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Dietary Intake and Plasma Metabolomic Analysis of Polyunsaturated Fatty Acids in Bipolar Subjects Reveal Dysregulation of Linoleic Acid Metabolism

Simon J. Evans¹, Rachel N. Ringrose¹, Gloria J Harrington¹, Peter Mancuso², Charles F Burant³, and Melvin G McInnis¹

¹Department of Psychiatry, University of Michigan

²Department of Environment Health Sciences, University of Michigan

³Department of Internal Medicine, University of Michigan

Abstract

Polyunsaturated fatty acids (PUFA) profiles associate with risk for mood disorders. This poses the hypothesis of metabolic differences between patients and unaffected healthy controls that relate to the primary illness or are secondary to medication use or dietary intake. However, dietary manipulation or supplementation studies show equivocal results improving mental health outcomes. This study investigates dietary patterns and metabolic profiles relevant to PUFA metabolism, in bipolar I individuals compared to non-psychiatric controls. We collected seven-day diet records and performed metabolomic analysis of fasted plasma collected immediately after diet recording. Regression analyses adjusted for age, gender and energy intake found that bipolar individuals had significantly lower intake of selenium and PUFAs, including eicosapentaenoic acid (EPA) (n-3), docosahexaenoic acid (DHA) (n-3), arachidonic acid (AA) (n-6) and docosapentaenoic acid (DPA) (n-3/n-6 mix); and significantly increased intake of the saturated fats, eicosanoic and docosanoic acid. Regression analysis of metabolomic data derived from plasma samples, correcting for age, gender, BMI, psychiatric medication use and dietary PUFA intake, revealed that bipolar individuals had reduced 13S-HpODE, a major peroxidation product of the n-6, linoleic acid (LA), reduced eicosadienoic acid (EDA), an elongation product of LA; reduced prostaglandins G2, F2 alpha and E1, synthesized from n-6 PUFA; and reduced EPA. These observations remained significant or near significant after Bonferroni correction and are consistent with metabolic variances between bipolar and control individuals with regard to PUFA metabolism. These findings suggest that specific dietary interventions aimed towards correcting these metabolic disparities may impact health outcomes for individuals with bipolar disorder.

Keywords

omega-3; omega-6; atypical antipsychotics; eicosadienoic acid; hydroperoxy octadecadienoic acid

Study Objectives and Background

Bipolar illness affects 2–3% of the US population (Kessler et al., 2005) and carries an average individual economic burden of nearly \$12,000 for a single manic episode to nearly \$625,000 for chronic unresponsive disease (Begley et al., 2001) and an estimated societal cost of \$25–45 billion per year (Begley et al., 2001; Wyatt & Henter, 1995). The lifetime prevalence of suicide attempts in bipolar patients is about 30%, which means that bipolar disorder is a large single risk factor for suicide and suicidal behavior (Novick, Swartz, & Frank, 2010). Pharmaceutical treatment of bipolar disorder is effective in short-term acute episodic management but often has a limited long-term success and often requires testing several approaches to find a treatment paradigm that approaches an acceptable level of effectiveness. This is likely due to multiple etiologies from the polygenic nature of this disorder and a plethora of environmental contributors.

A substantial environmental contributor may be dietary lipids since epidemiological studies have shown that higher incidences of bipolar and depressive illness associate with lower intake of n-3 polyunsaturated fatty acids (PUFA) (Noaghiul & Hibbeln, 2003). Furthermore, low serum levels of specific n-3 PUFA have been reported in bipolar subjects (Hibbeln, Nieminen, Blasbalg, Riggs, & Lands, 2006) and we have previously reported associations between both plasma n-3 and n-6 PUFA levels and measures of bipolar disease burden (Evans et al., 2012a; Evans et al., 2012b). Evidence suggests that these observations might be partially explained by genetic variants in specific PUFA-metabolizing enzymes that are differentially expressed in brains of bipolar and depressed subjects (Liu & McNamara, 2011). Also, dietary patterns may be different in bipolar patients, compared to non-psychiatric controls, due to emotional, personal, or socioeconomic reasons (Ellingrod et al., 2011; Jacka et al., 2011; Kilbourne et al., 2007). Thus, there may be gene-diet interactions that are reflected in the tissue levels of PUFAs that vary in bipolar patients relative to controls and effect burden of disease and treatment response.

The n-3 PUFA, alpha-linolenic acid (ALA) and n-6 PUFA, linoleic acid (LA) are essential fatty acids (EFAs) since they cannot be synthesized by mammals and must be obtained in the diet. These two are precursors for the longer chain PUFAs, notably docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) from ALA, and dihomogamma linolenic acid (DGLA) and arachidonic acid (AA) from LA (diagramed in Fig 1). However, controversy exists around the efficiency of conversion from the EFAs to the longer chain PUFA in humans and the role of dietary intake in regulating the levels of long chain PUFA directly. Plasma levels of EPA and DHA are much more responsive to dietary intake of these n-3 fatty acids and dietary supplementation with ALA does not result in significant accumulation of long chain n-3 fatty acids in plasma (Arterburn, Hall, & Oken, 2006). Conversely, AA seems to be tightly regulated and less responsive to dietary intake (Rett & Whelan, 2011). Furthermore, single nucleotide polymorphisms (SNPs) in FADS1 and FADS2 genes, which code for delta-5 and delta-6 desaturase enzymes, respectively, and are involved in the synthesis of long chain PUFA from ALA and LA, partially control levels of AA, DHA and EPA (Tanaka et al., 2009).

