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Bipolar Disorder Moderates Associations Between Linoleic Acid and Markers of Inflammation

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Abstract

Dietary polyunsaturated fatty acids (PUFA) and inflammatory proteins associate with immune activation and have been implicated in the pathophysiology of mood disorders. We have previously reported that individuals with bipolar disorder (BPD) have decreased PUFA intake, including eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and arachidonic acid (AA); and decreased PUFA concentration of plasma EPA and linoleic acid (LA). We have also reported an association between plasma LA and its metabolites and burden of disease measures in BPD. In the current cross-sectional study we collected blood samples and diet records from both bipolar (n=91) and control subjects (n=75) to quantify plasma cytokine concentrations and dietary LA intake, respectively. Using multiple linear regression techniques, we tested for case control differences in plasma cytokine levels and associations between cytokines and dietary LA intake, adjusting for sex, age, BMI, and total energy intake. We found significantly higher plasma levels of interleukin 18 (IL-18) (p=0.036), IL-18 binding protein (IL-18BP) (p=0.001), soluble tumor necrosis factor receptor (sTNFR) 1 (p=0.006), and sTNFR2 (p=0.007) in BPD compared with controls. Moreover, BPD significantly moderated the associations of dietary LA intake with plasma levels of IL-18, sTNFR1 and sTNFR2, which were inverse associations in bipolar individuals and positive associations in controls (p for dietary LA x BPD diagnosis interaction < 0.05 for all three). These findings suggest potential dysregulation of LA metabolism in BPD, which may extend to a modified influence of dietary LA on specific inflammatory pathways in individuals with BPD compared to healthy controls.

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Contributors:

YWC analyzed the data and wrote the manuscript; SA consulted on statistic models and edited the manuscript; ARP supervised the cytokine assay and edited the manuscript; LS performed the cytokine assay; MGM supervised the clinical aspects relevant to the study population; SJE advised on experimental design, data analysis, and writing the manuscript.

Keywords

linoleic acid; interleukin; tumor necrosis factor receptor; bipolar disorder

Introduction

Bipolar disorder is a medical illness characterized by episodic mood changes, each having potential deleterious effects on overall psychosocial functioning and increased risk of suicide (Rihmer & Kiss, 2002). Despite the high prevalence (Merikangas et al., 2011), little is known of the pathophysiology underlying bipolar disorder. While evidence suggests certain genetic variation enhances risk (Chen et al., 2013), the mechanistic underpinnings of bipolar disorder remain in need of clarification.

A body of evidence identifies mechanistic links between clinical depression and evidence of immune activation (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008). Emerging evidence suggests a potential mechanistic role for inflammasome activation in the underlying pathophysiology of bipolar disorder. Within cells sharing a common dendritic lineage, during the process of activation by various signals, the NLRP3 inflammasome potently induces activation of specific cytokines, most notably, interleukin-1β (IL-1β) and another more potent IL-1 family cytokine, interleukin-18 (IL-18) (Dinarello, 1999a; b; van de Veerdonk, Netea, Dinarello, & Joosten, 2011). In fact, many of medical illnesses that co-occur with bipolar disorder, such as heart diseases and obesity, have evidence of similar immune activation (Leboyer et al., 2012). For instance, patients with bipolar disorder and their descendants show abnormal inflammatory gene expression in monocytes, which play a critical role in inflammasome activation and subsequent production of various inflammatory cytokines (Padmos et al., 2008). A body of evidence identifies associations between elevated plasma concentrations of IL-18 and the presence of major depressive disorders (Al-Hakeim, Al-Rammahi, & Al-Dujaili, 2015; Merendino et al., 2002; Prossin et al., 2011) and more recent evidence suggests plasma IL-18 concentration is associated with affective state, elevated during sadness and relatively lower during neutral affective states (Prossin et al., 2015). Emerging evidence in individuals with bipolar disorder shows significant elevation of plasma concentrations of certain "pro-inflammatory" cytokines during mood episodes (Fiedorowicz et al., 2015; Goldstein, Kemp, Soczynska, & McIntyre, 2009). Given their potential buffering capacity of certain "pro-inflammatory" cytokines, efforts have been taken to investigate the role of "anti-inflammatory proteins" (including specific soluble cytokine receptors) in both Major Depressive Disorder (Myint, Leonard, Steinbusch, & Kim, 2005) and bipolar disorder. As compared to healthy control individuals, plasma concentration of soluble tumor necrosis factor receptor (sTNFR) 1 (Barbosa et al., 2011) and sTNFR2 have been found elevated in Bipolar Disordered patients, even during periods of euthymia (Doganavsargil-Baysal et al., 2013). However, associations between bipolar disorder and other so called "anti-inflammatory" proteins (i.e. interleukin-4 (IL-4), interleukin-10 (IL-10)), lack consistency across studies (Goldstein et al., 2009). These inconsistencies may potentially be explained by lack of control of the various potential confounders related to diet, exercise, medication, and other socio-demographic variances frequently found to confound research studies of bipolar disorder. Studies that attempt to control for these

confounds will help to elucidate the nature of the contribution of immune activation to the mechanistic underpinnings of bipolar disorder.

