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Validation of a food frequency questionnaire to assess intake of n-3 polyunsaturated fatty acids in subjects with and without Major Depressive Disorder

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Abstract

The role of n-3 polyunsaturated fatty acids (PUFAs) in psychiatric illness is a topic of public health importance. This report describes development and biomarker validation of a 21 item, self-report food frequency questionnaire (FFQ) intended for use in psychiatric research, to assess intake of α -linolenic acid (18:3n-3, ALA), docosahexaenoic acid (22:6n-3, DHA), and eicosapentaenoic acid (20:5n-3, EPA). In a cross-sectional study carried out from September, 2006 – September, 2008, 61 ethnically diverse adult participants with (n=34) and without (n=27) Major Depressive Disorder completed this n-3 PUFA FFQ and provided a plasma sample. Plasma levels of n-3 PUFAs EPA and DHA, and n-6 PUFA arachidonic acid (20:4n-6, AA) were quantified by gas chromatography. Using Spearman's ρ , FFQ-estimated intake correlated with plasma levels of DHA ($r=0.50$, $p<0.0001$) and EPA ($r=0.38$, $p=0.002$), but not with ALA levels ($r=0.22$, $p=0.086$). Participants were classified into quartiles by FFQ-estimated intake and plasma PUFA concentrations. Efficacy of the FFQ to rank individuals into same or adjacent plasma quartiles was 83% for DHA, 78.1% for EPA, and 70.6% for ALA; misclassification into extreme quartiles was 4.9% for DHA, 6.5% for EPA, and 8.2% for ALA. FFQ-estimated EPA intake and plasma EPA were superior to plasma AA levels as predictors of the plasma AA to EPA ratio. This brief FFQ can provide researchers and clinicians with valuable information concerning dietary intake of DHA and EPA.

Keywords

n-3 PUFA; mood disorders; docosahexaenoic acid; eicosapentaenoic acid; nutrition assessment

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INTRODUCTION

Polyunsaturated fatty acid (PUFA) status is increasingly recognized as an important physiological determinant of optimal mental health. Deficiency of n-3 PUFA, in particular, has been demonstrated in animal studies to be associated with increased aggression, vulnerability to stress, and depression-like behavior (1–3). In humans, the degree of n-3 PUFA intake has been shown to influence the development and severity of psychiatric disorders (4,5), including the transition of high-risk participants to psychotic disorder (6). Low levels of n-3 PUFA have also been associated with increased risk of suicide attempt (7,8). Thus, quantifying dietary intake of n-3 PUFAs is potentially important for research, clinical evaluation, and treatment of psychiatric patients.

The impetus for development of this FFQ was the need for a rapid assessment of n-3 intake for studies of PUFA in mood disorders being carried out at the New York State Psychiatric Institute. Research participants in these studies are assessed on multiple symptom domains and demographic characteristics. An n-3 PUFA dietary assessment measure was sought that would complement the existing research battery without creating an onerous burden of participant time and effort. At that time (2006), a literature search revealed a number of biomarker-validated PUFA FFQ measures, all of which comprised over 100 items (9–13). Because the majority of n-3 PUFA come from a relatively limited set of foods, it was thought that a much briefer valid dietary screener could be developed. This concept was exemplified by 2 existing brief, validated FFQs (14–15 items in length) (14,15) which, however, were not n-3 PUFA-specific. Therefore, a 21-item FFQ was designed for assessment of dietary intake of n-3 containing-foods over the previous 6 months. Given that depression is associated with problems with attention and memory, and that cognitive deficits can decrease FFQ reporting accuracy (16), brevity and clarity were important considerations in the FFQ design. In parallel to the development of this instrument, additional brief PUFA-specific FFQs have since been generated and validated in a number of settings (17–19), underlining the growing need for this type of instrument in current research.

To date, there are no reported studies of a FFQ validated in a psychiatric population. This brief FFQ was hypothesized to be a useful tool for quantification of PUFA intake in both depressed and healthy groups, as tested by biomarker validation against individual plasma PUFA levels.

