Omega-3 Fatty Acids for Major Depressive Disorder With High Inflammation: A Randomized Dose-Finding Clinical Trial

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ABSTRACT

Objective: This study compared the impact of 3 eicosapentaenoic acid (EPA) doses versus placebo on inflammatory biomarkers and depressive symptoms.

Methods: Sixty-one unmedicated adults (75% female; 45.5 ± 13.8 years) with *DSM-5* major depressive disorder (MDD), body mass index > 25 kg/m^2, and plasma high-sensitivity C-reactive protein (hs-CRP) ≥ 3.0 mg/L were randomly assigned to receive EPA 1 g/d, 2 g/d, or 4 g/d or placebo for 12 weeks. Prespecified endpoints were a ≥ 0.40 effect size decrease in plasma interleukin (IL)-6, peripheral blood mononuclear cell (PBMC) cytokines, and lipopolysaccharide-stimulated tumor necrosis factor (TNF) production. Response was defined as a ≥ 50% decrease of Inventory of Depressive Symptomatology, Clinician-Rated version (IDS-C30) scores. We compared outcomes for the 3 EPA doses versus placebo.

Results: In 45 completers, only median PBMC TNF decreased at 2 g/d EPA. No EPA dose produced a ≥ 0.35 effect size reduction in plasma IL-6 or mitogen-stimulated TNF. Response rates for EPA 4 g/d were 64%, versus 40% for placebo (odds ratio [OR] = 2.63; Cohen *d* = 0.53), 38% for EPA 1 g/d, and 36% for EPA 2 g/d (all *P* > .05). EPA 4 g/d showed a significant correlation between percent decrease in plasma hs-CRP and IDS-C30 symptom reduction at 12 weeks (Spearman ρ = 0.691, *P* = .019).

Conclusions: EPA 4 g/d demonstrated a medium effect size for response rates versus placebo. This dose may alleviate MDD in overweight individuals with elevated inflammatory markers, and change in hs-CRP may be correlated with clinical response.

Trial Registration: ClinicalTrials.gov identifier: NCT02553915

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Omega-3 polyunsaturated fatty acids (PUFA) have been studied in many conditions including cardiovascular disease,[1] hypertriglyceridemia,[2] hyperlipidemia,[1] risk of psychosis,[3] attention deficit disorder,[4] bipolar disorder,[5] and major depressive disorder (MDD).[6] Although meta-analyses for cardiovascular disease and MDD suggest that PUFA treatment may be effective, findings overall are mixed.[1,6] Furthermore, we have yet to determine the appropriate dosage and duration of PUFA treatment and the right populations to be treated with PUFA. These challenges stimulated us to develop with the National Center for Complementary and Integrative Health (NCCIH) the first UG3-funded multicenter 12-week pilot experimental therapeutics trial investigating 3 different doses of PUFA versus placebo in a specific population with inflammatory depression (including high-sensitivity C-reactive protein [hs-CRP] ≥ 3 and body mass index [BMI] > 25) and gather preliminary data on impact of different PUFA doses on hypothesized biomarkers, specifically plasma interleukin (IL)-6 and lipopolysaccharide stimulated macrophage production of tumor necrosis factor (TNF).

Aims of the Study

We examined 3 doses of an eicosapentaenoic acid (EPA)-enriched PUFA preparation with the following goals:

1. Evaluating whether a dose-response relationship exists between EPA and decrease in either plasma IL-6 or peripheral blood mononuclear cell (PBMC) lipopolysaccharide (LPS)-stimulated tumor necrosis factor (TNF) production compared with placebo. Omega-3s have been shown to reduce inflammation by reducing IL-6 and TNF,[7] and long chain omega-3s reduce IL-6 in obese individuals.[8] Several healthy volunteer studies involving fish oil supplementation have demonstrated decreased TNF production by LPS-stimulated monocytes or PBMC.[9–13] We hypothesized that doses of 1 g/d, 2 g/d, or 4 g/d of EPA would

Clinical Points

- Omega-3 fatty acids are thought to be effective for treating depression, but optimal doses and appropriate subpopulations of depressed individuals for this treatment remain to be determined.
- In a controlled study of 3 dosing regimens (1 g/d, 2 g/d, and 4 g/d) of eicosapentaenoic acid (EPA) in overweight adults with major depressive disorder (MDD) and elevated inflammatory biomarkers including C-reactive protein (CRP), EPA 4 g/d produced the most encouraging clinical response compared to placebo.
- Doses of EPA higher than the usually recommended 1–2 g/d may alleviate MDD in overweight individuals with elevated inflammatory markers, and CRP may be correlated with clinical response.

demonstrate a ≥ 0.40 effect size (ES) at weeks 8 and 12 for the decrease in plasma IL-6 and/or PBMC TNF production compared with placebo.

2. Evaluating whether EPA treatment decreases depression severity compared to placebo and whether changes in hs-CRP, IL-6, or mitogen-stimulated PBMC TNF production were associated with clinical improvement. We hypothesized that subjects receiving EPA versus placebo would demonstrate a ≥ 0.35 ES at weeks 8 and 12 for decrease in Inventory of Depressive Symptomatology, Clinician-Rated version (IDS-C30) scores or a sustained effect on IDS-C30 response rates at weeks 8 and 12 and that changes in hs-CRP, IL-6, and LPS-stimulated PBMC TNF production would be associated with depressive improvement.

METHODS

This parallel group, double-blind randomized controlled trial (RCT) was performed at Emory University and Massachusetts General Hospital (MGH). Institutional review board approval was obtained at both sites. The study was registered at ClinicalTrials.gov (identifier: NCT02553915). Subjects were recruited between April 7, 2016, and July 13, 2018, through our psychiatric programs and practices, weight management and bariatric centers, primary care offices, and advertisements. Subjects provided written informed consent prior to entry.

Inclusion criteria included men and women aged 18–80 years with current *Diagnostic and Statistical Manual of Mental Disorders,* Fifth Edition (*DSM-5*)–defined MDD without psychotic features, ascertained by a licensed psychiatrist or psychologist using the Mini-International Neuropsychiatric Interview (MINI) v.7.0[14]; a BMI > 25 (kg/m^2)[15]; plasma hs-CRP ≥ 3 mg/L[16]; and IDS-C30[17] total scores ≥ 25 at screening and baseline. We originally required BMI > 30 and waist circumference ≥ 100 cm for men and ≥ 93 cm for women. To increase recruitment after 19 randomizations (January 2017), we removed waist circumference requirements and decreased BMI. We also allowed telephone administration of baseline IDS-C-30, 2 days before randomization, to eliminate commutes for patients ineligible due to decreased severity.

