each family was informed by a sealed letter whether the youth had received Ω3 or PBO capsules without telling the staff involved in treatment and assessment.

Study assessments

Trained graduate students or postdoctoral researchers interviewed youth and caregivers separately.

Youth diagnoses.

At screen, psychiatric disorders were assessed using the structured Children's Interview for Psychiatric Syndromes Child and Parent Versions (ChIPS/P-ChIPS) (Fristad et al. 1998a, 1998b; Weller et al. 1999a, 1999b; Swenson et al. 2007). The K-SADS Depression Rating Scale (KDRS) and Mania Rating Scale (KMRS) (Chambers et al. 1985; Ambrosini et al. 1989; Geller et al. 2001) assisted in diagnosing a *DSM-IV-TR* mood disorder. All diagnoses were finalized during a clinical consensus conference.

Youth mood symptom severity.

The Children's Depression Rating Scale-Revised (CDRS-R) measures depression in youth ages 6–17 years. It has 17 items rated on a Likert scale in direction of increasing severity. Scores range from 17 to 113 with a score of ≥40 indicating a clinical elevation (Poznanski et al. 1984). The CDRS-R was completed at each study assessment. The 11-item

Young Mania Rating Scale (YMRS) was administered at each assessment and was a primary outcome measure for manic symptoms. The CDRS-R and YMRS provide ''unfiltered'' ratings, or ratings of symptom severity as it presented in the last 2 weeks, regardless of the presence of a mood episode, whereas the KDRS and KMRS are filtered, rated only during a mood episode. Both are reliable and valid measures (Young et al. 1978; Poznanski et al. 1984; Fristad et al. 1992, 1995; Youngstrom et al. 2003). (For a thorough discussion of ''filtered'' versus ''unfiltered'' ratings, see Yee et al. 2015).

Youth global function. The *Children's Global Assessment Scale (CGAS)* is a clinician rating of overall functioning at home, school, and in society; scores range from 1 to 100 with higher scores indicating better function (Shaffer et al. 1983). CGAS scores were rated by the interviewer—and confirmed in consensus conferences with a licensed psychologist or psychiatrist—based on information from the semi-structured interview.

Plasma fatty acid analysis

Total lipids were extracted from plasma samples with 2:1 (v/v) chloroform:methanol and washed with 0.88% KCl (Folch et al. 1957). Fatty acid methyl esters were prepared using 5% hydrochloric acid in methanol heated overnight at 76°C and extracted with hexane (Stoffel et al. 1959). Analysis of fatty acid methyl esters was completed by gas chromatography using a 30-m Omegawax TM 320 fused silica capillary column (Supelco, Bellefonte, PA). Oven temperature started at 175°C and increased at a rate of 3°C/min until reaching 220°C. Flow rate of the carrier gas helium was 30 mL/min. Retention times of samples were compared to standards for fatty acid methyl esters (Matreya, LLC, Pleasant Gap, PA, Supelco, Bellefonte, PA, and Nu-Check Prep, Inc., Elysian, MN). In keeping with the standard method in the field, *fatty acids are reported as percent of total identified fatty acids* (Belury and Kempa-Steczko 1997).

Statistical analyses

To assess whether youth who received the supplement experienced greater changes in PUFA levels pre- to post-treatment (increases in EPA and DHA, relative decreases in proportion of omega-6 fatty acids), regression analyses modeled the effects of treatment group (Ω3 supplementation vs. PBO) on endpoint PUFA levels, controlling for baseline PUFA levels. A correlation matrix including PUFA levels and clinical variables (KDRS, CDRS-R, YMRS, KMRS, and CGAS scores) was calculated to test the hypothesis that PUFAs would be cross-sectionally related to clinical outcomes.

To determine whether baseline PUFA levels moderated treatment effects, linear mixed effects (LMEs) models were fit to each of the main outcome variables (KDRS, CDRS-R, YMRS, KMRS, CGAS), using the intent-to-treat sample (only excluding five youth without baseline PUFA data). (KMRS scores were examined only among youth with a bipolar disorder diagnosis because those items pertain to symptoms occurring in the context of a manic episode, unlike the YMRS, which assesses symptom presentation regardless of manic episode status.) Intercepts and slopes were modeled as random effects; fixed effects were treatment group (dummy-coded relative to PBO+monitoring), time (weeks since randomization), baseline PUFA level, group × time, group × baseline PUFA level, baseline PUFA level × time, and group × time × baseline PUFA level. Significant three-way interactions indicated moderation effects, which were probed in simple slopes analyses using methods described by Preacher et al. (2006) and Hayes and Matthes (2009).

Similarly, among youth who had both baseline and endpoint PUFA data, the mediating effects of change in PUFA levels (endpoint–baseline level) on treatment outcomes were modeled using LME with a significant group × time × PUFA change interaction being indicative of mediation (Kraemer et al. 2002). Analyses were conducted using IBM SPSS Statistics version 22 or 23. For this exploratory study an alpha of 0.05 was used for significance.