METHODS

Questionnaire

This 21-item self-report was developed using the National Cancer Institute's Diet History Questionnaire (20) as a model. The FFQ, which took participants approximately 5 minutes to complete, assessed average n-3 intake over the last 6 months. Items in the n-3 FFQ included an extensive list of specific seafood and fish available in the metropolitan New York area, as well as walnuts, flaxseed, flaxseed oil, cod liver oil and canola oil. Questions about fish did not distinguish between farmed versus wild growing conditions. Eggs supplemented with n-3 were not included as an FFQ item because they were not widely available in stores in 2006 in the New York area. Sushi was included in the FFQ as a currently popular local source of fish intake. The questionnaire also included specific questions about type and dosage of n-3 PUFA dietary supplements, however for purposes of this study, individuals taking supplements were excluded.

Portion sizes were listed on the FFQ as a range. For example, portion sizes for fish and shellfish were described as small (2 ounces or less of fish or shellfish or less than 4 pieces of

sushi), medium (2–7 ounces of fish or 4–14 pieces of sushi), and large (more than 7 ounces of fish or more than 14 pieces of sushi). The gender-specific portion sizes (small, medium and large) in grams per serving were derived from parallel items in the National Cancer Institute's Diet History Questionnaire (DHQ) Nutrient Database (21). The DHQ database is derived from the United States Department of Agriculture (USDA) survey nutrient food codes found in the 1994–96 Continuing Survey of Food Intake by Individuals (CSFII) (22).

For each item, participants also were asked about frequency of intake over the last 6 months, with categories ranging from never to number of times each month, each week, or each day.

Intakes of docosahexaenoic acid (22:6n-3, DHA), α -linolenic acid (18:3n-3, ALA), and eicosapentaenoic acid (20:5n-3, EPA) were individually calculated according to an algorithm taking into account portion size, frequency of consumption, sex, and the average n-3 PUFA content of the participant's food choices. Since the DHQ does not distinguish nutrient values between different types of fish, PUFA values for specific fish and shellfish were obtained from the USDA Addendum A: EPA and DHA Content of Fish Species, a table within the USDA National Nutrient Database for Standard Reference {United States Department of Agriculture Agricultural Research Service, 2009 #1510}, supplemented when necessary with n-3 values from Bowes and Church's Food Values of Portions Commonly Used (24).

To assess the effects of recent food consumption on plasma PUFA levels, participants were also asked at the time of the blood draw how many hours had elapsed since their last meal.

Sample

Participants (n=61) were adults with a diagnosis of MDD (n=34) or healthy volunteers (n=27), aged 18–73, from the greater metropolitan New York City area, who responded to advertisements to participate in mood disorders studies. Participants signed informed consent for all procedures prior to study participation. Study approvals were obtained from the New York State Psychiatric Institute Institutional Review Board. In participants with MDD, depression severity was evaluated with the 17-item Hamilton Depression Rating Scale (25). Participants were not taking psychotropic medications for at least 2 weeks prior to blood drawing. Due to inconsistency in dosage recall, all participants who were taking n-3 supplementation (n=9) were excluded from this study.

Demographic information was collected from participants concerning sex, age, years of education, race, and smoking status. Height and weight were measured and used to calculate the Body-Mass Index (BMI) according to the formula ($\text{BMI kg/m}^2 = (\text{weight in pounds} * 703) / (\text{height in inches})^2$).

Blood Collection

Blood samples and FFQ data were obtained on the same day, after participants gave informed consent for the procedures. Blood samples were collected into two EDTA-containing tubes and maintained in an ice-water bath until refrigerated centrifugation for 10 min, followed by transfer to cryotubes and storage at -80°C until analysis.

Plasma PUFA Assay

The analytical procedures described herein were validated for the separation and quantification of plasma fatty acids DHA, EPA, and arachidonic acid (20:4n-6, AA) as fatty acid methyl esters (FAMES). The procedure is essentially as described previously (26), involving direct transesterification of all classes of lipids in a one-step procedure using 0.1ml of plasma. Separation of FAMES was accomplished via gas chromatography/flame

ionization detector using a capillary column DB-FFAP- 30m × 0.25mm × 0.25μm, with hydrogen carrier gas.

A retention time lock program allowed elution of an internal standard with a specific retention time. Methyl ester retention times and response factors were determined from a known equal-weight mixture of 28 FAMES commercially available as GLC462 from Nu-Chek Prep (Elysian MN). Retention times were virtually constant between chromatographic runs. Samples were run in duplicate. Plasma FAMES were identified by retention time; data were automatically quantified. Intra- and inter-assay reproducibility (CV%) for ALA, EPA, and DHA was < 5% and <9% for each analyte respectively.

