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Original Article

Low unesterified:esterified eicosapentaenoic acid (EPA) plasma concentration ratio is associated with bipolar disorder episodes, and omega-3 plasma concentrations are altered by treatment

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Objectives: Omega (n)-3 and n-6 polyunsaturated fatty acids (PUFAs) are molecular modulators of neurotransmission and inflammation. We hypothesized that plasma concentrations of n-3 PUFAs would be lower and those of n-6 PUFAs higher in subjects with bipolar disorder (BD) compared to healthy controls (HCs), and would correlate with symptom severity in subjects with BD, and that effective treatment would correlate with increased n-3 but lower n-6 PUFA levels. Additionally, we explored clinical correlations and group differences in plasma levels of saturated and monounsaturated fatty acids.

Methods: This observational, parallel group study compared biomarkers between HCs (n = 31) and symptomatic subjects with BD (n = 27) when ill and after symptomatic recovery (follow-up). Plasma concentrations of five PUFAs [linoleic acid (LA), arachidonic acid (AA), alpha-linolenic acid (ALA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA)], two saturated fatty acids (palmitic acid and stearic acid) and two monounsaturated fatty acids (palmitoleic acid and oleic acid) were measured in esterified (E) and unesterified (UE) forms. Calculated ratios included UE:E for the five PUFAs, ratios of n-3 PUFAs (DHA:ALA, EPA:ALA and EPA:DHA), and the ratio of n-6:n-3 AA:EPA. Comparisons of plasma fatty acid levels and ratios between BD and HC groups were made with Student *t*-tests, and between the BD group at baseline and follow-up using paired *t*-tests. Comparison of categorical variables was performed using chi-square tests. Pearson's *r* was used for bivariate correlations with clinical variables, including depressive and manic symptoms, current panic attacks, and psychosis.

Results: UE EPA was lower in subjects with BD than in HCs, with a large effect size (Cohen's $d = 0.86$, $p < 0.002$); however, it was not statistically significant after correction for multiple comparisons. No statistically significant difference was seen in any plasma PUFA concentration between the BD and HC groups after Bonferroni correction for 40 comparisons, at $p < 0.001$. Neither depressive severity nor mania severity was correlated significantly with any PUFA concentration. Exploratory comparison showed lower UE:E EPA in the BD than the HC group ($p < 0.0001$). At follow-up in the BD group, UE, E DHA:ALA, and UE EPA:ALA were decreased ($p < 0.002$).

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Exploratory correlations of clinical variables revealed that mania severity and suicidality were positively correlated with UE:E EPA ratio, and that several plasma levels and ratios correlated with panic disorder and psychosis. Depressive severity was not correlated with any ratio. No plasma fatty acid level or ratio correlated with self-reported n-3 PUFA intake or use of medication by class.

Conclusions: A large effect size of reduced UE EPA, and a lower plasma UE:E concentration ratio of EPA in the symptomatic BD state may be important factors in vulnerability to a mood state. Altered n-3 PUFA ratios could indicate changes in PUFA metabolism concurrent with symptom improvement. Our findings are consistent with preclinical and postmortem data and suggest testing interventions that increase n-3 and decrease n-6 dietary PUFA intake.

Bipolar disorder (BD) is an episodic illness affecting 1.0–4.4% of the population (1). It is a highly heritable polygenic disorder (2), which has a complex, incompletely understood etiology (3). BD type I is characterized by manic episodes of elevated mood, energy and cognition, and by major depressive episodes of lowered mood, energy and cognition. BD type II is characterized by hypomanic episodes of lower severity than manic episodes, and by major depressive episodes that are often longer and more difficult to treat than in BD type I (4–7). Improvements in acute and maintenance treatments are needed to prevent relapses and reduce the burden of disability.

Alterations of metabolism of lipids and resultant changes in cell signaling pathways have been hypothesized to perturb neurotransmitter systems in mood disorders (8, 9). Arachidonic acid [(AA) 20:4n-6] and docosahexaenoic acid [(DHA) 22:6n-3] are polyunsaturated fatty acids (PUFAs) that compose over 90% of essential fatty acids in the mammalian brain (10). AA and DHA are derived from the diet or are synthesized in the liver from their respective, nutritionally essential, shorter-chain PUFAs, linoleic acid [(LA) 18:2n-6] and alpha-linolenic acid [(ALA) 18:3n-3]. AA, DHA, and their metabolites function as intracellular second messengers during neurotransmission and as modulators of neuroinflammation and other pathological processes (8, 11).

Finding changes in neurobiological systems that are common to mood-stabilizing medications proven effective in phase III trials, is a potential window into understanding etiological and treatment mechanisms in BD. One suggested common mechanism of action of mood stabilizers, derived from pre-clinical studies, is downregulation of brain AA metabolism (12–14). Epidemiological and clinical data support the hypothesis that altered PUFA metabolism is present in BD, including reduced omega (n)-3 PUFA metabolism (15–21).

We hypothesized that plasma concentrations of n-3 PUFAs would be lower and plasma concentrations of n-6 PUFAs would be higher in subjects with BD who experience manic or depressive symptoms than in healthy controls (HCs), and that n-3 PUFAs would increase and n-6 PUFAs decrease after naturalistic treatment. We also hypothesized that lower n-3 and higher n-6 PUFA concentrations would be associated with manic and depressive symptoms, regardless of medication status. To test these hypotheses, we studied subjects with BD who were in a symptomatic episode while on medication.

In an exploratory analysis, we investigated associations between plasma n-3 and n-6 PUFA concentrations and clinical symptoms of panic, psychosis, and suicidality. We measured both unesterified (UE) and esterified (E) concentrations of nine fatty acids in plasma. We studied plasma concentrations because the UE form of the PUFAs passes through the blood–brain barrier into the brain more readily than does the E form (22, 23); thus the UE plasma concentration is the major peripheral form that represents PUFA metabolism in the brain.

Methods

Participants and measures

Participants were recruited when presenting for care during mood episodes at the Pennsylvania Psychiatric Institute, under Institutional Review Board Protocol No. 39164EP and NIH Office of Human Subject Research Exemption #11509 (7/16/2012). Participants were screened and consented to participate in the study. Patients with BD (i.e., bipolar disorder type I and bipolar disorder type II) and HCs with no personal or family history of mood or psychotic disorder were included. Exclusion criteria included daily use of anti-inflammatory medications, active substance

intoxication or withdrawal, or pregnancy. The Mini Neuropsychiatric Interview (MINI) (24), a DSM-IV-TR-based structured interview, was performed, and current mood state was assessed with the Hamilton Depression Rating Scale-21 (HDRS) plus Atypical (25) and the Clinician-Administered Rating Scale for Mania (CARS-M) (26). Subjects completed rating scales including a Food Frequency Questionnaire (FFQ) for assessment of intake of n-3 PUFAs (27). Demographic variables of interest included age and sex. Clinical variables of interest included depression severity (HDRS-21 + Atypical) and mania severity (CARS-M). Use of tobacco by smoking in the past month and alcohol use in the past month above recommended sex-specific limits (as recommended by the National Institute of Alcohol Abuse and Alcoholism: men, >14 drinks/week or four drinks per occasion, and women, >7 drinks/week or three drinks per occasion) were recorded from subject response (28). Use of antidepressant, antipsychotic, or mood-stabilizing medications was recorded from subject response. Height and weight were measured and recorded. Clinical features of illness, including the presence of current panic disorder, current suicidality, and current psychosis, were obtained from the relevant MINI modules. Current suicidality accounted for history of suicidal attempts, and current intent and plan. A dietary report of ALA, DHA, and EPA was calculated from self-reported intake of n-3-rich foods recorded on the FFQ.

Subjects were followed and treated as usual. At the start of the study, subjects were taking mood stabilizers (n = 21, 78%: lithium, n = 14; carbamazepine, n = 3; valproic acid, n = 3; and lamotrigine, n = 3), antipsychotics (n = 15, 56%: aripiprazole, n = 1; haloperidol, n = 2; risperidone, n = 6; quetiapine, n = 6; perphenazine, n = 1; and olanzapine, n = 2), antidepressants (n = 13, 48%), and/or sedatives (n = 17, 63%). At follow-up, subjects were taking mood stabilizers (n = 9, 69%: lithium, n = 4; carbamazepine, n = 1; valproic acid, n = 1; and lamotrigine, n = 1), antipsychotics (n = 7, 54%: aripiprazole, n = 2; risperidone, n = 3; perphenazine, n = 1; and olanzapine n = 2), antidepressants (n = 4, 31%), and/or sedatives (n = 6, 46%). After discharge from the hospital, subjects were followed each week by phone and assessed for clinical improvement. A return visit was scheduled with repeat measures when the subject was asymptomatic, or after three months had elapsed. However, due to irregular contact with some subjects, the maximum number of days for follow-up was 187 (median length of follow-up = 22 days; mean length of follow-up = 52 days).

