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Brain Lipid Concentrations in Bipolar Disorder

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Abstract

Reduced concentrations of docosahexaenoic acid (DHA, 22:6n-3) and arachidonic acid (AA, 20:4n-6) have been reported in the postmortem bipolar disorder (BD) brain. Additionally, an increased prevalence of BD has been related to low dietary intake of fish, and dietary supplements containing fish products or DHA have been reported to ameliorate BD symptoms. These observations suggest that brain lipid metabolism, particularly involving DHA, is disturbed in BD. To test this suggestion, concentrations of different lipids were measured using internal standards in postmortem frontal cortex from eight BD patients and six matched controls. Compared with control cortex, the BD cortex showed no statistically significant difference in mean concentrations (per gram wet weight) of "stable" lipids (total lipid, total phospholipid, individual phospholipids, or cholesterol), of unesterified fatty acids, or of esterified DHA or AA within stable lipids. Fractional esterified AA and DHA concentrations also did not differ significantly between groups. Some fatty acid concentration differences were found in low-abundant cholesteryl ester. These results do not support the hypothesis of disturbed brain lipid concentrations, including concentrations of AA and DHA, in BD. Positron emission tomography might be used, however, to see if brain AA or DHA kinetics are disturbed in the disease.

Keywords

bipolar disorder; brain; docosahexaenoic; fatty acid; human; n-3 PUFA; lipid

Introduction

Bipolar disorder (BD) is characterized by recurrent manic (or hypomanic) and depressive episodes. It afflicts 1.5% of the United States population (Judd & Akiskal, 2003), and increases the risk of suicide 5- to 17-fold relative to the general population (Bostwick & Pankratz, 2000). Although mechanisms of BD are not agreed on, evidence has suggested disturbed lipid metabolism in the disease. Reduced concentrations of n-3 polyunsaturated fatty acids (PUFAs) were reported in erythrocytes from BD patients under treatment (Chiu et al., 2003; Ranjekar et al., 2003), an increased disease prevalence was correlated with reduced dietary intake of marine products containing n-3 PUFAs (Noaghiul & Hibbeln, 2003), and dietary

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supplementation with long-chain n-3 PUFAs, including docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3), has been reported to ameliorate BD symptoms (Frangou, Lewis, & McCrone, 2006; Stoll et al., 1999). Reduced arachidonic acid (AA, 20:4n-6) and DHA concentrations (as fractions of the total fatty acid concentration) were reported in the postmortem BD brain (McNamara et al., 2008; Svennerholm, Bostrom, & Jungbjer, 1997).

Measurements of lipid concentrations in postmortem brain can be affected by collection and storage procedures, differences in lipid separation and analytical methodology, postmortem interval (PMI), drug treatment history, substance abuse exposure, age, diet and disease (Deutsch, Rapoport, & Purdon, 1997; McNamara et al., 2008; Svennerholm et al., 1997). We therefore thought it of interest to reconsider lipid metabolism in BD by measuring lipid concentrations in postmortem brain tissue from BD and control subjects, when clinical characteristics and drug history were well defined, when age, postmortem interval, and tissue pH were matched, and when standardized collection conditions were used (Benes, 2007). Fatty acid concentrations were determined per gram brain wet weight using internal standards, which is not usually done in postmortem studies (McNamara et al., 2008; Svennerholm et al., 1997), and as fractional (percent of total fatty acid) concentrations. We chose to study the prefrontal cortex (Brodmann area 9) because this region appears particularly involved in BD (Buchsbaum et al., 1986; Lyoo et al., 2006).

Materials and Methods

Postmortem brain samples

This study was approved by the Institutional Review Board of the McLean Hospital (Belmont, MA) and by the NIH Office of Human Subjects Research (Protocol No. #4380). It was performed on frozen prefrontal cortex (Brodmann area 9) from eight diagnosed BD patients and six age-matched controls, whose reported cause of death and medication history are given in Table 1. The tissue was provided by the Harvard Brain Tissue Resource Center (McLean Hospital, Belmont, MA; <http://www.brainbank.mclean.org/>) under PHS grant R24MH068855 awarded to J. Rao. pH of brain samples was measured by the method of Harrison et al. (Harrison et al., 1995). Mean values of age, PMI and brain pH did not differ significantly between the BD and control groups (Table 2).