Statistical Analyses

Statistical analyses were performed using SPSS (Release 17.0.0, 2008, SPSS Inc., Chicago, IL, for Mac [Apple, Inc., Cupertino, CA]). All tests were performed with each individual PUFA species (DHA, EPA, and ALA). Nonparametric correlations between plasma PUFA levels and FFQ-PUFA estimates were determined using Spearman's *rho*, since the distributions of plasma and FFQ estimates were highly skewed in the direction of very low n-3 PUFA, with a relatively large number of zero responses on the FFQ (11% for DHA and EPA), not easily correctable by log transformation. In interpreting correlations between plasma and FFQ-derived PUFA levels, a significance cutoff of $\alpha < 0.025$ was used rather than the more conservative Bonferroni approach, since two of the n-3 PUFA FFQ scores were highly correlated (correlation-adjusted significance cutoff of 0.027)(27). Binary logistic regression was performed with PUFA levels as independent variables and FFQ responses of "zero" or "non-zero" as dependent variable, to provide a measure of the ability of low plasma PUFA levels to predict zero FFQ responses. Participants were classified into quartiles according to FFQ-estimated intake and plasma concentrations of PUFAs. Sensitivity and specificity of the FFQ were calculated for classifying individuals into same or adjacent plasma-determined quartiles.

The following exploratory analyses were also performed: Independent regression analyses used FFQ intake as the first predictor variable, along with presence or absence of psychiatric diagnosis, age, and sex, in that order, testing for main effects, with plasma PUFA levels as the dependent variable, and for interactions between FFQ-estimated intake and diagnostic status. Evaluation of additional factors or interactions was precluded by the sample size. Regression analyses were used to examine plasma AA, plasma EPA and dietary EPA as predictors of the plasma ratio of AA to EPA, a potentially important index of brain functioning. The sensitivity of the FFQ to detect group differences with regard to presence or absence of MDD was evaluated for each plasma and FFQ-estimated PUFA measurement, using nonparametric Mann-Whitney U-tests. As the design of the parent studies precluded consistently obtaining fasting blood samples, t-tests were performed to compare effects of fasting (last reported meal greater than 8 hours previous) on plasma PUFA levels within the entire sample, and within the MDD and healthy groups.

RESULTS AND DISCUSSION

Sample characteristics

Demographic and clinical characteristics of the study population are presented in Table 1. Participants with MDD were on average moderately depressed and had a higher rate of smoking than healthy volunteers. No significant group differences were seen for other characteristics tested. FFQ-estimated intakes and plasma levels of DHA, EPA, and ALA are summarized in Table 2. Distributions of plasma ALA and EPA and all FFQ PUFA estimates

were found to be highly skewed to the left, with zero intake as the distribution mode (data not shown).

Questionnaire validity

The FFQ detected significant correlations between mean dietary intakes of DHA and EPA and their respective mean plasma levels, using Spearman's r (DHA, $r=0.50$, $p<0.0001$; EPA, $r=0.38$, $p=0.002$) (see Table 2). The strength of these correlations compares favorably to other validation studies in which FFQ estimates of n-3 intake were evaluated against short- and medium-term n-3 biomarkers ($r = 0.28$ to 0.48 for DHA and 0.19 to 0.54 for EPA). (13,17–19,28)