Dietary polyunsaturated fatty acids (PUFA) are important factors that regulate inflammation (Calder, 2002). The n-6, linoleic acid (LA) and the n-3, alpha linolenic acid (LNA), are essential PUFA. Mammals cannot synthesize these *de novo*, but can synthesize all other required PUFA from these two dietary substrates through shared enzymes, Δ6-desaturase, elongases and Δ5 desaturase (Pischon et al., 2003). Eicosanoids derived from n-6 PUFA, including leukotriene B_4 (LTB_4) and prostaglandins E_2 (PGE_2), have potent inflammatory properties when compared with those from n-3 PUFA, including leukotriene B_5 (LTB_5) and prostaglandins E_3 (PGE_3). These opposing inflammatory activities of n-3 and n-6 derived eicosanoids may underlie the importance of the complement of dietary PUFA intake on controlling inflammation. Many studies identified beneficial effects of n-3 PUFA on inflammation, suggesting inverse associations between n-3 PUFA dietary intake and plasma cytokine concentration (Kalogeropoulos et al., 2010; Lopez-Garcia et al., 2004); however, the effects of n-6 PUFA on inflammation are still under debate. Some studies highlight the ratio of n-6 PUFA to n-3 PUFA as important, showing a strong positive association with inflammatory markers, including C-reactive protein, and IL-6, and an inverse association with anti-inflammatory markers, including IL-10 (Ferrucci et al., 2006). However, other studies find inverse associations between both n-3 and n-6 PUFA consumption, and inflammation, suggesting that n-6 PUFA have similar anti-inflammatory roles as n-3 PUFA (Julia et al., 2013). Furthermore, in healthy adult men, diets containing 10.5% energy from LA associated with higher plasma LA concentrations than diets with only 3.8% total energy from LA, but no significant changes on AA concentration were observed. These data suggest that higher LA intake does not cause increased plasma AA, which is the direct precursor to downstream inflammatory eicosanoids (Angela Liou & Innis, 2009). Also, LA supplementation had no effect on either EPA or DHA levels, and the authors concluded the effects of LA on conversion of LNA to EPA or DHA do not reduce anti-inflammatory eicosanoid production (Minihane et al., 2005). Finally, a systematic review of randomized controlled trials concluded that there was not enough evidence to support that dietary LA intake would increase inflammatory cytokines levels (Johnson & Fritsche, 2012). Thus, further studies to understand the relationship between dietary PUFA and inflammation are warranted.

PUFA may also play a key role in mood (Liu et al., 2013). Higher serum n-6 PUFA and lower n-3 PUFA have been found to associate with depressive symptoms (Conklin et al., 2007). Results from plasma and erythrocyte phospholipid in patients with severe depression showed significant and positive correlation between the ratio of AA to EPA, and severity of depression (Adams, Lawson, Sanigorski, & Sinclair, 1996) and suicidal behavior (Evans et al., 2012b). However, plasma LA inversely associates with burden of disease measures in bipolar patients, including severity of depression and self-reported life functioning (Evans et al., 2015; Evans et al., 2012a; Evans et al., 2014). Other studies of bipolar disorder found higher n-3 and n-6 PUFA levels in the human postmortem superior temporal gyrus, a cortical area related to emotion (McNamara, Rider, Jandacek, & Tso, 2014), but no association in the postmortem entorhinal cortex (Hamazaki, Hamazaki, & Inadera, 2013). Furthermore, Jadoon et al. identified inverse associations between residual depression and levels of the n-6 PUFA,

LA, but not levels of n-3 PUFA, in both erythrocyte and plasma. In this study, the inverse association between LA and depression may have been due to inefficient conversion of LA to AA, with decreased production of AA by inhibition of Δ6-desaturase activity (Jadoon et al., 2012). Although several studies have evaluated the effect of PUFA on mood function (Sublette et al., 2007), the results are still inconsistent. Moreover, few have studied the association between PUFA and inflammation in bipolar disorder.

Our previous studies found decreased dietary PUFA intake and PUFA plasma concentrations in bipolar individuals compared to healthy controls (Evans et al., 2014). Furthermore, we found several LA-derived metabolites associated with bipolar disorder. Results from our studies indicate that LA metabolism may be dysregulated in bipolar disorder. Based on these data, the aim of the current study was to identify whether dietary LA intake would predict plasma cytokine levels differently in bipolar individuals compared to controls.

To accomplish this aim, we quantified concentrations of specific inflammatory cytokines from plasma of individuals with a confirmed diagnosis of bipolar disorder for comparison against plasma concentrations in healthy control individuals, following prospective collection of 7-day diet records. Inflammatory cytokines were selected based on evidence from the extant literature and included IL-1β, interleukin-6 (IL-6), IL-10, IL-18, IL-18 binding protein (IL-18BP), IL-1 receptor antagonist (IL-1RA), IL-6 receptor alpha (IL-6RA), sTNFR1, sTNFR2, and C-reactive protein (CRP). Results from these assays were coalesced with dietary intake data, and plasma n-3 and n-6 PUFA concentrations from the same individuals.