Chemical Analysis

Materials—Di-heptadecanoate phosphatidylcholine (di-17:0 PC), free heptadecanoic acid (17:0), and thin layer chromatography (TLC) standards for cholesterol, triglycerides, and cholesteryl esters were purchased from Sigma-Aldrich (St. Louis, MO, USA). Fatty acid methyl ester (FAME) standards for gas chromatography (GC) were purchased from NuChek Prep (Elysian, MN, USA). Identification standards for individual phospholipids were purchased from Avanti® Polar Lipids (Alabaster, AL, USA). 6-p-Toluidine-2-naphthalene sulfonic acid was purchased from Acros Organics (Fair Lawn, NJ, USA). Other chemicals and reagents were purchased from Sigma-Aldrich or Fisher Scientific.

Brain lipid extraction and separation of lipid classes: Total lipids from frozen brain were extracted by the method by Folch et. al. (Folch, Lees, & Sloane Stanley, 1957). They were separated into phospholipid classes and neutral lipid subclasses using TLC on silica gel-60 plates (EM Separation Technologies, Gibbstown, NJ, USA). The phospholipid classes were separated using the solvent system: chloroform:methanol:glacial acetic acid:water (60:50:1:4, by vol) (Skipski, Good, Barclay, & Reggio, 1968). This method separates ethanolamine glycerophospholipid (EtnGpl), phosphatidylinositol (PtdIns), phosphatidylserine (PtdSer), and choline glycerophospholipid (ChoGpl). The neutral lipid subclasses (cholesteryl esters,

triacylglycerol, unesterified fatty acids, cholesterol, and total phospholipids) were separated using the solvent system heptane: diethylether: glacial acetic acid (60:40:3, by vol) (Skipski et al., 1968). Authentic standards of phospholipids, triacylglycerol, cholesterol, cholesteryl ester and unesterified fatty acids were run on the plates to identify the lipids. The plates were sprayed with 0.03% 6-p-Toluidine-2-naphthalene sulfonic acid in 50 mM Tris-HCl buffer (pH 7.4) (w/v), and the lipid bands were visualized under ultraviolet light. The bands were scraped, and the silica gel was directly used to prepare FAMES and to determine phosphorus concentration.

Brain fatty acids: Fatty acid concentrations (nmol/g brain wet wt) were determined as described, with slight modification (Makrides, Neumann, Byard, Simmer, & Gibson, 1994). Unesterified and esterified fatty acids, which were separated by TLC as described above, were methylated with 1% H₂SO₄-methanol for 3 h at 70°C (DeMar, Ma, Bell, & Rapoport, 2004). To analyze fatty acid concentrations in total lipids, total lipid extracts were directly used for methylation. Before methylation, an internal standard (di-17:0 PC for phospholipids or 17:0 for unesterified fatty acids) was added to each tube. The prepared FAME samples were analyzed using a GC (6890N, Agilent Technologies, Palo Alto, CA, USA) equipped with an SPTM-2330 fused silica capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness) (Supelco, Bellefonte, PA, USA) and a flame ionization detector. Concentrations of fatty acids were calculated by proportional comparison of peak areas of samples to the area of the 17:0 internal standard.

Brain phospholipids: Total and individual phospholipid concentrations were quantified using a phosphorus assay (Rouser, Fkeischer, & Yamamoto, 1970). Total and individual phospholipids were digested in 0.5 ml water and 0.65 ml perchloric acid (70%) at 180 °C for 1 h. After the samples were cooled to room temperature, 0.5 ml of ascorbic acid (10%, w/v), 0.5 ml of ammonium molybdate (2.5%, w/v), and 3.0 ml of water were added. The mixture was boiled for 5 min to develop color. Absorbance was read at 797 nm. Phospholipid concentrations were determined using standard curves as described (Ma, Langenbach, Rapoport, & Basselin, 2007).

Brain plasmalogens: Concentrations of the plasmalogens, PlsEtn and PlsCho, were determined by the iodine uptake method (Gottfried & Rapport, 1962). Phospholipid classes in total lipid extracts were separated by TLC, as described above. The EtnGpl and ChoGpl bands were scraped and added to a tube containing a mixture of methanol (0.5 ml) and iodine reagent (0.5 ml) (6.0 × 10⁻⁴ N iodine in 3% aqueous KI). The mixture was mixed vigorously, then left at room temperature for 20 min. Absorbances of the samples and of iodide (control) were measured at 355 nm after adding 4.0 ml of 95% ethanol. Plasmalogen concentrations were calculated with absorbance coefficients: plasmalogen µmol/tube = (absorbance of iodine control – absorbance of sample)/27500 × 10⁻⁶.