Exclusion criteria included lifetime neurocognitive disorder, psychotic or bipolar disorder, or anorexia nervosa; substance use disorder ≤ 3 months prior to screening; current obsessive-compulsive disorder or bulimia nervosa; serious suicidal or homicidal risk; serious or unstable medical illness; malignancy not in remission for at least 1 year; active autoimmune disorder or inflammatory bowel disease; insulin-dependent diabetes mellitus; breastfeeding or pregnant women; sensitivity to soy, fish products, or PUFA; failure to respond during current major depressive episode (MDE) to > 4 adequate antidepressant trials; having taken ≥ 1 g/d omega-3 for ≥ 6 weeks during current MDE or any use ≤ 60 days before screening visit; concomitant antidepressant use; electroconvulsive therapy during current MDE or ≤ 6 months before screening visit; concomitant psychotropic agents within 2 weeks of baseline visit, except prescription hypnotics, diphenhydramine, or stable daily benzodiazepine; medications that might confound biomarker findings within 1 week of baseline visit and during the trial, including nonsteroidal anti-inflammatory drugs (NSAIDs) or cyclooxygenase (COX)-2 inhibitors, oral steroids, immunosuppressants, interferon, chemotherapy, or anticoagulants (as-needed NSAID use was monitored and recorded and was disallowed ≤ 24 hours prior to blood biomarker testing; some visits were excluded from analysis because of them); and consuming > 3 g/d of omega-3 or > 3 meals of fatty fish per week (monitored by a food diary).

Omega-3 capsules and matching placebos were provided by Nordic Naturals (Watsonville, California). Each medication capsule contained approximately 823 mg omega-3 fatty acids, with an EPA:docosahexaenoic acid (DHA) ratio of 3.9:1 (about 590 mg of EPA, 152 mg DHA), verified by an independent laboratory.[18] Matched placebos contained soybean oil (about 54% omega-6 and 6% omega-3, no EPA or DHA). Patients were randomized equally (permuted blocks of 4 or 8, per pharmacy) to one of 4 arms: (1) EPA 1.18 g/d, 254 mg/d DHA; (2) EPA 2.36 g/d, 508 mg/d DHA; (3) EPA 4.72 g/d, 1.16 g/d DHA; or (4) placebo. Participants took 4 capsules twice daily (8 capsules/d).

The screening period lasted up to 28 days. The first screening visit (V1) established an MDE; failed antidepressant trials per MGH Antidepressant Treatment Response Questionnaire[19]; IDS-C30 score ≥ 25; BMI > 25 kg/m^2; vital signs; urine drug screen; concomitant medications; and phlebotomy for hs-CRP. Patients received a diary to document food consumption for 3 days prior to second screening visit (V2).

The second study visit (V2) occurred 3–7 days after V1 and included the following: beginning washout of prohibited medications, completed at least 2 weeks prior to baseline visit; completing Food Processor Report (ESHA Research Inc, Salem, OR)[20] to measure omega-3 consumption; remaining MINI components; Columbia-Suicide Severity Rating Scale (C-SSRS)[21]; medical history; physical examination; electrocardiogram; and laboratory testing.

Table 1. Demographic and Clinical Characteristics at Intake, by Treatment Group for n = 61 Randomized Subjects[a]

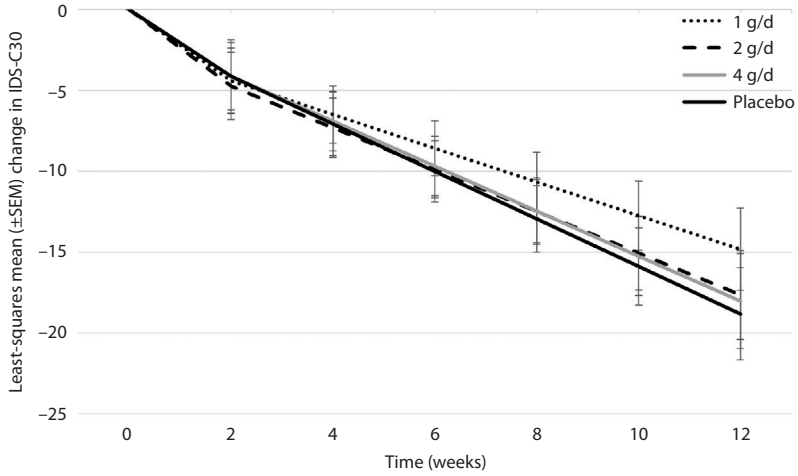
	1 g/d EPA (n = 15)	2 g/d EPA (n = 15)	4 g/d EPA (n = 16)	Placebo (n = 15)	Total (n = 61)
Demographic characteristics					
Study site, n (%)					
Emory University	7 (46.7)	7 (46.7)	8 (50.0)	7 (46.7)	29 (47.5)
Massachusetts General Hospital	8 (53.3)	8 (53.3)	8 (50.0)	8 (53.3)	32 (52.5)
Age, y					
Mean (SD)	41.6 (14.6)	44.5 (15.2)	45.8 (13.0)	50.3 (12.0)	45.5 (13.8)
Range	23–76	18–73	22–62	27–68	18–76
Sex, n (%)					
Female	11 (73.3)	12 (80.0)	11 (68.8)	12 (80.0)	46 (75.4)
Male	4 (26.7)	3 (20.0)	5 (31.2)	3 (20.0)	15 (24.6)
Race, n (%)					
Caucasian	12 (80.0)	9 (60.0)	6 (37.5)	7 (46.7)	34 (55.7)
African American	2 (13.3)	5 (33.3)	7 (43.8)	7 (46.7)	21 (34.4)
Other	0 (0.0)	1 (6.7)	2 (12.5)	0 (0.0)	3 (4.9)
Prefer not to say	1 (6.7)	0 (0.0)	1 (6.2)	1 (6.7)	3 (4.9)
Ethnicity, n (%)					
Hispanic	1 (6.7)	3 (20.0)	1 (6.2)	3 (20.0)	8 (13.1)
Non-Hispanic	14 (93.3)	12 (80.0)	15 (93.8)	12 (80.0)	53 (86.9)
Education, n (%)					
High school or less	1 (6.7)	3 (20.0)	2 (12.5)	3 (20.0)	9 (14.8)
Some college or more	14 (93.3)	12 (80.0)	14 (87.5)	12 (80.0)	52 (85.2)
Marital status, n (%)					
Married/living together	4 (26.7)	2 (13.3)	5 (31.2)	3 (20.0)	14 (22.9)
Separated/divorced/widowed	4 (26.7)	4 (26.7)	7 (43.8)	4 (26.7)	19 (31.2)
Never married	7 (46.7)	9 (60.0)	4 (25.0)	8 (53.3)	28 (45.9)
Clinical characteristics					
IDS-C30 score					
Mean (SD)	39.2 (7.9)	34.7 (7.7)	34.5 (6.8)	36.3 (7.3)	36.1 (7.5)
Range	28–60	25–49	25–52	28–53	25–60
hs-CRP mg/L					
Median (IQR)	6.6 (11.2)	6.2 (2.8)	4.3 (3.1)	5.9 (4.6)	5.9 (4.1)
Range	3.1–30.1	3.0–12.9	3.2–11.3	3.4–23.1	3.0–30.1
Body mass index					
Median (IQR)	33.2 (6.9)	34.6 (10.2)	34.6 (7.2)	37.9 (10.4)	34.8 (5.1)
Range	29.3–63.4	28.0–48.4	26.9–42.7	33.9–53.8	26.9–63.4
Recurrent major depressive disorder, n (%)					
Yes	7 (46.7)	9 (60.0)	9 (56.2)	11 (73.3)	36 (59.0)
No	8 (53.3)	6 (40.0)	7 (43.8)	4 (26.7)	25 (41.0)
Current anxiety disorder,[b] n (%)					
Yes	7 (46.7)	7 (46.7)	3 (18.8)	2 (13.3)	19 (31.2)
No	8 (53.3)	8 (53.3)	13 (81.2)	13 (86.7)	42 (68.8)
Current PTSD (in past month), n (%)					
Yes	4 (26.7)	1 (6.7)	2 (12.5)	2 (13.3)	9 (14.8)
No	11 (73.3)	14 (93.3)	14 (87.5)	13 (86.7)	52 (85.2)
Recent alcohol or substance use disorder,[c] n (%)					
Yes	2 (13.3)	0 (0.0)	0 (0.0)	0 (0.0)	2 (3.3)
No	13 (86.7)	15 (100.0)	16 (100.0)	15 (100.0)	59 (96.7)

