Original Research

Baseline Blood Levels of Omega-3 and Depression Remission:
A Secondary Analysis of Data From a Placebo-Controlled Trial of Omega-3 Supplements

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ABSTRACT

Objective: Depression is associated with low red blood cell (RBC) levels of 2 omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), suggesting that omega-3 supplements might improve depression. However, clinical trials have produced mixed results. The purpose of this secondary analysis of data from a randomized controlled trial was to determine whether baseline blood levels of omega-3, which are known to vary widely among individuals, predict depression outcomes.

Method: The percentages of EPA, DHA, and the omega-6 arachidonic acid (AA) were measured in RBCs at baseline and posttreatment in 122 participants with *DSM-IV* major depression who were randomly assigned between May 2005 and December 2008 to receive either 50 mg/d of sertraline and a daily dosage of 930 mg EPA/750 mg DHA or sertraline plus placebo. Associations between baseline omega-3 RBC levels and remission of depression (17-item Hamilton Depression Rating Scale score ≤ 7) were analyzed by treatment arm.

Results: Among participants in the omega-3 arm, baseline RBC levels of EPA + DHA ($P = .002$) and the EPA + DHA:AA ratio ($P = .003$) were significantly higher among those whose depression subsequently remitted compared with those whose depression did not remit. No associations were detected in the sertraline plus placebo arm. Baseline levels of EPA ($P = .03$) and the EPA + DHA:AA ratio ($P = .04$) moderated the relationship between treatment arm and depression outcomes.

Conclusions: High baseline RBC levels of EPA and DHA and a high EPA + DHA:AA ratio predict favorable depression outcomes in patients receiving omega-3 supplements. Omega-3 supplementation may be an effective treatment for depression, but the requisite dosage and duration of treatment may depend on the patient's baseline level of omega-3 fatty acids.

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Depression is associated with low dietary intake and low plasma phospholipid and erythrocyte levels of 2 essential omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), in patients with[1–4] and without[5–11] coronary heart disease. As a result, there has been considerable interest in determining whether omega-3 supplements could be used to treat depression. Most of the placebo-controlled clinical trials that have investigated this question have tested either omega-3 supplements as monotherapy or omega-3 augmentation of antidepressants. The results of these trials have been mixed and have been the subject of numerous qualitative and quantitative reviews.[12–21] Because of the heterogeneity of the trial results, these reviews have attempted to identify patient characteristics, methods of administration, and the types and dosages of omega-3 that predict treatment outcomes. Several conclusions can be drawn from this literature.

First, omega-3 augmentation of antidepressants appears to be a more promising approach than omega-3 monotherapy for depression.[13] Second, omega-3 seems to be more efficacious for major depressive disorder (MDD) than it is for subclinical depression symptoms.[13,18,19] Third, trials that have tested only or mostly EPA tended to show positive results, whereas those that have tested only or mostly DHA do not.[18,21–23] Trials of omega-3 supplements that include > 60% EPA have yielded a mean depression treatment effect size (d) of 0.53, whereas those with < 60% EPA relative to DHA have produced a mean effect size of −0.03.[21]

The optimal dosages and blood levels of EPA and DHA for treatment of depression have received little direct study. However, in a retrospective analysis of multiple clinical trials, Sublette and colleagues[21] found a nonlinear relationship in the form of a U-shaped quadratic function between depression improvement and the dosage of EPA in excess of DHA within the range of 200 to 2,200 mg/d of EPA. The blood levels of EPA and DHA that are associated with improvement in depression symptoms remain unclear.

In most pharmaceutical trials, the baseline (pre-randomization) blood level of the active drug is almost always zero. In contrast, pretreatment blood levels of omega-3 vary considerably due to individual differences in dietary intake, the rate of incorporation into blood and tissues, and genetic variation in omega-3 fatty acid metabolism.[24] Thus, whether the necessary therapeutic level of omega-3 is actually achieved during a trial may depend not only on the dosage of omega-3 and the duration of the trial but also on the participants' pretreatment levels of omega-3. Higher baseline blood levels of omega-3 may make it easier to reach a therapeutic level within the time constraints of a placebo-controlled, randomized depression treatment trial. Furthermore, a higher baseline level would allow the therapeutic blood level to be achieved at a

- Studies evaluating the efficacy of omega-3 supplements for depression have produced mixed results. As blood levels of these essential fatty acids may vary from person to person before beginning treatment, this study asked whether initial blood levels predict final depression outcomes.