Results

Sample characteristics

Characteristics of the sample are shown in Table 1. Because only 65 participants had the second blood draw (compared to 90 for baseline) and only 64 had blood drawn at both timepoints, we compared clinical outcome for those with missing plasma level data ($n = 31$) to the 64 with complete plasma levels. Compared to those with complete plasma data, those with only partial data were less likely to have attention-deficit/hyperactivity disorder (ADHD) (42% vs. 72%, $p = 0.008$), and, although not significantly older or taller, were heavier (58.9 kg vs. 45.2 kg, $p = 0.012$, with higher body mass index (BMI): 23.8 vs. 20.0, $p = 0.003$); they were marginally more likely to be girls (57% vs. 36%, $p = 0.058$).

Of the 26 with baseline but not endpoint plasma data, 21 did not come for final assessment and 5 declined or had an unsuccessful blood draw at final assessment. Compared to PBO, those with missing plasma data responded better to Ω3 supplementation on the KDRS ($p = 0.045$) than those with complete data, who showed similar improvement with both Ω3 and PBO. This does not affect analyses of baseline levels, but does affect analyses that involve changes in plasma levels relating to clinical results.

Change of plasma Ω3 proportions with supplementation (Hypothesis 1)

Compared to PBO (essentially no change), supplementation with 2 g Ω3 (1.4 g EPA, 200 mg DHA) per day nearly doubled mean Ω3 from 3.4% to 6.6% ($p < 0.001$) while decreasing mean Ω6 levels from 45.5% to 42.5% ($p = 0.001$). More specifically, supplementation with 2 g Ω3 increased mean EPA plasma levels sevenfold (0.3% to 2.2%) and DHA levels by half (2.0% to 2.8%) (Table 2), both $p < 0.001$, while decreasing mean AA levels from 7.5% to 6.6% ($p = 0.003$). Table 2 shows details.

Baseline correlations (Hypothesis 2)

Total saturated fatty acids constituted 30.8% and PUFAs 49% of the total measured plasma fatty acids. Total PUFAs correlated inversely with age ($r = -0.24$, $p = 0.024$) and weight ($r = -0.27$, $p = 0.010$). Baseline Omega-3 total, EPA, and DHA did not correlate significantly with clinical measures, but baseline DHA correlated inversely with ALA ($r = -0.23$, $p = 0.029$; $r = -0.24$, $p = 0.024$ after covarying age; $r = -0.23$, $p = 0.030$ after covarying weight).

Total Ω6 (LA, GLA, DHGLA, and AA) level correlated inversely with age ($r = -0.22$, $p = 0.036$; N.S. after covarying weight), weight ($r = -0.28$, $p = 0.008$; N.S. after covarying age), height ($r = -0.325$, $p = 0.002$; N.S. after covarying age or weight), and diastolic blood pressure ($r = -0.31$, $p = 0.006$; $r = -0.31$, $p = 0.008$ after covarying age; $r = -0.27$, $p = 0.020$ after covarying weight). AA correlated negatively with baseline CGAS (global function) ($r = -0.24$, $p = 0.022$; $r = -0.22$, $p = 0.037$ after covarying for age; $r = -0.22$, $p = 0.043$ after covarying for weight). Details are shown in Table 3.

Table 1. Characteristics of Sample (N=95; 90 with Baseline Plasma Fatty Acids, 65 with Endpoint Plasma Fatty Acids)