Given the metabolic importance of PUFAs, disturbances in this system likely impact several other metabolic pathways as well. For example, a plethora of eicosanoids and related molecules are produced from PUFAs that regulate many physiological functions, a review of which is beyond the scope of this manuscript. Furthermore, medications in the class of mood stabilizers (lithium or valproate) that are prescribed to many bipolar patients to prevent episode recurrence, selectively inhibit AA cleavage from neuronal membranes and concomitant downstream production of inflammatory eicosanoids (Rapoport, Basselin, Kim, & Rao, 2009). This suggests that the biology of PUFA metabolism may be involved in the neurochemistry of bipolar disorder. Bipolar individuals are frequently prescribed medications from the class of atypical antipsychotics for both acute and longer-term management of the illness. These medications have been extensively reported to interfere with various aspects of metabolism, including blood glucose and lipid regulation, and weight management (Cerit et al., 2010). This presents substantial complexity and confounding elements to the metabolic profiles in this disorder. We recently reported a positive association between n-3 PUFA dietary intake and endothelial function that is attenuated by atypical antipsychotic therapy (Ellingrod et al., 2011). The biology involving dietary, genetic and medication interactions that control neurochemical and metabolic manifestations of bipolar illness is clearly challenging and intertwined and will require detailed analyses of dietary patterns and metabolic profiles in well-characterized individuals with the disorder compared to healthy unaffected individuals.

Bipolar and healthy unaffected control individuals were recruited from an ongoing longitudinal study of bipolar disorder, from which we have deep psychiatric phenotyping data, medication history and genetic samples from all subjects. The analyses of dietary and metabolic profiles clearly indicate the importance of diet in the study and management of bipolar disorder.

Materials and Methods

Human Subjects

All subjects in the current study were recruited from the Heinz C. Prechter Longitudinal Study of Bipolar Disorder at the University of Michigan Depression Center (Langenecker, Saunders, Kade, Ransom, & McInnis, 2010). All individuals were diagnosed using the Diagnostic Interview for Genetic Studies (DIGS) (Nurnberger et al., 1994) and included in this current study are bipolar I individuals with no current substance abuse and healthy unaffected controls who were willing to complete daily dietary log records for 7 days. All protocols were approved by the Internal Review Board for Human Studies at the University of Michigan.

Dietary Analysis

One hundred fifty four nutrients were extracted from seven-day diet records using the Nutrition Data System for Research (NDSR) software, 2011 version. All study subjects met with a dietician prior to the seven-day recording period for instruction on how to track foods and estimate portion sizes. Upon returning the food record, all subjects again met with a dietician who personally curated the seven-day record in the presence of the subject to

clarify any misunderstood or under-detailed entries. For the purpose of the current analysis, nutrient data were collapsed to represent average daily intake and statistically analyzed as detailed below. One subject was omitted from the analysis with an excessive average daily caloric intake of approximately 6,000 kCal that was skewing the regression models and 6 subjects were omitted due to insufficient dietary data (e.g. unrealistically low caloric intake or missing diet records).

Metabolomics Analysis

Proteins were precipitated from 100ul of fasted plasma samples by the addition of methanol/acetonitrile/acetone (1:1:1) with a plasma:solvent ratio of 1:4. Proteins were pelleted by centrifugation and samples were dried under nitrogen. Dried samples were reconstituted in distilled water containing injection standards (tBOC-amino acids). Analysis was performed on an Agilent 1200 LC/6530 QTOF LC-MS system in both positive and negative ESI mode. Samples were first resolved on a UPLC HSS-T3 column with an Acquity VanGuard HSS T-3 guard column. Effluent from the LC was directed to the Agilent JetStream source and 6530 QTOF Mass Spectrometer. Initial data processing was performed using Agilent MassHunter 5.0 software. Molecular Feature Extraction (MFE) algorithm was used initially and consistently identified features were used to create library entries for “Known Unknowns” (KU). The “Find by Formula” (FbF) algorithm was used in conjunction with in-house standard-based library and KU library to reliably detect and identify mass spectral features. MS features (components) obtained with both algorithms and associated information were stored in a database and further processed using in-house developed software. Statistical analysis was performed using the standard R package (cran.r-project.org) as a part of integrated server-client data curation and analysis system and as a standalone program.

Lipomic Analysis

Total lipids were extracted from 75ul of fasted plasma according to the method of Bligh and Dyer (Bligh & Dyer, 1959). Heptadecanoic acid internal standards for lipid sub-classes were added to each sample prior to extraction. After hydrolysis, lipids were methylated and analyzed on an Omega Wax 250 capillary column (Supelco) using an Agilent 6890 gas chromatograph. Relative abundance of 22 different naturally occurring fatty acid species were done by comparison of retention times with known standards. Fatty acids were reported as nmol concentration terms and normalized to percent of total for statistical analysis of each of the 22 species.