[a]Out of subjects who were screened and eligible to participate. Treatment groups did not differ significantly from one another on any variable, at $P \leq .05$.
[b]Current generalized anxiety disorder, panic disorder, agoraphobia, or social phobia.
[c]Substance use in the past year, but not during the past 3 months.
Abbreviations: EPA = eicosapentaenoic acid, hs-CRP = high-sensitivity C-reactive protein, IDS-C30 = Inventory of Depressive Symptomatology, Clinician-Rated version, IQR = interquartile range, PTSD = posttraumatic stress disorder, SD = standard deviation.

Eligible patients were randomized at baseline visit (V3) and seen biweekly to assess pill compliance, depressive symptoms, and adverse events (AEs) through treatment week 12 (V9). At each visit, a clinician administered the IDS-C30, C-SSRS, Clinical Global Impression-Severity[22] and, from V4 onward, the Clinical Global Impression-Improvement.[22]

Phlebotomy for fasting biomarkers was performed at baseline (V3), week 4 (V5), week 8 (V7), and week 12 (V9) between 7:30–11:00 AM, after 30 minutes of rest, with avoidance of NSAIDs or cyclooxygenase-2 (COX-2) inhibitors ≤ 24 hours before draws. Plasma IL-6 and TNF from PBMC culture supernatant were measured with fluorokine MAP assays (R&D Systems, Minneapolis, MN).[23] Plasma CRP was assessed with a high sensitivity turbidimetric assay,[24] with assay sensitivity 0.18 mg/L, range of measure 0.2–80 mg/L, and functional sensitivity (at 20% CV) 0.2 mg/L. All samples from each subject were assayed together to reduce interassay variability.

Figure 1. Mixed Model Repeated Measures (MMRM) Analysis of Change in IDS-C30 Scores Over 12 Weeks of Treatment by Treatment Group for n = 51 Per Protocol Evaluable Subjects[a]



[a]Includes 6 evaluable subjects without usable data to treatment week 12 (early terminators). For EPA 1 g/d group, n = 14; for EPA 2 g/d group, n = 13; for EPA 4 g/d group, n = 13; for placebo group, n = 11. The overall treatment-by-time effect was nonsignificant ($F_3 = 2.16$; $P = .097$).
Abbreviations: EPA = eicosapentaenoic acid, IDS-C30 = Inventory of Depressive Symptomatology, Clinician-Rated version, MMRM = mixed model repeated measures, SEM = standard error of the mean.

Statistical Analyses

We intended to recruit 100 subjects (25/arm), expecting a per protocol (PP) sample of 80 (20% early termination) with power to detect prespecified ES. Study enrollment fell short (N = 61) but was judged by the NCCIH and authors as sufficient to meet project goals.

Safety analysis (AEs) was based on all 61 randomized patients. Two PP samples included data for all visits without a serious protocol violation due to visit timing, treatment noncompliance, or prohibited medication use. Analysis of main aims (change in inflammatory biomarkers, clinical efficacy and its relationship to biomarker change) was based on a modified intent-to-treat (mITT) PP completer sample of 45 completer subjects with PP data at baseline and 12 treatment weeks. A second mITT PP evaluable sample for clinical efficacy consisted of 51 randomized subjects who had a baseline and at least 1 post-baseline PP assessment of IDS-C30.

We tested whether EPA demonstrated a ≥ 0.40 ES at weeks 8 and 12 for decrease in plasma IL-6 or mitogen-stimulated PBMC TNF production compared with placebo. Raw and percent change in these biomarkers from baseline to week 8 or 12 were non-normally distributed. As log transformation did not normalize the distributions enough for parametric statistics, we used non-parametric rank-order statistics across the 4 treatment groups (Kruskal-Wallis test) and for each of 6 pairs of treatment group comparisons (Wilcoxon rank sum test).

We next tested (*a*) whether subjects receiving EPA vs placebo demonstrated at weeks 8 and 12 a ≥ 0.35 ES for decrease in IDS-C30 score or a sustained effect on IDS-C30 response rates and (*b*) whether IL-6 and PBMC TNF production changes were associated with depressive severity changes.

For subaim (*a*), we performed mixed model repeated measures (MMRM) analysis on change in IDS-C30 from baseline through all biweekly assessments for the next 12 weeks, on the evaluable 51 PP subjects. An auto-regressive Type 1 (AR-1) covariance structure fit the repeated measures data best. No covariate was incorporated into the MMRM model because no baseline demographic, clinical, or biological measure (including IDS-C30 score) met criteria of at least a moderate correlation with the dependent variable and a difference with $P \leq .10$ across treatment groups. Empirical testing with Akaike and Bayesian information criterion was performed, and covariates did not improve the model. As a sensitivity analysis, we performed analysis of variance (ANOVA) on change from baseline to week 12 across treatment groups in the 45 PP completers, without baseline covariates because none altered the findings.

To test whether subjects receiving EPA vs placebo would demonstrate a sustained ≥ 0.35 ES on IDS-C30 response rates at weeks 8 and 12, we computed relative risk (RR) and odds ratio (OR) for obtaining ≥ 50% decrease in IDS-C30 score from baseline for each EPA dose vs placebo and vs other doses. Simple RR was a direct measure of response rate comparison. OR and 95% confidence interval were computed for each of the 6 paired-group comparisons; OR was converted to Cohen *d* using an R language calculator.[25]

For subaim (*b*), 2 analyses were conducted using non-parametric methods because change in biomarkers was non-normally distributed (highly skewed): (1) Spearman

rank order correlations (ρ) between change in depression vs change in the 2 biomarkers over 12 weeks were examined, within each treatment group in the 45 PP completers, using raw change for each measure and percent change from baseline. A correlation of $\rho \geq 0.50$ indicated at least moderate association of change between a biomarker and depressive improvement; (2) changes in each of the 2 primary biomarkers were compared between IDS-C30–defined responders and nonresponders in each treatment group. We also plotted individual subjects' change in IDS-C30 against change in each biomarker, to determine whether a quasi-linear relationship was evident for most of the subjects in a particular treatment group.