- The study found that initial blood levels of omega-3 fatty acids do predict response to treatment. Baseline levels of omega-3 may need to be considered when determining omega-3 dose and the duration of treatment to maximize effectiveness.

lower dose, thereby minimizing side effects and treatment dropouts.[25] Thus, pre-randomization differences in omega-3 blood levels may explain some of the differences in the outcomes of previous trials.

The physiological pathways through which omega-3 fatty acids improve depression are unclear, but it is known that during their metabolism, omega-3 fatty acids help to reduce inflammatory activity, which may lead, in turn, to improvement in depression symptoms.[18,21,26] For example, studies have shown that DHA and EPA suppress the production of the proinflammatory cytokines interleukin-1 beta, interleukin 6, and tumor necrosis factor alpha by monocytes and endothelial cells.[27–29] Eicosapentaenoic acid inhibits the synthesis of an omega-6 fatty acid, arachidonic acid (AA), from linoleic acid and competes with AA for its enzymatic conversion to proinflammatory molecules.[30,31] Omega-3 may therefore reduce depression symptoms by favorably shifting the systemic inflammatory balance toward lower inflammation. Thus, the EPA + DHA:AA ratio may also affect depression outcomes, and these ratios may vary considerably among trial participants at baseline.

The purpose of this study was to examine the relationship between depression outcomes and baseline red blood cell (RBC) levels of DHA and EPA and the EPA + DHA:AA ratio in depressed patients with coronary heart disease who were administered omega-3 supplements in a clinical trial.[32]

METHOD

The data for this study came from a randomized, double-blinded, placebo-controlled trial (NCT00116857) to determine the efficacy of 50 mg/d of sertraline plus 2 capsules containing a total daily dosage of 930 mg of EPA and 750 mg of DHA omega-3 (2 pills of the prescription supplement Lovaza), compared to sertraline plus corn oil placebo capsules.[32] The dosage of omega-3 was based on Peet and Horrobin's[25] finding that 1 g/d of EPA produced more improvement in depression than did higher doses. Eicosapentaenoic acid constituted 55% of the omega-3 supplement, just under the 60% level reported by Sublette and colleagues[21] to be associated with optimal improvement in depression.

Patients were recruited between May 2005 and December 2008 from cardiology practices and services affiliated with Washington University School of Medicine in St Louis,

Missouri. The medical inclusion criteria were documented coronary heart disease defined as ≥ 50% stenosis in ≥ 1 major coronary artery, a history of revascularization, or hospitalization for an acute coronary syndrome. The exclusion criteria were cognitive impairment; psychosis or other severe psychiatric comorbidities; high risk of suicide; current substance abuse; acute coronary syndrome within the previous 2 months; left ventricular ejection fraction < 30%; advanced malignancy; physical inability to participate; current use of an antidepressant, anticonvulsant, lithium, or omega-3 supplement; known sensitivity to sertraline or omega-3; and physician or patient refusal. Consenting patients who met the *DSM-IV* criteria for a current major depressive episode and scored ≥ 16 on the Beck Depression Inventory-II (BDI-II)[33] were enrolled in the study. A blood sample was obtained, and the 17-item Hamilton Depression Rating Scale (HDRS-17) was administered at baseline and after 10 weeks of treatment to assess treatment outcomes.[34] The study was approved by the Human Research Protection Office at Washington University, and all participants signed an approved informed consent form.

Unused medications were counted and subtracted from the number provided to determine adherence. Measures of total RBC membrane EPA, DHA, and AA were obtained at baseline and posttreatment assessments and assayed by capillary gas chromatography (GC2010, Shimadzu Corp, Columbia, Maryland) after extraction and conversion to fatty acid methyl esters.[35] Red blood cell levels of each fatty acid were expressed as the percentage of the total fatty acids. Dietary intake of fish containing high levels of omega-3 fatty acids was estimated before and during the trial using a brief structured dietary recall interview that asked participants to report the number of servings (3.5 oz) of any of 30 fatty fishes that are known to be high in EPA and DHA.