Statistical Analyses

SPSS 20.0.0 was used to perform all statistical analyses. First, partial correlations were run to determine associations between dietary intake of PUFA relative to plasma PUFA and PUFA-derived metabolites, correcting for total caloric intake, to identify important dietary covariates for metabolomic regression models. Second, for dietary data analysis, we ran linear regression with average daily nutrient intakes (natural log transformed) as the dependent variables (run independently for each nutrient) and diagnosis, gender, age (centered) and average daily caloric intake (centered) as predictors. False discovery rate multiple testing corrections were applied to calculate adjusted p values for the BP covariate

based on the 154 tests run using an online tool (<http://users.ox.ac.uk/~npike/fdr/>) (Pike, 2011). Effect sizes of each covariate were back calculated from coefficients from the log-linear model by using $(\exp^{\beta}-1)*100$, to express effect size as percentage change per given unit of the covariate (or ratio of case:reference for binary covariates). Third, metabolomic and lipomic data were also analyzed by linear regression. Dependent variables were the relative metabolite levels (as natural log transformed normalized chromatographic peak area values from metabolomics dataset) or lipid levels (as natural log transformed percent total lipids analyzed from lipomics dataset). Covariates included age, gender, BMI, psychiatric medication use and PUFA dietary intake (natural log transformed) when dietary intake was directly relevant to the metabolite of interest as determined by the correlation analyses; or if inclusion of the dietary precursor to the metabolite improved the fit of the regression model based on the adjusted r-squared. Medication uses were coded as binary variables (taking or not taking), independently for three major classes of medications: 1) SSRI or SNRI antidepressants (duloxetine, fluoxetine, sertraline, escitalopram, desvenlafaxine, venlafaxine), 2) mood stabilizers or anticonvulsants (lithium, lamotrigine, carbamazepine, topiramate, divalproex, gabapentin) or 3) atypical antipsychotics (clozapine, olanzapine, aripiprazole, risperidone, paliperidone, quetiapine, ziprasidone). No other classes of psychiatric medications were represented in more than 2 research subjects. To minimize multiple testing errors and remain focused on our initial hypothesis, we only analyzed the seven metabolites from the metabolomics data set that are direct metabolites of PUFA metabolism and the eight PUFA species assayed by lipomics. Bonferroni corrections were used to calculate adjusted p values in the metabolomic and lipomic regressions.

Results

There are 100 individuals (54 bipolar and 46 health controls) included in these analyses and the demographics are described in Table 1. All were included in the lipomic analyses and 91 had sufficient dietary data to include in the diet analysis. The metabolomic data set (64 individuals) is derived from a subset of plasma analyzed by lipomics. The bipolar group had greater average BMIs and lower percent males than the control group. Both of these factors were included as covariates in the regression models, except that analysis of dietary data used total energy intake as a covariate in lieu of BMI as it is more appropriate. In an ad hoc test we regressed BMI against diagnosis, age, gender, AAP, MS, AD and total caloric intake. The model returned a significant fit ($p=0.032$), however, only the diagnosis covariate approached significance ($p=0.089$), leaving the difference in BMI unexplained by these covariates. The power of the current study may not be sufficient to detect these potential effects on BMI.

Dietary Data

Significant case-control differences and magnitudes of nutrient intakes are reported in Table 2. We observed a significant decreased intake of selenium and of specific n-3 and n-6 PUFA, including EPA (n-3, 20:5), docosapentaenoic acid (DPA) (n-3/n-6 mix, 22:5), DHA (n-3, 22:6) and AA (n-6, 20:4). We also observed a significant increased intake of the saturated fatty acids, eicosanoic (20:0) and docosanoic (22:0) acid. Of the 154 nutrients

analyzed, these were the only observations that remained significant at the $p < 0.05$ level following FDR corrections.

In order to build the statistical models correctly to test for an effect of having bipolar disorder on the assayed PUFA and PUFA metabolites we wanted to understand which dietary PUFA intakes associated with these metabolites. We performed partial correlations, adjusting for total caloric intake, for the associations between dietary PUFA and serum PUFA and PUFA metabolites (Table 3). These data guide the design of linear regression models discussed below.

Metabolomics and Lipomics Data

Metabolomics analysis reliably detected (in greater than 75% of the samples), 333 unique known molecules and 4,096 “known unknown” molecules. Of the known molecules, seven were metabolic products directly related to PUFA metabolism. Given our *a priori* hypothesis that PUFA metabolism is dysregulated in bipolar subjects, for this analysis we focused on these seven metabolites to minimize the possibility of type I error. Table 4a lists the metabolites, the effect sizes and significance levels for the covariates, as well as Bonferroni corrected p-values for the diagnosis covariate. Lipomics analysis assayed 22 naturally occurring saturated, monounsaturated and polyunsaturated fatty acids. The results of our analysis on the eight n-3 and n-6 PUFA are shown in Table 4b. Additionally, analyses of delta-5 and delta-6 desaturase estimated activities (using product to substrate ratios defined in the table legend) as dependent variables in the regression models are also given in the table. For the statistical models, all covariates given in Table 4 were used in all models and additionally, dietary intake levels for specific PUFA were added to models as follows to correct for the observed associations between intake and plasma levels: the regression of 13(S)-HpODE was additionally corrected for intake of LA (as its biosynthetic precursor) due to an improved model fit (adjusted R^2); the regressions of EPA and DHA were additionally corrected for EPA intake; the regression of LA was additionally corrected for LA intake; and the regression of GLA was additionally corrected for EPA and LA intake; and the regression of DGLA was additionally corrected for EPA and AA intake. We did not further include dietary ALA, DPA or DHA into the models for those metabolites that showed significant correlations with these intakes due to the high collinearity between ALA and LA intakes ($r = 0.59$, $p < 0.001$), EPA and DHA intakes ($r = 0.87$, $p < 0.001$) and EPA and DPA intakes ($r = 0.80$, $p < 0.001$). Therefore, only LA and EPA were added to the models where appropriate.