Materials and Methods

Participants

Individuals were recruited from the Heinz C. Prechter Longitudinal Study of Bipolar Disorder at the University of Michigan Depression Center previously described (Langenecker, Saunders, Kade, Ransom, & McInnis, 2010). Briefly, all individuals were diagnosed using the Diagnostic Interview for Genetic Studies (DIGS) (Nurnberger et al., 1994), and were recruited carrying a diagnosis of bipolar I disorder (BP I) with history of treated BP I mania, or schizoaffective manic type or BP II disorder with history of treated major depression, or were non-psychiatric controls. Individuals with current and active substance abuse or suffer from a medical illness specifically associated with depression (including terminal cancers, Cushing’s disease, or stroke) were excluded. For the current analysis, existing Prechter Longitudinal Study of Bipolar Disorder Subjects (cases and controls) willing to participate in a dietary analysis and living within commuting distance of the University of Michigan were invited to enroll. Inflammatory conditions were not considered when recruiting individuals and no further inclusion/exclusion criteria were imposed beyond those included in the parent Prechter Longitudinal Study of Bipolar Disorder as described by Langenecker et al (Langenecker, Saunders, Kade, Ransom, & McInnis, 2010). Ninety one Bipolar individuals and 75 healthy individuals provided daily diet records for 7 days as well as a fasted blood sample immediately following the diet recording period. The Institutional Review Board for Human Studies at the University of Michigan approved this cross-sectional study.

Dietary intake

Dietary intake was assessed using 7-day diet records. Nutrients were extracted using the Nutrition Data System for Research (NDSR) software (University of Minnesota, 2011). A trained dietitian guided all subjects in this study in recording of foods and portion sizes before the 7-day recording period. After receiving the returned food record, the dietitian curated the entries in the presence of the research subject to clarify any missing or misunderstood information. Seven subjects were dropped out from the analysis due to excessively and improbably high or low average reported daily caloric intake, or incomplete diet records.

Inflammatory markers

Concentrations of specific inflammatory proteins were quantified from plasma samples derived from fasted whole blood. All the plasma samples were collected between 8 to 10 am with at least 8 hours fasting duration. Volunteers' fresh whole blood samples were obtained intravenously in a fasted stated and centrifuged at 4250 rpm for 15 minutes. Plasma was extracted and 1mL aliquots were frozen at −80 degrees Celsius. Plasma concentrations of specific proteins were quantified with standard Enzyme Linked Immunosorbent Assay. Specific cytokine assay kits were obtained from different manufacturers. The IL-1b, IL-1ra, IL-6, IL-6ra and IL-10 assays were purchased from R & D Systems (Minneapolis, MN). The IL-18 ELISA kit was obtained from Medical and Biological laboratories (Japan), the IL18Bp ELISA kit was obtained from R&D Systems (Minnesota, USA), and remaining assay kits (i.e. Bio-Plex® x-Plex Assays) were obtained from Bio-Rad Laboratories (Hercules, CA). With regards to the Bio-Plex kits, we used one Bio-Plex kit for cytokine quantification and one for cytokine receptor quantification so as to reduce likelihood of analyte cross-reactivity. In general, plasma samples were thawed, diluted, and assays completed according to manufacturer provided instructions of the particular inflammatory assay kit. All samples provided to the lab were coded. As such, no lab staff had access to any clinical and/or demographic information associated with the samples. Samples were assayed in duplicate pairs on manufacturer provided 96 well plates together with standards of known concentrations. Raw absorbance data obtained from assays was compared against a standard curve of known concentrations, yielding final plasma concentrations for each specific inflammatory protein.

Statistical methods

The SPSS statistics software (Version 22, IBM Corp) was used for multiple linear regression statistical analysis. Covariates in the models included sex, age, BMI, daily caloric intake, and plasma batch. The latter factor was introduced due to a modification of blood collection procedure that included retaining buffy coat after plasma was transferred from spun blood. The importance of this factor became apparent after unsupervised clustering revealed an effect of batch, which was removed by adding the factor to the statistical model. Furthermore, there was equal representation of control and bipolar subjects across batches so diagnostic confounding was not a concern. To improve normality of the data, dietary intake and plasma cytokines levels were natural log transformed. We report means and standard deviations (SD) for continuous measures including age, BMI, daily caloric intake, and

plasma cytokines levels. In the exploratory analyses, first, we ran linear regression with cytokines levels as dependent variables, and bipolar disorder as a predictor, adjusting for all covariates. Second, we tested for an effect of psychiatric medications in a separate linear regression model with cytokines levels as dependent variables, bipolar disorder, and medication use as predictors. For this analysis medications were binned by class as antidepressants (duloxetine, fluoxetine, sertraline, escitalopram, desvenlafaxine, venlafaxine), atypical antipsychotics (clozapine, olanzapine, aripiprazole, risperidone, paliperidone, quetiapine, ziprasidone), or mood stabilizers (lithium, lamotrigine, carbamazepine, topiramate, divalproex, gabapentin). No other class of psychiatric medication was represented in more than 2 research subjects. Third, we tested associations between dietary LA intake and cytokines levels in another linear regression model, adjusting for all covariates. We then repeated linear regressions separately for bipolar subjects and the healthy controls, to determine and compare associations between dietary LA intake and plasma cytokines levels independently by diagnostic group. Finally, based on our previous findings, which suggested the dysregulated LA metabolism and associations with burden of disease measures in bipolar disorder (Evans et al., 2014), we introduced an interaction term for bipolar diagnosis x dietary LA intake in the entire pooled data set. Results from multiple linear regression analysis were output as standardized beta coefficients, standard errors, and associated p-values.