Brain total cholesterol: Total cholesterol was determined with a commercial kit, according to the manufacturer's instructions (BioVision Research Products, Mountain View, CA). Total lipid extract was dried under a SpeedVac, and the residue was dissolved in 0.1% Triton X-100. The concentration was expressed in µmol/g brain wet weight.

Statistical analysis: We screened for outliers and tested assumptions of normality and homogeneity of variance with the Shapiro-Wilk and Levene tests, respectively, using SPSS 12.0 (SPSS Inc, Chicago, IL, USA). A Mann-Whitney U test was applied if the normality assumption was violated and an Aspin-Welch test was used if there was significant heterogeneity of variance. An unpaired Student's t-test was applied if statistical assumptions were met. For all statistical tests, the alpha level was set at 0.05.

Results

Mean concentrations of total phospholipid, individual phospholipids (EtnGpl, ChoGpl, PtdIns and PtdSer), and total cholesterol did not differ significantly between BD and control frontal cortex (Table 3). Additionally, mean PlsEtn and PlsCho concentrations did not differ significantly between the two groups. Concentrations of cholesteryl ester were too low to be measured with our method.

Table 4 summarizes mean esterified fatty acid concentrations in total lipids, total phospholipid and cholesteryl ester, as well as mean unesterified fatty acid concentrations, in BD and control frontal cortex. Statistically significant differences between the two groups were found only in cholesteryl ester. Concentrations of linoleic acid (18:2n-6) and of oleic acid (18:1n-9) were elevated significantly by 122% and 46%, respectively, in cholesteryl ester of BD frontal cortex, whereas concentrations of total saturated and monounsaturated fatty acid were increased significantly by 58% and 95%, respectively. There was no significant group difference in the AA or DHA concentration in total lipids, total phospholipids, cholesteryl esters, or unesterified fatty acids.

Some studies have reported reduced brain AA and DHA concentrations (as percent or fraction of the net fatty acid concentration) in BD brain (McNamara et al., 2008; Svennerholm et al., 1997). To compare our results with these reports, we transformed absolute fatty acid concentrations (per gram brain wet wt) into fractional concentrations by dividing by the net fatty acid concentration. As shown in Supplementary Table 1, there was no statistically significant group difference in any fractional fatty acid concentration in total lipids, total phospholipids, or unesterified fatty acids between BD and control brain. Fractional concentrations of docosapentaenoic acid (DPA, 22:5n-3) and of total n-3 PUFAs in cholesteryl ester were increased significantly by 50 % and 43 %, respectively.

Table 5 summarizes mean esterified fatty acid concentrations per gram wet wt in EtnGpl, ChoGpl, PtdIns, and PtdSer of BD and control frontal cortex. The esterified palmitate (16:0) concentration was decreased in PtdIns and PtdSer of BD compared with control cortex, but there was no statistically significant difference between the two groups in mean concentrations of other fatty acids.

Fractional concentrations of fatty acids in individual glycerophospholipids were calculated based on the absolute concentrations in Table 5. As shown in Supplementary Table 2, the fractional concentration of 16:0 was lower and the fractional concentration of 18:0 was higher in PtdIns and PtdSer of BD compared with control brain. The fractional concentration of DPAn-6 (22:5n-6) also was higher in PtdIns of the BD brain. There was no statistically significant difference between the two groups in mean fractional concentrations of other fatty acids, including AA or DHA, in brain phospholipid.

Discussion

This study indicates few statistically significant differences in lipid composition in BD compared with control postmortem frontal cortex, and no evidence for a reduced brain AA or DHA concentration. A statistically significant difference was not noted in the concentration (per gram brain wet weight) of total phospholipid, individual glycerophospholipids (EtnGpl, ChoGpl, PtdIns or PtdSer), individual plasmalogens (PlsEtn or PlsCho), or total cholesterol between BD and control cortex. Significant differences also were absent in concentrations of unesterified and esterified fatty acids in total lipids, total phospholipids, EtnGpl or ChoGpl. Palmitic acid (16:0) was reduced significantly in PtdIns and PtdSer, while 18:2, 18:1n-9, total saturated and monounsaturated fatty acids in these glycerophospholipids were increased in BD compared with control frontal cortex. Total saturated and monounsaturated fatty acid

concentrations were increased significantly in cholesteryl ester, which represented only a small fraction of brain lipid.

