Published in final edited form as:
PharmaNutrition. 2014 April 1; 2(2): 38–46. doi:10.1016/j.phanu.2014.02.002.

Detection and Treatment of Long-Chain Omega-3 Fatty Acid Deficiency in Adolescents with SSRI-Resistant Major Depressive Disorder

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Abstract

Residual depressive symptoms are commonly observed in adolescents with major depressive disorder (MDD) following treatment with selective serotonin reuptake inhibitors (SSRIs). This study combined a case-control analysis and an open-label fish oil (FO) trial to investigate the relationship between long-chain omega-3 (LC*n*-3) fatty acid status and residual depressive symptoms in SSRI-resistant adolescent MDD patients. Baseline erythrocyte docosahexaenoic acid (DHA)(-28%, p=0.0003), but not eicosapentaenoic acid (EPA)(-18%, p=0.2), was significantly lower in patients (n=20) compared with healthy controls (n=20). Patients receiving 10-week low-dose (2.4 g/d, n=7) and high-dose (16.2 g/d, n=7) FO exhibited significant increases in erythrocyte EPA and DHA composition. In the intent-to-treat sample, depressive symptoms decreased significantly in the high-dose group (n=7, -40%, p<0.0001), and there was a trend in the low-dose group (n=10, −20%, p=0.06). Symptom remission was observed in 40% of patients in the low-dose group and 100% of patients in the high-dose group. There were no significant changes in vital signs and adverse events were rated as mild or moderate in severity. These preliminary findings demonstrate that adolescents with SSRI-resistant depression exhibit robust DHA deficits, and suggest that adjunctive FO supplementation is well-tolerated and effective for increasing LC*n*-3 fatty acid status and augmenting SSRI antidepressant effects.

Keywords

Omega-3 fatty acids; Docosahexaenoic acid; Eicosapentaenoic acid; Adolescents; Erythrocyte; Major depressive disorder (MDD)

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1. INTRODUCTION

Converging evidence suggests that a deficiency in long-chain omega-3 (LC*n*-3) fatty acids, including eicosapentaenoic acid (EPA, 20:5*n*-3) and docosahexaenoic acid (DHA, 22:6*n*-3), during brain development may represent a modifiable risk factor for mood disorders including major depressive disorder (MDD)[1]. Cross-national epidemiological data suggest that greater per capita intake of fish, a principal dietary source of preformed EPA+DHA, is associated with reduced lifetime prevalence rates of MDD [2,3]. Independent meta-analyses of controlled trials suggest that fish oil (FO) supplementation is superior to placebo for reducing depressive symptoms in adult MDD patients [4-8]. The initial onset of MDD frequently occurs during adolescence [9,10], and a large percentage of adolescents residing in western countries consume low quantities of LC*n*-3 fatty acids in their diet [11-13]. Preliminary FO supplementation trials have observed reductions in depressive symptoms in children and adolescents with established mood disorders [14-16]. These and other data suggest that greater dietary fish intake may be protective against the development of depressive symptoms, and that FO supplementation has antidepressant effects in patients with MDD.

A valid technique for determining an individuals LC*n*-3 fatty acid 'status' is gas chromatographic analysis of erythrocyte (red blood cell) fatty acid composition [17,18]. Erythrocyte EPA+DHA levels are positively correlated with fish intake frequency [19,20], and increase in a dose-dependent manner following FO supplementation [22,23]. Additionally, normative population data are beginning to emerge [20,21], and low erythrocyte EPA+DHA composition (termed the 'omega-3 index') has been proposed as a risk biomarker for coronary heart disease mortality [24]. Specifically, erythrocyte EPA +DHA composition of ≤4% is considered a high risk zone whereas >8% is a low risk zone [24]. Although this index has not been systematically evaluated in the context of MDD, a meta-analysis of fourteen cross-sectional studies found significantly lower erythrocyte EPA and DHA levels in patients with MDD [25]. More recent case-control studies found that erythrocyte EPA+DHA composition of ≤4.0% is significantly more prevalent among adolescent and adult MDD patients [26,27]. These and other data suggest that erythrocyte EPA+DHA composition may serve as a biomarker relevant to the pathophysiology and potentially etiology of MDD [28].

Multiple lines of evidence suggest that a dysregulation in serotonin neurotransmission is central to the pathophysiology and treatment of MDD [29,30]. Emerging translational evidence suggests that LC*n*-3 fatty acid status influences the development of central serotonin systems. Specifically, dietary *n*-3 fatty acid insufficiency during perinatal development is associated with systemic LC*n*-3 fatty acid deficits and impaired serotonin release [31], as well as elevated behavioral indices of depression and aggression [32], in young adulthood. In contrast, FO supplementation during development increases serotonin concentrations in rat frontal cortex [33], attenuates reductions in frontal cortex serotonin content in response to chronic stress [34], and decreases behavioral indices of depression [35]. Furthermore, FO supplementation augments the antidepressant-like effects of selective serotonin reuptake inhibitors (SSRIs) in rodents [36,37]. Similarly, controlled trials have observed greater reductions in depressive symptoms by combining FO with SSRIs compared

with either treatment alone [38,39]. Together these data suggest that LC*n*-3 fatty acid status influences the maturation and resilience of serotonin neurotransmitter systems which may impact SSRI antidepressant effects.