Results

Table 1 shows demographic characteristics of the study subjects and statistics for plasma inflammatory cytokine concentrations by diagnostic group. The mean BMIs were significantly different between two groups ($p<0.001$). There was no significant difference between the groups in either daily calories or LA intake ($p>0.05$ for all). For cytokine concentrations, plasma concentrations of IL-18, IL-18BP, sTNFR1, and sTNFR2 in individuals with bipolar disorder were significantly higher than those in healthy controls ($p<0.05$ for all).

In the first set of analyses we tested for a main effect of bipolar disorder on plasma cytokine levels, after adjusting for sex, age, and BMI. We also entered for total caloric intake and plasma batch into the regressions to adjust for experimental artifacts but don't report these in the tables as they are not biologically meaningful. Table 2 shows the positive association between bipolar disorder and IL-18BP (Standardized B= 0.17, $p<0.05$) remained after adjusting for covariates. However, including medication classes as covariates in the regression models (Table S1) reveal that the effect of bipolar disorder on IL-18BP might be partially explained by atypical antipsychotics or mood stabilizers use.

In a second set of analysis we tested for a main effect of dietary LA intake on cytokine levels and potential moderation by a bipolar diagnosis. Table 3 shows that dietary LA intake inversely associated with IL-1RA and IL-18BP concentrations (Standardized B= −0.39, $p<0.01$; standardized B=−0.34, $p<0.01$, respectively), after adjusting for the given covariates. Separating the analyses by diagnostic group or adding an interaction term for diagnosis x LA intake suggested moderation of dietary LA – cytokine associations by bipolar diagnosis (Table 4). In pooled sample analyses (including both bipolar and control

subjects in the model) with an interaction term for bipolar diagnosis x LA intake, there was a significant negative interaction between bipolar diagnosis and dietary LA intake on plasma levels of IL-18, sTNFR1, and sTNFR2 (Standardized B=−0.29, p<0.05; standardized B= −0.38, p<0.01; standardized B= −0.32, p<0.05, respectively). While not all of these cytokines significantly associated with LA intake in analyses separated by diagnostic group, the trends for associations with IL-18, sTNFR1, and sTNFR2 were all positive in the control group (standardized B= 0.328, p=0.071; standardized B= 0.371, p=0.037; standardized B= 0.335, p=0.068; respectively) and negative in the bipolar group (standardized B= −0.269, p=0.153; standardized B= −0.154, p=0.326; standardized B= −0.158, p=0.345, respectively) as reported in tables 4b and 4c. The opposite signs for main effect coefficients and the significant negative interaction coefficient terms imply an effect of bipolar diagnosis on the relationship between dietary LA intake and some cytokine plasma levels.

Separate models for bipolar disorder and healthy controls supported several interactions between dietary LA intake and bipolar diagnosis. In bipolar individuals but not controls, LA intake inversely associated with lower IL-18BP (Standardized B= −0.55, p<0.01); and in controls but not bipolar individuals, LA intake was inversely associated with IL-1β, IL-1RA, and IL-10 (Standardized B= −0.48, p<0.05; standardized B= −0.56, p<0.01; standardized B= −0.50, p<0.05, respectively) and positively associated with sTNFR1(Standardized B= 0.37, P<0.05). Furthermore, while the diagnosis x LA intake interaction term did not reach significance thresholds for association with IL-18, TNFR1 and TNFR2, analyses separated by diagnosis showed opposite signs of association trends. Additionally, compared to the results from control samples, bipolar disorder attenuated the associations of dietary LA with sTNFR1 and sTNFR2 but strengthened the inverse association with IL-18BP. Taken together these data support differential effects of dietary LA intake on plasma cytokine systems in bipolar relative to control individuals.

Discussion

In this exploratory study, we analyzed dietary LA intake and plasma cytokine concentrations in bipolar and healthy individuals to test the hypothesis that dietary LA would be differentially associated with inflammatory markers in bipolar disorder, compared to controls. This is based on our previous studies suggesting dysregulated LA metabolism in bipolar disorder by metabolomics analysis following dietary monitoring (Evans et al., 2014), and association with with burden of disease measures (Evans et al., 2015).

In our previous study, decreased dietary PUFA intake (including EPA, DHA and AA) and decreased plasma PUFA concentration (including n-6 eicosadienoic acid (EDA), EPA, and LA) were found in bipolar individuals, compared to healthy controls (Evans et al., 2014). Moreover, plasma metabolites of LA were lower in bipolar subjects, after adjusting for age, sex, BMI, and psychiatric medication. We also found that psychiatric medication use, including mood stabilizers and anti-depressants associated with differential levels of some, but not all, plasma LA metabolites. These findings suggest that individuals with bipolar disorder have dysregulated LA metabolism that is not completely explained by psychiatric medication use, but may be secondary to reduced dietary intake of other PUFA.