	Total (N=*95*)	*OATS-depression sample* (n=*72*)	*OATS-bipolar sample* (n=*23*)	*Blood draw status*[a]		p-*Value blood draw status difference*
				Baseline only (n=*26*)	*Baseline+ endpoint* (n=*64*)	
Baseline age, M±SD	11.3 ± 2.2	11.6±2.1	10.2±2.2	11.8±2.2	11.1±2.3	0.212
Sex: male, *n* (%)	54 (56.8)	41 (56.9)	13 (56.5)	11 (43.2)	41 (64.1)	0.058
Race, *n* (%)						
White	58 (61.1)	41 (56.9)	17 (73.9)	14 (53.8)	40 (62.5)	
Black/African American	25 (26.3)	22 (30.6)	3 (13.0)	10 (38.5)	14 (21.9)	0.368
Asian	1 (1.1)	0	1 (4.3)	0	1 (1.6)	
Bi- or multiracial	11 (11.6)	9 (12.5)	2 (8.7)	2 (7.7)	9 (14.1)	
Ethnicity: Hispanic, *n* (%)	7 (7.4)	7 (9.7)	0	1 (3.8)	6 (9.4)	0.375
Insurance: Medicaid, *n* (%)	32 (33.7)	23 (31.9)	9 (39.1)	10 (38.5)	22 (34.4)	0.714
Household income, *n* (%)						
<$20,000	18 (18.9)	13 (18.3)	5 (21.7)	6 (23.1)	11 (17.5)	
$20,000–40,000	22 (23.2)	19 (26.8)	3 (13.0)	7 (26.9)	14 (22.2)	
$40,000–60,000	17 (17.9)	13 (18.3)	4 (17.4)	3 (11.5)	14 (22.2)	0.648
$60,000–80,000	12 (12.5)	8 (11.3)	4 (17.4)	2 (7.7)	10 (15.9)	
$80,000–100,000	7 (7.4)	3 (4.2)	4 (17.4)	2 (7.7)	5 (7.9)	
>$100,000	18 (18.9)	15 (21.1)	3 (13.0)	6 (23.1)	9 (14.3)	
Comorbid disorders, *n* (%)						
Anxiety disorder	75 (78.9)	56 (77.8)	19 (82.6)	21 (80.8)	49 (76.6)	0.663
ADHD	58 (61.1)	41 (56.9)	17 (73.9)	11 (42.3)	46 (71.9)	0.008
DBD	37 (39.0)	22 (30.6)	15 (65.2)	10 (38.5)	25 (39.1)	0.958
Height (cm), M±SD	149.8±14.6	150.9±14.5	146.4±14.8	154.1±15.0	147.8±14.6	0.068
Weight (kg), M±SD	49.7±19.6	50.3±20.3	44.8±17.1	58.9±23.9	45.2±16.8	0.012
Body mass index, M±SD	21.0±5.5	21.2±5.7	20.2±4.8	23.8±6.6	20.0±4.7	0.003
Fatty acid levels, M±SD (*n*=90, baseline values)						
Total PUFA	49.0±4.2	49.0±4.3	48.8±4.2	48.2±4.5	49.3±4.1	0.288
Total Ω-3	3.4±0.5	3.4±0.5	3.3±0.5	3.4±0.6	3.3±0.5	0.814
Total Ω-6	45.5±4.1	45.6±4.1	45.4±4.2	44.8±4.4	45.8±4.0	0.260
Total SFA	30.8±2.0	30.7±2.0	31.2±2.1	31.3±2.1	30.7±2.0	0.155
Treatment group, *n* (%)						
Ω-3 supplements	45 (47.4)	35 (48.6)	10 (43.5)	14 (53.8)	28 (43.8)	0.384
Placebo	50 (52.6)	37 (51.4)	13 (56.5)	12 (46.2)	36 (56.2)	

Sex, race, ethnicity, insurance status, household income, and comorbid disorders were assessed/recorded at the screening assessment; all other variables were observed at baseline.

[a]One child had endpoint but not baseline blood draw.

ADHD, attention-deficit/hyperactivity disorder; DBD, Disruptive Behavior Disorder; OATS, Omega-3 and Therapy Studies; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

Table 2. Baseline and Changes in Plasma Fatty Acid Levels (Percent/Proportion of Total Fatty Acids) by Treatment Condition

		Participants with baseline+endpoint levels (n=*64*)						
	Total baseline sample (n=*90*)	*All with baseline+ endpoint levels* (n=*64*)	*Ω-3 supplement* (n=*28*)			*Placebo* (n=*36*)		
Fatty acid	*Baseline (M ± SD)*	*Baseline (M ± SD)*	*Baseline (M ± SD)*	*Endpoint (M ± SD)*	p	*Baseline (M ± SD)*	*Endpoint (M ± SD)*	p
Ω-3	3.4±0.5	3.3±0.5	3.4±0.5	6.6±2.2	<0.001	3.3±0.5	3.3±0.5	0.331
EPA	0.3±0.1	0.3±0.1	0.3±0.1	2.2±1.4	<0.001	0.3±0.1	0.3±0.1	0.736
DHA	2.0±0.5	2.0±0.5	2.0±0.4	2.8±0.6	<0.001	2.0±0.5	1.9±0.5	0.609
ALA	0.6±0.2	0.6±0.2	0.6±0.2	0.6±0.2	0.842	0.6±0.2	0.6±0.2	0.786
Ω-6	45.5±4.1	45.8±4.0	45.5±3.9	42.5±3.0	0.001	46.1±4.1	45.2±3.9	0.243
LA	35.4±3.7	35.6±3.6	35.3±3.6	33.7±2.5	0.026	35.8±3.7	35.3±3.4	0.487
AA	7.4±1.6	7.6±1.5	7.5±1.4	6.6±1.3	0.003	7.6±1.6	7.3±1.5	0.102
GLA	0.5±0.2	0.5±0.2	0.5±0.2	0.4±0.2	0.008	0.6±0.2	0.6±0.2	0.879

AA, arachidonic acid; ALA, alpha-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GLA, gamma linolenic acid; LA, linoleic acid; Ω-3, omega-3; Ω-6, omega-6.