Although SSRI medications have become the primary treatment for adolescent depression, approximately 30-40 percent of adolescent MDD patients exhibit residual symptoms following standard SSRI treatment [40,41]. Because untreated depression is associated with poor outcomes and increased risk of suicide [42,43], there is an urgent need to identify risk biomarkers and adjunctive treatments for adolescents with SSRI-resistant MDD to inform clinical practice [28]. Based on the translational evidence reviewed above, low LC*n*-3 fatty acid status may represent a modifiable risk factor for SSRI-resistance. To evaluate this, the present study first investigated erythrocyte LC*n*-3 fatty acid status of adolescents with SSRI-resistant MDD in a case-control analysis, and then determined the effects of open-label FO supplementation on erythrocyte LC*n*-3 fatty acid composition and residual depressive symptoms. Our specific prediction was that adolescents with SSRI-resistant MDD would exhibit erythrocyte EPA+DHA deficits compared with healthy adolescent controls, and that FO supplementation would dose-dependently increase erythrocyte EPA+DHA levels and decrease residual depressive symptoms.

2. Materials and Methods

2.1. Subjects

Male or female adolescents (8-24 years of age) diagnosed with MDD (*DSM-IV-TR* criteria) were recruited by advertisement, word of mouth, and referral from existing pediatric/adolescent recruitment infrastructure within the Department of Psychiatry, University of Cincinnati College of Medicine. The MDD diagnosis was confirmed with the Kiddie Schedule for Affective Disorders and Schizophrenia (K-SADS)[44]. All adolescents were assessed by a board-certified child and adolescent psychiatrist. Written informed consent and assent were provided by a legal guardian and the subject, respectively. Participants were required to have a baseline score of >28 and <40 on the Children Depression Rating Scale-Revised (CDRS-R) despite being administered a standard therapeutic dose of an SSRI for a minimum of 6 weeks (i.e., SSRI-resistant). Patients were maintained on their baseline SSRI dose over the course of the trial. Patients were excluded by a positive urine pregnancy test, a history of seizures, traumatic brain injury, or major medical illness, having a urine drug screen which was positive for illicit substance use, were greater than 1 year outside appropriate age/grade level, required treatment with any psychotropic drug which might obscure the action of the study treatment, or had a seafood allergy. A dietary omega-3 intake questionnaire was administered at baseline to estimate current habitual dietary fish/seafood intake and to exclude patients currently taking omega-3 fatty acid supplements. A nested group of healthy controls with no personal history of a *DSM-IV-TR* Axis I disorder were recruited from the greater Cincinnati area. This trial was approved by the University of Cincinnati Institutional Review Board, and was registered at clinicaltrials.gov as NCT00511810.

2.2. Gas chromatography

Whole venous blood (10 ml) was collected into EDTA-coated BD Vacutainer tubes, and centrifuged at 4°C for 20 min (1,500 ×*g*). Plasma and buffy coat were removed and erythrocytes washed 3 times with 0.9% NaCl and stored at −80°C. Total erythrocyte membrane fatty acid composition was determined with a Shimadzu GC-2010 equipped with an auto-injector (Shimadzu Scientific Instruments Inc., Columbia MD), as previously described [26]. The column was a DB-23 (123-2332): 30m (length), I.D. (mm) 0.32 wide bore, film thickness of 0.25 μM (J&W Scientific, Folsom CA). The carrier gas was helium with a column flow rate of 2.5 ml/min. Fatty acid identification was determined using retention times of authenticated fatty acid methyl ester standards (Matreya LLC Inc., Pleasant Gap PA). Analysis of fatty acid methyl esters was based on areas calculated with Shimadzu Class VP 4.3 software. Data are expressed as weight percent of total fatty acids (mg fatty acid/100 mg fatty acids). Based on studies which added a known mass of heptadecanoic acid (17:0, 99%, Matreya LLC Inc., Pleasant Gap PA) to samples, the lower limit of detection with a threshold area of 500 and a 1 μl injection volume is approximately 200 ng of an individual fatty acid. All samples were processed by a technician blinded to group and treatment assignment. The primary measures of interest were EPA and/or DHA composition and the ratio of arachidonic acid to EPA and/or DHA.

2.3. Fish oil supplementation

Patients were randomized (stratified by gender) to open-label FO at a fixed EPA+DHA dose of either 2.4 g/day (Low-Dose: EPA 1.6 g + DHA 0.8 g; 4 capsules/d) or 16.2 g/day (High-Dose: EPA, 10.8 g + DHA 5.4 g; 2 tablespoons/day) for 10 weeks. In order to achieve high-dose FO while avoiding the burden of taking the equivalent of 27 capsules, high-dose FO was administered as a liquid. The FO is an ethyl ester formulation, derived from anchovies and sardines, and was generously supplied by The Inflammation Research Foundation. Compliance was evaluated by determining capsule counts (low-dose) or bottle volumes (high-dose) and self-reports at weekly visits. The fatty acid composition of the FO was independently confirmed by gas chromatography as described above. The low dose (2.4 g/d) was selected based on previous studies findings that similar doses were safe and efficacious in pediatric and adolescent patients with mood disorders [14-16], and the high dose (16.2 g/d) based on efficacy and safety data in pediatric and adolescent ADHD patients [45]. Patients were requested to maintain their current dietary habits over the course of the trial. To minimize gastrointestinal adverse events associated with FO supplementation, patients were instructed to take their supplements with meals. Based on effect sizes observed in prior FO intervention trials in pediatric and adolescent patients with mood disorders [14-16], the target sample size of n=10-15/dose group was estimated to have 80% power to detect large effect sizes.

2.4. Depression symptom ratings

At weekly visits depression symptom severity was determined with the Children's Depression Rating Scale-Revised (CDRS-R), a 17-item observer-rated questionnaire [46,47]. All patients were rated by a board-certified child and adolescent psychiatrist with established inter-rater reliabilities (kappa > 0.9). Remission was defined as an endpoint

CDRS-R score of ≤28 [41]. Response was defined as a ≥50% baseline-endpoint decrease in CDRS-R total score. If a patient's depressive symptoms worsened over the course of the trial (defined as 30% worsening relative to baseline on two consecutive visits using CDRS-R total score), they were withdrawn from study participation and referred for alternate treatment.