Sample collection and biochemical analysis

Participants fasted for at least six hours, and blood was drawn into vacutainers containing EDTA for biomarkers in the morning. After centrifugation for 10 min at 654.03 *g*, the plasma supernatant was transferred to plastic tubes and maintained at −80°C or on dry ice until processed. Samples were processed for fatty acids after thawing in a blinded fashion at the Brain Physiology and Metabolism Section, National Institute on Aging, National Institutes of Health, Bethesda, Maryland.

Each plasma sample (0.5 mL) was extracted using the method described by Folch et al. (29) and Sublette (17). The plasma was mixed with a partition system of 3.0 mL chloroform:methanol (2:1) and extracted with 0.6 mL of 0.1 M KCl. Organic extracts were concentrated under N_2 at 45°C, and then suspended in 0.5 mL of chloroform. Standards and samples were applied to Silica gel 60 Thin Layer Chromotography plates (Electron Microscopy Science, Gibbstown, NJ, USA) and the lipids were separated using heptane:diethyl ether:acetic acid (60:40:3) (30). Plates were sprayed with 0.03% 6-p-toluidine-2-naphthalene sulfonic acid (TNS) in 50 mM Tris buffer (pH 7.4) and lipid bands were visualized under UV light. Bands corresponding to UE fatty acids were scraped off and then directly methylated using 1% H_2SO_4 in methanol (v/v) and fatty acid methyl esters (FAMEs) were extracted with heptane (31). Prior to methylation, heptadecanoic acid (17:0) was added as an internal standard. For E fatty acid determinations, 50 μL of plasma extract was concentrated under N_2 at 45°C, then directly methylated as above. FAMEs were separated using a gas chromatograph (GC) (Model 6890 N; Agilent Technologies, Palo Alto, CA, USA) with a capillary column (SP 2330, 30 m × 0.32 mm i.d.; Supelco, Bellefonte, PA, USA) and a flame ionization detector (32). Plasma fatty acid concentrations (nmol/mL) were calculated by direct proportional comparison of GC peak areas with that of the added 17:0 internal standard.

Data and statistical analysis

SPSS version 20, IBM SPSS Statistics (IBM Corporation, Armonk, NY, USA) was used for all statistical calculations. Values of continuous variables were compared between the BD and HC groups using two-sample *t*-tests, and categorical variables were compared using the Pearson chi-square test. Paired *t*-tests were used for baseline and follow-up measurements.

Hypothesis-driven analysis. Outcome variables included the E and UE plasma forms of five PUFAs, including LA, AA, ALA, DHA, and eicosapentaenoic acid (EPA, 22:5n-3). The following comparisons were performed: (i) plasma fatty acid levels were compared between the BD and HC groups using Student *t*-tests; (ii) plasma fatty acid concentrations in the BD group were compared between baseline and follow-up using paired *t*-tests, and (iii) Pearson's *r* correlations between mean PUFA concentrations and mania severity, and between mean PUFA concentrations and depression severity, also were performed. Thus, 40 comparisons were investigated in a hypothesis-driven manner. An alpha = 0.05/40 = 0.001 was used as a significance level for these comparisons.

In an *exploratory analysis*, comparisons were made between the BD and HC groups, and between baseline and follow-up, of plasma concentrations of the UE and E forms of the saturated fatty acids palmitic acid (16:0) and stearic acid (18:0), and of the monounsaturated fatty acids palmitoleic acid (16:1 n-7) and oleic acid (18:1 n-9). Additionally, the ratios of UE to E concentrations of the five PUFAs were calculated to determine the relations between unbound and bound forms; the unbound form crosses the blood–brain barrier more easily (22, 23). Ratios of n-3 PUFA concentrations (DHA to ALA, EPA to ALA and EPA to DHA) in the UE and E forms were constructed to estimate progression in the n-3 metabolism pathway. The UE and E AA:EPA ratios were calculated to estimate the n-6/n-3 ratio, an indication of the balance of the overall body PUFA cascade and their bioactive metabolites involved in neurotransmission and the resolution of inflammation (11, 33, 34). All calculated ratios were compared between the HC and BD groups, and between baseline and follow-up.

Additional exploratory analysis was used to investigate associations between demographic and clinical variables and the fatty acid concentrations and ratios described above. Pearson's *r* was used for bivariate correlations. Bivariate correlations were made between the fatty acid UE to E concentration ratios, ratios of DHA to ALA, EPA to ALA, EPA to DHA, and AA to EPA, and the following demographic and clinical variables: age, sex, depression severity (HDRS-21 + Atypical), mania severity (CARS-M), smoking (past month), alcohol (use in the past month above recommended guidelines), current panic disorder (MINI), current suicidality (MINI), dietary report of ALA, DHA and EPA (FFQ), and use of antidepressant, antipsychotic or mood-stabilizing medications.

Results

Demographic and clinical description of the sample

A cohort of 27 patients with BD was recruited while in a symptomatic mood episode, and a parallel HC group was recruited for this study. Patients with BD had an average age of 35 years at entry to the study and did not differ in age, gender distribution, race, or ethnicity from the HC group (n = 31) (Table 1). The subjects with BD were less likely to be married, and less likely to be employed or a student than the HC group (Table 1).

Table 1. Demographics

	Healthy controls (n = 31)	Patients with bipolar disorder (n = 27)[a]	p-value
Sex, female, n (%)	18 (60)	12 (44)	0.30
Race, n (%)			
Asian	4 (13)	0 (0)	0.19
Black	1 (3)	1 (3.7)	
White	26 (84)	25 (93)	
Mixed	0 (0)	1 (3.7)	
Ethnicity, n (%)			0.18
Hispanic	2 (6)	0 (0)	
Not Hispanic	29 (94)	27 (100)	
Marital status, n (%)			0.03
Married	16 (52)	5 (19)	
Separated	1 (3)	1 (3)	
Divorced	0 (0)	3 (11)	
Never married	14 (46)	18 (67)	
Employment, n (%)			<0.01
Unemployed	2 (6)	14 (54)	
Disabled	0 (0)	5 (19)	
Employed	17 (55)	6 (23)	
Student	11 (36)	1 (4)	
Retired	1 (3)	0 (0)	
Smoking, n (%)	1 (3)	14 (56)	NA
Heavy alcohol use, n (%)	1 (3)	4 (15)	
Drug use, n (%)	1 (3)	10 (39)	
Suicidality, n (%)	0 (0)	22 (82)	
Psychosis, n (%)	0 (0)	14 (52)	
Panic, n (%)	0 (0)	5 (19)	
Age, years, mean (SD)	32 (11)	35 (11)	0.31
BMI, mean (SD)	24.9 (4.7)	30.5 (6.0)	1.7×10^{-4}
Mania (CARS-M), mean (SD)	15.3 (12.5)	0 (0)	
Depression (HDRS-21 + Atypical), mean (SD)	34.4 (14.5)	0.3 (0.6)	

BMI = body mass index; CARS-M = Clinician-Administered Rating Scale for Mania; HDRS = Hamilton Depression Rating Scale-21; SD = standard deviation.
[a]At baseline.

The subjects with BD were significantly heavier than the HC subjects, with a mean body mass index (BMI) in the obese range [BD: 30.5 (6.0), HC: 24.9 (4.7); $p = 1.7 \times 10^{-4}$]. At baseline, the BD group had a severe level of depressive symptoms [mean HDRS-21 + Atypical questions = 34.4 (14.5)] and a moderate level of manic symptoms [mean CARS-M = 15.3 (12.5)]. Severity of manic and depressive symptoms was significantly reduced at follow-up compared with entry [mean HDRS-21 + Atypical = 9.00 (8.98), $p < 0.01$; mean CARS-M 3.46 (5.36), $p = 0.01$]. However, more than 50% of the subjects with BD were lost to follow-up. Many subjects had an unstable living situation upon discharge from the hospital and this may have contributed to poor follow-up. Self-report of n-3 EPA, DHA, or ALA consumption using the FFQ did not differ between the HC and BD groups at baseline [*EPA*, HC: 0.03 (0.03), BD: 0.03 (0.04), $p = 0.88$; *DHA*, HC: 0.06 (0.07), BD: 0.04 (0.06), $p = 0.36$; *ALA*, HC: 0.33 (0.50), BD: 0.32 (0.65), $p = 0.96$] or in the BD group from baseline (BL) to follow-up (FU): [*EPA*, BD BL: 0.02 (0.03), BD FU: 0.02 (0.03), $p = 0.28$; *DHA*, BD BL: 0.04 (0.05), BD FU: 0.04 (0.05), $p = 0.22$; *ALA*, HC: 0.28 (0.64), BD: 0.28 (0.23), $p = 0.83$].