TABLE 3. CORRELATIONS OF BASELINE AND ENDPOINT PLASMA FATTY ACID (MEASURED AS PERCENT OF TOTAL PLASMA FATTY ACIDS) AND CLINICAL CHARACTERISTICS

	Variables	M	SD	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1	BL age	11.32	2.23	–																								
2	BL weight (kg)	48.96	19.61	.68***	–																							
3	BL height (cm)	149.77	14.59	.85***	.81***	–																						
4	BL BMI	20.97	5.49	.45***	.92***	.54***	–																					
5	BL EPA	.31	.13	−.05	.07	.01	.09	–																				
6	Endpt EPA	1.14	1.32	−.27*	−.22	−.18	−.23	.01	–																			
7	BL DHA	1.98	.46	−.10	−.05	−.10	−.02	.18	.04	–																		
8	Endpt DHA	2.32	.68	−.33**	−.23	−.26*	−.19	.00	.76***	.31*	–																	
9	BL ALA	.61	.20	−.03	.14	.02	.19	−.04	.03	−.24*	.03	–																
10	Endpt ALA	.62	.18	.21	.14	.28*	−.01	−.16	−.06	−.14	−.19	.22	–															
11	BL LA	35.44	3.66	−.11	−.19	−.21	−.15	−.16	.11	.05	.03	.17	−.06	–														
12	Endpt LA	34.63	3.10	−.12	−.07	−.14	−.05	.05	−.17	−.19	−.05	.08	−.10	.32*	–													
13	BL AA	7.44	1.56	−.30**	−.23*	−.34**	−.11	.20	.00	.54***	.21	−.37***	−.43***	.14	−.11	–												
14	Endpt AA	7.01	1.46	−.11	.00	−.19	.16	.39**	−.23	.19	.14	−.13	−.56***	.00	.16	.60***	–											
15	BL GLA	.52	.20	−.10	−.19	−.11	−.19	.36***	−.02	−.41***	−.17	−.02	−.20	−.27**	.09	−.06	.17	–										
16	Endpt GLA	.48	.23	.09	−.07	−.03	−.06	.38**	−.37**	.12	−.44***	−.04	−.18	.02	−.13	.32**	.26*	.28*	–									
17	BL KDRS	13.95	6.76	.23*	.20	.13	.20*	.08	−.18	.12	−.10	.05	−.06	−.02	.11	.09	.20	−.21	.12	–								
18	Endpt KDRS	6.33	5.66	−.05	−.03	−.09	.04	.15	−.08	.12	−.06	−.07	−.23	.09	.21	.43***	.26*	.11	.24	.31***	–							
19	BL KMRS	4.97	5.54	−.14	−.17	−.08	−.19	−.02	.17	−.04	.09	−.06	.19	−.05	−.25*	.06	−.09	.09	−.08	−.13	−.09	–						
20	Endpt KMRS	2.68	4.77	−.18	−.21	−.18	−.17	−.03	.15	.03	.22	−.13	.01	.04	−.11	.11	.08	−.13	−.19	.01	−.05	.50***	–					
21	BL CDRS	40.53	10.27	.20	.12	.10	.10	−.04	−.15	.10	−.05	.02	−.02	.00	.24	.12	.19	−.14	.11	.89***	.361**	−.11	.01	–				
22	Endpt CDRS	29.11	8.83	−.06	−.04	−.10	.04	.09	−.03	.17	.04	−.09	−.20	.01	.10	.45***	.30*	.06	.15	.35**	.90***	.04	.11	.44***	–			
23	BL YMRS	14.61	6.99	−.33**	−.27**	−.29**	−.21*	−.10	.19	−.05	.15	−.13	.14	−.02	−.14	.07	−.04	.11	.01	−.10	−.02	.73***	.54***	−.05	.10	–		
24	Endpt YMRS	10.75	6.90	−.19	−.24*	−.17	−.23*	−.08	.04	.03	.13	−.21	.02	.02	−.17	.28*	.16	.02	−.04	−.01	.01	.43***	.71***	.10	.24*	.63***	–	
25	BL CGAS	51.95	7.78	.08	.13	.12	.12	.01	−.08	−.13	−.10	.03	−.08	−.04	−.05	−.24*	−.02	.00	.08	−.44***	−.25*	−.27**	−.31**	−.61***	−.40***	−.35***	−.39**	–
26	Endpt CGAS	61.56	12.09	.22	.18	.19	.13	−.06	−.07	−.13	−.12	.12	.10	−.03	.11	−.35**	−.19	−.09	−.11	−.09	−.47***	−.34**	−.39**	−.20	−.65***	−.48***	−.62***	.51***

AA, arachidnonic acid; ALA, alpha-linolenic acid; CDRS, Children's Depression Rating Scale-Revised; CGAS, Child Global Assessment Scale; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GLA, gamma linolenic acid; KDRS, KSADS Depression Rating Scale; KMRS, KSADS Mania Rating Scale; LA, linoleic acid; YMRS, Young Mania Rating Scale Ω-3, omega-3; Ω-6, omega-6. *p < .05, **p < .01, ***p < .001.