Eight PUFA or PUFA metabolites were significantly different between bipolar and controls and three remained significant after Bonferroni multiple testing correction, with another three showing strong trends toward significance with corrected p-values between 0.05–0.09. Bipolar subjects had 42% lower plasma levels of the LA peroxidation product, 13S-hydroperoxy-9Z,11E-octadecadienoic acid (13S-HpODE); 37% less plasma levels of the LA elongation product, EDA; 48% lower plasma levels of PGE1, synthesized from DGLA; and 47% lower plasma levels of prostaglandin F2 alpha (PGF2a), and 71% lower plasma prostaglandin G2, both synthesized from AA. Bipolar subjects also had decreased LA and the desaturation product GLA, however these no longer showed trends after Bonferroni

correction. The n-3, EPA was 54% lower in bipolar subjects and remained significant after multiple testing correction. Delta-6 desaturase activity, as estimated by the DGLA:LA ratio was significantly predicted by the regression model, however, no individual covariates, including diagnosis, were independently significant. These data are given in Tables 4a and 4b and support our hypothesis that PUFA metabolism is dysregulated in bipolar subjects. Metabolomics and lipomics data are also summarized schematically in figure 1.

Discussion

Analyses of dietary intakes and metabolomic profiles of bipolar I and healthy unaffected control individuals were focused on PUFA metabolism as there is epidemiological evidence for an association at the population level (Noaghiul et al., 2003). In individuals with bipolar disorder we found decreased dietary intakes of the n-3 PUFA, EPA and DHA, the n-6 PUFA, AA and the n-3/n-6 mix of DPA (the NDSR database does not distinguish between the n-3 and n-6 isomers of DPA); as well as decreased intake of selenium. We also found increased intakes of the SFAs, eicosanoic (20:0) and docosanoic acid (22:0). Analysis of food level data, not discussed, largely explains the decreased PUFA intake with decreased intake of fish. We did not explore the association between fish intake and bipolar illness, but previous studies reported this specific relationship (Noaghiul et al., 2003) as well as general poorer nutrition patterns in bipolar subjects (Davison & Kaplan, 2012; Kilbourne et al., 2007). Therefore our observations in our study population are consistent with previous findings. The high quality of our dietary data is supported by the expected associations between dietary intake and plasma levels of PUFA given in Table 3. Psychiatric practice does not commonly include discussions about diet as a component of the treatment paradigm and these data and results contribute to an accumulating literature suggesting that management of bipolar illness and comorbid metabolic disorders may benefit by incorporating nutritional counseling and dietary education into mental health services. Metabolomic and lipomic profiling of our subjects revealed several significant differences between bipolar and control subjects relevant to PUFA metabolism that remained significant or strongly trended towards significance after correcting for relevant covariates and multiple testing. The lipoxygenase product of LA, 13S-HpODE was lower in plasma from bipolar subjects compared to controls. It is unknown at this point how the observed decreased plasma levels of this metabolite may relate to the biology of bipolar illness, however, several possibilities are considered. First, 15-lipoxygenase (15-LO), responsible for production of 13S-HpODE, has complicated roles in the control of endothelial function with both pro- and anti-atherogenic functions (Wittwer & Hersberger, 2007). Decreased levels of 13SHpODE may be indicative of reduced 15-LO activity in bipolar subjects and of broader biological consequences. Second, 13S-HpODE has been reported to attenuate the production of hydrogen peroxide in thyrocytes (Luci, Bettzieche, Brandsch, & Eder, 2006), the rate-limiting step in synthesis of thyroid hormones. It is known that the mood stabilizer, lithium, alters thyroid function (Lazarus, 2009). However, no differences in 13S-HpODE levels between Li-treated and untreated subjects were identified, suggesting that the observed decrease in 13S-HpODE is not secondary to medication.

We observed decreased PGF2a, PGE1 and PGG2 in bipolar individuals relative to controls. The implications of this are unclear, since tissue specific effects of these prostaglandins on

inflammation are known (Basu, 2010; Kalinski, 2012). Urinary analysis of prostaglandin metabolites provides a more accurate indication of prostaglandin activity and should be considered in future studies.