The subjects with BD predominantly were diagnosed with bipolar disorder type I (n = 21, 78%) and a smaller number were diagnosed with bipolar disorder type II (n = 6, 22%). About one-half of the subjects with BD had psychosis with the current episode, the majority (n = 22, 82%) had suicide risk, and 19% (n = 5) had current panic disorder. At the time of assessment, more than one-half of the BD sample smoked (n = 14, 56%), 15% (n = 4) drank above the sex-specific recommended limit within the month prior to assessment, and 39% (n = 10) used an illicit drug within the month prior to assessment. At baseline presentation, 78% (n = 21) of the BD subjects were taking mood-stabilizing medication, 48% (n = 13) were taking antidepressants, and 56% (n = 15) were taking antipsychotic medication. Treatment was not controlled for this study, and, on follow-up, 69% of the subjects were taking a mood-stabilizing medication, 54% were taking an antipsychotic, and 31% were taking an antidepressant.

We report our results below categorized by fatty acid or fatty acid group. Unesterified (UE) forms of the FA were measured because they are better able to cross the blood–brain barrier (22, 23), and a difference in the UE:E ratio may signal a difference in the availability of the PUFA to the brain, or a slowed production of the UE from the E plasma PUFA due to hydrolysis by circulating or tissue lipases.

Plasma n-3 concentrations, UE:E ratios, n-3 ratios, and clinical correlations

EPA. The mean concentration of UE EPA was lower in the BD than HC group. Although the effect size was large (Cohen's $d = 0.86$), the difference bordered on statistical significance after consideration of multiple testing ($p = 0.002$, alpha set at 0.001). No statistically significant difference was found in the E EPA levels at baseline, nor in either form of EPA in the BD group between baseline and follow-up (Table 1). Neither mania nor depression severity correlated with UE or E EPA plasma concentrations at baseline in the BD group.

Exploratory analysis revealed that, at baseline, the mean ratio of UE:E EPA was significantly lower in the BD than the HC group (Table 2 and Fig. 1) ($p = 9 \times 10^{-5}$), but did not differ in the BD group between baseline and follow-up.

Significant exploratory clinical correlations for U or E EPA or UE:E EPA ratios included a positive correlation between age and a higher mean UE EPA concentration ($r = 0.46$, $p = 0.02$). Panic attacks and suicidality correlated negatively and significantly with mean UE EPA plasma concentrations (panic attacks: $r = -0.41$, $p = 0.03$; suicidality: $r = -0.49$, $p = 0.009$). However, mania was positively correlated with the UE:E EPA ratio (mania: $r = 0.51$, $p = 0.006$).

DHA. No significant difference was found in UE or E DHA plasma concentrations between the BD and HC groups at baseline or in the BD group between follow-up and baseline, using an adjusted alpha for multiple testing ($p = 0.001$) (Table 2). Neither mania nor depression severity correlated with UE or E DHA plasma concentrations at baseline in the BD group.

Exploratory analysis revealed no differences in mean ratios of UE:E DHA at baseline, nor in the BD group between baseline and follow-up (Table 2).

Significant exploratory clinical correlations for U or E DHA or UE:E DHA included a positive correlation between panic attacks and UE DHA ($r = 0.46$, $p = 0.02$), and between panic attacks and UE:E DHA ($r = 0.42$, $p = 0.03$).

ALA. No significant difference was found in UE or E ALA plasma concentrations between the BD and HC groups at baseline or in the BD group

Table 2. n-3 polyunsaturated fatty acid plasma concentrations and ratios in the bipolar disorder and healthy control groups at baseline and follow-up (nmol/mL)

	Baseline			Bipolar disorder, follow-up		
	Healthy controls (n = 31)	Patients with bipolar disorder (n = 27)		Baseline (n = 13)	Follow-up (n = 13)	
	Mean (SD)	Mean (SD)	p-value	Mean (SD)	Mean (SD)	p-value
ALA						
E	19.89 (25.64)	18.86 (12.81)	0.85	18.48 (17.30)	25.45 (15.42)	0.18
UE	2.10 (2.80)	1.40 (1.77)	0.27	0.88 (1.06)	2.28 (1.72)	0.02
DHA						
E	220.93 (93.79)	221.37 (83.10)	0.99	227.46 (84.10)	177.71 (161.19)	0.28
UE	1.07 (0.64)	1.04 (1.04)	0.89	0.73 (0.38)	0.55 (0.26)	0.11
EPA						
E	8.91 (6.94)	13.91 (13.39)	0.07	14.62 (18.98)	12.68 (6.86)	0.75
UE	0.32 (0.18)	0.20 (0.08)	0.002	0.21 (0.06)	0.31 (0.26)	0.16
UE:E						
ALA	0.14 (0.13)	0.08 (0.10)	0.05	0.08 (0.13)	0.12 (0.10)	0.27
DHA	0.006 (0.005)	0.005 (0.003)	0.34	0.004 (0.003)	0.005 (0.003)	0.32
EPA	0.05 (0.03)	0.02 (0.01)	9×10^{-5}	0.02 (0.02)	0.04 (0.07)	0.24
DHA:ALA						
E	18.20 (11.34)	14.27 (6.05)	0.10	16.15 (6.05)	7.44 (5.36)	0.001
UE	0.92 (0.86)	1.13 (0.77)	0.33	1.12 (0.43)	0.31 (0.17)	4×10^{-5}
EPA:ALA						
E	0.61 (0.23)	0.71 (0.18)	0.07	0.72 (0.19)	0.54 (0.20)	0.06
UE	0.28 (0.18)	0.26 (0.17)	0.78	0.36 (0.17)	0.19 (0.16)	0.002
EPA:DHA						
E	0.05 (0.03)	0.08 (0.12)	0.13	0.09 (0.17)	0.10 (0.07)	0.86
UE	0.39 (0.29)	0.30 (0.21)	0.18	0.37 (0.24)	0.58 (0.29)	0.03

ALA = alpha-linolenic acid; DHA = docosahexaenoic acid; E = esterified; EPA = eicosapentaenoic acid; SD = standard deviation; UE = unesterified.

between follow-up and baseline, using an adjusted alpha for multiple testing ($p = 0.001$) (Table 2). Neither mania nor depression severity correlated with UE or E ALA plasma concentrations at baseline in the BD group.

Exploratory analysis revealed no differences in mean ratios of UE:E ALA at baseline, nor in the BD group between baseline and follow-up (Table 2).

Significant exploratory clinical correlations for U or E ALA or UE:E ALA included a negative correlation between psychosis and E ALA ($r = -0.46$, $p = 0.03$).

n-3 ratios (DHA:ALA, EPA:ALA and EPA:DHA)

Differences in the ratios of n-3 species between the BD and HC groups, and in the BD group between baseline and follow-up were tested in an exploratory fashion (Table 2). No group difference was found at baseline in the three ratios. At the follow-up time-point, investigation of PUFA ratios revealed that the UE EPA:ALA was decreased significantly ($p = 0.002$), and the UE EPA:DHA increased significantly ($p = 0.03$). Additionally, the E and UE DHA:ALA ratios both decreased significantly ($p \leq 0.001$).

Clinical correlations with these ratios revealed that panic attacks correlated negatively and significantly with the EPA:DHA ratio ($r = -0.40$, $p = 0.04$), and panic attacks were positively correlated with UE DHA:ALA ($r = 0.47$, $p = 0.01$).

Plasma n-6 PUFA concentrations and clinical correlations

No significant difference was found in UE or E LA or UE or E AA concentrations between the BD and HC groups at baseline or in the BD group between follow-up and baseline using an adjusted alpha for multiple testing ($p = 0.001$) (Table 3). There were no significant correlations between UE or E LA, or UE or E AA and mania or depression severity.

In exploratory analysis, group differences in the LA UE:E ratio significantly decreased between follow-up and baseline in members of the BD group who completed follow-up (Table 3).