Moderation of Ω3 supplementation effect by baseline plasma levels (Hypothesis 3)

Baseline ALA moderated CDRS-R response to Ω3 supplementation such that those with baseline ALA levels above the sample mean had significantly better response to supplement (compared to PBO) than those with baseline levels below the mean (time × group × baseline ALA $b=1.6$, $t(87.3)=2.1$, $p=0.04$; Fig. 1a). Among youth in the bipolar sample, baseline EPA marginally moderated KMRS response to Ω3 supplementation (time × group × baseline EPA $b=5.5$, $t(20.2)=2.1$, $p=0.051$) such that those with baseline EPA levels above the sample mean experienced significant improvements in manic symptoms with supplement ($p=0.002$) but not PBO; however, the difference in slopes did not reach statistical significance (Fig. 1b). Baseline AA (Ω6 series) marginally moderated YMRS response to Ω3 supplementation such that those with baseline AA levels above the sample mean had better response to supplement (compared to PBO) than those with baseline levels below the mean ($p=0.051$).

Mediation of clinical response by change in plasma fatty acid levels (Hypothesis 4)

Some background to understanding the mediation analyses: Body weight correlated inversely with increase in plasma levels of EPA ($r=-0.52$, $p=0.004$) and DHA ($r=-0.54$, $p=0.003$), as did BMI: EPA, $r=-0.64$, $p<0.001$; DHA, $r=-0.46$, $p=0.01$. Age also correlated inversely with EPA increase ($r=-0.26$, $p=0.035$). Baseline BMI also moderated clinical depression response to Ω3 on both the KDRS ($p=0.037$) and CDRS ($p=0.011$) such that overweight and obese participants had a better response relative to PBO than did those with normal weight (Fig. 2). Neither age nor weight correlated significantly with LA change.

Mediation effects were investigated using criteria described by Kraemer et al. (2002): (1) observed change in fatty acid levels occurred in the course of treatment (Table 3), (2) change in fatty acid levels (EPA, DHA, and LA) was correlated with treatment group (see Results section for Hypothesis 1, Table 3), (3) there was a main effect of the mediator OR significant interactive effect with the treatment group.