Plasma DHA levels significantly predicted FFQ responses of zero intake ($\chi^2=4.48$, $p=0.03$), whereas plasma EPA levels did not ($\chi^2=0.72$, $p=0.40$). Thus, reported non-intake of DHA was a good indicator of low plasma levels, but the same was not true for EPA. This is unlikely to be due to poor participant estimation, since both were calculated from the same responses with regard to portion size and frequency. When zero-reporters were excluded, FFQ-plasma EPA correlations improved from Spearman's $r = 0.38$ ($p=0.002$) to $r = 0.47$ ($p<0.001$). This suggests that other sources specifically of EPA intake were not covered by the FFQ. Other explanations are possible, however. The relationship between dietary intake and plasma PUFA levels may be affected by physiological processes, such as polymorphisms in fatty acid delta-5 and delta-6 desaturase (FAD) genes that have been shown to affect variation in EPA but not DHA (29). Another theoretically important factor is intake of n-6 PUFA, which can competitively inhibit the conversion of ALA into EPA by FAD. (30) However, participant quantification of n-6-containing foods would have been more complex and time-consuming, and thus estimation of n-6 PUFA intake was not included in this FFQ. Moreover, n-3 PUFA deficiency is common in the United States, where n-3 PUFA intake is derived predominantly from fish, which may not be available, affordable, or palatable to some people, whereas n-6 PUFA-containing foods are in superabundance. Thus, perturbations in n-3 PUFA have been observed to have a larger impact than n-6 PUFA changes on n-6 to n-3 PUFA ratios (31). In this study, the plasma AA to EPA ratio was likewise strongly predicted by both plasma EPA levels ($F=110$, $R^2=0.652$, $p<0.00001$) and FFQ-estimated EPA intake ($F=11.39$, $R^2=0.16$, $p=0.001$), but not by AA plasma levels ($F=1.71$, $R^2=0.028$, $p=0.196$), showing that intake of EPA was the major dietary determinant of the AA to EPA balance.

FFQ-estimated ALA intake was not a significant predictor of plasma ALA levels (Spearman's $r = 0.22$ $p=0.086$). It is possible that this brief FFQ may not effectively estimate ALA intake. However, these findings are consistent with other reports of no (9,32,33) or modest (10,13,34) ($r = 0.22$ to 0.34) correlations of FFQ-estimated ALA intake with plasma or erythrocyte levels. A prospective study of ALA supplementation (35) likewise found only minor resulting changes in plasma phospholipid ALA levels. This has been suggested to be due to competition with linoleic acid (36) and to a high rate oxidation of ALA (35). The notion of loss of dietary ALA is consistent with the findings of this study (see Table 2) in which each mg of DHA or EPA intake translates into approximately 1 mg per liter of plasma (DHA, 50 mg/d : 54 mg/L; EPA, 26 mg/d : 20 mg/L), whereas the ALA diet:plasma correspondence is on the order of 10:1 (ALA, 234 mg/d : 20 mg/L). In contrast, more robust correlations (Pearson's $r=0.62$ to 0.68) of ALA intake with adipose tissue levels have been seen (12). Taken together, these results suggest that short- and medium-term biomarkers may be unsuitable for validation of ALA intake status.

Plasma PUFA levels positively correlated with each another (Spearman's r , all comparisons had $p<0.001$): DHA-EPA, $r = 0.64$, DHA-ALA, $r = 0.46$, EPA-ALA, $r = 0.40$. As expected, FFQ-estimated DHA and EPA intakes were even more strongly correlated: DHA-EPA, r

=0.98, $p<0.001$. However, correlations of FFQ-derived ALA with DHA and EPA intakes were non-significant: EPA-ALA $r=0.18$, $p=0.164$; DHA-ALA, $r=0.18$, $p=0.166$.

Sensitivity of classification of FFQ-estimated levels into the same plasma-determined quartile ranged from 25–50% for DHA, 37.5–47% for EPA, and 12.5–25% for ALA. Specificity ranges were 72–85% for DHA, 76–85% for EPA, and 67–80% for ALA. Efficacy of the FFQ to rank individuals into the same or adjacent plasma quartiles was 83% for DHA, 78.1% for EPA, and 70.6% for ALA, while misclassification into extreme quartiles was 4.9% for DHA, 6.5% for EPA, and 8.2% for ALA.