Based on our previous findings, we analyzed and compared the relationships between dietary LA and inflammatory cytokines between bipolar subjects and healthy controls in this current study. First, we examined differences in plasma cytokine levels between bipolar and control individuals, and found significantly higher levels of IL-18, IL-18BP, sTNFR1 and sTNFR2 in plasma from bipolar subjects. However, after correcting for age, sex, and BMI, only IL-18BP remained significant, with the others largely explained by the difference in BMI between bipolar and control individuals. Some studies showed the relationships between inflammation and bipolar disorder might be affected by the mood state, identifying no significant differences in inflammatory cytokine levels in euthymic bipolar subjects, compared to healthy controls (Brietzke et al., 2009; Guloksuz et al., 2010). In our study, blood samples from bipolar subjects were collected during euthymic state, which might explain the lack of significant differences of inflammatory cytokines in the two groups. When further including medications as covariates in the models, IL-18BP showed significant associations with atypical antipsychotics or mood stabilizers use, potentially explaining the main effect of bipolar diagnosis.

Focusing on associations between cytokine levels and dietary LA intake revealed several moderating effects of bipolar diagnosis. After controlling for diagnosis, sex, age and BMI, we found that dietary LA intake inversely associated with plasma IL-1RA and IL-18BP. When we tested for an interaction between BP diagnosis and LA intake on associations with plasma cytokine levels we found a significant inverse interaction between bipolar disorder and LA intake on the associations with plasma levels of IL-18, sTNFR1 and sTNFR2. These data showed that important associations with LA were only evident after controlling for the interaction term. When the data were split into separate analyses for BP and control individuals, we found several significant associations between dietary LA intake and cytokines that were not evident in the pooled analyses, further supporting a moderating effect of bipolar diagnosis on the effect of dietary LA. From our previous studies, we found higher plasma LA associates with better clinical outcomes in bipolar subjects. In the current study, we identified potential interactions between dietary LA intake and inflammatory systems that may differ in bipolar relative to controls. These data indicate that bipolar disorder might confer a reduced role for dietary LA in the expression of inflammatory markers, by inversely impacting the associations between dietary LA and the cytokines IL-18BP, sTNFR1 and sTNFR2. To our knowledge, this the first report of potential differences in dietary effects of essential nutrients on inflammatory profiles in bipolar individuals relative to controls.

Concerns as to potential health risks of high dietary LA intake in western society have been raised (Lands, 2014), primarily from the perspective that humans can convert LA to AA (Salem, Pawlosky, Wegher, & Hibbeln, 1999), and consequently, a high LA diet could potentially increase AA-derived inflammatory eicosanoids (Choque, Catheline, Rioux, & Legrand, 2014). However, the role of dietary LA intake on the inflammatory system remains controversial. Recent systematic reviews conclude that there is an absence of strong evidence supporting a relationship between LA intake and tissue levels of AA or inflammatory markers (Johnson et al., 2012). The effect of dietary LA intake on inflammatory state requires more direct studies that consider various aspects of the sample

population, such as BMI, medication use and other important nutrient intakes that may alter LA metabolism.

Of the inflammatory proteins we identified in the current study as being elevated in bipolar individuals, IL-1β is known as a pro-inflammatory cytokine (Dinarello, 1998), whereas IL-1RA performs anti-inflammatory properties by binding to IL-1 receptor and thus inhibiting biological responses of IL-1 (Arend, 1993; Arend, Malyak, Guthridge & Gabay, 1998). IL-10 acts as an anti-inflammatory cytokine, inhibiting immune responses from leukocytes (Ouyang et al., 2011). IL-18 exhibits pro-inflammatory properties, while IL-18BP is likely anti-inflammatory by preventing IL-18 from binding to its receptor (Dinarello, 1999a). The soluble TNF receptors, sTNFR1 and sTNFR2, may be considered as inhibitors of the inflammatory effects of TNF-α by competing with membrane-associated receptors to reduce TNF-α activity (Engelmann, Novick, & Wallach, 1990). Thus, the increase in both inflammatory and anti-inflammatory markers in bipolar disorder and the associations with dietary LA intake are complex and further reinforce the importance of understanding dysregulation of LA metabolism in this illness. Careful dietary manipulation studies are needed to elucidate the subtleties of LA intake on inflammatory state, which may vary in healthy relative to diseased populations.

The current study is limited by difficulty in determining the effects of psychiatric medication use on the relationship between dietary LA intake and inflammation in bipolar disorder. The polypharmacy among research subjects makes it impossible to completely control for medication use. Previous animal studies have shown that atypical antipsychotic use up-regulates mRNA expression of Δ6-desaturase, and increased biosynthesis of n-3 and n-6 PUFA in plasma (McNamara et al., 2011). Our own human studies suggest that some products of LA metabolism may positively associate with psychiatric medication use (Evans et al., 2014). Dosage, and time of taking psychiatric medication, and interactions between different medications all need to be considered in further investigations. Furthermore, we binned research subjects by medications prescribed, which does not guarantee adherence to medications. A second limitation is the cross-sectional nature of our study and the inability to draw causal conclusions regarding relationship between LA intake and inflammation in bipolar disorder.