Statistical Analysis

Chi-square tests and analysis of variance (ANOVA) models were used to compare prespecified baseline fatty acids and other clinical, medical, and demographic variables between patients whose depression subsequently remitted (HDRS-17 ≤ 7) and those who did not achieve remission. Analysis of covariance was used to determine whether baseline RBC levels of EPA and DHA or the EPA + DHA:AA ratio moderated the relationship between treatment assignment and depression outcomes. Posttreatment HDRS-17 scores were regressed on the percentage of the fatty acid in RBCs, a dichotomous indicator for treatment arm assignment; the HDRS-17 score at baseline; and the interaction between treatment assignment and the percentage of the fatty acid in RBCs. A similar analysis was conducted with the number of fatty fish portions reportedly consumed in the previous month.

Distributional assumptions were verified, and residual analyses were performed on each statistical model. All statistical tests were 2-sided with a type I error rate set at the .05 level. All analyses were performed using SAS statistical software, version 9.3 (SAS Institute).

Table 1. Demographic and Medical Characteristics by Posttreatment Remission Status (HDRS-17 ≤ 7)

Variable[a]	Intervention Group No (n = 31)	Intervention Group Yes (n = 27)	P	Placebo Group No (n = 28)	Placebo Group Yes (n = 27)	P
Demographics						
Sex (% female)	9 (29.0)	10 (37.0)	.52	9 (32.1)	8 (29.6)	.84
Age, y	58.0 ± 10.2	59.0 ± 7.9	.69	57.9 ± 9.8	58.9 ± 7.6	.69
White	22 (71.0)	24 (88.9)	.09	23 (82.1)	22 (81.5)	.95
Medical						
Current cigarette smoker	11 (35.5)	5 (18.5)	.15	7 (25.0)	4 (14.8)	.35
Body mass index, kg/m^2	33.2 ± 7.0	34.8 ± 8.0	.41	32.1 ± 6.0	33.4 ± 8.5	.53
Diabetes mellitus	14 (45.2)	4 (14.8)	.01	11 (39.3)	13 (48.1)	.51
History revascularization	24 (77.4)	23 (85.2)	.45	24 (85.7)	24 (88.9)	.72
History of hypertension	24 (77.4)	20 (74.1)	.77	23 (82.1)	22 (81.5)	.95
Creatinine, mg/dL	0.95 ± 0.24	0.93 ± 0.28	.76	0.97 ± 0.32	0.92 ± 0.23	.48
Medications and biomarkers						
β blocker	24 (77.4)	21 (77.8)	.97	24 (85.7)	21 (77.8)	.45
ACE inhibitor	18 (58.1)	11 (40.7)	.19	13 (46.4)	12 (44.4)	.88
Aspirin	23 (74.2)	19 (70.4)	.75	23 (82.1)	26 (96.3)	.09
Statins	19 (61.3)	22 (81.5)	.09	21 (75.0)	21 (77.8)	.81
Insulin	6 (19.4)	2 (7.4)	.26	8 (28.6)	3 (11.1)	.11

[a]Continuous variables are reported as mean ± SD; categorical variables represent number of patients (%).

Abbreviations: ACE = angiotensin-converting enzyme, HDRS-17 = 17-item Hamilton Depression Rating Scale.

RESULTS

As previously reported, there were no differences between the sertraline plus placebo (n = 55) and sertraline plus omega-3 (n = 58) arms between baseline and posttreatment HDRS-17 change scores (9.4 [placebo] vs 9.3 [omega-3]; $P = .90$) or in pre-post BDI-II change scores (14.8 [placebo] vs 16.1 [omega-3]; $P = .44$).[32] Furthermore, the groups did not differ after treatment on depression remission (HDRS-17 ≤ 7). Remission occurred in 27 (46.6%) of the participants in the omega-3 arm, and 27 (49.1%) of the patients in the placebo arm. The demographic and medical characteristics of the remitters and nonremitters are presented by treatment arm in Table 1. The prevalence of diabetes differed between remitters and nonremitters in the omega-3 arm. However, diabetes was unrelated to either baseline EPA/DHA levels ($P = .32$) or to change in EPA/DHA levels following treatment ($P = .77$). There were no differences between remitters and nonremitters on any variable in the placebo arm.