2.5. Safety and tolerability assessments

Body weight (kg) and height (cm), a complete blood count (white blood cells, WBC; red blood cells, RBC; platelets), thyroid stimulating hormone (TSH) concentrations, and vital signs (pulse, blood pressure, and temperature) were obtained at baseline and endpoint. Sex- and age-adjusted body mass index (BMI, kg/m^2) percentile and *z* scores were calculated. The frequency and severity of adverse events were assessed at each visit using the Side Effects Form for Children and Adolescents (SEFCA)[48]. In view of the potential risk for developing hypomanic symptoms following FO supplementation [49], manic symptoms were evaluated biweekly with the Young Mania Rating Scale (YMRS), an 11-item observer-rated questionnaire [50].

2.6. Statistical analyses

Statistical analyses were performed using Statistical Analysis System (SAS) software, version 9.0 (SAS Institute, Cary, NC, USA). Differences between patients and controls in demographic measures were evaluated using unpaired *t*-tests (2-tail, α=0.05) for continuous variables and Chi-square tests (2-tailed, α=0.05) for dichotomous variables. For the case-control fatty acid analysis, we employed Bonferroni correction for multiple comparisons (α=0.05/17 fatty acids and ratios = 0.003). Categorical assessments were used to determine the percentage of subjects with EPA+DHA levels ≤4.0 percent of total fatty acids (2-tailed Chi-square test, α=0.05). Baseline-endpoint changes in vital signs and labs were evaluated with a two-way ANOVA, with dose (low-dose, high-dose) and study time point (baseline, endpoint) as the main factors. Efficacy and tolerability analyses were performed on the intent-to-treat (ITT) sample, which included all patients who received at least one dose of study medication and also had at least one post-baseline efficacy and tolerability assessment. For mood symptom scores obtained at weekly (CDRS-R) or biweekly (YMRS) visits, a mixed-effects regression model (PROC MIXED) that included terms for dose, time, and dose-by-time interaction was used. AIC was used to select the variance-covariance structure for this model (inclusion or exclusion of subject-level random intercepts and slopes and autoregressive structure for the residual covariances). Pearson correlation coefficients were used to evaluate relationships between primary outcome measures (2-tail, α=0.05). For primary outcome measures, effect sizes were calculated using Cohen's *d*, with small, medium, and large effect sizes being equivalent to *d*-values of 0.30, 0.50, and 0.80, respectively.

3. Results

3.1. Case-control analysis

For demographic variables there were no significant differences between MDD patients and healthy controls (Table 1). For patients, mean daily SSRI doses were: fluoxetine 26±16.5

mg; citalopram 26.7±28.9 mg; sertraline 70.8±43.1 mg; escitalopram 20±0 mg. The case-control comparison of total erythrocyte fatty acid composition is presented in Table 2. Erythrocyte DHA composition (−28%, p=0.0003, *d* = 1.3), but not EPA (−18%, p=0.2) or DPA (−8%, 22:5*n*-3)(p=0.07), was significantly lower in patients compared with controls. EPA+DHA composition (−28%, p=0.0001, *d* = 1.4) and the sum of LC*n*-3 fatty acids (EPA +DPA+DHA, −21%, p=0.0001) were significantly lower in patients. A significantly greater proportion of patients (90%) exhibited EPA+DHA composition ≤4.0% compared with controls (40%)(Chi-Square: p=0.002). Erythrocyte AA composition did not differ between patients and controls, and the AA/DHA (+27%, p=0.0001) and AA/EPA+DHA (+25%, p=0.0001) ratios, but not the AA/EPA ratio (+13%, p=0.48), were significantly greater in patients. Among all MDD patients (n=20), EPA+DHA (*r* = +0.36, p=0.11), DHA (*r* = +0.35, p=0.13), and the AA/EPA+DHA (*r* = −0.35, p=0.12) and AA/EPA (*r* = −0.24, p=0.29) ratios, were not significantly correlated with baseline CDRS-R total scores. Other major fatty acids did not differ between patients and controls after correcting for multiple comparisons.

3.2. Open-label intervention

3.2.1. Subject recruitment and attrition—A flow diagram illustrating the sequence of subject recruitment and attrition is illustrated in Figure 1. A total of 14 patients completed the 10 week open-label intervention (low-dose, n=7; high-dose, n=7). A total of 3 patients were lost to follow-up post-randomization, and there were 3 patients randomized to low-dose that terminated study participation early (2 patients (weeks 3 and 6) due to a worsening of depressive symptoms, and 1 patient (week 9) declined to complete endpoint study procedures). At baseline, low-dose and high-dose groups did not differ in age (p=0.30), gender (p=1.0), race (p=0.99), BMI (p=0.94), or age at onset of MDD (p=0.92). At baseline, the majority of patients consumed fish ≤1 times/month (70%), and monthly fish intake frequency did not differ between low-dose and high-dose groups (p=0.29).