In summary, the current study provides further support for our previous finding that LA metabolism is dysregulated in bipolar disorder and new data suggesting this may extend to the regulation of inflammatory systems. Dietary intervention studies are required to determine if modifying dietary LA or other PUFA is beneficial in bipolar disorder to reduce inflammatory profiles and burden of disease measures.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Subject descriptives

	Bipolar (n=91)	Control (n=75)	p
Female (%)	52(57.1)	44 (58.7)	0.844
Age (year) (SD)	43.0 (12.2)	44.03 (16.43)	0.656
BMI (kg/m^2) (SD)	30.0 (7.2)	26.22 (4.93)	**<0.001**
Daily caloric intake (kcal) (SD)	2159.9 (734.6)	1969.4 (524.7)	0.061
Dietary LA (g) (SD)	18.2 (10.8)	15.8 (6.0)	0.094
IL-1β (pg/ml) (SD)	628.8 (384.3)	549.8 (271.3)	0.872
IL-1RA (pg/ml) (SD)	2267.0 (663.2)	2990.9 (767.2)	0.474
IL-6 (pg/ml) (SD)	1066.8 (510.4)	311.6 (211.9)	0.187
IL-6RA (pg/ml) (SD)	7544.4 (6006.6)	6586.8 (4908.6)	0.270
IL-10 (pg/ml) (SD)	2020.6 (829.0)	1387.8 (800.6)	0.589
IL-18 (pg/ml) (SD)	269.8 (162.5)	219.8 (132.7)	**0.036**
IL-18BP (pg/ml) (SD)	2140.9 (1400.6)	1515.8 (738.5)	**0.001**
sTNFR1 (pg/ml) (SD)	2048.4 (1103.8)	1612.9 (843.3)	**0.006**
sTNFR2 (pg/ml) (SD)	6391.1 (5341.1)	4302.8 (4276.8)	**0.007**
CRP (ug/ml) (SD)	4.3 (0.5)	3.6 (0.4)	0.243

BMI= body mass index; LA= linoleic acid; IL-1β= interleukin 1 beta; IL-1RA= interleukin 1 receptor antagonist; IL-6= interleukin 6; IL-6RA= interleukin 6 receptor alpha; IL-10= interleukin 10; IL-18= interleukin 18; IL-18BP= interleukin 18 binding protein; TNFR1= tumor necrosis factor receptor 1; TNFR2= tumor necrosis factor receptor 2; CRP= C-reactive protein; p= p-value.

Table 2

Summary of linear regression models on the associations between BP, BMI, and cytokines

Ln (Cytokine levels)= β1×Age + β2×Sex + β3×BMI + β4×BP

	IL-1β	IL-1RA	IL-6	IL-6RA	IL-10	IL-18	IL-18BP	sTNFR1	sTNFR2	CRP
Age (SE)	0.030 (0.020)	−0.056 (0.012)	0.024 (0.019)	0.000 (0.004)	0.044 (0.019)	0.161 (0.005)	**0.221 (0.003)** **	**0.216 (0.003)** **	0.148 (0.005)	0.122 (0.005)
Female (SE)	−0.175 (0.625)	−0.018 (0.402)	−0.139 (0.612)	0.077 (0.117)	−0.178 (0.617)	−0.069 (0.140)	0.031 (0.094)	−0.028 (0.080)	0.061 (0.148)	**0.158 (0.151)** *
BMI (SE)	−0.223 (0.059)	−0.064 (0.030)	−0.177 (0.047)	0.023 (0.009)	−0.152 (0.052)	0.105 (0.011)	0.117 (0.007)	**0.252 (0.006)** **	**0.215 (0.011)** *	**0.537 (0.011)** ***
BP (SE)	0.156 (0.603)	−0.063 (0.382)	0.088 (0.572)	−0.015 (0.112)	0.145 (0.579)	0.118 (0.135)	**0.169 (0.091)** *	0.108 (0.076)	0.158 (0.143)	−0.056 (0.145)
df	(6, 69)	(6, 118)	(6, 84)	(6,141)	(6, 90)	(6, 139)	(6,141)	(6, 141)	(6,141)	(6, 140)
F-value	0.904	0.991	1.234	0.492	0.864	2.091	**6.550** ***	**5.755** ***	**3.857** **	**11.220** ***

The table shows the standardized beta coefficients for the covariates as given in the linear regression model above the table. Each column represents and independent model with the given cytokine as the dependent variable. BMI= body mass index; BP= bipolar disorder; IL-1β= interleukin 1 beta; IL-1RA= interleukin 1 receptor antagonist; IL-6= interleukin 6; IL-6RA= interleukin 6 receptor alpha; IL-10= interleukin 10; IL-18= interleukin 18; IL-18BP= interleukin 18 binding protein; TNFR1= tumor necrosis factor receptor 1; TNFR2= tumor necrosis factor receptor 2; CRP= C-reactive protein; Degrees of freedom (df) and F-value for each model is listed in the bottom 2 rows;