As expected, RBC levels of omega-3 fatty acids rose significantly in the omega-3 group following treatment, but changed very little in the placebo arm (Table 2). Baseline RBC levels of EPA + DHA were higher in the remitted than the nonremitted participants who had received omega-3 ($P = .002$), and the remitted participants tended to have higher RBC levels of EPA + DHA following treatment than did the nonremitters ($P = .07$). There were no differences in RBC levels of EPA + DHA at baseline or posttreatment between remitted and nonremitted participants in the placebo arm. Adherence to the omega-3 or placebo capsule and sertraline regimens exceeded 97% in both groups, with no significant between-group differences.

The baseline level of AA did not differ between remitters and nonremitters in either arm, but the ratio of EPA + DHA:AA was significantly higher among the remitters

than the nonremitters in the omega-3 arm ($P = .003$). There was also a trend toward a difference in the EPA + DHA:AA ratio between remitters and nonremitters following treatment in the omega-3 arm ($P = .09$). In contrast, there were no differences in baseline or posttreatment EPA + DHA:AA ratios between remitters and nonremitters in the placebo arm. Consistent with the baseline RBC levels of omega-3, the self-reported number of fatty fish servings consumed in the month prior to study enrollment was higher in the remitters than in the nonremitters in the omega-3 arm ($P = .02$) and tended to be higher in those who remitted in the placebo arm ($P = .07$). The correlation between self-reported consumption of fatty fish and baseline levels of EPA + DHA was 0.28 ($P = .002$).

Analyses of covariance were conducted to determine whether baseline RBC levels of EPA and DHA, the EPA + DHA:AA ratio, or the consumption of fatty fish in the prior month moderated the relationship between treatment arm and depression outcome. The interactions between treatment arm and baseline RBC level of EPA ($P = .03$) and the baseline EPA + DHA:AA ratio ($P = .04$) were significant. Both baseline EPA and baseline EPA + DHA:AA therefore moderated the relationship between treatment arm and depression outcomes. There was also a trend toward an interaction between treatment arm and baseline EPA + DHA ($P = .08$) and DHA alone ($P = .07$). The interaction between treatment assignment and the number of fatty fish portions consumed in the prior month to baseline was not significant ($P = .17$).

There was a significant linear relationship between baseline and posttreatment omega-3 (EPA + DHA) blood levels ($r = 0.71$; $P < .0001$) in the omega-3 arm. Predicted probabilities from a logistic regression model (probability of remission regressed on omega-3 blood levels and baseline HDRS-17 score) were used to classify patients as having

Table 2. Red Blood Cell (RBC) Omega-3 and Omega-6 Fatty Acid Levels and Adherence by Posttreatment Remission Status

Variable	Intervention Group Fatty Acid Level[a]			Placebo Group Fatty Acid Level[a]		
	No (n = 31)	Yes (n = 27)	P	No (n = 28)	Yes (n = 27)	P
EPA + DHA						
Baseline	4.1 ± 0.9	5.3 ± 1.9	.002	4.7 ± 1.5	4.6 ± 1.3	.75
Posttreatment	7.2 ± 1.5	8.0 ± 2.0	.07	4.6 ± 1.3	4.5 ± 1.0	.74
EPA						
Baseline	0.47 ± 0.11	0.61 ± 0.31	.02	0.60 ± 0.22	0.49 ± 0.23	.10
Posttreatment	1.5 ± 0.60	1.6 ± 0.70	.51	0.56 ± 0.23	0.45 ± 0.26	.18
DHA						
Baseline	3.7 ± 1.0	4.7 ± 1.7	.004	4.2 ± 1.4	4.1 ± 1.1	.75
Posttreatment	5.7 ± 1.1	6.4 ± 1.5	.048	4.1 ± 1.1	4.0 ± 1.0	.77
Arachidonic acid (omega-6)						
Baseline	18.4 ± 1.7	17.8 ± 2.2	.31	17.7 ± 2.5	17.7 ± 2.1	.99
Posttreatment	16.5 ± 3.1	16.0 ± 2.5	.48	17.7 ± 1.8	17.7 ± 2.3	.94
EPA + DHA:AA ratio						
Baseline	0.23 ± 0.06	0.30 ± 0.12	.003	0.28 ± 0.12	0.27 ± 0.10	.67
Posttreatment	0.45 ± 0.12	0.52 ± 0.16	.09	0.27 ± 0.09	0.26 ± 0.09	.72
Omega-3/placebo compliance, %	97.6 ± 3.1	97.3 ± 5.5	.78	98.1 ± 2.4	97.1 ± 3.0	.20
Sertraline compliance, %	99.0 ± 2.3	98.2 ± 3.9	.35	98.5 ± 3.2	98.7 ± 1.9	.86
Fish intake (mean servings/wk)	0.3 ± 0.3	1.0 ± 1.5	.02	0.5 ± 0.6	0.9 ± 1.2	.07
Number of reported side effects	0.6 ± 1.8	0.8 ± 2.2	.62	1.4 ± 3.3	0.5 ± 1.0	.21