Effects of other factors

Although plasma PUFA levels can theoretically be influenced by short-term variations in dietary intake, no difference was found in individual PUFA levels between 8-hour fasting ($n=20$) and non-fasting ($n=40$) participants in the whole sample or within MDD and healthy groups (data not shown). Use of the short-term plasma biomarker to validate FFQ assessment of intake over several months is consistent with precedent set by other studies (using different FFQ instruments) (19,37). In one study (19), plasma levels were found to correlate as well as or better than the medium-term marker, erythrocyte membrane concentrations, the premise being that intake is relatively consistent over time with respect to fish intake. An otherwise valid dietary measure might be less able to predict plasma PUFA levels in depressed patients, due to illness-related changes in appetite and food consumption, decreased recall of food intake, or physiologic differences affecting the processing of dietary PUFA into circulating plasma PUFA levels. However, when diagnostic status (presence or absence of MDD diagnosis), sex, and age were included as covariates in a regression model, FFQ estimates and plasma levels remained significantly associated with each other for both DHA ($F=9.22$, $p<0.004$) and EPA ($F=4.18$, $p=0.046$), although both diagnosis and sex explained a significant portion of the variability in plasma DHA (diagnosis, $F=6.51$, $p=0.014$ [lower in depression]; sex, $F=10.26$, $p=0.002$ [lower in males]). None of the covariates apart from the FFQ EPA predictor explained additional variability in plasma EPA. There was no interaction between FFQ estimates and diagnostic status in either the DHA or EPA model (the only interaction tested). Thus, after controlling for age and sex, the FFQ estimates were valid predictors of plasma levels in the depressed as well as healthy participants, more significantly so for DHA than EPA. In the case of ALA, since there was no main effect, covariates and interactions were not tested.

Clinical relevance

Supplementation with n-3 PUFA for patients with depression has recently been endorsed by a task force of the American Psychiatric Association (4), and one expert has recommended that in general, adult Americans should increase n-3 PUFA intake to reduce the risk of developing mood disorders (38). Consistent with previous findings (39–41), this study found that mean levels of plasma levels of DHA and ALA were significantly lower and that plasma EPA and all FFQ-estimated values were numerically lower in participants with MDD than healthy volunteers (see Table 2).

Limitations

This study tested the parameters of validity for the FFQ; however, reproducibility was not tested. It is unclear whether use of this FFQ would be valid for populations with psychiatric disorders other than MDD or for depressed patients in clinical as opposed to research settings.

Illness-specific influences on FFQ validity, including effects of recent appetite changes on n-3 intake and effects of cognitive dysfunction on response reliability, were not specifically

addressed in this small pilot study. Likewise, effects of other covariates, such as smoking, educational status, or race, on the ability of dietary intake to predict plasma PUFA levels are important concepts that might be addressed in larger studies.

This questionnaire may not retain validity across different cultures and over time. One major factor affecting n-3 PUFA intake is local food industry practices concerning the diets of livestock, poultry, and farm-raised fish; e.g., meat from pasture-fed cows contains higher amounts of n-3 PUFA compared to grain-fed livestock. (42) Other factors include economic change, public perception of what is healthy, and food fashions.

CONCLUSIONS

The significant association of FFQ estimated intake of DHA and EPA with a plasma biomarker supports the validity of this instrument in healthy and in depressed populations. This FFQ can be useful for research studies seeking to perform brief and inexpensive measurement of long-chain n-3 PUFA intake. It may also have potential as a clinical screening tool to help identify individuals with poor intake who would most benefit from n-3 PUFA supplementation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References

1. Fedorova I, Salem N Jr. Omega-3 fatty acids and rodent behavior. *Prostaglandins Leukot Essent Fatty Acids* 2006;75:271–289. [PubMed: 16973342]
2. Mathieu G, Denis S, Lavialle M, Vancassel S. Synergistic effects of stress and omega-3 fatty acid deprivation on emotional response and brain lipid composition in adult rats. *Prostaglandins Leukot Essent Fatty Acids* 2008;78:391–401. [PubMed: 18579362]
3. Demar J. One generation of n-3 polyunsaturated fatty acid deprivation increases depression and aggression test scores in rats. *Journal of Lipid Research* 2005;47:172–180. [PubMed: 16210728]
4. Freeman MP, Hibbeln JR, Wisner KL, Davis JM, Mischoulon D, Peet M, Keck PE Jr, Marangell LB, Richardson AJ, Lake J, Stoll AL. Omega-3 fatty acids: Evidence basis for treatment and future research in psychiatry. *J Clin Psychiatry* 2006;67:1954–1967. [PubMed: 17194275]
5. Mcnamara R, Carlson S. Role of omega-3 fatty acids in brain development and function: Potential implications for the pathogenesis and prevention of psychopathology. *Prostaglandins, Leukotrienes and Essential Fatty Acids* 2006;75:329–349.
6. Amminger GP, Schafer MR, Papageorgiou K, Klier CM, Cotton SM, Harrigan SM, Mackinnon A, McGorry PD, Berger GE. Long-chain omega-3 fatty acids for indicated prevention of psychotic disorders: A randomized, placebo-controlled trial. *Arch Gen Psychiatry* 67:146–154. [PubMed: 20124114]
7. Sublette ME, Hibbeln JR, Galfalvy H, Oquendo MA, Mann JJ. Omega-3 polyunsaturated essential fatty acid status as a predictor of future suicide risk. *Am J Psychiatry* 2006;163:1100–1102. [PubMed: 16741213]
8. Huan M, Hamazaki K, Sun Y, Itomura M, Liu H, Kang W, Watanabe S, Terasawa K, Hamazaki T. Suicide attempt and n-3 fatty acid levels in red blood cells: A case control study in China. *Biol Psychiatry* 2004;56:490–496. [PubMed: 15450784]
9. Fawzi WW, Rifas-Shiman SL, Rich-Edwards JW, Willett WC, Gillman MW. Calibration of a semi-quantitative food frequency questionnaire in early pregnancy. *Ann Epidemiol* 2004;14:754–762. [PubMed: 15519898]
10. Garland M, Sacks FM, Colditz GA, Rimm EB, Sampson LA, Willett W, Hunter DJ. The relation between dietary intake and adipose tissue composition of selected fatty acids in us women. *Am J Clin Nutr* 1998;67:25–30. [PubMed: 9440371]