Similarly, we examined possible moderating influence of all baseline inflammatory biomarker levels on depression response in all completers in the 3 EPA doses. We performed non-parametric analyses consisting of Spearman rank-order correlation of baseline biomarkers in relation to raw change and percent change in IDS-C30 over 12 weeks of treatment in relation to baseline, as well as raw change and percent change in 7 inflammatory markers (hs-CRP plus plasma IL-6, PBMC and gene expression of IL-6 and TNF) and mol% of 4 fatty acids (EPA, DHA, arachidonic acid, and docosapentaenoic acid). We also performed non-parametric rank-order comparison of EPA responders vs EPA nonresponders based on the combination of all completers in the 3 EPA dose groups.

Statistical analyses were performed using SAS Version 8.2 (SAS; Cary, NC).

RESULTS

We obtained informed consent from 147 subjects and randomized 61 to treatment. Treatment groups did not differ significantly on baseline demographic or clinical characteristics (Table 1). Supplementary Figure 1 (CONSORT) shows 51 subjects evaluable for clinical efficacy and 45 completers with adequate adherence and IDS-C30 and biomarker data.

Biomarker Outcomes

Baseline plasma IL-6 and PBMC TNF concentrations had highly non-normal distributions within and across treatment groups and could not be transformed to meet assumptions of normal distribution so were analyzed by non-parametrics. The only baseline difference in biomarkers was that the EPA 2 g/d group had a significantly lower baseline plasma IL-6 than the placebo group ($P = .034$; Table 2). EPA failed to demonstrate a ≥ 0.40 ES at weeks 8 and 12 for decrease in either plasma IL-6 or PBMC TNF production compared with placebo (Table 2).

After 8 weeks, the 4 g/d EPA group had a median decrease in hs-CRP significantly different from the large median increase at 2 g/d EPA ($P = .022$). After 12 weeks, median plasma hs-CRP levels decreased in a dose-dependent manner with the 3 EPA regimens, compared to a minimal decrease in the placebo group (Table 2). Both the 2 g/d (Cohen $d = 0.77$) and 4 g/d (Cohen $d = 0.68$) EPA doses yielded a higher proportion of participants with ≥25% reduction of plasma hs-CRP by 12 weeks than placebo.

Clinical Outcomes

MMRM analysis (Figure 1) shows that within the PP evaluable sample (n = 51), IDS-C30 least-squares mean scores decreased significantly in all 4 treatment arms starting at week 4, with further improvement in all groups until 12 weeks. No 2 groups differed significantly during treatment. ANOVA of the n = 45 PP completers also found no clinically meaningful or statistically significant difference between the 4 groups.

Among the n = 45 PP completers, the EPA 4 g/d group had the highest IDS-C30 response rate at 12 weeks (64%) compared to the other 3 groups, whose rates were 36%–40% (Table 3). The resulting OR was 2.625 for EPA 4 g/d versus placebo, equivalent to Cohen *d* of 0.532 (Table 4), but the 95% confidence interval for ORs was very wide. (A binary treatment outcome requires an OR ≥ 1.89 for equivalence to the prespecified Cohen *d* of 0.35.[25]) This threshold was not met for any EPA dose vs placebo at 8 weeks, and only for the 4 g/d EPA dose at 12 weeks. All subjects who responded to any EPA dose at week 8 remained responders at week 12 (100%), while only 2/5 placebo responders at week 8 (40%) remained responders at week 12. Treatment response at *both* week 8 and week 12 was highest (55%) for EPA 4 g/d and lowest (20%) for placebo (Table 3) (OR = 4.80, Cohen $d = 0.865$) (Table 4).

We compared baseline depressive severity and inflammation for clinical responders vs nonresponders within each treatment group. Placebo responders had notably lower baseline IDS-C30 scores than nonresponders (mean [SD] = 29.50 [7.14], n = 4 vs 41.33 [10.11], n = 6; $t_8 = -2.17$, $P = .075$ with adjustment for unequal group variances). Placebo responders also had significantly lower baseline plasma IL-6 than nonresponders (mean rank 2.75 vs 7.33, Kruskal-Wallis $P = .019$).

Test for Association

IDS-C30 responders did not differ significantly from nonresponders within any treatment group on absolute or percent change in plasma IL-6 or PBMC TNF. Spearman rank order correlations between 12-week change in IDS-C30 scores and change in plasma IL-6 or PBMC TNF were not statistically significant. A linear relationship between treatment response and plasma IL-6 and PBMC TNF for the 4 g/d EPA group was suggested.

Baseline level of hs-CRP had a low correlation with change in IDS-C30, within each EPA dose group (all ρ values smaller than −0.26). EPA 4 g/d resulted in a significant correlation between percent change in plasma hs-CRP and percent change in IDS-C30 at 12 weeks (Spearman $\rho = 0.691$, $P = .019$ [Supplementary Figure 2]). An additional exploratory analysis found that responders to 4 g/d had a significantly greater percent decrease in hs-CRP compared to nonresponders ($P = .038$; Table 5). Additionally, rank sum

Table 2. Primary Inflammatory Biomarker Levels at Baseline and Percent Change From Baseline to Treatment Weeks 8 and 12 by Treatment Group, for n = 45 Per Protocol Study Completers[a]