Finally, we observed significantly decreased EDA and EPA plasma levels in bipolar subjects. EDA has previously been negatively associated with waist circumference in humans (Mocking et al., 2013) and conjugated forms of EDA can reduce body fat and increase lean mass in animal studies (Park, Storkson, Albright, Liu, & Pariza, 2005). EDA negatively associates with sleep disturbances in depressed subjects (Irmisch, Schlafke, Gierow, Herpertz, & Richter, 2007) and is decreased in post-mortem brain tissue (caudate) from schizophrenics (Yao, Leonard, & Reddy, 2000). All of these findings are in the direction of positive health benefits associated with increased EDA; consistent with the direction of our own findings of reduced EDA in bipolar subjects, relative to controls. EPA is a PUFA concentrated in fish oil and has a large literature relevant to depression and bipolar disorder, and has been recently reviewed extensively (Balanza-Martinez et al., 2011; Montgomery & Richardson, 2008; Turnbull, Cullen-Drill, & Smaldone, 2008). Controversy remains regarding the efficacy of therapeutic doses of EPA, however several studies suggest decreased intake and tissue levels of EPA are associated with mood disorders.

We did not find a difference in delta-6 desaturase activity in bipolar individuals, although previous reports found increased expression of the cognate FADS2 mRNA in this population (Liu et al., 2011). Our data are derived from estimated enzyme activities in plasma while the previous study examined mRNA expression in brain. However, when we eliminated psychiatric medication use from the regression model (data not shown), we found a significant 20% ($p=0.009$) increase in estimated delta-6 desaturase activity (using the DGLA:LA ratio). These results suggest either the current study was underpowered to observe a residual diagnosis effect after correcting for medication use or that psychiatric medication use may explain the previously observed effect.

With the exception of EPA, all of the metabolites discussed above are either direct or indirect products of LA metabolism. LA was significantly decreased in bipolar subjects, however the result did not survive the conservative Bonferroni multiple testing correction. Plasma levels of LA synthesis products, EDA GLA and DGLA, were also associated with psychiatric medication use. Atypical antipsychotic use significantly positively associated with GLA levels; mood stabilizer use positively associated with EDA levels; and anti-depressants positively associated with EDA and DGLA. Previous studies in rodents have demonstrated that atypical antipsychotics can decrease liver mRNA expression of FADS1 and increase expression of FADS2 with concomitant decreases in plasma concentrations LA and ALA and increases of the respective desaturation products AA and DHA (McNamara et al., 2011). Although the directionality and precise effects of our data are not entirely consistent with this previous report, the prior study was in rodents treated with controlled doses of single medications, while our study evaluated humans on polypharmacy medication regimens, making direct comparisons difficult. However, a commonality in the research is a finding that psychiatric medications effect PUFA metabolism, suggesting a need for further investigation in medicated bipolar individuals.

The observational nature of this study precludes our ability to establish causative relationships between bipolar illness and PUFA serum levels. This could be addressed by a prospective intervention component to examine the efficacy of dietary manipulation to effect lipid levels and physical or mental health measures in medicated bipolar subjects.

In summary, LA metabolism may be dysregulated in bipolar disorder and some, but not all, differences may be influenced by both diet and psychiatric medications. Dietary LA intake has controversial roles in the regulation of inflammatory systems and the risk of related health problems that are often comorbid with bipolar disorder, such as cardiovascular disease and obesity. It has previously been reported that excessive LA intake increases inflammation by driving AA synthesis, a direct precursor to several inflammatory signaling molecules. However, others have shown positive relationships between dietary LA intake or plasma LA levels and markers of metabolic health. Recent reviews debate this issue (Choque, Catheline, Rioux, & Legrand, 2014; Fritsche, 2008) as well as the importance of the form and composition of dietary LA (Anton, Heekin, Simkins, & Acosta, 2013). Data and results herein suggest that psychiatric medications may affect LA metabolism, adding another potentially important variable to dietary LA recommendations. The amount of necessary dietary LA intake is an active area of study in cardiovascular research (Vedtofte, Jakobsen, Lauritzen, & Heitmann, 2012) and the current findings emphasizes that the same considerations should apply to bipolar illness.

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References

- Anton SD, Heekin K, Simkins C, Acosta A. Differential effects of adulterated versus unadulterated forms of linoleic acid on cardiovascular health. *J Integr Med*. 2013; 11:2–10. [PubMed: 23464640]
- 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]
- Balanza-Martinez V, Fries GR, Colpo GD, Silveira PP, Portella AK, Tabares-Seisdedos R, Kapczinski F. Therapeutic use of omega-3 fatty acids in bipolar disorder. *Expert Rev Neurother*. 2011; 11:1029–1047. [PubMed: 21721919]
- Basu S. Bioactive eicosanoids: role of prostaglandin F(2alpha) and F(2)-isoprostanes in inflammation and oxidative stress related pathology. *Mol Cells*. 2010; 30:383–391. [PubMed: 21113821]
- Begley CE, Annegers JF, Swann AC, Lewis C, Coan S, Schnapp WB, Bryant-Comstock L. The lifetime cost of bipolar disorder in the US: an estimate for new cases in 1998. *Pharmacoeconomics*. 2001; 19:483–495. [PubMed: 11465308]
- Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. *Can J Biochem Physiol*. 1959; 37:911–917. [PubMed: 13671378]
- Cerit C, Vural M, Bos Gelmez SU, Ozten E, Aker AT, Yildiz M. Metabolic syndrome with different antipsychotics: a multicentre cross-sectional study. *Psychopharmacol Bull*. 2010; 43:22–36. [PubMed: 21240150]