In terms of their fractional fatty acid concentrations (as percent to total), DPAn-3 and total n-3 PUFAs were reduced in cholesteryl ester, whereas 18:1n-7 was increased in BD compared with control cortex. The fractional concentration of 16:0 was reduced in PtdIns and PtdSer, while the fractional concentration of 18:0 was increased. In any case, the few significant results that we found should be interpreted with caution, given the high false positive risk associated with the large number of tests conducted without adjustment for multiple comparisons.

The control brain fatty acid concentrations in this paper are comparable to published human brain concentrations per gram wet weight, and per gram protein or DNA, in which internal standards were employed (Martinez, 1990; Prasad, Lovell, Yatin, Dhillon, & Markesbery, 1998). A study in which brain phospholipid concentrations were determined using phospholipid standards and a phosphorus assay (Nitsch et al., 1992) also provided brain glycerophospholipid concentrations comparable to ours, and our fatty acid concentrations in cholesteryl ester agree with reported values (Alling & Svennerholm, 1969).

Clinical studies have reported increased hydrolysis of serum phospholipids in BD patients (Hibbeln, Palmer, & Davis, 1989; Lieb, Karmali, & Horrobin, 1983; Sublette, Russ, & Smith, 2004), and increased levels of prostaglandins (metabolites of AA) in saliva (Nishino, Ueno, Ohishi, Sakai, & Hayaishi, 1989), cerebrospinal fluid (Linnoila et al., 1983) and serum (Lieb et al., 1983) from BD patients, suggesting disturbed whole body and brain AA metabolism. In BD patients, furthermore, genetic studies have identified a variant of the secretory phospholipase (*sPLA₂*) gene, which hydrolyzes AA and other fatty acids from the stereospecifically numbered (*sn*)-2 position of phospholipids (Jacobsen, Franks, Owen, & Craddock, 1999). Also, the postmortem BD frontal cortex was reported to have upregulated expression of calcium-dependent AA-selective cytosolic phospholipase A₂ (cPLA₂) Type IVA, sPLA₂ Type IIA and cyclooxygenase (COX)-2 (Rao et al., 2007), enzymes involved in AA metabolism (Rapoport, 2008), as well as reduced fractional concentrations of AA (McNamara et al., 2008; Svennerholm et al., 1997).

Suggesting disturbed DHA metabolism in BD are reports that dietary n-3 PUFA supplementation was beneficial (Frangou et al., 2006; Stoll et al., 1999), that the prevalence of BD correlated inversely with dietary fish intake (Noaghiul et al., 2003), that fractional DHA concentrations were reduced in the postmortem BD brain (McNamara et al., 2008; Svennerholm et al., 1997), and other observations (Chiu et al., 2003; Ranjekar et al., 2003; Stahl, Begg, Weisinger, & Sinclair, 2008; Sublette et al., 2007). However, the results in this paper are inconsistent with this suggestion, as is a report of no significant beneficial effect of dietary EPA supplementation in the disease (Keck et al., 2006). Supporting our negative finding regarding DHA is evidence in rats that dietary n-3 PUFA deprivation, which leads to DHA loss from brain, causes a reciprocal increment in the brain DPAn-6 concentration (DeMar et al., 2004; Igarashi et al., 2007). Not only was the DHA concentration unchanged in the BD brain, but the DPAn-6 concentration was unchanged as well.

In contrast to the absence of a statistically significant difference between BD and control frontal cortex in either absolute (per g wet wt) or fractional (as percent of net) DHA or AA concentrations in the present study, significant reductions have been reported in the fractional concentrations of DHA (−24%), AA (−14%), and stearic acid (18:0) (−4.5%) of postmortem orbitofrontal cortex from 18 BD compared with 19 control subjects, as well as a significant elevation in the fractional concentration of cis-vaccenic acid (18:1n-7) (+12.5%) (McNamara et al., 2008). Reductions in fractional AA and DHA concentrations brain also were reported in another study (Svennerholm et al., 1997).