	1 g/d EPA completers (n = 13)	2 g/d EPA completers (n = 11)	4 g/d EPA completers (n = 11)	Placebo completers (n = 10)
Plasma IL-6				
Baseline (pg/mL)	n = 12[b]	n = 11	n = 11	n = 10
Mean (SD)	3.64 (1.82)	2.86 (1.16)	3.18 (0.84)	4.11 (1.37)
Median (IQR)	3.12 (1.32)	2.95 (1.52)	2.95 (1.61)	3.70 (0.67)
Low, high	2.02, 8.92	1.41, 5.25	2.15, 4.87	2.52, 7.13
Percent change at treatment week 8	n = 11[b]	n = 11	n = 10[b]	n = 10
Mean (SD)	4.74 (22.98)	10.15 (47.47)	1.37 (20.02)	−6.09 (27.82)
Median (IQR)	0.00 (44.29)	0.00 (48.30)	0.00 (28.99)	0.00 (50.33)
Low, high	−26.12, 36.14	−42.03, 134.13	−25.57, 32.58	−42.40, 38.66
Median of EPA minus placebo group[c]	0.00	0.00	0.00	–
Percent change at treatment week 12	n = 12[b]	n = 11	n = 11	n = 10
Mean (SD)	0.46 (28.91)	7.19 (32.24)	3.40 (40.46)	1.92 (30.24)
Median (IQR)	0.28 (37.73)	13.90 (17.33)	0.00 (32.01)	6.25 (42.99)
Low, high	−37.80, 57.61	−60.76, 70.92	−29.88, 118.31	−38.57, 60.54
Median of EPA minus placebo group[c]	−5.97	+7.65	−6.25	–
PBMC TNF-α production				
Baseline (pg/mL)	n = 13	n = 11	n = 11	n = 10
Mean (SD)	1805.81 (1,113.53)	1792.60 (1014.05)	1748.01 (708.34)	1568.09 (597.20)
Median (IQR)	1480.00 (703.00)	1629.00 (1507.00)	1520.00 (597.00)	1682.50 (607.00)
Low, high	877.55, 5052.00	505.93, 3632.00	741.16, 3572.00	586.10, 2659.00
Percent change at treatment week 8	n = 12[b]	n = 11	n = 10[b]	n = 9[b]
Mean (SD)	7.52 (37.35)	25.37 (23.79)	22.51 (80.77)	20.09 (51.79)
Median (IQR)	0.84 (51.75)	33.51 (28.18)	−13.88 (40.32)	15.54 (47.57)
Low, high	−61.82, 72.43	−19.37, 50.08	−29.91, 225.59	−53.54, 117.71
Median of EPA minus placebo group[c]	−14.70	+17.97	−29.42	–
Percent change at treatment week 12	n = 13	n = 11	n = 11	n = 10
Mean (SD)	−0.87 (41.16)	10.38 (80.36)	28.01 (56.62)	8.59 (45.82)
Median (IQR)	1.93 (43.63)	−8.31 (93.45)	7.07 (45.31)	3.38 (22.89)
Low, high	−52.45, 95.20	−87.92, 166.84	−28.91, 150.78	−74.66, 82.49
Median of EPA minus placebo group[c]	−1.45	−11.69	+3.69	–
hs-CRP[d]				
Baseline (mg/L)	n = 12[b]	n = 11	n = 11	n = 10
Mean (SD)	6.35 (5.05)	5.16 (3.39)	5.72 (3.05)	7.91 (5.33)
Median (IQR)	3.55 (9.07)	4.79 (1.39)	3.86 (5.27)	6.90 (5.02)
Low, high	2.05, 14.67	0.82, 12.97	2.25, 10.35	2.51, 18.24
Percent change at treatment week 8	n = 11[b]	n = 11	n = 10[b]	n = 10
Mean (SD)	23.35 (61.41)	84.84 (133.30)	−8.59 (38.93)	19.77 (36.68)
Median (IQR)	18.02 (94.94)	35.11 (161.58)	−15.85 (43.48)	24.94 (65.50)
Low, high	−64.88, 148.07	−16.77, 427.82	−59.08, 57.94	−32.02, 74.66
Median of EPA minus placebo group[c]	−6.92	+10.17	−40.79	–
Percent change at treatment week 12	n = 12[b]	n = 11	n = 11	n = 10
Mean (SD)	−8.28 (56.07)	1.09 (74.46)	−15.79 (37.57)	7.22 (54.27)
Median (IQR)	−15.44 (61.38)	−19.12 (36.74)	−24.93 (50.43)	−1.12 (63.80)
Low, high	−77.30, 110.95	−65.98, 197.56	−66.22, 54.04	−79.14, 89.98
Median of EPA minus placebo group[c]	−14.32	−18.00	−23.81	–

[a]The Kruskal-Wallis test was used to evaluate the significance of differences across the 4 treatment groups; none were significant at $P \leq .05$. The Wilcoxon rank sum test was used to evaluate the significance of differences in mean rank within each of 6 pairs of treatment groups. The 2 g/d EPA group had a lower baseline plasma IL-6 level than the placebo group ($P = .034$), and the 4 g/d EPA group had a greater decrease in hs-CRP than the 2 g/d EPA group at treatment week 8 ($P = .022$).
[b]Some subjects did not have a usable sample at a specific visit (baseline, treatment week 8, or treatment week 12).
[c]Median percent change for each EPA dose is expressed relative to median percent change in the placebo group over 8 or 12 weeks of treatment.
[d]Although hs-CRP was defined as a secondary outcome variable, it is included here because median change over 12 weeks indicates a possible EPA dose effect in reducing hs-CRP relative to placebo.
Abbreviations: EPA = eicosapentaenoic acid, hs-CRP = high-sensitivity C-reactive protein, IL-6 = interleukin-6, IQR = interquartile range, PBMC = peripheral blood mononuclear cell, SD = standard deviation, TNF-α = tumor necrosis factor α.

comparison of n = 16 responders vs n = 19 nonresponders to all doses of EPA combined (ie, among n = 35 subjects who completed 1, 2, or 4 g/d of EPA) did not show a significant difference in baseline CRP for EPA responders vs nonresponders ($P = .285$). There was no significant difference between responders and nonresponders on any baseline or change measure of inflammatory markers or fatty acids based on the combination of EPA doses. Likewise, the highest Spearman ρ correlation value was 0.359 for the 3 EPA doses combined, which is well below the 0.50 threshold generally considered indicative of a "moderate" association.

Tolerability

Thirty-six subjects (59%) reported at least 1 AE; most were mild. No serious AEs were reported (Supplementary Table 1). Overall AE rate was highest for placebo, and the EPA arms did not differ substantially from each other. No discontinuations were due to AEs.

Table 3. IDS-C30 Response[a] at Treatment Weeks 8 and 12, for n = 45 Per Protocol Study Completers

IDS-C30 response status	1 g/d EPA	2 g/d EPA	4 g/d EPA	Placebo
Treatment week 8				
N	12[b]	11	11	10
Response, n (%)	3 (25.00)	4 (36.36)	6 (54.55)	5 (50.00)
Nonresponse, n (%)	9 (75.00)	7 (63.64)	5 (45.45)	5 (50.00)
Treatment week 12				
N	13	11	11	10
Response, n (%)	5 (38.46)	4 (36.36)	7 (63.64)	4 (40.00)
Nonresponse, n (%)	8 (61.54)	7 (63.64)	4 (36.36)	6 (60.00)
Response at both treatment weeks 8 and 12				
N	12[b]	11	11	10
Yes, n (%)	3 (25.00)	4 (36.36)	6 (54.55)	2 (20.00)
No, n (%)	9 (75.00)	7 (63.64)	5 (45.45)	8 (80.00)

[a]Clinical response as of treatment week 8 or 12 is defined using the conventional threshold of 50% or greater improvement (in this study, reduction in IDS-C30 scores) since baseline.
[b]One subject was missing usable IDS-C30 score at treatment week 8.
Abbreviations: EPA = eicosapentaenoic acid, IDS-C30 = Inventory of Depressive Symptomatology, Clinician-Rated version.