- Choque B, Catheline D, Rioux V, Legrand P. Linoleic acid: Between doubts and certainties. *Biochimie*. 2014; 96:14–21. [PubMed: 23900039]
- Davison KM, Kaplan BJ. Food intake and blood cholesterol levels of community-based adults with mood disorders. *BMC Psychiatry*. 2012; 12:10. [PubMed: 22333556]
- Ellingrod VL, Taylor SF, Brook RD, Evans SJ, Zollner SK, Grove TB, Gardner KM, Bly MJ, Pop-Busui R, Dalack G. Dietary, lifestyle and pharmacogenetic factors associated with arteriole endothelial-dependent vasodilatation in schizophrenia patients treated with atypical antipsychotics (AAPs). *Schizophr Res*. 2011; 130:20–26. [PubMed: 21504842]
- Evans SJ, Kamali M, Prossin AR, Harrington GJ, Ellingrod VL, McInnis MG, Burant CF. Association of plasma omega-3 and omega-6 lipids with burden of disease measures in bipolar subjects. *J Psychiatr Res*. 2012a
- Evans SJ, Prossin AR, Harrington GJ, Kamali M, Ellingrod VL, Burant CF, McInnis MG. Fats and Factors: Lipid Profiles Associate with Personality Factors and Suicidal History in Bipolar Subjects. *PLoS One*. 2012b; 7:e29297. [PubMed: 22253709]
- Fritsche KL. Too much linoleic acid promotes inflammation-doesn't it? *Prostaglandins Leukot Essent Fatty Acids*. 2008; 79:173–175. [PubMed: 18990555]
- Hibbeln JR, Nieminen LR, Blasbalg TL, Riggs JA, Lands WE. Healthy intakes of n-3 and n-6 fatty acids: estimations considering worldwide diversity. *Am J Clin Nutr*. 2006; 83:1483S–1493S. [PubMed: 16841858]
- Irmisch G, Schlafke D, Gierow W, Herpertz S, Richter J. Fatty acids and sleep in depressed inpatients. *Prostaglandins Leukot Essent Fatty Acids*. 2007; 76:1–7. [PubMed: 17123808]
- Jacka FN, Pasco JA, Mykletun A, Williams LJ, Nicholson GC, Kotowicz MA, Berk M. Diet quality in bipolar disorder in a population-based sample of women. *J Affect Disord*. 2011; 129:332–337. [PubMed: 20888648]
- Kalinski P. Regulation of immune responses by prostaglandin E2. *J Immunol*. 2012; 188:21–28. [PubMed: 22187483]
- Kessler RC, Berglund P, Demler O, Jin R, Merikangas KR, Walters EE. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. *Arch Gen Psychiatry*. 2005; 62:593–602. [PubMed: 15939837]
- Kilbourne AM, Rofey DL, McCarthy JF, Post EP, Welsh D, Blow FC. Nutrition and exercise behavior among patients with bipolar disorder. *Bipolar Disord*. 2007; 9:443–452. [PubMed: 17680914]
- Langenecker SA, Saunders EF, Kade AM, Ransom MT, McInnis MG. Intermediate: cognitive phenotypes in bipolar disorder. *J Affect Disord*. 2010; 122:285–293. [PubMed: 19800130]
- Lazarus JH. Lithium and thyroid. *Best Pract Res Clin Endocrinol Metab*. 2009; 23:723–733. [PubMed: 19942149]
- Liu Y, McNamara RK. Elevated Delta-6 desaturase (FADS2) gene expression in the prefrontal cortex of patients with bipolar disorder. *J Psychiatr Res*. 2011; 45:269–272. [PubMed: 20615514]
- Luci S, Bettzieche A, Brandsch C, Eder K. Research paper effects of 13-HPODE on expression of genes involved in thyroid hormone synthesis, iodide uptake and formation of hydrogen peroxide in porcine thyrocytes. *Int J Vitam Nutr Res*. 2006; 76:398–406. [PubMed: 17607960]
- 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. [PubMed: 21458237]
- Mocking RJ, Lok A, Assies J, Koeter MW, Visser I, Ruhe HG, Bockting CL, Schene AH. Ala54Thr Fatty Acid-Binding Protein 2 (FABP2) Polymorphism in Recurrent Depression: Associations with Fatty Acid Concentrations and Waist Circumference. *PLoS One*. 2013; 8:e82980. [PubMed: 24340071]
- Montgomery, P.; Richardson, AJ. *Cochrane Database Syst Rev*. 2008. Omega-3 fatty acids for bipolar disorder; p. CD005169
- Noaghiul S, Hibbeln JR. Cross-national comparisons of seafood consumption and rates of bipolar disorders. *Am J Psychiatry*. 2003; 160:2222–2227. [PubMed: 14638594]
- Novick DM, Swartz HA, Frank E. Suicide attempts in bipolar I and bipolar II disorder: a review and meta-analysis of the evidence. *Bipolar Disord*. 2010; 12:1–9. [PubMed: 20148862]