3.2.2. Erythrocyte fatty acid composition—Based on capsule counts (low-dose) or bottle volumes (high-dose) determined at weekly visits, there was a compliance rate of 92% for the low-dose group and 97% for the high-dose group. At baseline, erythrocyte EPA +DHA composition did not differ between low-dose and high-dose groups (p=0.75), and among all patients was positively correlated with fish intake frequency but this did not reach significance (*r* = +0.28, p=0.12). Change in erythrocyte fatty acid compositions for patients with baseline and endpoint values are presented in Table 3. For each of the primary measures, the main effect of Time was significant, and the main effect of Dose and the Time × Dose interaction were not significant. EPA+DHA composition increased significantly in low-dose (+57%, p=0.0001) and high-dose (+62%, p=0.003) groups. Endpoint EPA+DHA composition was 7.3%±0.6% for low-dose and 7.9%±1.3% for high-dose (p=0.70), and all patients exhibited EPA+DHA composition ≥4.0%. Endpoint EPA+DHA composition in low-dose (+42%, p<0.0001) and high-dose (+46%, p=0.0002) groups were significantly greater than healthy controls. The AA/DHA ratio decreased significantly in low-dose (−57%, p<0.0001) and high-dose (−53%, p=0.0002) groups, and the AA/EPA+DHA ratio decreased significantly in low-dose (−64%, p<0.0001) and high-dose (−58%, p=0.0002) groups.

3.2.3. Depression symptom ratings—Baseline CDRS-R total scores in low-dose and high-dose groups did not differ significantly (p=0.94). Change in CDRS-R total scores over the 10 week trial are presented in Figure 2. For the CDRS-R mixed-effects model, the dose by time interaction was not significant (p=0.670). The baseline-last available CDRS-R total score declined significantly in the high-dose group (−40%, p=0.0001, d = 5.2) and there was a trend for a decrease in the low-dose group (−20%, p=0.063, d = 0.93). After removal of the n=3 patients in the low-dose group that did not complete the 10 week treatment trial, baseline CDRS-R total scores decreased significantly at endpoint (−27%, p=0.002, d = 2.2). At Week 10, 60% of patients in the low-dose group and 100% of patients in the high-dose group met criteria for symptom remission (CDRS-R total score ≤28), and 40% of patients in the low-dose group and 100% of patients in the high-dose group met criteria for response (≥50% baseline-endpoint decline in CDRS-R total score). Among all completers (n=14), the baseline-endpoint change in CDRS-R total scores was not significantly correlated with baseline erythrocyte EPA+DHA (r = +0.17, p=0.56) and DHA (r = −0.03, p=0.92), or AA/EPA+DHA (r = −0.03, p=0.89) and AA/EPA (r = −0.01, p=0.98) ratios. Moreover, the baseline-endpoint change in CDRS-R total scores were not significantly correlated with baseline-endpoint change in erythrocyte EPA+DHA (r = −0.04, p=0.88), DHA (r = −0.01, p=0.95), or AA/EPA+DHA (r = −0.13, p=0.66) and AA/EPA (r = −0.02, p=0.93) ratios.

3.2.4. Tolerability and safety assessments—Baseline-endpoint changes in vital signs and labs are presented in Table 4. The main effects of Time and Dose, and the Time x Dose interaction, were not significant for any measure. The most commonly reported adverse events at any point post-baseline over the course of the 10 week trial are presented in Table 5. Among all patients, the most frequently reported adverse events were headache, nasal congestion, decreased appetite, nausea, and lethargy which were rated as mild or moderate in severity. Headache, abdominal pain, and vomiting were more frequently reported (p<0.01) by subjects in the high-dose group than the low-dose group, and difficulty staying asleep, dizziness and dizziness when standing, increased appetite, and joint aches were more frequently reported by subjects in the low-dose group. Baseline YMRS total scores in low-dose and high-dose groups did not differ significantly (p=0.86). YMRS total scores declined significantly in the high-dose group (−77%, p=0.004) and there was a similar trend in the low-dose group (−23%, p=0.37).

4. Discussion

This study investigated the relationship between LC*n*-3 fatty acids status and residual depressive symptoms in adolescents with SSRI-resistant MDD. We found that SSRI-resistant patients exhibited robust DHA deficits compared with healthy adolescents. Furthermore, 10-week adjunctive FO supplementation significantly increased erythrocyte EPA+DHA composition and decreased depressive symptom severity scores in the high-dose group, and there was a trend for a decrease in the low-dose group. In the intent-to-treat sample, symptom remission was observed in 40% of patients in the low-dose group and 100% of patients in the high-dose group. There were no significant baseline-endpoint changes in BMI, blood counts, thyroid stimulating hormone concentrations, or vital signs. Adverse events including headache and gastrointestinal symptoms were rated as mild to

moderate and did not result in discontinuation of treatment. Neither dose was associated with the development of manic or hypomanic symptoms as assessed by YMRS total score. Together, these findings demonstrate that adolescents with SSRI-resistant MDD exhibit robust DHA deficits, and suggest that FO supplementation is safe and efficacious for increasing erythrocyte EPA+DHA levels and augmenting SSRI antidepressant effects.

This preliminary study has several important limitations. First, although we observed statistically significant baseline-endpoint changes with large effect sizes for our primary outcome measures, the relatively small number of subjects randomized to each treatment group may not be a representative sample of adolescent SSRI-resistant MDD patients. Second, the intervention trial design was open-label, and the observed baseline-endpoint changes in primary outcome measures should be viewed as preliminary given the potential for a placebo effect. However, the present findings are consistent with previous placebo-controlled trials finding that adjunctive LC*n*-3 fatty acid supplementation augmented SSRI efficacy in adult MDD patients [38,39]. Third, the duration of FO supplementation was relatively short (10 weeks), and greater changes in primary outcome measures may occur with a longer supplementation period. However, the 10 week duration was selected based in part on a non-human primate study finding that approximately 10 weeks of FO supplementation was required to normalize cortical DHA levels from a deficient state [52]. Nevertheless, a larger and longer placebo-controlled study is warranted to extend and confirm the present findings.