11. Hunter DJ, Rimm EB, Sacks FM, Stampfer M, Colditz GA, Litin LB, Willett W. Comparison of measures of fatty acid intake by subcutaneous fat aspirate, food frequency questionnaire, and diet records in a free-living population of US men. *Am J Epidemiol* 1992;135:418–427. [PubMed: 1550093]
12. Knutsen SF, Fraser GE, Beeson WL, Lindsted KD, Shavlik DJ. Comparison of adipose tissue fatty acids with dietary fatty acids as measured by 24-hour recall and food frequency questionnaire in black and white adventists: The adventist health study. *Ann Epidemiol* 2003;13:119–127. [PubMed: 12559671]
13. Parra M, Schnaas L, Meydani M, Perroni E, Martínez S, Romieu I. Erythrocyte cell membrane phospholipid levels compared against reported dietary intakes of polyunsaturated fatty acids in pregnant Mexican women. *Public Health Nutr* 2002;5:931–937. [PubMed: 12633518]
14. Laviolle B, Froger-Bompas C, Guillo P, Sevestre A, Letellier C, Pouchard M, Daubert JC, Paillard F. Relative validity and reproducibility of a 14-item semi-quantitative food frequency questionnaire for cardiovascular prevention. *Eur J Cardiovasc Prev Rehabil* 2005;12:587–595. [PubMed: 16319550]
15. Svilaas A, Ström EC, Svilaas T, Borgejordet A, Thoresen M, Ose L. Reproducibility and validity of a short food questionnaire for the assessment of dietary habits. Nutrition, metabolism, and cardiovascular diseases: NMCD 2002;12:60–70.
16. Pope SK, Kritchevsky SB, Morris MC, Block G, Tylavsky FA, Lee JS, Stewart S, Harris T, Rubin SM, Simonsick EM. Cognitive ability is associated with suspected reporting errors on food frequency questionnaires. *J Nutr Health Aging* 2007;11:55–58. [PubMed: 17315081]
17. McNaughton SA, Hughes MC, Marks GC. Validation of a FFQ to estimate the intake of PUFA using plasma phospholipid fatty acids and weighed foods records. *Br J Nutr* 2007;97:561–568. [PubMed: 17313719]
18. Mina K, Fritschi L, Knuiman M. A valid semiquantitative food frequency questionnaire to measure fish consumption. *Eur J Clin Nutr* 2007;61:1023–1031. [PubMed: 17299496]
19. Sullivan BL, Williams PG, Meyer BJ. Biomarker validation of a long-chain omega-3 polyunsaturated fatty acid food frequency questionnaire. *Lipids* 2006;41:845–850. [PubMed: 17152921]
20. Subar AF, Thompson FE, Kipnis V, Midthune D, Hurwitz P, McNutt S, McIntosh A, Rosenfeld S. Comparative validation of the Block, Willett, and National Cancer Institute food frequency questionnaires: The Eating at America's Table Study. *Am J Epidemiol* 2001;154:1089–1099. [PubMed: 11744511]
21. National Cancer Institute Applied Research Program. DHQ nutrient database. Retrieved from <http://riskfactor.cancer.gov/DHQ/database/>
22. Subar AF, Midthune D, Kulldorff M, Brown CC, Thompson FE, Kipnis V, Schatzkin A. Evaluation of alternative approaches to assign nutrient values to food groups in food frequency questionnaires. *Am J Epidemiol* 2000;152:279–286. [PubMed: 10933275]
23. United States Department of Agriculture Agricultural Research Service. USDA national nutrient database for standard reference, release 22. 2009. Retrieved from <http://www.ars.usda.gov/nutrientdata>
24. Pennington, JA.; Douglass, JS. Bowes and Church's food values of portions commonly used. Lippincott Williams & Wilkins; Philadelphia: 2004.
25. Hamilton M. A rating scale for depression. *J Neurol Neurosurg Psychiatry* 1960;23:56–62. [PubMed: 14399272]
26. Lepage G, Levy E, Ronco N, Smith L, Galéano N, Roy CC. Direct transesterification of plasma fatty acids for the diagnosis of essential fatty acid deficiency in cystic fibrosis. *J Lipid Res* 1989;30:1483–1490. [PubMed: 2614252]
27. Sankoh AJ, Huque MF, Dubey SD. Some comments on frequently used multiple endpoint adjustment methods in clinical trials. *Stat Med* 1997;16:2529–2542. [PubMed: 9403954]
28. Mikkelsen TB, Osler M, Olsen SF. Validity of protein, retinol, folic acid and n-3 fatty acid intakes estimated from the food-frequency questionnaire used in the Danish national birth cohort. *Public Health Nutr* 2006;9:771–778. [PubMed: 16925883]