These differences from our results may reflect differences in the brain regions analyzed, in methodology, tissue sources or collection, disease severity and diet and drug history, and remain to be explored. Of relevance however the fact that the BD brains that were analyzed by McNamara et al. (McNamara et al., 2008) were obtained (14 samples) from the Stanley Research Foundation Neuropathology Consortium (www.stanleyresearch.org) (Torrey, Webster, Knable, Johnston, & Yolken, 2000) and (4 samples) from the Harvard Brain Tissue Resource Center, the source of our tissue (see Introduction). Reanalysis by McNamara et al. of their data (R. K. McNamara, Personnel Communication, Supplementary information) revealed that their Stanley Research but not the Harvard Resource brains exhibited the significantly lower DHA concentration compared with control. Thus, our results on brain DHA and those of McNamara et al. on Harvard Resource Center brains are not inconsistent.

Mean concentrations of stable lipids did not differ significantly between BD and control brains in this study. We did not find a significant difference in the total cholesterol concentration, consistent with a prior observation (Beasley et al., 2005). Increased concentrations of 18:2n-6 and 18:1n-9, total saturated and monounsaturated fatty acids in cholesteryl ester suggest abnormal metabolism of this lipid in BD, but the concentration of cholesteryl ester was low, 0.1 to 0.2% of total cholesterol (Alling et al., 1969), and we could not measure it. Clinical investigations have noted an association between low serum cholesterol and violent suicide attempts (Vevera, Zukov, Morcinek, & Papezova, 2003) and manic episodes in BD patients (Atmaca et al., 2002; Swartz, 1990).

Several factors, such as pH, PMI and tissue quality, which could influence measurements, did not differ between the BD and control brain tissue. There was no significant difference in the mean PMI (Table 2), and a PMI of 18 hours does not alter brain phospholipid composition (Pearce & Komoroski, 2000). Brain pH was unrelated to the PMI when both groups were combined ($n=14$) (Pearson product moment correlation analysis; $r=-0.068$, $p=0.82$). We reported previously that the RNA integrity number, a marker of tissue quality (Stan et al., 2006), did not differ significantly between the cortical tissue from the two groups, and that PMI and RNA integrity number were not correlated significantly (Rao, Harry, Rapoport, & Kim, 2009; Rao, Rapoport, & Kim, 2009). These considerations, plus the fact that our concentrations in control brain are comparable to prior reports (see above), further support the conclusions of this paper.

Diet, drug exposure, substance abuse, gender, pregnancy, age, and liver disease, among other factors, may alter brain concentrations of fatty acids and stable lipids (Biagi, Hrelia, Stefanini, Zunarelli, & Bordoni, 1990; Bourre, Durand, Pascal, & Youyou, 1989; Burdge, Finnegan, Minihane, Williams, & Wootton, 2003; Holman, 1986; Innis, 2000; Levant et al., 2008; Simopoulos, Leaf, & Salem, 2000). We do not have data on the diets of the BD and control subjects or on their general health status, and our samples were too small to identify effects related to different drug exposures. Thus, our results remain to be replicated and extended using larger sample sizes. In any case, the present study is inconsistent with their being significant disturbances in brain fatty acid or stable lipid concentrations in the BD frontal cortex, with the exception of cholesteryl ester composition. Furthermore, it remains possible that brain AA or DHA metabolic rates are altered in patients with BD. This could be tested directly by using *in vivo* positron emission tomography and [$1-^{11}\text{C}$]AA or [$1-^{11}\text{C}$]DHA to quantitatively image metabolic consumption rates (Giovacchini et al., 2004; Umhau et al., 2008).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

AA	arachidonic acid
BD	bipolar disorder
COX	cyclooxygenase
DHA	docosahexaenoic acid
DPA	docosapentaenoic acid
EPA	eicosapentaenoic acid
FAME	fatty acid methyl ester
GC	gas chromatography
cPLA ₂	cytosolic phospholipase A ₂
sPLA ₂	secretory phospholipase A ₂
EtnGpl	ethanolamine glycerophospholipid
PC	phosphatidylcholine
PtdIns	phosphatidylinositol
PtdSer	phosphatidylserine
ChoGpl	choline glycerophospholipid
PlsEtn	plasménylethanolamine
PlsCho	plasménylcholine
PMI	postmortem interval
PUFA	polyunsaturated fatty acid
<i>sn</i>	stereospecifically numbered
TLC	thin layer chromatography

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Table 1

Characteristics of control and bipolar disorder subjects and of their frontal cortex samples