DISCUSSION

This is the first dose-finding trial of EPA in MDD to focus on inflammatory biomarkers as primary outcome and to specifically select overweight/obese subjects with elevated hs-CRP to operationalize a definition of inflammatory MDD. Findings did not support our hypothesis that EPA would decrease plasma IL-6 or LPS-stimulated TNF levels with an ES ≥ 0.40 compared to placebo. However, the secondary finding that EPA 4 g/d was associated with increased sustained IDS-C response rates and concomitant hs-CRP decreases is encouraging. Recent studies suggest that EPA 4 g/d demonstrated significant cardioprotective effects[26] and in one case led to the breakdown of coronary artery plaques.[27] These findings are particularly important, given our recent demonstration that EPA 4 g/d is associated with production of proresolving mediators of inflammation, particularly resolvins,[28] which have shown antidepressant effects in animal models of MDD.[29]

Regarding clinical efficacy, the study was designed without expectation that comparisons between treatment arms would be significant, but that a meaningful effect size would be obtained. We proposed that the IDS ES of 0.35 would be the minimum required to produce a clinically meaningful effect. No EPA dose produced the hypothesized ES ≥ 0.35 for decrease in IDS-C30 vs placebo. However, regarding the binary outcome of clinical response vs nonresponse, EPA 4 g/d demonstrated an ES = 0.865 over

Table 4. Statistics for Comparison of Pairs of Treatment Groups on IDS-C30 Response at Treatment Weeks 8 and 12, for n = 45 per Protocol Study Completers

	Groups compared	Relative risk	OR (95% confidence interval)[a]	OR *P* value	Effect size[b]
Week 8 response	1 g/d EPA vs PBO	0.500	0.333 (0.055–2.019)	0.232	−0.601
	2 g/d EPA vs PBO	0.727	0.571 (0.100–3.273)	0.628	−0.309
	4 g/d EPA vs PBO	1.091	1.200 (0.216–6.676)	0.208	0.101
	2 g/d vs 1 g/d EPA	1.454	1.714 (0.285–10.303)	0.556	0.297
	4 g/d vs 1 g/d EPA	2.182	3.600 (0.616–21.034)	1.555	0.706
	4 g/d vs 2 g/d EPA	1.500	2.100 (0.380–11.589)	0.395	0.409
Week 12 response	1 g/d EPA vs PBO	0.961	0.938 (0.173–5.070)	0.940	−0.036
	2 g/d EPA vs PBO	0.909	0.857 (0.147–5.000)	0.864	−0.086
	4 g/d EPA vs PBO	1.591	2.625 (0.450–15.311)	0.283	0.532[c]
	2 g/d vs 1 g/d EPA	0.945	0.914 (0.174–4.812)	0.916	−0.049
	4 g/d vs 1 g/d EPA	1.654	2.800 (0.532–14.736)	0.224	0.568
	4 g/d vs 2 g/d EPA	1.750	3.062 (0.537–17.402)	0.207	0.617
Response at both week 8 and 12	1 g/d EPA vs PBO	1.250	1.333 (0.176–10.121)	0.781	0.159
	2 g/d EPA vs PBO	1.818	2.286 (0.316–16.512)	0.413	0.456[c]
	4 g/d EPA vs PBO	2.727	4.800 (0.682–33.799)	0.115	0.865[c]
	2 g/d vs 1 g/d EPA	1.454	1.714 (0.285–10.303)	0.556	0.297
	4 g/d vs 1 g/d EPA	2.182	3.600 (0.616–21.034)	0.155	0.706
	4 g/d vs 2 g/d EPA	1.500	2.100 (0.380–11.589)	0.395	0.409

[a]The 95% confidence interval is very wide due to the small number of subjects per treatment group.
[b]Cohen *d* determined as described in Lenhard and Lenhard.[25] A negative effect size occurs if the second group in the comparison had a higher response rate than the first group in the pair.
[c]Greater than the predicted effect size of 0.35 for superiority of some EPA dose over placebo.
Abbreviations: EPA = eicosapentaenoic acid, IDS-C30 = Inventory of Depressive Symptomatology, Clinician-Rated version, OR = odds ratio, PBO = placebo.

Table 5. Percent Change in hs-CRP From Baseline to Treatment Week 12 for Responders vs Nonresponders to 4 g/d EPA

	Responders (n = 7)	Nonresponders (n = 4)	Statistic
Mean rank[a]	4.43	8.75	Kruskal-Wallis $P = .038$
Mean (SD)	−33.12 (31.96)	+14.52 (27.13)	
Median (IQR)	−36.59 (40.09)	+4.98 (34.83)	
Low	−66.22	−5.90	
High	+28.90	+9.84	
N with decrease	6 of 7	1 of 4	

[a]Rank from low (1) to high (11) within 4 g/d EPA group, with non-parametric Kruskal-Wallis test, as appropriate for non-normal distribution.
Abbreviations: EPA = eicosapentaenoic acid, hs-CRP = high-sensitivity C-reactive protein, IQR = interquartile range, SD = standard deviation.

placebo at 8 and 12 weeks and an ES ≥ 0.35 over the 1 and 2 g/d doses of EPA (Table 4). Thus, EPA 4 g/d produced a sustained effect exceeding our prespecified minimum ES. It must be noted that placebo outperformed the 1 g/d and 2 g/d regimens of EPA, albeit nonsignificantly. This might be explained by heterogeneity in the sample or by random effects. It was partly due to concerns about such a finding that we examined effects at weeks 8 and 12. This finding, however, lends further support to the 4-g dose as the more effective one.

Placebo responders had lower baseline IDS-C30 scores than nonresponders, which agrees with previous findings that placebo response in MDD decreases with increasing baseline severity.[30] By contrast, clinical responders within each EPA dose group had similar mean baseline IDS-C30 scores as respective nonresponders. Also consistent with our prior report,[31] the significantly lower levels of baseline plasma IL-6 in placebo responders versus nonresponders suggests that placebo may be more clinically effective in subjects with MDD who have lower baseline inflammation on certain biomarkers.

This study has limitations. First, this modestly funded UG3 exploratory trial requiring a relatively small sample was not designed or powered to produce definitive results, or to permit substantive analysis by gender or ethnicity. Moderator analyses and tests for association were similarly limited. However, large within-group variation, indicated by standard errors of means (Figure 1), suggests that further analysis should focus on individual biological or other characteristics that may account for differences in antidepressant response within and across treatment groups. Second, the superiority of EPA 4 g/d over placebo was based on a dichotomous definition of treatment response that is prone to false positives with small sample sizes. Third, we were not budgeted to analyze red blood cell or plasma phospholipid levels. Responders may have had low baseline levels of PUFAs, or perhaps their levels increased more than in nonresponders. This information would have enhanced the selected phenotype beyond high BMI and high CRP. Fourth, recruitment was a challenge, with less than the 100 projected subjects, which limited statistical power to detect prespecified ES differences for biomarker and clinical outcomes. Yet, experimental therapeutics trials should enrich subject cohorts based on hypothesized mechanisms of action, in this case anti-inflammatory effects of EPA. Future studies of inflammatory depression should include more sites to achieve appropriate sample size.