- Nurnberger JI Jr, Blehar MC, Kaufmann CA, York-Cooler C, Simpson SG, Harkavy-Friedman J, Severe JB, Malaspina D, Reich T. Diagnostic interview for genetic studies. Rationale, unique features, and training. NIMH Genetics Initiative Arch Gen Psychiatry. 1994; 51:849–859. discussion 863–844.
- Park Y, Storkson JM, Albright KJ, Liu W, Pariza MW. Biological activities of conjugated fatty acids: conjugated eicosadienoic (conj. 20:2delta(c11,t13/t12,c14)), eicosatrienoic (conj. 20:3delta(c8,t12,c14)), and heneicosadienoic (conj. 21:2delta(c12,t14/c13,t15)) acids and other metabolites of conjugated linoleic acid. Biochim Biophys Acta. 2005; 1687:120–129. [PubMed: 15708360]
- Pike N. Using false discovery rates for multiple comparisons in ecology and evolution. Methods in Ecology and Evolution. 2011; 2:278–282.
- Rapoport SI, Basselin M, Kim HW, Rao JS. Bipolar disorder and mechanisms of action of mood stabilizers. Brain Res Rev. 2009; 61:185–209. [PubMed: 19555719]
- Rett BS, Whelan J. Increasing dietary linoleic acid does not increase tissue arachidonic acid content in adults consuming Western-type diets: a systematic review. Nutr Metab (Lond). 2011; 8:36. [PubMed: 21663641]
- Tanaka T, Shen J, Abecasis GR, Kisiailiou A, Ordovas JM, Guralnik JM, Singleton A, Bandinelli S, Cherubini A, Arnett D, Tsai MY, Ferrucci L. Genome-wide association study of plasma polyunsaturated fatty acids in the InCHIANTI Study. PLoS Genet. 2009; 5:e1000338. [PubMed: 19148276]
- Turnbull T, Cullen-Drill M, Smaldone A. Efficacy of omega-3 fatty acid supplementation on improvement of bipolar symptoms: a systematic review. Arch Psychiatr Nurs. 2008; 22:305–311. [PubMed: 18809123]
- Vedtofte MS, Jakobsen MU, Lauritzen L, Heitmann BL. The role of essential fatty acids in the control of coronary heart disease. Curr Opin Clin Nutr Metab Care. 2012; 15:592–596. [PubMed: 23037902]
- Wittwer J, Hersberger M. The two faces of the 15-lipoxygenase in atherosclerosis. Prostaglandins Leukot Essent Fatty Acids. 2007; 77:67–77. [PubMed: 17869078]
- Wyatt RJ, Henter I. An economic evaluation of manic-depressive illness--1991. Soc Psychiatry Psychiatr Epidemiol. 1995; 30:213–219. [PubMed: 7482006]
- Yao JK, Leonard S, Reddy RD. Membrane phospholipid abnormalities in postmortem brains from schizophrenic patients. Schizophr Res. 2000; 42:7–17. [PubMed: 10706981]

Highlights

- We evaluated dietary intake in bipolar and control individuals.
- We evaluated metabolomic profiles in bipolar and control individuals.
- Polyunsaturated fatty acid intake was lower in bipolar individuals.
- Linoleic acid metabolism was variant in bipolar individuals.
- There was an effect of psychiatric medication and diagnosis on linoleic acid metabolism

Figure 1.

Schematic representation of PUFA metabolism from the essential fatty acids, LA and ALA. PUFA metabolites found significantly different between bipolar and controls in the current analyses are highlighted with a box, showing the cluster of differences around LA metabolism.

Table 1

Subject Descriptives

Subjects	Lipomics (n=100)			Diet Analysis (n=91)			Metabolomics (n=64)		
	BPI (54)	HC (46)		BPI (47)	HC (44)		BPI (37)	HC (26)	
Diagnosis (n)									
% prescribed AAP	49%	0		52%	0		46%	0	
% prescribed MS	64%	0		63%	0		62%	0	
% prescribed AD	44%	0		44%	0		38%	0	
% Male	27%	50%		27%	53%		21%	46%	
Age (sd)	45.0 (12.5)	43.1 (15.4)		44.7 (12.5)	43.5 (15.4)		43.2 (12.1)	43.0(15.5)	
BMI (sd)	30.0 (7.2)	25.7 (6.0)		30.0 (7.3)	25.7 (7.3)		30.3 (8.4)	25.7 (4.2)	
Daily kCal (sd)	-	-		2024 (702)	1955 (513)		-	-	

The subjects included in the metabolomics dataset are a subset of the diet analysis cohort, who are a subset of the lipomics data set subjects.

Table 2

Differences in nutrient intake between bipolar and control subjects

Effect size (as predicted % change in dependent variable)						
	Age(1yr)	Male	kCal(x100)	BP	adj. R ² (model)	BP p value (FDR)
Selenium (mcg)	0.2	5.87	5.60***	-16.5***	0.504***	0.021
eicosadienoic acid (g)	0.50	-3.44	8.70***	35.8**	0.350***	0.021
docosanoic acid (g)	0.50	-1.69	10.0***	73.9***	0.221***	0.021
AA (g)	1.41**	-1.19	6.75**	-38.9***	0.221***	0.024
DPA (g)	2.02	13.7	3.35	-67.8***	0.149***	0.024
EPA (g)	3.46**	2.94	7.04	-65.9***	0.201***	0.024
DHA (g)	2.74*	-19.8	5.81	-69.0***	0.144**	0.041

The table includes all models where significant nutrient intake differences were found between bipolar and controls by regression models after false discovery rate multiple testing correction (FDR). The right columns show the model adjusted R² and FDR corrected p values for the BP coefficient, correcting for the 154 nutrient intake tests (non significant tests not shown). The left columns show the effect sizes as expected percent change in the dependent variable for: each year increase in age, being male relative to female, increasing caloric intake by 100 kCal, or being bipolar relative to controls; with significance indicated by *0.05 p>0.01, **0.01 p>0.001, ***0.001 p.