* p <0.05;

** p <0.01;

*** p <0.001.

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Table 3

Summary of linear regression models on the association between BP, BMI, dietary LA, and cytokines

Ln (Cytokine levels)= β1×Age + β2×Sex + β3×BMI + β4×BP + β5×Dietary LA

	IL-1β	IL-1RA	IL-6	IL-6RA	IL-10	IL-18	IL-18BP	sTNFR1	sTNFR2	CRP
Age (SE)	0.017 (0.020)	−0.057 (0.012)	0.018 (0.019)	−0.001 (0.004)	0.046 (0.019)	0.161 (0.005)	**0.222 (0.003)** **	**0.216 (0.003)** **	0.148 (0.005)	0.122 (0.005)
Female (SE)	−0.110 (0.651)	0.047 (0.402)	−0.097 (0.620)	0.065 (0.120)	−0.134 (0.619)	−0.069 (0.144)	0.084 (0.094)	−0.037 (0.082)	0.054 (0.153)	**0.170 (0.155)** *
BMI (SE)	−0.236 (0.058)	−0.042 (0.029)	−0.154 (0.047)	0.020 (0.009)	−0.121 (0.052)	0.105 (0.011)	0.129 (0.007)	**0.250 (0.006)** **	**0.213 (0.011)** *	**0.540 (0.011)** ***
BP (SE)	0.161 (0.597)	−0.067 (0.372)	0.089 (0.566)	−0.015 (0.113)	0.132 (0.571)	0.118 (0.135)	**0.165 (0.088)** *	0.109 (0.077)	0.158 (0.143)	−0.057 (0.145)
Dietary LA (SE)	−0.269 0.415)	**−0.389 (0.261)** **	−0.256 (0.371)	0.073 (0.083)	−0.312 (0.393)	0.001 (0.100)	**−0.338 (0.065)** **	0.058 (0.057)	0.047 (0.106)	−0.076 (0.107)
df	(7, 68)	(7,117)	(7, 83)	(7,140)	(7, 89)	(7, 138)	(7, 140)	(7, 140)	(7, 140)	(7,139)
F-value	1.128	2.013	1.461	0.463	1.327	1.779	**7.141** ***	**4.939** ***	**3.306** **	**9.649** ***

The table shows the standardized beta coefficients for the covariates as given in the linear regression model above the table. Each column represents and independent model with the given cytokine as the dependent variable. BMI= body mass index; BP= bipolar disorder; LA= linoleic acid IL-1β= interleukin 1 beta; IL-1RA= interleukin 1 receptor antagonist; IL-6= interleukin 6; IL-6RA= interleukin 6 receptor alpha; IL-10= interleukin 10; IL-18= interleukin 18; IL-18BP= interleukin 18 binding protein; TNFR1= tumor necrosis factor receptor 1; TNFR2= tumor necrosis factor receptor 2; CRP= C-reactive protein; Degrees of freedom (df) and F-value are given in the bottom 2 rows for each model;

* p <0.05;

** p <0.01;

*** p <0.001.

Table 4

Summary of linear regression models on the association between BD, BMI, dietary LA intake, cytokines, and drugs in the (a) pooled sample, (b) bipolar subjects, and (c) healthy controls

(a) Ln (Cytokine levels)= β1×Age + β2×Sex + β3×BMI + β4×Dietary LA + β5×BP + β6×BP*LA

	IL-1β	IL-1RA	IL-6	IL-6RA	IL-10	IL-18	IL-18BP	sTNFR1	sTNFR2	CRP
Pooled										
Age (SE)	0.014 (0.020)	−0.058 (0.012)	0.015 (0.019)	−0.002 (0.004)	0.044 (0.019)	0.160 (0.005)	**0.221 (0.003)** **	**0.214 (0.003)** **	0.146 (0.005)	0.122 (0.005)
Female (SE)	−0.115 (0.655)	0.046 (0.401)	−0.099 (0.622)	0.061 (0.119)	−0.131 (0.620)	−0.075 (0.142)	0.079 (0.093)	−0.045 (0.080)	0.048 (0.150)	**0.169 (0.156)** *
BMI (SE)	−0.240 90.058)	−0.047 (0.029)	−0.159 (0.047)	0.027 (0.009)	−0.133 (0.052)	0.114 (0.010)	0.136 (0.007)	**0.261 (0.006)** **	**0.223 (0.011)** **	**0.541 (0.011)** ***
Dietary LA (SE)	−0.352 (0.517)	**−0.517 (0.345)** **	−0.343 (0.498)	0.267 (0.112)	**−0.438 (0.528)** *	0.242 (0.133)	−0.135 (0.087)	0.380 (0.074)	0.315 (0.140)	−0.050 (0.145)
BP (SE)	0.173 (0.607)	−0.059 (0.373)	0.093 (0.569)	−0.026 (0.112)	0.141 (0.574)	0.103 (0.134)	**0.153 (0.088)** *	**0.090 (0.075)** *	0.143 (0.141)	−0.059 (0.146)
BPxLA (SE)	0.118 (0.566)	0.159 (0.349)	0.115 (0.533)	−0.230 (0.011)	0.155 (0.541)	**−0.288 (0.132)** *	−0.241 (0.086)	**−0.383 (0.074)** **	**−0.319 (0.139)** *	−0.031 (0.144)
df	(8, 67)	(8,116)	(8, 82)	(8, 139)	(8, 88)	(8, 137)	(8, 139)	(8, 139)	(8, 139)	(8, 138)
F-value	1.0303	1.903	1.317	0.736	1.259	**2.138** *	**6.849** ***	**5.726** ***	**3.720** **	**8.394** ***