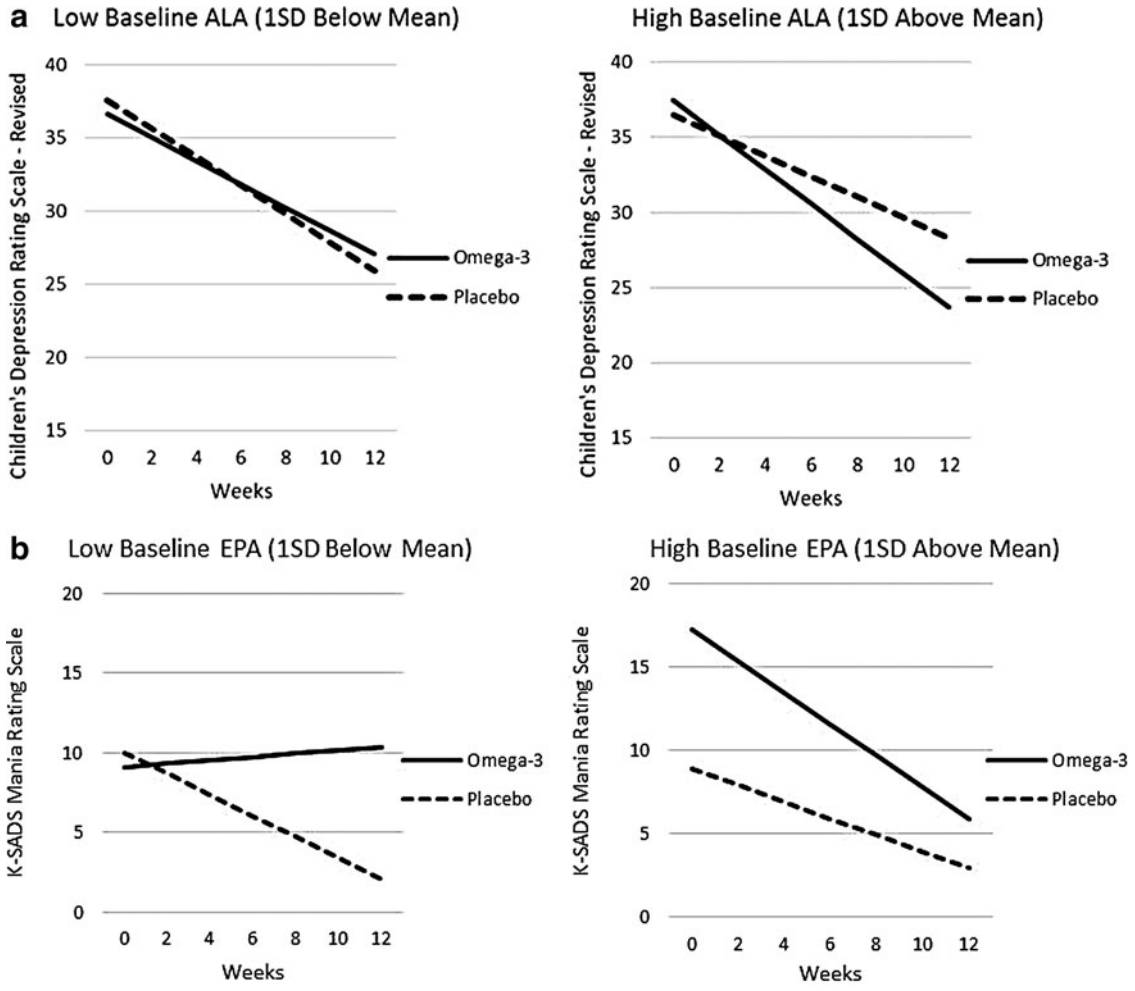

FIG. 1. Baseline fatty acid Moderation of response to Ω3 versus Placebo. Lower score is better. **(a)** Moderation of depression response by baseline alpha-linolenic acid in the full sample ($N=90$, $p=0.040$). At 1 SD below the sample mean, both group slopes (Ω3: $b=-0.8$, SE = 0.2; placebo: $b=-1.0$, SE = 0.1) were significantly different from zero ($p<0.001$), but not significantly different from each other; at 1 SD above the mean, although trajectories for Ω3 ($b=-1.1$, SE = 0.1) and placebo ($b=-0.7$, SE = 0.2) were again both significantly different from zero ($p<0.001$), Ω3 demonstrated a more favorable trajectory relative to placebo ($p=0.031$). **(b)** Marginal moderation of manic symptom response by EPA in the bipolar subgroup ($n=23$, $p=0.052$); at 1 SD below the subgroup mean, slope of Ω3 group was NS; placebo $b=-0.7$ (SE = 0.3, $p=0.011$), difference in slopes was NS; at 1 SD above the mean, Ω3 $b=-1.0$, SE = 0.3, $p=0.002$, placebo $b=-0.5$, SE = 0.3, $p=0.085$; difference in slopes was NS. EPA, eicosapentaenoic acid.

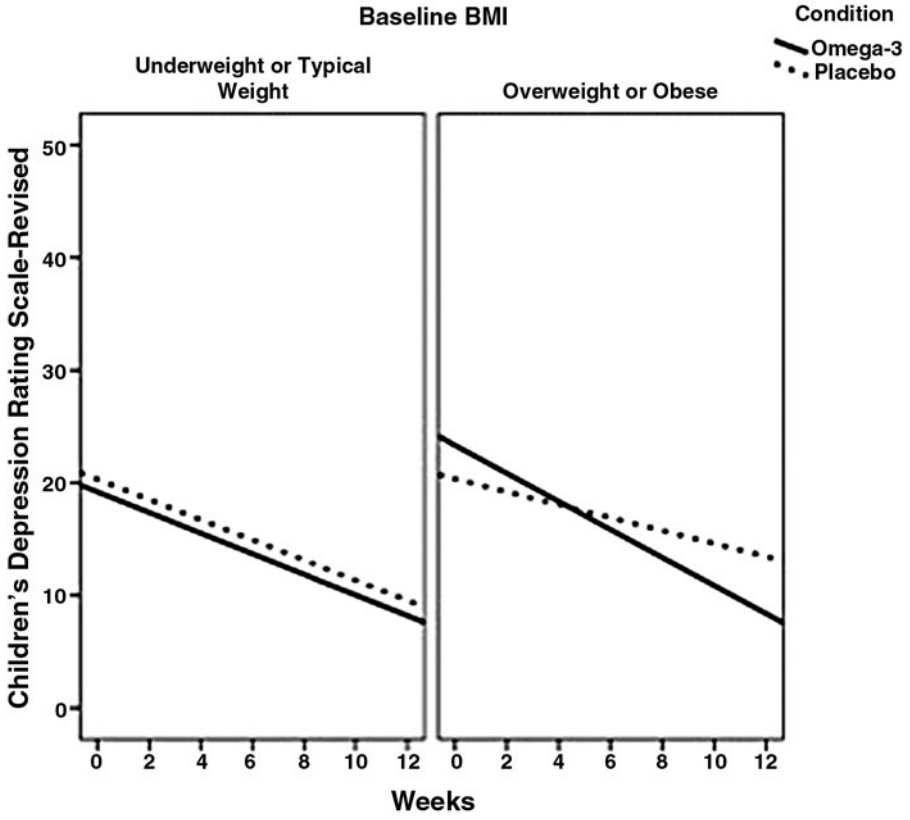

FIG. 2. Moderation of clinical response by BMI. Children's Depression Rating Scale (CDRS); lower score is better. On the left, improvement was almost identical for omega-3 and placebo in underweight and typical BMI children. On the right, improvement was significantly better for omega-3 than placebo in the overweight and obese subgroup, who had a lower mg/kg dose. BMI, body mass index.