The mean erythrocyte EPA+DHA composition observed in healthy adolescents was 4.3%, which is similar to that observed in a large cohort of healthy subjects residing in the United States (4.5%)[21]. The mean erythrocyte EPA+DHA composition observed at baseline in adolescent MDD patients was 3.1%, and 90% of MDD patients exhibited an erythrocyte EPA+DHA composition of ≤4.0% compared with 40% of healthy controls. The latter finding is consistent with previous studies finding that erythrocyte EPA+DHA composition of ≤4.0% is more prevalent among adult and adolescent MDD patients than controls [26,27]. The observed EPA+DHA deficit was primarily attributable to robust DHA deficits, also consistent with previous observations [26,27]. This finding may take on additional significance because erythrocyte DHA composition is positively correlated with prefrontal cortex DHA composition [51,52], and lower DHA levels have been observed in the postmortem prefrontal cortex and anterior cingulate of adult MDD patients [53-55]. Moreover, imaging studies suggest that erythrocyte DHA levels are positively correlated with functional prefrontal activity [56] and neurometabolic integrity in the anterior cingulated [57] of healthy developing youth. Together these data suggest that the robust erythrocyte DHA deficits observed in SSRI-resistant MDD patients may be relevant to central neuropathological processes associated with MDD.

While the etiology of the lower DHA levels observed in adolescent MDD patients may be multifactorial [19], this deficit could not be attributed to group differences in age, BMI, or smoking status. Moreover, we previously found that chronic treatment with fluoxetine, resulting in clinically-relevant plasma fluoxetine concentrations, did not alter rat erythrocyte EPA or DHA levels [58]. Here we demonstrate that 10-week dietary FO supplementation is sufficient to increase erythrocyte DHA and EPA to levels similar to those observed in

healthy adults residing in Japan [20]. This finding suggests that increasing the intake of preformed EPA+DHA is sufficient to correct the low EPA+DHA status observed in adolescent MDD patients. It is notable that the baseline-endpoint increase in erythrocyte EPA+DHA composition was similar in both low- and high-dose FO groups. This finding is not consistent with prior studies finding that FO supplementation increases erythrocyte EPA +DHA levels in a dose-dependent manner [22,23]. Therefore, it is possible that compliance rates in the high-dose group were lower than indicated by self-report and bottle volumes. It is also notable that two patients in the low-dose group, and none in the high-dose group, experienced a worsening of depressive symptoms. Together these findings suggest that future FO supplementation trials in SSRI-resistant MDD patients should consider employing a flexible dose design with upward titration based on treatment response, as previously demonstrated in pediatric bipolar patients [16].

Consistent with some previous studies [26,59,60], but not others [61-63], DHA, EPA+DHA and the AA/EPA ratio were not significantly correlated with baseline CDRS-R total scores. However, this relationship may have been confounded by concomitant SSRI treatment and the fact that all MDD patients exhibited similar low EPA and DHA levels. Furthermore, baseline-endpoint change in CDRS-R total scores were not correlated with baseline or baseline-endpoint change in EPA+DHA, DHA, and the AA/EPA ratio. While these findings suggest that erythrocyte LC*n*-3 fatty acid status and depressive symptoms and treatment response are poorly correlated in SSRI-resistant MDD patients, larger studies are required to further evaluate this relationship.

In conclusion, this study found that adolescents with SSRI-resistant MDD exhibit robust erythrocyte DHA deficits compared with healthy adolescent controls. This study also demonstrated that FO supplementation is efficacious and well-tolerated for increasing erythrocyte EPA+DHA levels in adolescents with SSRI-resistant MDD, and is associated with reductions in residual depressive symptoms. Although this trial was open-label and employed a small sample size, the present findings suggest that low DHA status may represent a modifiable risk factor for SSRI-resistance in adolescent MDD patients. Larger placebo-controlled trials are warranted to further evaluate adjunctive FO as an option for treating residual depressive symptoms in SSRI-resistant MDD patients.

Acknowledgments

Supported in part by an investigator-initiated research grant from the Inflammation Research Foundation (IRF) to R.K.M., and DK59630 to P.T. The IRF and NIH did not have any role in the design, implementation, analysis or interpretation of the research. R.K.M. has received research support from Martek Biosciences Inc, Ortho-McNeil Janssen, NARSAD, and NIH, and was a member of the IRF scientific advisory board. M.P.D. has received research support from AstraZeneca, Eli Lilly, Johnson & Johnson, Shire, Ortho-McNeil Janssen, Pfizer, Otsuka, Shire, Amylin, Lundbeck, Novartis., Somerset, Sumitomo, Thrasher Foundation, GlaxoSmithKline, NARSAD, and NIMH, NIDA, NIAAA, and is a consultant for GlaxoSmithKline, Eli Lilly, Pfizer, and Merck. J.R.S. has received research support from Eli Lilly, Shire, Forest Research Laboratories, Lundbeck, and the American Academy of Child and Adolescent Psychiatry. The other authors do not have any conflicts to declare.

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Highlights

- SSRI-resistant adolescent MDD patients exhibit robust erythrocyte long-chain omega-3 (LC*n*-3) fatty acid deficits.
- Fish oil supplementation significantly increases erythrocyte LC*n*-3 fatty acid levels in MDD patients.
- Fish oil supplementation is safe and well-tolerated and may augment SSRI efficacy.
- Additional studies are warranted to further evaluate adjunctive fish oil supplmentation as an option for SSRI-resistant MDD patients.