Investigation for clinical correlations revealed that panic attacks were significantly positively cor-

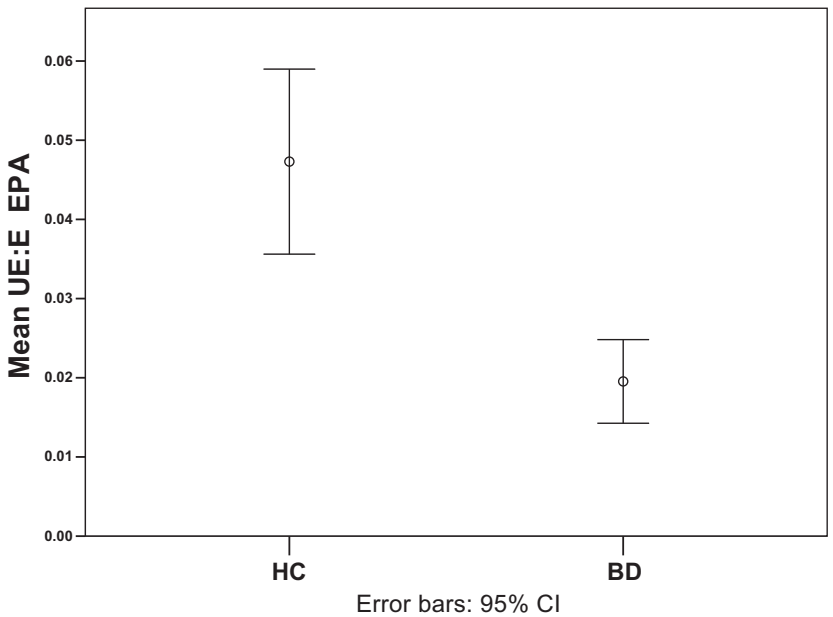

Fig. 1. At baseline, the mean ratio of unesterified (UE) to esterified (E) concentration of eicosapentaenoic acid (EPA) was significantly lower in the bipolar disorder group when compared to the healthy control group. CI = confidence interval.

Table 3. n-6 and n-6:n-3 polyunsaturated fatty acid plasma concentrations and ratios in the bipolar disorder and healthy control groups at baseline and follow-up (nmol/mL)

	Baseline			Bipolar disorder, follow-up		
	Healthy controls (n = 31)	Patients with bipolar disorder (n = 27)		Baseline (n = 13)	Follow-up (n = 13)	
	Mean (SD)	Mean (SD)	p-value	Mean (SD)	Mean (SD)	p-value
LA						
E	2,684.88 (794.62)	2,624.80 (638.07)	0.75	2,548.78 (724.90)	3,808.82 (2,030.68)	0.02
UE	54.07 (30.18)	62.81 (57.70)	0.48	53.45 (47.66)	36.66 (27.46)	0.07
AA						
E	676.94 (202.42)	720.07 (196.35)	0.42	739.90 (244.02)	950.11 (449.19)	0.03
UE	2.73 (1.64)	2.85 (2.15)	0.82	2.28 (1.67)	1.98 (0.84)	0.46
UE:E						
LA	0.02 (0.01)	0.03 (0.02)	0.39	0.02 (0.02)	0.01 (0.01)	0.02
AA	0.004 (0.002)	0.004 (0.003)	0.85	0.003 (0.002)	0.002 (0.001)	0.18
AA: EPA						
E	100.69 (55.32)	68.05 (26.48)	0.005	78.68 (31.02)	88.05 (39.86)	0.54
UE	10.96 (7.59)	18.59 (22.13)	0.10	11.83 (9.62)	9.65 (7.18)	0.28

AA = arachidonic acid; E = esterified; EPA = eicosapentaenoic acid; LA = linoleic acid; SD = standard deviation; UE = unesterified.

related with UE AA (r = 0.43, p = 0.03) and mean UE:E ratios of LA (r = 0.42, p = 0.03) and of AA (r = 0.44, p = 0.02). Psychosis was negatively correlated with E LA (r = −0.45, p = 0.02).

n-6 to n-3 ratio and clinical correlations

The mean ratio of E AA:EPA was significantly lower in the BD group compared to the HC group (Table 3) (p = 0.005). There was a medium effect size difference of 0.46 calculated using Cohen's d in the group difference between the HC and BD groups for UE:E AA:EPA, but the difference was not statistically significant (p = 0.10). Panic (r = 0.59, p = 0.001) and psychosis (r = 0.43, p = 0.03) were significantly and positively correlated with the UE AA:EPA ratio.

Saturated and monounsaturated fatty acids

No significant difference was found in saturated or monounsaturated fatty acid concentrations

between the BD and HC groups at baseline, or in the BD group between follow-up and baseline (see *Supplementary Table 1*). Panic attacks were positively correlated with mean concentrations of several UE fatty acids, including palmitic ($r = 0.46$, $p = 0.03$), palmitoleic ($r = 0.46$, $p = 0.02$), and oleic ($r = 0.44$, $p = 0.022$) acids. Psychosis was negatively correlated with mean E concentrations of palmitic ($r = -0.53$, $p = 0.004$) and stearic ($r = -0.44$, $p = 0.02$) acids.

Clinical correlations of PUFA concentrations and ratios were explored with age, sex, smoking, alcohol, current panic disorder, current suicidality, and use of antidepressant, antipsychotic, or mood-stabilizing medications. No fatty acid level or ratio correlated significantly with sex ($p > 0.07$), depression ($p > 0.14$), smoking ($p > 0.06$), or alcohol use ($p > 0.07$). There was no significant correlation between any fatty acid level and use of a medication class (antidepressant, $p > 0.24$; mood stabilizer, $p > 0.12$; or antipsychotic, $p > 0.08$).

Discussion

We determined differences in the plasma concentrations and ratios of nine esterified (E) and unesterified (UE) long-chain fatty acids, five of which were PUFAs, between 27 BD patients and 31 HCs. Two PUFAs belonged to the n-6 series, AA and its nutritionally essential shorter chain precursor LA, and three to the n-3 series, EPA, DHA, and their nutritionally essential shorter chain precursor ALA (illustrated in Fig. 2).

Based on the literature, we hypothesized that (i) plasma concentrations of n-3 PUFAs would be lower and concentrations of n-6 PUFAs would be higher in patients with BD with manic or depressive symptoms; (ii) n-3 PUFAs would increase and

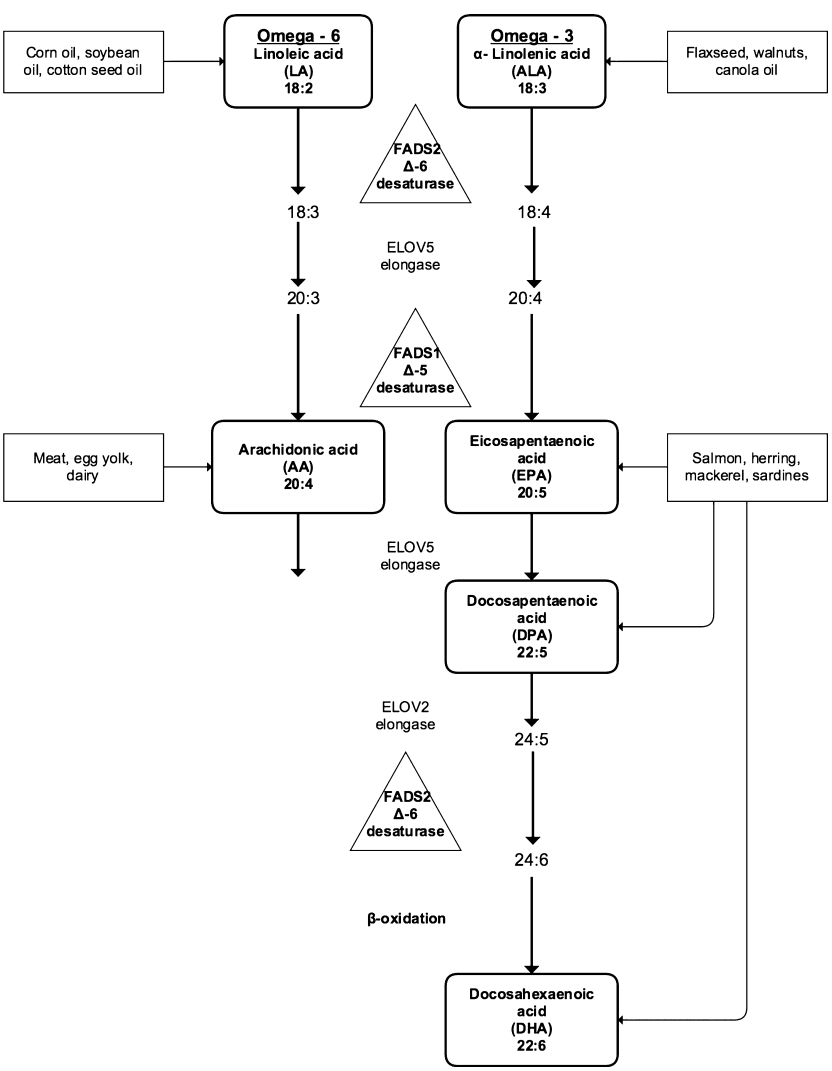

Fig. 2. The metabolic pathway of the polyunsaturated fatty acids linoleic acid (the n-6 pathway) and alpha-linoenic acid (the n-3 pathway) to arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA).

n-6 PUFAs decrease after naturalistic treatment of the patients; and (iii) lower n-3 and higher n-6 PUFA concentrations would be associated with manic and depressive symptoms, regardless of medication status.