[a]Continuous variables are reported as mean ± SD % in red blood cells.
Abbreviations: AA = arachidonic acid, DHA = docosahexaenoic acid, EPA = eicosapentaenoic acid.

Table 3. Logistic Regression Model Predicting Probability of Remission Based on Baseline and Posttreatment Levels of EPA + DHA

EPA + DHA Level	Low Probability (< 60%), n = 44	High Probability (≥ 60%), n = 18
Baseline EPA + DHA (%)		
Mean ± SD	3.9 ± 0.8	6.3 ± 1.6
25th percentile	3.3	5.2
Median	3.9	6.0
75th percentile	4.6	7.3
(Minimum, maximum)	(2.4, 5.5)	(4.1, 9.7)
Posttreatment EPA + DHA (%)		
Mean ± SD	7.1 ± 1.4	8.7 ± 2.0
25th percentile	6.3	7.7
Median	7.1	8.2
75th percentile	8.3	9.1
(Minimum, maximum)	(3.8, 9.9)	(5.5, 14.1)

Abbreviations: DHA = docosahexaenoic acid, EPA = eicosapentaenoic acid.

either a "high" (≥ 60%) or a "low" (< 60%) probability of remission and to compare the distribution of baseline and posttreatment omega-3 blood levels between these 2 groups (Table 3). In general, the higher the baseline EPA + DHA level, the higher the posttreatment EPA + DHA level and the greater the probability of remission.

DISCUSSION

We previously reported the primary results of this double-blind, randomized, placebo-controlled trial of omega-3 augmentation of sertraline.[32] Although we found that this combination of EPA and DHA had some cardiovascular benefits,[36] it was not efficacious for depression.[32] As hypothesized for this secondary analysis, baseline RBC levels of EPA and DHA, the EPA + DHA:AA ratio, and the reported number of servings of fatty fish rich in omega-3 consumed in the month prior to enrollment in

the clinical trial were significantly higher among patients in the omega-3 arm whose depression subsequently remitted than in those whose depression did not remit. In addition, we found that the percentage of EPA in RBCs at baseline and the EPA + DHA:AA ratio moderated the relationship between treatment group and depression outcomes. There was also a trend for baseline RBC levels of DHA to moderate improvement in depression. These results suggest that if all participants in the trial had begun with higher RBC levels of omega-3, especially EPA, there might have been a significant between-group, posttreatment difference in depression.

Omega-3 and omega-6 are essential fatty acids that must be obtained through dietary intake. While the blood level of a study drug can ordinarily be assumed to be zero before a trial begins, this assumption is not warranted in trials of food supplements such as omega-3 fatty acids. Higher baseline blood levels of omega-3 may make it easier to reach a therapeutic level within the normal duration of a placebo-controlled depression trial.

High baseline levels of EPA and DHA may result from a diet high in these omega-3 fatty acids. It is also possible that participants with higher omega-3 levels at baseline incorporate these fatty acids into RBCs and other tissues more efficiently than do those with lower levels of omega-3 and are therefore better able to utilize the omega-3 provided by the supplements. There are large individual differences in the plasma and RBC uptake of omega-3, even with an identical diet. In both short-term and long-term studies, a fixed daily dose of omega-3 produces substantial individual variability in omega-3 blood levels,[37] which may be explained in part by genetic polymorphisms.[38] Lower blood and tissue uptake of omega-3 may help to explain the lower levels of EPA and DHA found in depressed patients and may even have etiologic significance.