Layman Summary

An accumulating body of evidence has implicated dietary essential long-chain omega-3 (LC*n*-3) fatty acids in the pathophysiology and treatment of major depressive disorder (MDD). This study demonstrates that adolescents with SSRI-resistant MDD exhibit significant LC*n*-3 fatty acid deficits compared with healthy controls. It also demonstrates that fish oil supplementation is safe, well-tolerated, and effective for increasing LC*n*-3 fatty acid and is associated with reductions in depressive symptoms.

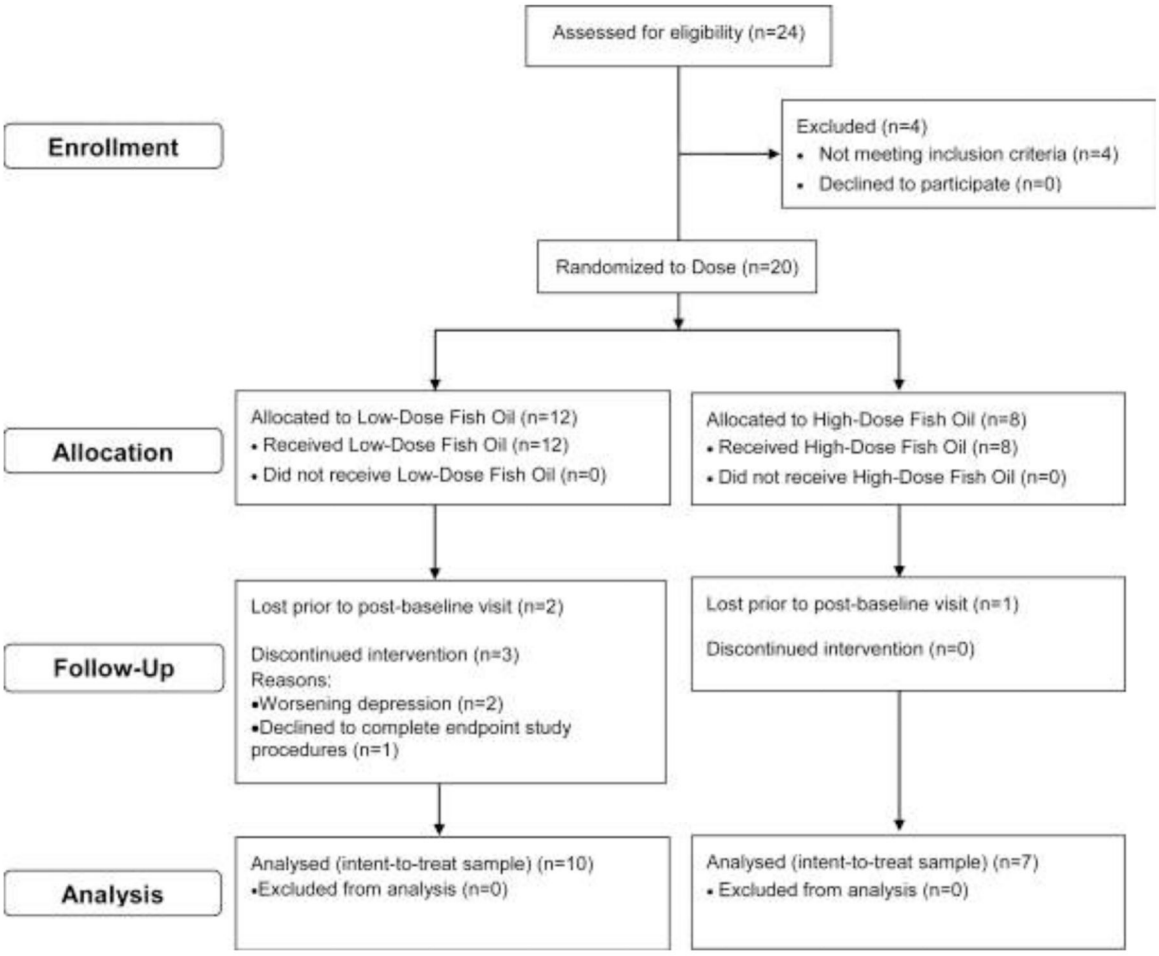

Figure 1.
Flow diagram illustrating the sequence of subject recruitment and attrition.

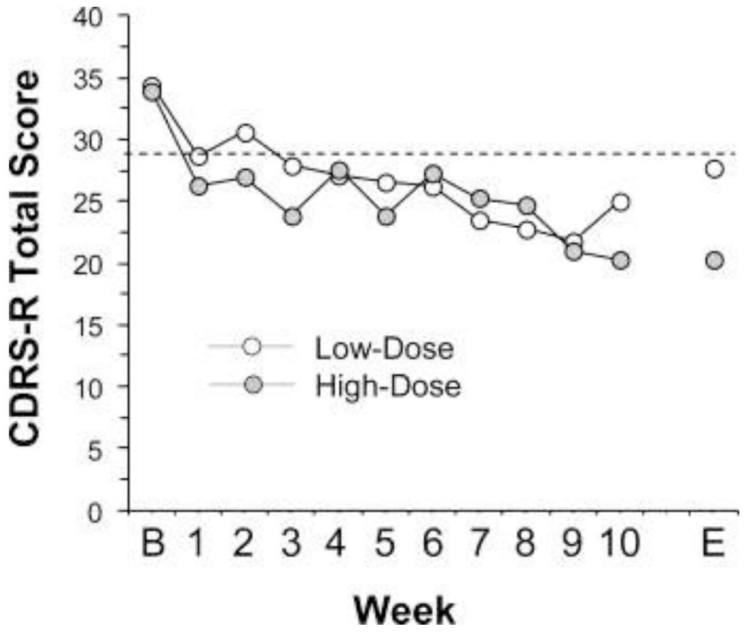

Figure 2.