After correcting for 40 comparisons, we did not find a significant difference in the plasma E or UE concentration of any of the fatty acids, although the effect size indicating a reduced EPA in BD patients compared with HCs was high and the p-value was 0.002, suggesting a robust effect. This suggestion is particularly relevant because this study should be considered exploratory in a unique patient cohort, and the multiple comparison restriction should be taken with reservations considering the likelihood of a Type 2 error (35).

Some evidence indicates that EPA is the active n-3 PUFA responsible for mood-altering effects in n-3 PUFA supplementation studies of depression (36, 37). Low EPA may be a vulnerability factor for experiencing mood episodes, and alteration of its elongation from ALA may influence effective treatment. Other studies of patients with BD also have reported reduced plasma EPA (38), and efficacy of nutritional supplementation with EPA (27, 39), although the issue is far from settled.

EPA metabolites include bioactive cyclopentenone-IsoP molecules, termed A(3)/J(3)-IsoPs, formed by EPA peroxidation (40), and anti-inflammatory resolvin E1 (41). EPA has positive effects in mitochondria (42), a protective effect in cardiac myocytes (43), and a regulatory effect on brain capillary tight junctions (44) and other capillary endothelium (45). In this regard, while unesterified EPA is largely β-oxidized after entering the rat brain, as compared with the incorporation of intact DHA into brain membrane phospholipids (46), EPA's mechanism of action in BD may be at the capillary endothelium, which has a high mitochondrial content (47) and may be disturbed due to neuroinflammation in BD (48).

Lower n-3 PUFAs have been seen in a number of clinical studies of BD, particularly studies that have investigated erythrocyte concentrations. McNamara et al. (19) found differences in erythrocyte EPA + DHA levels, and three studies reported lower erythrocyte DHA in symptomatic BD groups (18, 19, 49). However, Sublette et al. (17) reported on plasma concentrations of UE and E PUFAs, and found no between-group differences in UE PUFA levels or the UE AA:EPA ratio. The critical differences may lie in the different methods of measuring PUFAs, in the different symptomatic states of the subject populations, and in the different dietary intake statuses.

While plasma PUFA concentrations may vary more over time than erythrocyte concentrations, due to the four-month mean lifetime of circulating erythrocytes, studying plasma concentrations allows us to evaluate circulating UE and E PUFAs separately. Additionally, plasma concentrations reflect immediate consequences of changing diet and liver metabolism. Esterase activity on circulating lipoproteins releases UE PUFAs, the form that preferably enters the brain, likely by a diffusional mechanism (50, 51). After entering the brain, the largest percentage of UE shorter chain PUFAs (e.g. LA, ALA and EPA) are rapidly beta-oxidized by mitochondria (38), whereas the elongated AA and DHA forms preferentially enter the *sn*-2 position of brain membrane phospholipids to replace the respective PUFAs that have been hydrolyzed and lost by metabolism.

The suggested beneficial effects of certain PUFAs may be peripheral as well as central. Increased UE fatty acids triggered by fasting have been shown in rodents to drive immunosuppression (52). Alternately, conversion of EPA to DHA in the liver (46) may drive the treatment effect. One intervention study using UE PUFAs showed that administration of fish oil increased brain UE DHA and a neuroprotective DHA metabolite, neuroprotectin D-1, in a rodent model of Alzheimer's disease (53). Additionally, phospholipase A_2 genes and enzyme levels may differ between BD and HC groups, which may affect production of plasma PUFAs (52–58). A recent trial of n-3 PUFAs for reduction of plasma triglycerides showed a phospholipase A_2 genotype × treatment interaction (59). Therefore, investigation of UE and E PUFAs provides a different window of investigation from that of PUFAs within the red blood cell membrane into the role of PUFA metabolism in BD.

Changes at follow-up in n-3 PUFA metabolism

We engaged participants during an active mood episode, and monitored symptoms during recovery, asking them to return when they no longer met threshold criteria for mania and depression. Treatment was not altered from usual care during this time. During this follow-up period, we lost approximately half of our participants due to an inability to contact them. Therefore, our results in investigative comparisons between baseline and follow-up are exploratory and only suggestive due to the small sample size. We found no difference in plasma levels of PUFAs when comparing mean baseline concentrations to concentrations at follow-up; however, ratios of some plasma n-3 PUFA concentrations were significantly different at

follow-up from baseline in BD subjects. There was no change in self-reported n-3 PUFA intake at follow-up. The ratio changes indicate *decreases* in UE DHA relative to UE ALA, in UE EPA relative to UE ALA, and in E DHA relative to E ALA. Since ALA once ingested can be elongated and desaturated, particularly in the liver, first to EPA and then to DHA (60), these results suggest some limitation to these metabolic conversions. One possibility is drug effects on the liver (61). Though we cannot comment on mechanism from our data, the ratio data suggest abnormal metabolic relations among the three measured n-3 PUFAs.

Symptom and clinical correlations with fatty acid levels and ratios

Symptom severity has been correlated with circulating PUFA biomarkers in the Sublette et al.(17) and Evans et al. (20) studies, but not in the Chiu et al. (18) and McNamara et al. (19) studies. Sublette et al. found a positive correlation between manic symptoms and the UE AA:EPA ratio. In our study, however, the UE AA:EPA ratio was not correlated with mania, but was positively correlated with psychosis. Two factors that could affect this ratio differed in our trial from the Sublette trial: symptomatic state of our subjects (we had few subjects with pure mania in our study), and the number of patients currently on psychiatric medication. We found that the UE:E ratio of EPA was positively correlated with mania, which would not be expected given that a lower UE:E EPA was found in BD patients compared to HCs. This will bear further investigation.

In our study, subjects with panic attacks as part of the current mood episode showed lower n-3 and higher n-6 concentrations, and a higher n-6 to n-3 ratio. This pattern suggests activation of an inflammatory pathway or reduction of an anti-inflammatory pathway. The noradrenergic surge seen in panic attacks has been hypothesized to be related to the dopaminergic surge seen in mania in BD, and perhaps panic identifies a subset of mania or is a severity marker of psychiatric illness (62–65). Together, ours and others' findings suggest an altered PUFA metabolism in BD, in accord with pre-clinical data.

We found a positive correlation between UE saturated fatty acids and panic, and a negative association between E saturated fatty acids and psychosis. The differentiating factor may be the esterification status, as the UE form preferentially enters the brain. UE palmitic acid was shown to increase anxiety-like behaviors and immunosuppression in rodent models (66, 67). UE fatty acids may be associated with clinical phenotype through an immunological link.

Medication status

While both Sublette et al. (17) and McNamara et al. (19) studied PUFAs in unmedicated patients, the Chiu et al. study (18) found no difference between unmedicated and medicated patients. Our approach differed, as we studied relations between effective treatment and biological markers. We included patients who were ill though medicated, and those with comorbid psychiatric illness, specifically looking for differences before and after treatment. We had no unmedicated subjects in our sample, but we did not find that concentrations or ratios of PUFAs were correlated with antidepressants, antipsychotics, or mood stabilizers as medication groups.

Dietary report

We found no correlations between self-report of ALA, EPA, and DHA intake and plasma fatty acid levels, which could occur for several reasons. The subjects in this study may have been unable to accurately fill out the questionnaire given the severity of mood symptoms. A more controlled dietary regimen would be useful in this regard (68). Additionally, individual metabolic differences may mask correlations with reported intake. The memory difficulty inherent in mood episodes may have interfered with the ability to report food intake accurately. Further study with this instrument would be beneficial to understand barriers to its use.

Limitations

Limitations include the use of patients who are taking medication in a study of biomarkers. While the heterogeneity of the subjects with BD with regard to medication use at baseline and in follow-up and comorbid psychiatric illness may limit the precision with which the findings of PUFA differences can be related to bipolar neuropathology, this study design maximizes the generalizability of the findings to a real-world clinical population.