29. Schaeffer L, Gohlke H, Muller M, Heid IM, Palmer LJ, Kompauer I, Demmelmaier H, Illig T, Koletzko B, Heinrich J. Common genetic variants of the FADS1 FADS2 gene cluster and their reconstructed haplotypes are associated with the fatty acid composition in phospholipids. *Hum Mol Genet* 2006;15:1745–1756. [PubMed: 16670158]
30. Goyens PL, Spilker ME, Zock PL, Katan MB, Mensink RP. Conversion of alpha-linolenic acid in humans is influenced by the absolute amounts of alpha-linolenic acid and linoleic acid in the diet and not by their ratio. *Am J Clin Nutr* 2006;84:44–53. [PubMed: 16825680]
31. Sublette ME, Bosetti F, DeMar JC, Ma K, Bell JM, Fagin-Jones S, Russ MJ, Rapoport SI. Plasma free polyunsaturated fatty acid levels are associated with symptom severity in acute mania. *Bipolar Disord* 2007;9:759–765. [PubMed: 17988367]
32. Astorg P, Bertrais S, Laporte F, Arnault N, Estaquio C, Galan P, Favier A, Hercberg S. Plasma n-6 and n-3 polyunsaturated fatty acids as biomarkers of their dietary intakes: A cross-sectional study within a cohort of middle-aged French men and women. *Eur J Clin Nutr* 2008;62:1155–1161. [PubMed: 17622261]
33. Olsen SF, Hansen HS, Sandstrom B, Jensen B. Erythrocyte levels compared with reported dietary intake of marine n-3 fatty acids in pregnant women. *Br J Nutr* 1995;73:387–395. [PubMed: 7766562]
34. Holmes, MD.; Powell, JJ.; Campos, H.; Stampfer, MJ.; Giovannucci, EL.; Willett, WC. *Eur J Clin Nutr*. 2007. Validation of a food frequency questionnaire measurement of selected nutrients using biological markers in African-American men.
35. Arterburn LM, Hall EB, Oken H. Distribution, interconversion, and dose response of n-3 fatty acids in humans. *Am J Clin Nutr* 2006;83:1467S–1476S. [PubMed: 16841856]
36. Emken EA, Adlof RO, Gulley RM. Dietary linoleic acid influences desaturation and acylation of deuterium-labeled linoleic and linolenic acids in young adult males. *Biochim Biophys Acta* 1994;1213:277–288. [PubMed: 7914092]
37. Woods RK, Stoney RM, Ireland PD, Bailey MJ, Raven JM, Thien FC, Walters EH, Abramson MJ. A valid food frequency questionnaire for measuring dietary fish intake. *Asia Pac J Clin Nutr* 2002;11:56–61. [PubMed: 11892722]
38. McNamara RK. Evaluation of docosahexaenoic acid deficiency as a preventable risk factor for recurrent affective disorders: Current status, future directions, and dietary recommendations. *Prostaglandins Leukot Essent Fatty Acids* 2009;81:223–231. [PubMed: 19515544]
39. Edwards R, Peet M, Shay J, Horrobin D. Omega-3 polyunsaturated fatty acid levels in the diet and in red blood cell membranes of depressed patients. *J Affect Disord* 1998;48:149–155. [PubMed: 9543204]
40. Maes M, Smith R, Christophe A, Cosyns P, Desnyder R, Meltzer H. Fatty acid composition in major depression: Decreased omega 3 fractions in cholesteryl esters and increased c20:4 omega 6/ c20:5 omega 3 ratio in cholesteryl esters and phospholipids. *J Affect Disord* 1996;38:35–46. [PubMed: 8735157]
41. Mamalakis G. Depression and adipose essential polyunsaturated fatty acids. *Prostaglandins Leukot Essent Fatty Acids* 2002;67:311–318. [PubMed: 12445491]
42. Howe P, Meyer B, Record S, Baghurst K. Dietary intake of long-chain omega-3 polyunsaturated fatty acids: Contribution of meat sources. *Nutrition* 2006;22:47–53. [PubMed: 16289978]