Our findings have potential public health implications. Given the limitations of traditional antidepressants,[32–34] it is important to know whether complementary interventions represent safe and effective alternatives for individuals with MDD and high peripheral inflammation. Furthermore, personalized/precision treatment approaches are important, and others have studied biomarkers in PUFA treatment of other psychiatric conditions,[35] which supports our approach, especially considering the overall mixed findings regarding clinical efficacy and biomarker effects associated with omega-3s. This study represents a step toward characterizing possible mechanisms of omega-3 fatty acids' effects on immune function and physiology of MDD and may pave the way for broader treatment options.

In conclusion, doses of EPA higher than the usually recommended 1–2 g/d may be more effective for depression in some patients with obesity and chronic inflammation, and reduction in hs-CRP may play a role in this process. Replication in larger samples is necessary, and a larger trial of 4 g/d EPA-enriched supplementation in medication-stable patients with more resistant depression and hs-CRP ≥ 3 mL/L seems a logical next step to better characterize the potential of this intervention.

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Supplementary Material

Article Title: Omega-3 Fatty Acids for Major Depressive Disorder With High Inflammation: A Randomized Dose-Finding Clinical Trial

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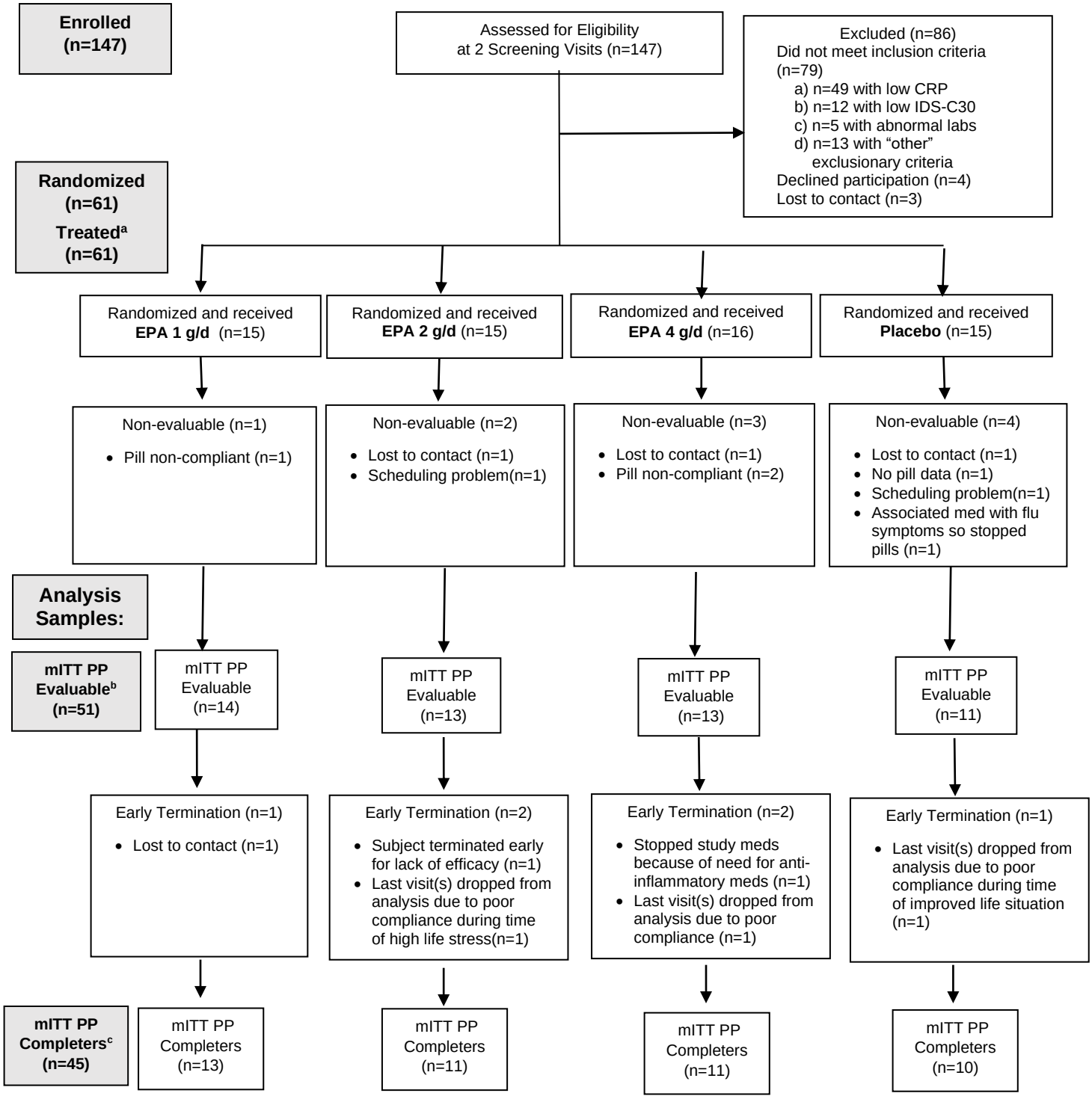
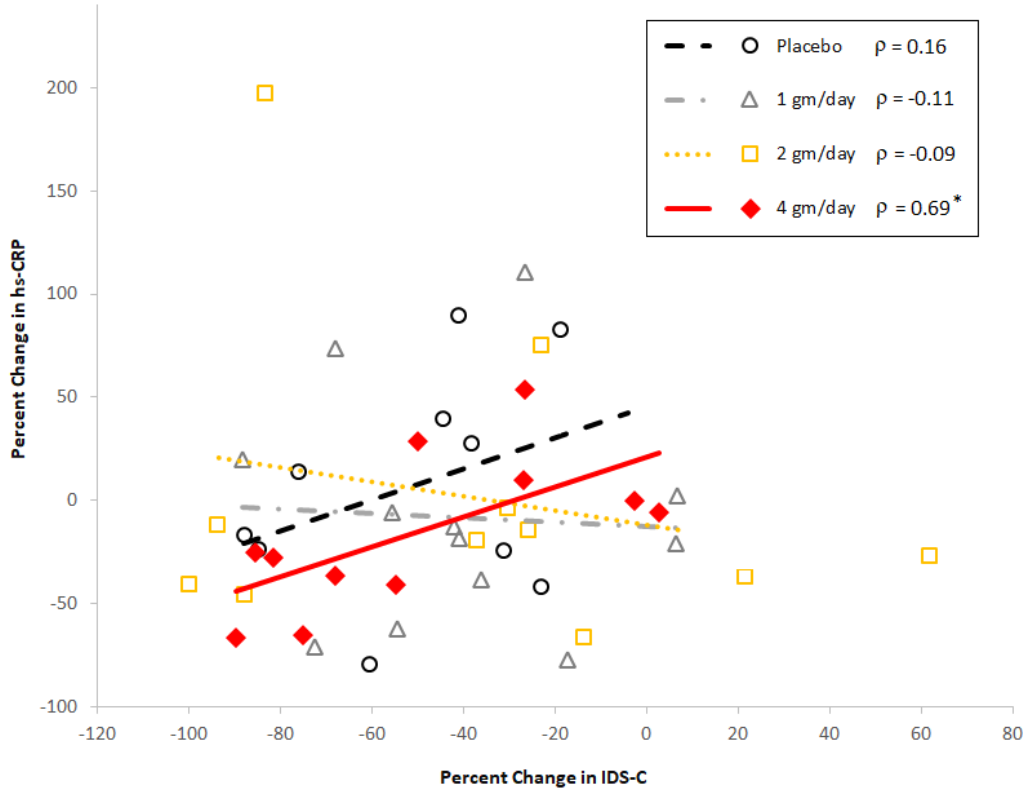
Supplementary Figure 1: CONSORT Statement Flow Diagram