If EPA metabolism differs in patients who are symptomatic and is altered with successful treatment, regardless of the medication class, this suggests that effective medications in practice have a similar biological impact on the n-3/n-6 metabolic balance, mirroring pre-clinical findings (12). Additionally, our results at follow-up are only suggestive, as (i) treatment was not delivered as a

controlled intervention, and (ii) less than 50% of the sample completed the follow-up. While baseline factors did not differ between subjects who did or did not complete a follow up visit, we could not measure treatment response in those who did not complete the study. The patients had a high rate of unstable housing and contact information, which may have been a factor in their inability to complete the study. Other limitations include self-report of dietary n-3 input only. Baseline dietary intake of PUFAs may indeed influence the response of the system to intervention. In addition, this study was not large enough to look for subgroups of subjects who may have PUFA alterations, while others may not.

Conclusions

We found (i) a lower plasma UE:E concentration ratio of EPA, (ii) a trend toward lower U EPA in the symptomatic BD state, and (iii) altered n-3 PUFA ratios upon follow-up in a subset of the BD sample. Altered plasma circulating n-3 PUFA concentrations may influence vulnerability to a mood state, and altered n-3 PUFA ratios could indicate changes in PUFA metabolism concurrent with symptom improvement. Panic attacks, which may be a marker of clinical severity, were correlated with lower n-3 and higher n-6 PUFA concentrations and a higher n-6 to n-3 ratio. Our findings are consistent with preclinical and postmortem data and suggest testing interventions that increase n-3 and decrease n-6 dietary PUFA intake.

Implications

While initial n-3 PUFA supplementation studies in BD demonstrated efficacy in open trials (69–72), and subsequent randomized controlled trials (73, 74) failed to replicate these findings (75–79), preclinical and postmortem data implicate PUFAs in the pathophysiology of BD (12, 13). Interpretation of the varied responses seen in randomized controlled trials is confounded by a number of factors, including heterogeneity of diagnosis, design of trials, compliance to study drug, and composition and dose of supplements (36, 80–84).

One reason that supplement studies may have not shown effect may be that, in the past century, the n-6 PUFA precursor LA has increased in the average US diet, resulting in decreasing tissue concentrations of n-3 EPA and DHA and increasing concentrations of AA and LA and their metabolites (85–87). Addition of an n-3 supplement without concurrent reduction in dietary n-6 LA may not produce clinically meaningful benefit. A recent randomized trial investigated PUFA dietary intervention in chronic migraine headache, a condition with considerable overlap with BD in pathophysiology, comorbidity, and treatment (34, 88–101). Clinical efficacy and biochemical effects of a high n-3 EPA + DHA plus low n-6 LA (H3-L6) diet were compared to a low n-6 PUFA diet (L6). The H3-L6 group experienced a significantly greater reduction in headache hours per day, headache days per month, headache-related quality of life and psychological distress than the L6 group, although both groups experienced improvement from the run-in phase (68). Interestingly, both the L6 and the H3-L6 groups had increases in UE EPA, specifically, among PUFAs (102). Based on the clinical and neuroinflammatory links between BD and migraine, concurrent lowering of dietary n-6 in BD may also be necessary for effective treatment of bipolar disorder with n-3 PUFA supplementation (103). Future studies of the specific signaling pathways and lipid mediators linking n-3 and n-6 PUFAs to BD pathogenesis may lead to development of targeted dietary and medication strategies for treating BD.

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Disclosures

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References

1. Merikangas KR, Akiskal HS, Angst J et al. Lifetime and 12-month prevalence of bipolar spectrum disorder in the National Comorbidity Survey replication. Arch Gen Psychiatry 2007; 64: 543–552.

2. Craddock N, Sklar P. Genetics of bipolar disorder. Lancet 2013; 381: 1654–1662.
3. Goodwin FK, Jamison KR. Manic-Depressive Illness: Bipolar Disorders and Recurrent Depression. New York: Oxford University Press, 2007: 1262.
4. American Psychiatric Association. DSM-5 Task Force. Diagnostic and Statistical Manual of Mental Disorders: DSM-5, 5th edn. Washington, DC: American Psychiatric Association, 2013: 947.
5. Ghaemi SN, Bauer M, Cassidy F et al. Diagnostic guidelines for bipolar disorder: a summary of the International Society for Bipolar Disorders Diagnostic Guidelines Task Force Report. Bipolar Disord 2008; 10: 117–128.
6. Tohen M, Frank E, Bowden CL et al. The International Society for Bipolar Disorders (ISBD) Task Force report on the nomenclature of course and outcome in bipolar disorders. Bipolar Disord 2009; 11: 453–473.
7. Vieta E, Suppes T. Bipolar II disorder: arguments for and against a distinct diagnostic entity. Bipolar Disord 2008; 10: 163–178.
8. Hibbeln JR, Palmer JW, Davis JM. Are disturbances in lipid-protein interactions by phospholipase-A2 a predisposing factor in affective illness? Biol Psychiatry 1989; 25: 945–961.
9. Allison JH, Stewart MA. Reduced brain inositol in lithium-treated rats. Nature 1971; 233: 267–268.
10. Svennerholm L. Distribution and fatty acid composition of phosphoglycerides in normal human brain. J Lipid Res 1968; 9: 570–579.
11. Rapoport SI. Brain arachidonic and docosahexaenoic acid cascades are selectively altered by drugs, diet and disease. Prostaglandins Leukot Essent Fatty Acids 2008; 79: 153–156.
12. Rapoport SI. Lithium and the other mood stabilizers effective in bipolar disorder target the rat brain arachidonic acid cascade. ACS Chem Neurosci 2014; 5: 459–467.
13. Rapoport SI, Basselin M, Kim HW, Rao JS. Bipolar disorder and mechanisms of action of mood stabilizers. Brain Res Rev 2009; 61: 185–209.
14. Rapoport SI, Bosetti F. Do lithium and anticonvulsants target the brain arachidonic acid cascade in bipolar disorder? Arch Gen Psychiatry 2002; 59: 592–596.
15. Freeman MP. Omega-3 fatty acids and perinatal depression: a review of the literature and recommendations for future research. Prostaglandins Leukot Essent Fatty Acids 2006; 75: 291–297.
16. Noaghiul S, Hibbeln JR. Cross-national comparisons of seafood consumption and rates of bipolar disorders. Am J Psychiatry 2003; 160: 2222–2227.
17. Sublette ME, Bosetti F, DeMar JC et al. Plasma free polyunsaturated fatty acid levels are associated with symptom severity in acute mania. Bipolar Disord 2007; 9: 759–765.
18. Chiu CC, Huang SY, Su KP et al. Polyunsaturated fatty acid deficit in patients with bipolar mania. Eur Neuropsychopharmacol 2003; 13: 99–103.
19. McNamara RK, Jandacek R, Rider T, Tso P, Dwivedi Y, Pandey GN. Selective deficits in erythrocyte docosahexaenoic acid composition in adult patients with bipolar disorder and major depressive disorder. J Affect Disord 2010; 126: 303–311.
20. Evans SJ, Kamali M, Prossin AR et al. Association of plasma omega-3 and omega-6 lipids with burden of disease measures in bipolar subjects. J Psychiatr Res 2012; 46: 1435–1441.
21. Evans SJ, Prossin AR, Harrington GJ et al. Fats and factors: lipid profiles associate with personality factors and suicidal history in bipolar subjects. PLoS ONE 2012; 7: e29297.
22. Purdon D, Arai T, Rapoport S. No evidence for direct incorporation of esterified palmitic acid from plasma into brain lipids of awake adult rat. J Lipid Res 1997; 38: 526–530.
23. Ouellet M, Emond V, Chen CT et al. Diffusion of docosahexaenoic and eicosapentaenoic acids through the blood–brain barrier: an in situ cerebral perfusion study. Neurochem Int 2009; 55: 476–482.
24. Sheehan DV, Lecrubier Y, Sheehan KH et al. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J Clin Psychiatry 1998; 59 (Suppl. 20): 22–33.
25. Hamilton M. A rating scale for depression. J Neurol Neurosurg Psychiatry 1960; 23: 56–62.
26. Altman EG, Hedeker DR, Janicak PG, Peterson JL, Davis JM. The Clinician-Administered Rating Scale for Mania (CARS-M): development, reliability, and validity. Biol Psychiatry 1994; 36: 124–134.
27. Sublette ME, Segal-Isaacson CJ, Cooper TB et al. Validation of a food frequency questionnaire to assess intake of n-3 polyunsaturated fatty acids in subjects with and without major depressive disorder. J Am Diet Assoc 2011; 111: 117–123.e1-2.
28. NIAAA Alcohol Facts and Statistics [webpage]. National Institute on Alcohol Abuse and Alcoholism, 2015. Available from: http://www.niaaa.nih.gov/alcohol-health/overview-alcohol-consumption/alcohol-facts-and-statistics [accessed July 2015].
29. Folch J, Lees M, Sloan Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 1957; 226: 497–509.
30. Breckenridge WC, Kuksis A. Specific distribution of short-chain fatty acids in molecular distillates of bovine milk fat. J Lipid Res 1968; 9: 388–393.
31. Makrides M, Neumann MA, Byard RW, Simmer K, Gibson RA. Fatty acid composition of brain, retina, and erythrocytes in breast- and formula-fed infants. Am J Clin Nutr 1994; 60: 189–194.
32. DeMar JC Jr, Ma K, Bell JM, Rapoport SI. Half-lives of docosahexaenoic acid in rat brain phospholipids are prolonged by 15 weeks of nutritional deprivation of n-3 polyunsaturated fatty acids. J Neurochem 2004; 91: 1125–1137.
33. Serhan CN. Novel chemical mediators in the resolution of inflammation: resolvins and protectins. Anesthesiol Clin 2006; 24: 341–364.
34. Ramsden CE, Mann JD, Faurot KR et al. Low omega-6 vs. low omega-6 plus high omega-3 dietary intervention for chronic daily headache: protocol for a randomized clinical trial. Trials 2011; 12: 97.
35. Perneger TV. What's wrong with Bonferroni adjustments. BMJ 1998; 316: 1236–1238.
36. Sublette ME, Ellis SP, Geant AL, Mann JJ. Meta-analysis of the effects of eicosapentaenoic acid (EPA) in clinical trials in depression. J Clin Psychiatry 2011; 72: 1577–1584.
37. Sarris J, Mischoulon D, Schweitzer I. Omega-3 for bipolar disorder: meta-analyses of use in mania and bipolar depression. J Clin Psychiatry 2012; 73: 81–86.
38. Evans SJ, Ringrose RN, Harrington GJ, Mancuso P, Burant CF, McInnis MG. Dietary intake and plasma metabolomic analysis of polyunsaturated fatty acids in