Table 1

Comparison of major depressive disorder and healthy subgroups with respect to demographic and clinical characteristics, and estimated dietary intake and plasma levels of n-3 polyunsaturated fatty acids.

	Participant status			
	MDD n=34	Healthy n=27		
Demographic and clinical characteristics	%	%	<i>p</i>	
Sex (% male)	49	37	0.438	
Race (% white)	62	37	0.073	
Smokers (% yes)	50	19	*0.016	
	Mean (SD)	Mean (SD)	<i>p</i>	
Age (yrs)	36.3 (12.4)	38.56 (16.4)	0.547	
BMI (kg*m ⁻²)	25.1 (5.6)	24.4 (3.9)	0.605	
Education (yrs)	15.4 (2.5)	15.4 (2.5)	0.953	
Mean Hamilton Depression scores	16.6 (8.1)	N/A	N/A	
FFQ PUFA estimates (mg/day)	Mean (SD)	Mean (SD)	Z	<i>p</i>
ALA	188.3 (550.4)	292.0 (576.3)	-0.784	0.433
DHA	45.4 (74.3)	55.3 (67.6)	-0.748	0.454
EPA	22.9 (33.5)	30.9 (38.9)	-0.676	0.499
Plasma PUFA levels (mg/L)	Mean (SD)	Mean (SD)	Z	<i>p</i>
ALA	18.6 (8.4)	22.0 (9.2)	-1.982	0.047
DHA	48.2 (20.3)	62.2 (23.0)	-2.483	0.013
EPA	19.1 (11.1)	21.3 (14.7)	-0.007	0.994

Abbreviations: MDD, Major Depressive Disorder; SD, standard deviation; BMI, body-mass index; N/A, not applicable; FFQ, Food Frequency Questionnaire; PUFA, polyunsaturated fatty acid; ALA, α -linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.

Correlations between mean food frequency questionnaire estimates and plasma levels of n-3 polyunsaturated fatty acids, in total sample (n=61).

Table 2

	FFQ PUFA estimates (mg/day)			Plasma PUFA levels (mg/L)			Correlation of means of FFQ estimates and plasma levels	
	mean (SD)	median	range	mean (SD)	median	range	Spearman's r	p
ALA	234.2 (560.0)	45.9	0–3018.2	20.1 (8.8)	18.6	6.4–52.7	0.22	0.086
DHA	49.8 (71.0)	17.0	0–318.2	54.4 (22.5)	51.4	19.2–111.6	0.50	<0.0001
EPA	26.4 (35.9)	11.0	0–140.0	20.1 (12.8)	16.5	4.7–66.9	0.38	0.002

Abbreviations: FFQ, Food Frequency Questionnaire; PUFA, polyunsaturated fatty acid; SD, standard deviation; ALA, α -linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.