a. Used for safety analysis
b. Randomized subjects who had baseline and at least one post-baseline per protocol (PP) assessment of IDS-C30
c. Randomized subjects who had PP data at baseline and treatment week 12 (end of treatment)

Abbreviations: EPA: Eicosapentaenoic Acid; CRP: C-Reactive Protein; IDS-C30: Inventory of Depressive Symptomatology, Clinician-Rated version; mITT: modified Intent-to-Treat; PP: Per Protocol

Supplementary Figure 2: Spearman's Rho Correlation Between Percent Changes in hsCRP and IDS-C Scores, By Treatment Arm



* Statistically significant correlation, p=0.019.

Abbreviations: hsCRP: high-sensitivity C-Reactive Protein; IDS-C: Inventory of Depressive Symptomatology.

Supplementary Table 1: Adverse Events (AEs) Reported by n=61 Randomized Subjects, by Treatment Group

	EPA 1 g/day	EPA 2 g/day	EPA 4 g/day	Placebo
	Affected / Total (%)	Affected / Total (%)	Affected / Total (%)	Affected / Total (%)
Total	**8/15 (53.33%)**	**8/15 (53.33%)**	**9/16 (56.25%)**	**11/15 (73.33%)**
Gastrointestinal System				
Abnormal Propulsive movement of large bowel	0/15 (0%)	0/15 (0%)	0/16 (0%)	1/15 (6.67%)
Acute vomiting	0/15 (0%)	0/15 (0%)	1/16 (6.25%)	1/15 (6.67%)
Belching	0/15 (0%)	0/15 (0%)	1/16 (6.25%)	0/15 (0%)
Bloating	0/15 (0%)	0/15 (0%)	0/16 (0%)	1/15 (6.67%)
Constipation alternating with diarrhea	0/15 (0%)	0/15 (0%)	0/16 (0%)	1/15 (6.67%)
Diarrhea/Loose stool	1/15 (6.67%)	0/15 (0%)	0/16 (0%)	2/15 (13.33%)
Flatulence	0/15 (0%)	1/15 (6.67%)	0/16 (0%)	0/15 (0%)
Gastric reflux	2/15 (13.33%)	1/15 (6.67%)	1/16 (6.25%)	2/15 (13.33%)
Gastroenteritis	1/15 (6.67%)	0/15 (0%)	0/16 (0%)	0/15 (0%)
Increased frequency of bowel movements	0/15 (0%)	0/15 (0%)	1/16 (6.25%)	1/15 (6.67%)
Nausea	1/15 (6.67%)	1/15 (6.67%)	0/16 (0%)	0/15 (0%)
Constipation	2/15 (13.33%)	0/15 (0%)	0/16 (0%)	1/15 (6.67%)
Increased appetite	1/15 (6.67%)	0/15 (0%)	0/16 (0%)	0/15 (0%)
Renal and Urinary Tract				
Increased Urination	0/15 (0%)	0/15 (0%)	0/16 (0%)	1/15 (6.67%)
Acute Urinary Tract Infection	1/15 (6.67%)	1/15 (6.67%)	0/16 (0%)	0/15 (0%)
Nervous System				
Headache	0/15 (0%)	1/15 (6.67%)	0/16 (0%)	2/15 (13.33%)
Dizziness of unknown cause	0/15 (0%)	0/15 (0%)	0/16 (0%)	2/15 (13.33%)
General				
Increased thirst	0/15 (0%)	1/15 (6.67%)	0/16 (0%)	0/15 (0%)
Tiredness	0/15 (0%)	0/15 (0%)	0/16 (0%)	1/15 (6.67%)
Weight loss	0/15 (0%)	0/15 (0%)	0/16 (0%)	1/15 (6.67%)
Eye related				
Acute conjunctivitis	0/15 (0%)	0/15 (0%)	1/16 (6.25%)	0/15 (0%)
Immune System				
Allergy symptoms (environmental)	1/15 (6.67%)	0/15 (0%)	0/16 (0%)	1/15 (6.67%)
Hives	0/15 (0%)	0/15 (0%)	1/16 (6.25%)	0/15 (0%)
Musculoskeletal				
Back pain	2/15 (13.33%)	0/15 (0%)	0/16 (0%)	0/15 (0%)
Knee pain	0/15 (0%)	0/15 (0%)	0/16 (0%)	1/15 (6.67%)
Ligament strain	0/15 (0%)	0/15 (0%)	1/16 (6.25%)	0/15 (0%)
Muscle strain	1/15 (6.67%)	1/15 (6.67%)	1/16 (6.25%)	0/15 (0%)
Lower limb numbness	1/15 (6.67%)	0/15 (0%)	0/16 (0%)	0/15 (0%)
Hematoma (toe)	0/15 (0%)	0/15 (0%)	0/16 (0%)	1/15 (6.67%)
Hand laceration	0/15 (0%)	1/15 (6.67%)	0/16 (0%)	0/15 (0%)

Dermatological				
Burn injury (right thumb)	1/15 (6.67%)	0/15 (0%)	0/16 (0%)	0/15 (0%)
Reproductive System				
Ovarian cyst exacerbation	0/15 (0%)	0/15 (0%)	1/16 (6.25%)	0/15 (0%)
Vaginal discharge	0/15 (0%)	1/15 (6.67%)	0/16 (0%)	0/15 (0%)
Cardiovascular System				
Palpitations	0/15 (0%)	0/15 (0%)	1/16 (6.25%)	0/15 (0%)
infections				
Influenza	0/15 (0%)	1/15 (6.67%)	0/16 (0%)	0/15 (0%)
Shingles	0/15 (0%)	1/15 (6.67%)	0/16 (0%)	0/15 (0%)
Upper Respiratory Infection	3/15 (20%)	1/15 (6.67%)	4/16 (25%)	2/15 (13.33%)
Ear related				
Tinnitus	0/15 (0%)	0/15 (0%)	0/16 (0%)	1/15 (6.67%)
Respiratory				
Cough	0/15 (0%)	0/15 (0%)	0/16 (0%)	1/15 (6.67%)

Abbreviations: AE: Adverse Event; EPA: Eicosapentaenoic acid.