bipolar subjects reveal dysregulation of linoleic acid metabolism. J Psychiatr Res 2014; 57: 58–64.
39. Grosso G, Pajak A, Marventano S et al. Role of omega-3 fatty acids in the treatment of depressive disorders: a comprehensive meta-analysis of randomized clinical trials. PLoS ONE 2014; 9: e96905.
40. Brooks JD, Milne GL, Yin H, Sanchez SC, Porter NA, Morrow JD. Formation of highly reactive cyclopentenone isoprostane compounds (A3/J3-isoprostanes) in vivo from eicosapentaenoic acid. J Biol Chem 2008; 283: 12043–12055.
41. Arita M, Yoshida M, Hong S et al. Resolvin E1, an endogenous lipid mediator derived from omega-3 eicosapentaenoic acid, protects against 2,4,6-trinitrobenzene sulfonic acid-induced colitis. Proc Natl Acad Sci USA 2005; 102: 7671–7676.
42. Jeng JY, Lee WH, Tsai YH, Chen CY, Chao SY, Hsieh RH. Functional modulation of mitochondria by eicosapentaenoic acid provides protection against ceramide toxicity to C6 glioma cells. J Agric Food Chem 2009; 57: 11455–11462.
43. Leroy C, Tricot S, Lacour B, Grynberg A. Protective effect of eicosapentaenoic acid on palmitate-induced apoptosis in neonatal cardiomyocytes. Biochim Biophys Acta 2008; 1781: 685–693.
44. Yamagata K, Tagami M, Takenaga F, Yamori Y, Nara Y, Itoh S. Polyunsaturated fatty acids induce tight junctions to form in brain capillary endothelial cells. Neuroscience 2003; 116: 649–656.
45. Omura M, Kobayashi S, Mizukami Y et al. Eicosapentaenoic acid (EPA) induces Ca(2+)-independent activation and translocation of endothelial nitric oxide synthase and endothelium-dependent vasorelaxation. FEBS Lett 2001; 487: 361–366.
46. Igarashi M, Chang L, Ma K, Rapoport SI. Kinetics of eicosapentaenoic acid in brain, heart and liver of conscious rats fed a high n-3 PUFA containing diet. Prostaglandins Leukot Essent Fatty Acids 2013; 89: 403–412.
47. Oldendorf WH, Brown WJ. Greater number of capillary endothelial cell mitochondria in brain than in muscle. Proc Soc Exp Biol Med 1975; 149: 736–738.
48. Goldstein BI, Young LT. Toward clinically applicable biomarkers in bipolar disorder: focus on BDNF, inflammatory markers, and endothelial function. Curr Psychiatry Rep 2013; 15: 425.
49. Pomponi M, Janiri L, La Torre G et al. Plasma levels of n-3 fatty acids in bipolar patients: deficit restricted to DHA. J Psychiatr Res 2013; 47: 337–342.
50. Smith QR, Nagura H. Fatty acid uptake and incorporation in brain: studies with the perfusion model. J Mol Neurosci 2001; 16: 167–172.
51. Robinson PJ, Noronha J, DeGeorge JJ, Freed LM, Nariai T, Rapoport SI. A quantitative method for measuring regional in vivo fatty-acid incorporation into and turnover within brain phospholipids: review and critical analysis. Brain Res Brain Res Rev 1992; 17: 187–214.
52. Dawson E, Gill M, Curtis D et al. Genetic association between alleles of pancreatic phospholipase A2 gene and bipolar affective disorder. Psychiatr Genet 1995; 5: 177–180.
53. Jacobsen NJ, Franks EK, Owen MJ, Craddock NJ. Mutational analysis of phospholipase A2A: a positional candidate susceptibility gene for bipolar disorder. Mol Psychiatry 1999; 4: 274–279.
54. Ikenaga EH, Talib LL, Ferreira AS, Machado-Vieira R, Forlenza OV, Gattaz WF. Reduced activities of phospholipases A2 in platelets of drug-naïve bipolar disorder patients. Bipolar Disord 2015; 17: 97–101.
55. Meira-Lima I, Jardim D, Junqueira R, Ikenaga E, Vallada H. Allelic association study between phospholipase A2 genes and bipolar affective disorder. Bipolar Disord 2003; 5: 295–299.
56. Noponen M, Sanfilipo M, Samanich K et al. Elevated PLA2 activity in schizophrenics and other psychiatric patients. Biol Psychiatry 1993; 34: 641–649.
57. Ross BM, Hughes B, Kish SJ, Warsh JJ. Serum calcium-independent phospholipase A2 activity in bipolar affective disorder. Bipolar Disord 2006; 8: 265–270.
58. Kim HW, Rapoport SI, Rao JS. Altered arachidonic acid cascade enzymes in postmortem brain from bipolar disorder patients. Mol Psychiatry 2011; 16: 419–428.
59. Tremblay BL, Cormier H, Rudkowska I, Lemieux S, Couture P, Vohl MC. Association between polymorphisms in phospholipase A2 genes and the plasma triglyceride response to an n-3 PUFA supplementation: a clinical trial. Lipids Health Dis 2015; 14: 12.
60. Igarashi M, DeMar JC Jr, Ma K, Chang L, Bell JM, Rapoport SI. Upregulated liver conversion of alpha-linolenic acid to docosahexaenoic acid in rats on a 15 week n-3 PUFA-deficient diet. J Lipid Res 2007; 48: 152–164.
61. McNamara RK, Jandacek R, Rider T, Tso P, Cole-Strauss A, Lipton JW. Differential effects of antipsychotic medications on polyunsaturated fatty acid biosynthesis in rats: relationship with liver delta6-desaturase expression. Schizophr Res 2011; 129: 57–65.
62. MacKinnon DF, Zamoiski R. Panic comorbidity with bipolar disorder: what is the manic–panic connection? Bipolar Disord 2006; 8: 648–664.
63. Bailey JE, Argyropoulos SV, Lightman SL, Nutt DJ. Does the brain noradrenaline network mediate the effects of the CO_2challenge?JPsychopharmacol2003;17:252–259.
64. Bunney WE Jr, Goodwin FK, Murphy DL, House KM, Gordon EK. The "switch process" in manic-depressive illness. II. Relationship to catecholamines, REM sleep, and drugs. Arch Gen Psychiatry 1972; 27: 304–309.
65. Joyce PR, Fergusson DM, Woollard G, Abbott RM, Horwood LJ, Upton J. Urinary catecholamines and plasma hormones predict mood state in rapid cycling bipolar affective disorder. J Affect Disord 1995; 33: 233–243.
66. Joesting JJ, Moon ML, Gainey SJ, Tisza BL, Blevins NA, Freund GG. Fasting induces IL-1 resistance and free-fatty acid-mediated up-regulation of IL-1R2 and IL-1RA. Front Immunol 2014; 5: 315.
67. Moon ML, Joesting JJ, Lawson MA et al. The saturated fatty acid, palmitic acid, induces anxiety-like behavior in mice. Metabolism 2014; 63: 1131–1140.
68. Ramsden CE, Faurot KR, Zamora D et al. Targeted alteration of dietary n-3 and n-6 fatty acids for the treatment of chronic headaches: a randomized trial. Pain 2013; 154: 2441–2451.
69. Osher Y, Bersudsky Y, Belmaker RH. Omega-3 eicosapentaenoic acid in bipolar depression: report of a small open-label study. J Clin Psychiatry 2005; 66: 726–729.
70. Sagduyu K, Dokucu ME, Eddy BA, Craigen G, Baldassano CF, Yildiz A. Omega-3 fatty acids decreased irritability of patients with bipolar disorder in an add-on, open label study. Nutr J 2005; 4: 6.
71. Wozniak J, Biederman J, Mick E et al. Omega-3 fatty acid monotherapy for pediatric bipolar disorder: a prospective open-label trial. Eur Neuropsychopharmacol 2007; 17: 440–447.
72. Clayton EH, Hanstock TL, Hirneth SJ, Kable CJ, Garg ML, Hazell PL. Reduced mania and depression in