Table 3

Partial Correlations Between Dietary PUFA and Plasma PUFA and Metabolites.

Plasma Metabolite	Dietary Intake					
	ALA	EPA	DPA	DHA	LA	AA
ALA	-0.046	-0.092	-0.123	-0.08	0.044	-0.132
EPA	0.055	0.229*	0.174	0.232*	0.026	0.151
DPA	-0.145	-0.028	-0.043	-0.049	-0.084	-0.036
DHA	-0.116	0.196	0.142	0.173	-0.033	0.118
LA	0.184	0.065	-0.002	0.061	0.377***	-0.001
EDA	-0.154	0.048	0.11	-0.006	-0.002	0.07
GLA	-0.343***	-0.305**	-0.237*	-0.276***	-0.26*	-0.148
DGLA	-0.181	-0.332***	-0.308**	-0.313	-0.196*	-0.214*
AA	-0.213*	-0.126	-0.071	-0.116	0.009	0.012
13(S)-HPODE	-0.041	0.055	0.036	0.077	0.007	0.038
9(10)-EPOME	0.108	-0.037	0.084	0.04	0.152	0.08
15(S)-HETE	-0.106	-0.019	0.042	0.016	-0.026	0.096
LT4A	0.134	0.244	0.191	0.27	-0.049	0.081
PGE1	-0.246	0.037	0.159	0.117	-0.185	0.196
PGF2a	-0.198	-0.073	0.041	-0.053	-0.176	-0.05
PGG2	-0.004	0.094	0.019	0.037	0.024	0.017

The table gives the Pearson correlation coefficients between the dietary intake levels of PUFA (columns) and the plasma levels of PUFA and PUFA metabolites (rows), after correcting for total caloric intake. Significant associations are indicated by *0.05 p>0.01, **0.01 p>0.001, ***0.001 p.

Table 4

Table 4a. Difference in plasma metabolite levels related to PUFA metabolism between bipolar and control subjects.

	Effect size (as predicted % change in dependent variable)						
	age	male	BMI	MS	AAP	AD	BP
13(S)-HpODE	-0.50	20.3	-0.70	3.67	15.5	5.44	-42.0**
9(10)-EpOME	-0.30	11.4	0.00	-27.3	11.2	-4.21	29.4
15(S)-HETE	0.60	-16.5	0.10	-5.73	-22.1	-14.5	14.3
LTA4	0.10	-2.47	-0.10	68.4	-1.00	-33.4	-15.1
PGE1	-1.09	11.4	0.70	6.18	54.2	29.2	-48.1*
PGF2a	-1.00	27.38	0.50	14.34	41.20	27.76	-47.06*
PGG2	-0.30	-23.59	-1.19	122.78	45.21	84.97	-70.68*

Table 4b. Differences in plasma PUFA levels between bipolar and control subjects.

	Effect size (as predicted % change in dependent variable)						
	age	male	BMI	MS	AAP	AD	BP
ALA	-0.40	1.71	-0.40	5.23	4.60	35.26	-19.99
EPA	0.30	-32.3*	-1.19	11.3	72.6*	38.3	-54.1**
DHA	-0.10	-18.1	-1.00	20.7	19.4	40.1	-38.9
LA	-0.30	0.90	-0.20	-1.39	8.44	3.15	-9.97*
EDA	0.00	8.22	0.10	36.6***	-1.29	26.9**	-36.7***
GLA	0.50	-8.24	0.20	10.7	37.0*	18.7	-30.3*
DGLA	-0.20	-9.24	0.40	2.84	3.25	20.6*	-5.35
AA	-0.10	-4.78	-0.10	-6.39	4.19	2.33	-2.37
D5D	1.01	24.86	-5.64	82.58	42.90	-3.63	-68.30
D6D.1	0.01*	0.00	0.00	0.10	0.30	0.20	-0.20
D6D.2	0.01*	0.00	0.00	0.10	0.30	0.20	-0.20

The tables summarize regression results for metabolites related to PUFA metabolism contained within the metabolomics (4a, n=64) or lipomics (4b, n=91) data sets, respectively. The right columns show the model adjusted R² and the Bonferroni corrected p values for the BP coefficient. The left columns show the effect sizes for the covariates. D5D is and estimate of delta-5 desaturase activity given by the product to substrate ratio, AA:DGLA, D6D.1 and D6D.2 are activity estimates for delta-6 desaturase given by the product to substrate ratios, GLA:LA and DGLA:LA, respectively. Significance levels for each covariate are summarized by *0.01 p>0.05, **0.01 p>0.001, ***0.001 p. No asterisk means the covariate was not significant at the p<0.05 level. Column header abbreviations: BMI, body mass index; MS, mood stabilizers; AAP, atypical antipsychotics; AD, antidepressants; BP, bipolar diagnosis. Additional dietary intakes were entered as covariates into some models as explained in the text.