Mean depression symptom severity scores (CDRS-R total score) in MDD patients treated with low-dose (2.4 g/d, n=10) or high-dose (16.2 g/d, n=7) FO over the 10 week trial. B = baseline, E = mean endpoint score including the last available CDRS-R score carried forward for the n=3 patients in the low-dose group with early termination on weeks 3, 6, and 9. Dotted line demarks symptom remission (CDRS-R total score of 28).

Table 1
Demographic variables for healthy controls and MDD patients

	Controls (n=20)	MDD (n=20)	*P*-alue[2]
Age (yrs)	15.5 ± 2.4	15.6 ± 3.2	0.91
Gender (% male)	45	40	0.98
Race (*n*)			
Caucacian	17	19	0.61
African American	2	1	
Other	1	0	
Height (cm)	165.2 ± 10.1	163.2 ± 13.8	0.60
Weight (kg)	64.4 ± 19.7	62.3 ± 19.1	0.73
BMI (kg/m^2)	22.6 ± 5.5	24.2 ± 7.2	0.49
BMI z score	0.3 ± 1.2	0.6 ± 1.1	0.44
BMI percentile	59.0 ± 34.1	65.1 ± 32.4	0.56
Smoking status (current) (n)	1	2	1.0
Age at Onset (years)	–	13.1 ± 3.1	–
Duration of Illness (yrs)	–	2.4 ± 2.7	–
CDRS-R Total Score	–	34.7 ± 4.7	–
SSRI (*n*)			
Fluoxetine	–	10	–
Citalopram	–	3	–
Escitalopram	–	1	–
Sertraline	–	6	–

[1] Values are group mean ± S.D.

[2] Two-tailed t-test or Chi-square test

Table 2
Comparison of erythrocyte fatty acid composition in healthy controls and MDD patients

Fatty Acid[1]	Controls(n=20)	MDD (n=20)	*P*-value[2]
Palmitic acid (16:0)	17.29 ± 1.26	17.86 ± 0.98	0.12
Stearic acid (18:0)	17.49 ± 1.11	17.15 ± 0.45	0.21
Oleic acid (18:1 *n*-9)	11.81 ± 0.95	12.56 ± 0.85	0.01
Vaccenic acid (18:1 *n*-7)	1.09 ± 0.36	1.21 ± 0.15	0.17
Linoleic acid LA, 18:2*n*-6)(	11.78 ± 1.31	11.35 ± 0.81	0.23
Homo-γ-linolenic (HGLA, 20:3*n*-6)	1.69 ± 0.27	1.89 ± 0.31	0.03
Arachidonic acid (AA, 20:4*n*-6)	17.80 ± 1.40	18.10 ± 1.21	0.52
Docosatetraenoic acid (22:4*n*-6)	4.48 ± 0.53	4.75 ± 0.46	0.10
Eicosapentaenoic acid (EPA, 20:5*n*-3)	0.34 ± 0.16	0.28 ± 0.10	0.20
Docosapenaenoic acid (22:5*n*-3)	2.40 ± 0.43	2.20 ± 0.28	0.07
Docosahexaenoic acid (DHA, 22:6*n*-3)	3.90 ± 1.01	2.80 ± 0.59	0.0003
EPA±DHA	4.24 ± 1.03	3.10 ± 0.58	0.0002
AA:DHA	4.80 ± 1.20	6.60 ± 1.20	0.0001
AA:EPA	67.9 ± 42.3	77.8 ± 45.4	0.48
AA:EPA±DHA	4.40 ± 1.02	5.90 ± 0.93	0.0001

[1] Values are group mean fatty acid composition (wt % total fatty acids) ± S.D.

[2] Two-tailed t-test.

Table 3

Effects of 10 week fish oil supplementation on erythrocyte fatty acid composition in MDD patients

Fatty Acid[1]	Baseline	Week 10	*P*-value[2]
Low-Dose (n=7)			
Palmitic acid (16:0)	18.23 ± 1.03	18.45 ± 0.72	0.64
Stearic acid (18:0)	17.11 ± 0.62	17.04 ± 0.67	0.83
Oleic acid (18:1 *n*-9)	12.24 ± 0.50	12.11 ± 0.49	0.63
Vaccenic acid (18:1 *n*-7)	1.28 ± 0.15	1.23 ± 0.25	0.67
Linoleic acid (LA, 18:2*n*-6)	11.31 ± 0.69	10.57 ± 0.95	0.12
Homo-γ-linolenic (HGLA, 20:3*n*-6)	1.75 ± 0.16	1.55 ± 0.13	0.02
Arachidonic acid (AA, 20:4*n*-6)	18.04 ± 0.79	15.29 ± 1.74	0.002
Docosatetraenoic acid (22:4*n*-6)	4.64 ± 0.50	3.55 ± 0.55	0.01
Eicosapentaenoic acid (EPA, 20:5*n*-3)	0.28 ± 0.16	1.95 ± 0.76	0.0003
Docosapenaenoic acid (22:5*n*-3)	2.28 ± 0.33	3.45 ± 0.71	0.003
Docosahexaenoic acid (DHA, 22:6*n*-3)	2.81 ± 0.66	5.36 ± 0.83	0.0001
EPA+DHA	3.09 ± 0.63	7.32 ± 1.47	0.0001
AA:DHA	6.73 ± 1.45	2.93 ± 0.67	0.0001
AA:EPA	95.4 ± 71.4	9.63 ± 5.86	0.009
AA:EPA±DHA	6.04 ± 1.06	2.20 ± 0.67	0.0001
High-Dose (*n*=7)			
Palmitic acid (16:0)	17.55 ± 0.80	17.41 ± 0.54	0.71
Stearic acid (18:0)	17.26 ± 0.37	17.18 ± 0.43	0.72
Oleic acid (18:1 *n*-9)	12.61 ± 1.15	12.27 ± 0.70	0.52
Vaccenic acid (18:1 *n*-7)	1.23 ± 0.20	1.11 ± 0.22	0.31
Linoleic acid (LA, 18:2*n*-6)	11.50 ± 0.84	10.37 ± 0.88	0.03
Homo-γ-linolenic (HGLA, 20:3*n*-6)	1.86 ± 0.22	1.41 ± 0.42	0.03
Arachidonic acid (AA, 20:4*n*-6)	18.75 ± 0.85	16.12 ± 2.43	0.02
Docosatetraenoic acid (22:4*n*-6)	4.54 ± 0.34	3.55 ± 0.66	0.005
Eicosapentaenoic acid (EPA, 20:5*n*-3)	0.29 ± 0.06	2.27 ± 2.05	0.03
Docosapenaenoic acid (22:5*n*-3)	2.11 ± 0.22	3.20 ± 0.79	0.004
Docosahexaenoic acid (DHA, 22:6n-3)	2.70 ± 0.46	5.61 ± 1.79	0.001