juvenile bipolar disorder associated with long-chain omega-3 polyunsaturated fatty acid supplementation. Eur J Clin Nutr 2009; 63: 1037–1040.
73. Stoll AL, Severus WE, Freeman MP et al. Omega 3 fatty acids in bipolar disorder: a preliminary double-blind, placebo-controlled trial. Arch Gen Psychiatry 1999; 56: 407–412.
74. Frangou S, Lewis M, McCrone P. Efficacy of ethyl-eicosapentaenoic acid in bipolar depression: randomised double-blind placebo-controlled study. Br J Psychiatry 2006; 188: 46–50.
75. Chiu CC, Huang SY, Chen CC, Su KP. Omega-3 fatty acids are more beneficial in the depressive phase than in the manic phase in patients with bipolar I disorder. J Clin Psychiatry 2005; 66: 1613–1614.
76. Keck PE Jr, Mintz J, McElroy SL et al. Double-blind, randomized, placebo-controlled trials of ethyl-eicosapentanoate in the treatment of bipolar depression and rapid cycling bipolar disorder. Biol Psychiatry 2006; 60: 1020–1022.
77. Frangou S, Lewis M, Wollard J, Simmons A. Preliminary in vivo evidence of increased N-acetyl-aspartate following eicosapentanoic acid treatment in patients with bipolar disorder. J Psychopharmacol 2007; 21: 435–439.
78. Gracious BL, Chirieac MC, Costescu S, Finucane TL, Youngstrom EA, Hibbeln JR. Randomized, placebo-controlled trial of flax oil in pediatric bipolar disorder. Bipolar Disord 2010; 12: 142–154.
79. Murphy BL, Stoll AL, Harris PQ et al. Omega-3 fatty acid treatment, with or without cytidine, fails to show therapeutic properties in bipolar disorder: a double-blind, randomized add-on clinical trial. J Clin Psychopharmacol 2012; 32: 699–703.
80. Ross BM, Seguin J, Sieswerda LE. Omega-3 fatty acids as treatments for mental illness: which disorder and which fatty acid? Lipids Health Dis 2007; 6: 21.
81. Martins JG. EPA but not DHA appears to be responsible for the efficacy of omega-3 long chain polyunsaturated fatty acid supplementation in depression: evidence from a meta-analysis of randomized controlled trials. J Am Coll Nutr 2009; 28: 525–542.
82. Martins JG, Bentsen H, Puri BK. Eicosapentaenoic acid appears to be the key omega-3 fatty acid component associated with efficacy in major depressive disorder: a critique of Bloch and Hannestad and updated meta-analysis. Mol Psychiatry 2012; 17: 1144–1149.
83. Appleton KM, Rogers PJ, Ness AR. Updated systematic review and meta-analysis of the effects of n-3 long-chain polyunsaturated fatty acids on depressed mood. Am J Clin Nutr 2010; 91: 757–770.
84. Lin PY, Mischoulon D, Freeman MP et al. Are omega-3 fatty acids antidepressants or just mood-improving agents? The effect depends upon diagnosis, supplement preparation, and severity of depression. Mol Psychiatry 2012; 17: 1161–1163.
85. Blasbalg TL, Hibbeln JR, Ramsden CE, Majchrzak SF, Rawlings RR. Changes in consumption of omega-3 and omega-6 fatty acids in the United States during the 20th century. Am J Clin Nutr 2011; 93: 950–962.
86. Ramsden CE, Ringel A, Feldstein AE et al. Lowering dietary linoleic acid reduces bioactive oxidized linoleic acid metabolites in humans. Prostaglandins Leukot Essent Fatty Acids 2012; 87: 135–141.
87. Igarashi M, Gao F, Kim HW, Ma K, Bell JM, Rapoport SI. Dietary n-6 PUFA deprivation for 15 weeks reduces arachidonic acid concentrations while increasing n-3 PUFA concentrations in organs of post-weaning male rats. Biochim Biophys Acta 2009; 1791: 132–139.
88. Baptista T, Uzcategui E, Arape Y et al. Migraine life-time prevalence in mental disorders: concurrent comparisons with first-degree relatives and the general population. Invest Clin 2012; 53: 38–51.
89. Ortiz A, Cervantes P, Zlotnik G et al. Cross-prevalence of migraine and bipolar disorder. Bipolar Disord 2010; 12: 397–403.
90. Dilsaver SC, Benazzi F, Oedegaard KJ, Fasmer OB, Akiskal HS. Is a family history of bipolar disorder a risk factor for migraine among affectively ill patients? Psychopathology 2009; 42: 119–123.
91. McIntyre RS, Konarski JZ, Wilkins K, Bouffard B, Soczynska JK, Kennedy SH. The prevalence and impact of migraine headache in bipolar disorder: results from the Canadian Community Health Survey. Headache 2006; 46: 973–982.
92. McIntyre RS, Konarski JZ, Soczynska JK et al. Medical comorbidity in bipolar disorder: implications for functional outcomes and health service utilization. Psychiatr Serv 2006; 57: 1140–1144.
93. Fasmer OB. The prevalence of migraine in patients with bipolar and unipolar affective disorders. Cephalalgia 2001; 21: 894–899.
94. Fasmer OB, Oedegaard KJ. Clinical characteristics of patients with major affective disorders and comorbid migraine. World J Biol Psychiatry 2001; 2: 149–155.
95. Saunders EF, Nazir R, Kamali M et al. Gender differences, clinical correlates, and longitudinal outcome of bipolar disorder with comorbid migraine. J Clin Psychiatry 2014; 75: 512–519.
96. Antonova M, Wienecke T, Olesen J, Ashina M. Prostaglandin E(2) induces immediate migraine-like attack in migraine patients without aura. Cephalalgia 2012; 32: 822–833.
97. Moskowitz MA, Buzzi MG. Migraine general aspects. Handb Clin Neurol 2010; 97: 253–266.
98. Buzzi MG, Moskowitz MA. The pathophysiology of migraine: year 2005. J Headache Pain 2005; 6: 105–111.
99. Buzzi MG, Moskowitz MA. The trigemino-vascular system and migraine. Pathol Biol (Paris) 1992; 40: 313–317.
100. BALANCE Investigators and collaborators, Geddes JR, Goodwin GM et al. Lithium plus valproate combination therapy versus monotherapy for relapse prevention in bipolar I disorder (BALANCE): a randomised open-label trial. Lancet 2010; 375: 385–395.
101. Linde M, Mulleners WM, Chronicle EP, McCrory DC. Valproate (valproic acid or sodium valproate or a combination of the two) for the prophylaxis of episodic migraine in adults. Cochrane Database Syst Rev 2013; 6: CD010611.
102. Taha AY, Cheon Y, Faurot KF et al. Dietary omega-6 fatty acid lowering increases bioavailability of omega-3 polyunsaturated fatty acids in human plasma lipid pools. Prostaglandins Leukot Essent Fatty Acids 2014; 90: 151–157.
103. MacIntosh BA, Ramsden CE, Faurot KR et al. Low-n-6 and low-n-6 plus high-n-3 diets for use in clinical research. Br J Nutr 2013; 110: 559–568.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Saturated and monounsaturated fatty acid concentrations (nmol/mL).