PBO-controlled global function improvement (CGAS) with Ω3 was mediated by EPA increase from baseline to endpoint (treatment group × time × change in EPA slope $p = 0.036$ for Ω3 monotherapy; $p = 0.034$ for combined treatment), such that the greater the EPA increase, the less Ω3 improvement surpassed PBO improvement. PBO-controlled improvement in depression on the CDRS-R with Ω3 was mediated by EPA increase from baseline to endpoint ($p = 0.047$ for Ω3 monotherapy; $p = 0.048$ for combined treatment) such that the greater the EPA increase, the less Ω3 improvement surpassed PBO.

As weight and age were significantly associated with EPA change, these analyses were repeated covarying for weight and age separately. Mediation of depression severity (CDRS-R) improvement was minimally affected by covarying for weight ($p = 0.048$ for Ω3 monotherapy; $p = 0.050$ for combined treatment): greater EPA increase was still associated with less depression improvement. Similarly, EPA's mediation of the effects of treatment on global functioning appears robust when controlling for weight ($p = 0.036$ for Ω3 monotherapy; $p = 0.034$ for combined treatment) and age ($p = 0.034$ for Ω3 monotherapy; $p = 0.036$ for combined treatment): greater EPA increase is still associated with less global function improvement. Other examined mediator analyses (other clinical variables, DHA) were nonsignificant.

Because of the weight effect on clinical outcome, we checked the Ω3 dose-to-weight ratio (mg/kg) effect on CGAS and CDRS-R improvement in the supplemented group as a possible explanation for the unexpected direction of mediation. Although the mg/kg dose correlated inversely with CGAS improvement ($r = -0.34$, Fig. 3), the correlation did not reach statistical significance. Notably, only one supplemented patient deteriorated on CGAS. A negative correlation of mg/kg dose with CDRS-R improvement was nonsignificant.

Discussion

This study examined the relationship of baseline and end-of-study fatty acid composition in relation to clinical outcomes in two PBO-controlled pilot randomized trials of a fixed dose 2 g/day Ω3 supplementation. Thirty-one participants did not provide a blood sample at both timepoints and they exhibited a better PBO-controlled response to Ω3 than did subjects who provided blood samples at both times, possibly related to heavier weight, female sex, or lower number with ADHD.

Baseline ALA, EPA, and DHA blood levels were not associated with initial mood symptom severity or global function, but ALA and EPA moderated clinical responses during the trial. Specifically, baseline ALA moderated depression improvement (the higher the baseline ALA, the better the response to supplementation), possibly related to FADS activity variation. Similarly, there was a marginal trend ($p = 0.051$) of moderation of manic symptoms by baseline EPA and baseline AA, not significant because of the small number ($n = 23$) in the bipolar sample.

In this study, Ω3 supplementation with 1.6 g/day of EPA:DHA in a ratio of 7:1 raised blood levels of EPA (proportion of total measured lipids) sevenfold and DHA by almost half while decreasing the proportion of AA (Ω6 series). The increase in EPA blood levels mediated Ω3 improvement in global function, but in the unexpected direction of greater improvement with smaller EPA increase.