Fatty Acid[1]	Baseline	Week 10	*P*-value[2]
EPA±DHA	2.99 ± 0.47	7.88 ± 3.51	0.003
AA:DHA	7.08 ± 1.00	3.30 ± 1.62	0.0002
AA:EPA	66.7 ± 15.1	22.6 ± 31.2	0.006
AA:EPA±DHA	6.37 ± 0.84	2.65 ± 1.66	0.0002

[1] Values are group mean fatty acid composition (wt % total fatty acids) ± S.D.

[2] Two-tailed t-tests.

Table 4

Effects of 10 week fish oil supplementation on vital signs and laboratory measures in MDD patients

Variable[1]	Baseline	Week 10	P-value[2]
Low-Dose (n=7)			
Height (cm)	164.8 ± 12.2	165.0 ± 12.7	0.97
Weight (kg)	54.6 ± 3.8	55.5 ± 2.3	0.43
BMI (kg/m^2)	20.2 ± 2.3	20.8 ± 2.8	0.68
BMI *z* score	−0.04 ± 1.0	0.20 ± 0.9	0.66
BMI percentile	47.7 ± 32.0	54.1 ± 28.8	0.72
Heart rate (bpm)	79.1 ± 10.4	74.3 ± 13.1	0.84
Blood Pressure (mmHg)	□	□	
Systolic	112.6 ± 9.4	118.1 ± 9.5	0.26
Diastolic	64.9 ± 12.5	72.1 ± 8.9	0.21
Temperature (Celsius)	36.8 ± 0.4	37.0 ± 0.3	0.37
TSH (mIU/L)	1.9 ± 0.7	1.7 ± 0.5	0.72
WBC (K/uL)	6.3 ± 0.6	6.2 ± 1.0	0.84
RBC (M/uL)	4.5 ± 0.7	4.5 ± 0.6	0.97
Platelets (K/uL)	227.5 ± 61.2	229.0 ± 70.5	0.96
High-Dose (n=7)			
Height (cm)	162.7 ± 18.6	159.3 ± 15.3	0.72
Weight (kg)	67.1 ± 25.7	72.6 ± 26.3	0.67
BMI (kg/m^2)	24.8 ± 8.1	25.9 ± 7.9	0.79
BMI *z* score	0.9 ± 1.2	1.3 ± 0.7	0.49
BMI percentile	72.9 ± 34.9	86.4 ± 14.3	0.36
Heart rate (bpm)	80.4 ± 8.7	81.1 ± 4.9	0.85
Blood Pressure (mmHg)	□	□	
Systolic	117.6 ± 12.8	110.6 ± 10.3	0.28
Diastolic	70.0 ± 8.2	66.6 ± 6.9	0.42
Temperature (Celsius)	36.3 ± 0.6	36.8 ± 0.5	0.12
TSH (mlU/L)	1.8 ± 0.9	2.1 ± 1.1	0.52
WBC (K/uL)	7.4 ± 3.4	7.4 ± 2.4	0.97
RBC (M/uL)	4.6 ± 0.4	4.7 ± 0.3	0.87
Platelets (K/uL)	291.4 ± 48.7	289.3 ± 51.1	0.94

[1] Values are group mean ± S.D. (see Methods for abbreviations)

[2] Two-tailed t-test.

Table 5

Incidence of common adverse events during fish oil supplementation

Adverse Event[1]	Low+High (n=17)	Low-Dose (n=10)	High-Dose (n=7)	P-value[2]
Headache	86	100	71	0.001
Nasal congestion	75	78	71	0.33
Appetite Decrease	69	67	71	0.65
Nausea	63	56	71	0.04
Lethargy	63	56	71	0.04
Drowsiness	62	67	57	0.19
Abdominal pain	58	44	71	0.002
Difficulty concentrating	56	56	57	0.99
Difficulty staying asleep	55	67	43	0.001
Difficulty falling asleep	49	56	43	0.09
Diarrhea	37	44	29	0.04
Difficulty waking up	37	44	29	0.04
Vomitting	33	22	43	0.002
Dizziness when standing	29	44	14	0.001
Appetite Increase	29	44	14	0.001
Dizziness	29	44	14	0.001
Joint aches	29	44	14	0.001

[1] Percentage of subjects in the intent-to-treat sample with any post-baseline instance of the event during the 10 week trial.

[2] Two-tailed Chi-Square test (Low-dose vs. High-dose).