(b) Ln (Cytokine levels)= β1×Age + β2×Sex + β3×BMI + β4×Dietary LA

	IL-1β	IL-1RA	IL-6	IL-6RA	IL-10	IL-18	IL-18BP	sTNFR1	sTNFR2	CRP
BP										
Age (SE)	0.135 (0.028)	0.030 (0.019)	0.105 (0.030)	0.043 (0.007)	0.146 (0.029)	**0.241 (0.007)** *	**0.386 (0.005)** ***	**0.416 (0.004)** ***	**0.344 (0.007)** **	0.061 (0.007)
Female (SE)	−0.072 (0.851)	0.066 (0.555)	−0.126 (0.862)	0.156 (0.187)	−0.182 (0.883)	0.044 (0.196)	0.191 (0.135)	0.004 (0.108)	0.094 (0.202)	**0.260 (0.195)** *
BMI (SE)	−0.367 (0.068)	0.020 (0.035)	−0.162 (0.056)	0.040 (0.011)	−0.122 (0.066)	0.097 (0.012)	0.146 (0.008)	**0.334 (0.007)** **	**0.332 (0.012)** **	**0.623 (0.012)** ***
Dietary LA (SE)	−0.060 (0.522)	−0.238 (0.342)	−0.154 (0.497)	0.030 (0.120)	−0.177 (0.510)	−0.269 (0.128)	**−0.545 (0.087)** **	−0.154 (0.069)	−0.158 (0.130)	−0.039 (0.128)
df	(6, 30)	(6, 60)	(6, 43)	(6, 73)	(6, 44)	(6, 72)	(6, 73)	(6, 73)	(6, 73)	(6, 74)
F-value	0.777	0.579	0.821	0.539	0.839	1.531	**7.077** ***	**6.680** ***	**4.453** **	**9.288** ***

(c) Ln (Cytokine levels)= β1×Age + β2×Sex + β3×BMI + β4×Dietary LA

	IL-1β	IL-1RA	IL-6	IL-6RA	IL-10	IL-18	IL-18BP	sTNFR1	sTNFR2	CRP
HC										

(c) Ln (Cytokine levels)= β1×Age + β2×Sex + β3×BMI + β4×Dietary LA

	IL-1β	IL-1RA	IL-6	IL-6RA	IL-10	IL-18	IL-18BP	sTNFR1	sTNFR2	CRP
Age (SE)	−0.133 (0.029)	−0.099 (0.016)	−0.075 (0.027)	−0.027 (0.004)	−0.060 (0.026)	0.097 (0.006)	0.064 (0.004)	0.060 (0.003)	0.034 (0.006)	0.193 (0.008)
Female (SE)	−0.083 (0.012)	0.061 (0.594)	−0.041 (0.958)	−0.059 (0.147)	−0.081 (0.901)	−0.182 (0.209)	−0.036 (0.120)	−0.073 (0.113)	0.030 (0.215)	0.061 (0.253)
BMI (SE)	−0.028 (0.105)	−0.143 (0.056)	−0.148 (0.096)	−0.001 (0.015)	−0.075 (0.094)	0.161 (0.021)	0.164 (0.012)	0.141 (0.011)	0.026 (0.022)	**0.367 (0.025)** **
Dietary LA (SE)	**−0.477 (0.669)** *	**−0.557 (0.414)** **	−0.392 (0.598)	0.204 (0.114)	**−0.496 (0.646)** *	0.328 (0.161)	−0.051 (0.093)	**0.371 (0.088)** *	0.335 (0.167)	−0.104 (0.192)
df	(6, 32)	(6, 51)	(6, 34)	(6, 61)	(6, 39)	(6, 60)	(6, 61)	(6, 61)	(6, 61)	(6, 59)
F-value	1.240	**2.585** *	1.106	1.178	1,194	1.573	2.097	2.076	1.149	**3.019** *

The table shows the standardized beta coefficients for the covariates as given in the linear regression model above the table. Each column represents and independent model with the given cytokine as the dependent variable. BMI= body mass index; BP= bipolar disorder; LA= linoleic acid IL-1β= interleukin 1 beta; IL-1RA= interleukin 1 receptor antagonist; IL-6= interleukin 6; IL-6RA= interleukin 6 receptor alpha; IL-10= interleukin 10; IL-18= interleukin 18; IL-18BP= interleukin 18 binding protein; TNFR1= tumor necrosis factor receptor 1; TNFR2= tumor necrosis factor receptor 2; CRP= C-reactive protein; Degrees of freedom (df) and F-value are given in the bottom 2 rows for each model;

* p <0.05;

** p <0.01;

*** p <0.001.