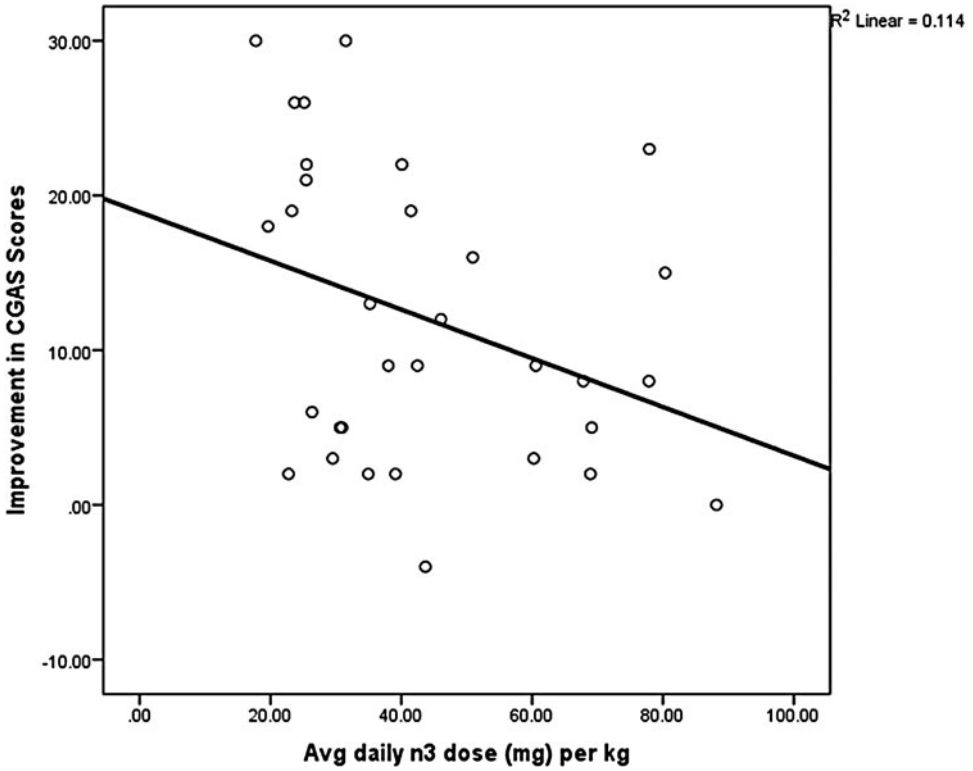

FIG. 3. Response on the Children's Global Assessment Scale by mg/kg doses of Ω3 within the supplemented participants. (Although higher mg/kg dose correlated with less improvement, only one supplemented youth declined in global function.)

Owen et al. (2008) posited four possible pathways for linking Ω3 depletion to depression: (1) Ratios of DHA to other fatty acids affect membrane fluidity and the functioning of enzymes, ion channels, and receptor binding. This pathway presumably requires integration of DHA into neural membranes; the delay needed for membrane integration might explain the absence of DHA effect in this 12-week study. Alternatively, perhaps the dosage ratio (7:1 of EPA:DHA) might explain the relative absence of DHA effect. (2) Ω3 concentrations impact neuroplasticity and cell survival. (3) Ω3 concentrations affect gene expression. (4) Ω3 availability affects production of proinflammatory and anti-inflammatory eicosanoids (prostaglandins, thromboxanes, and cytokines), which are elevated in depressed patients. For this pathway, EPA, the immediate precursor of the series 3 eicosanoids, would be more important.

Through several anabolic steps ALA can act as a precursor to EPA and DHA in small amounts, though this pathway contributes a relatively minor amount of EPA and DHA. The moderation of clinical depression response by ALA, with higher baseline ALA predicting better PBO-controlled response to Ω3 supplementation, could reflect a FADS deficiency backing up the ALA that should be desaturated into the next step toward anabolizing EPA and DHA. In this scenario, direct supplementation with EPA and DHA bypasses the FADS deficiency. The similar moderation by AA, the Ω6 counterpart of EPA, is compatible with this hypothesis because of the competition between Ω6 and Ω3 for FADS. However, the hypothesis is not supported by similar (although marginal) moderation of manic symptoms by baseline EPA.

The negative correlations of less clinical improvement with increase in EPA/DHA is unexpected and puzzling. A possible explanation may be that EPA increased less in those with higher BMI, who had a significantly better clinical response. Thus, the inverse correlation of clinical improvement with EPA increase could be an artifact of those with higher BMI, who may have greater inflammation, having both more room to show a response to the Ω3 anti-inflammatory effect and a greater body mass in which the standard dose was ''diluted,'' possibly even sequestered in adipose tissue. A more intriguing speculation could be a U-shaped dose–response curve, in which those with lower BMI may have had ''too much of a good thing'': As Paracelsus said, ''The dose alone makes the poison.'' Although it at first appears unlikely, the speculation is highly relevant to the fact that developmentally appropriate therapeutic dosing has not been established. Nemets et al. (2006) successfully used 1 g/day in their pediatric sample, compared to the 2 g/day used here.

Limitations

The study was not powered for moderator and mediator analyses. Further, there were significant differences in demographics and clinical treatment response between those with complete blood data and those with missing endpoint blood.

Conclusions

Supplementation increases blood levels of EPA and DHA, with more increase of Ω3 in lighter children. The increased level of plasma Ω3 mediates some of the clinical effects we measured, including improvement of global function and depression (although in the opposite direction of expected). Older and heavier children had a better PBO-controlled response to Ω3 and a smaller increase of blood EPA and DHA, highlighting the need to study Ω3 dosing and metabolism in children and adolescents potentially separately, as they have widely varying body masses. Our supplement was

much higher in EPA than DHA, and the relationships between plasma EPA and clinical and mood outcomes were more pronounced than DHA-related effects. A direct comparison of varying ratios of EPA:DHA is needed to, as well, elucidate whether EPA, DHA, or both are needed for improving mood in children with depression or bipolar disorder.

Clinical Significance

The fact that the larger youth, with effectively lower mg/kg dose of Ω3 and smaller increases in plasma Ω3, responded better, suggests that ''less might be more,'' that it is possible to get ''too much of a good thing.'' Although optimal dosage has not been established, the data presented here suggest that for younger children caution should be exercised if exceeding one gram of EPA+DHA per day. However, note that most fish oil is not 100% EPA and DHA. This caution does not negate the evidence for positive effects on symptoms of depression, attention-deficit/hyperactivity disorder, and disruptive behavior disorder.

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