However, in the group receiving the omega-3 supplement, the number of servings of fish rich in omega-3 consumed during the month before enrollment in the trial was also higher in the remitters compared to the nonremitters. Fish intake tended to be higher in the remitters compared to nonremitters in the sertraline plus placebo arm, but it did not moderate the effect of treatment assignment on depression outcomes. The correlation between the estimated number of fish servings and omega-3 blood levels was only moderate ($r = 0.28$), accounting for less than 10% of the variance in omega-3 levels. In addition to the wide variability in the uptake of omega-3 from food or supplements, this low correlation may be due to the difficulty in obtaining accurate and reliable estimates of diet using standard self-report or interview-based dietary assessments.[39] It is important to acknowledge, however, that the dietary interview used in this study considered only a list of fish known to be high in DHA and EPA, and for the purpose of adding fish servings (approximately 3.5 ounces), the interview did not distinguish between type of fish (eg, tuna vs salmon) although DHA + EPA levels in these fish vary considerably.

The optimal therapeutic blood (RBC or plasma) levels of omega-3 associated with successful omega-3 treatment of major depression, either alone or in combination with antidepressants, have received little study. However, studies have compared plasma or RBC levels of EPA + DHA between patients with major depression and healthy controls.[40] Although plasma and RBC omega-3 levels correlate, RBC levels are less sensitive to recent intake of omega-3, and therefore tend to provide more reliable, stable estimates of omega-3 levels over time.[41,42] In 3 studies[6,10,11] comparing EPA + DHA in RBCs in individuals with major depression and healthy controls, the mean percentages of RBC omega-3 in depressed patients were 3.6%, 3.6%, and 3.8% versus 6.3%, 5.4%, and 4.8% in the nondepressed participants. These findings suggest that an omega-3 level of 5% to 6% in RBCs might be a reasonable target for therapeutic efficacy. However, patients whose depression did not remit in the present study nevertheless increased their RBC omega-3 levels to an average of 7.2%, compared to 8% for the remitters. Further analysis of these data revealed that the probability of remission was highest in the participants in the omega-3 arm who had the highest blood levels after treatment, in the range of 9% to 10%, and these participants also had the highest blood levels before the trial began. This finding is similar to that reported in a review[38] of trials of omega-3 to improve cardiovascular outcomes, which concluded that the beneficial effects of omega-3 on cardiovascular outcomes in clinical trials were achieved by the patients who reached the highest blood levels of omega-3, which in many cases were higher than those typically observed in healthy controls.

The levels associated with depression reported in the studies of otherwise medically well participants[6,10,11] are similar to those found in patients with coronary heart disease.[38,43,44] Numerous clinical trials have investigated the effects of omega-3 on cardiovascular outcomes. These studies were recently reviewed by Superko and colleagues,[38] who found wide variability in blood levels of omega-3 in clinical trial participants who received identical dosages of omega-3. They concluded that, because of individual variability in blood levels following treatment, blood levels of omega-3 may be better markers of cardiovascular disease risk/benefit than simple assignment to a fixed dose of omega-3 supplementation. Furthermore, they noted that including subjects who achieved less than therapeutic blood levels may have diluted the beneficial effect of omega-3 on cardiovascular disease endpoints. These observations may also apply to depression clinical trials.

It is important to note that participants in this study had major depressive disorder and coronary heart disease. Cardiovascular disease and other comorbid medical diseases present in these patients may affect omega-3 metabolism and utilization and the relationship of RBC levels to depression outcomes. Thus, the generalizability of these findings to medically well psychiatric patients is not clear. It is also important to note that the present data were collected for a randomized clinical trial. The trial was not designed to determine therapeutic levels of omega-3 that might be needed to improve depression outcomes. Studies designed to determine the range of omega-3 blood levels and the duration of exposure to these levels that are required to maximize the probability of remission are needed. In addition, more work is needed to identify the genetic, metabolic, medical, and dietary factors that may moderate the dose/response relationship between omega-3 and depression improvement, similar to the research that has been reported concerning the dosing requirements for vitamin D supplementation.[45]

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