Group	Age, yr	Sex	PMI, hr	pH	Cause of death	Medications
Control	32	F	29	6.6	Cardiopulmonary attack	Antibiotics
Control	46	M	30	7.2	Cardiopulmonary attack	Insulin
Control	54	M	24	6.5	Cardiopulmonary attack	Insulin
Control	36	M	21	6.8	Electrocution	Vitamins
Control	41	M	30	6.6	Cardiopulmonary attack	None
Control	49	M	27	6.9	Cardiopulmonary attack	Vitamins
BD	29	M	20	6.5	Suicide	Paxil
BD	74	M	7	6.7	Pneumonia	Neurontin
BD	51	F	35	6.5	Ischemic heart disease	Ambien
BD	47	F	16	6.4	Major system failure	Lithium carbonate
BD	40	M	30	6.4	Suicide	Risperidone
BD	75	M	20	7.1	Myocardial infarction	Prozac, Avandia
BD	90	F	19	6.6	Ventricular tachycardia	Lithium carbonate, Prozac, Norpramine
BD	27	M	20	7.3	Suicide	Lithium carbonate, Restoril

PMI, postmortem interval

Table 2

Mean parameters for control and bipolar disorder subjects

	Control	Bipolar disorder
Age, years	43 ± 8	54 ± 23
Post-mortem interval, hours	27 ± 4	21 ± 8 ^a
pH	6.8 ± 0.3	6.7 ± 0.3

Mean ± SD, n = 6 for controls, 8 for BD patients

^aMann-Whitney test was performed. All other results were obtained with Student's *t*-test.

Table 3

Phospholipid and total cholesterol concentrations in postmortem frontal cortex from bipolar disorder and control subjects

Lipid	Control	Bipolar disorder
	$\mu\text{mol/g brain wet weight}$	
Total phospholipids	$57.4 \pm 13.8^{\dagger}$	53.3 ± 13.3
Ethanolamine glycerophospholipid	19.8 ± 3.6	23.3 ± 8.2
Choline glycerophospholipid	18.6 ± 2.3	22.4 ± 5.9
Phosphatidylserine	9.0 ± 3.2	10.4 ± 3.9
Phosphatidylinositol	3.7 ± 1.6	4.0 ± 0.9
Total cholesterol	$45.0 \pm 13.9^{\dagger}$	44.9 ± 21.0
Plasmalogens		
Plasmenylethanolamine	$9.2 \pm 3.9^{\dagger}$	7.4 ± 2.2
Plasmenylcholine	$3.5 \pm 1.7^{\dagger}$	4.1 ± 2.8

Mean $\pm$ SD, n = 6 († n = 5) for controls, 8 for BD patients

No BD mean differed from control ($p > 0.05$) by Student's *t*-test

Table 4
Esterified and unesterified fatty acid concentrations in postmortem cortex from bipolar disorder and control subjects

Fatty acid	Total lipids		Total phospholipid		Cholesteryl ester		Unesterified fatty acid	
	Control	BD	Control	BD	Control	BD	Control	BD
nmol/g brain wet weight								
16:0	17778 ± 2237	15370 ± 4023 ^a	17840 ± 2424	15486 ± 4641 ^a	12 ± 5	21 ± 9	496 ± 123	425 ± 218
16:1n-7	481 ± 125	414 ± 145	491 ± 141	421 ± 158	0.4 ± 0.1	0.8 ± 0.6 ^b	14 ± 6	13 ± 9
18:0	23082 ± 4174	19345 ± 5165	21969 ± 4375	18285 ± 5744	28 ± 11	42 ± 16	698 ± 350	749 ± 425
18:1n-9	19080 ± 8669	15202 ± 6719	21426 ± 7819	15242 ± 6414	17 ± 6	33 ± 14 ^{*,b}	367 ± 123	370 ± 221
18:1n-7	5284 ± 1988	4155 ± 1342	3998 ± 1280	3254 ± 1102	4.4 ± 1.6	6.5 ± 4.0	135 ± 53	110 ± 36
18:2n-6	623 ± 105	658 ± 270	602 ± 98	599 ± 282	2.7 ± 0.4	6.0 ± 3.4 ^{*,a}	35 ± 14	50 ± 39 ^a
20:3n-6	906 ± 162	665 ± 222	946 ± 159	701 ± 240	4.0 ± 2.2	3.6 ± 1.3	51 ± 20	46 ± 18
20:4n-6 (AA)	7751 ± 923	6664 ± 1748	7873 ± 1047	6746 ± 2051	11 ± 4	12 ± 7	449 ± 97	495 ± 270
20:5n-3	246 ± 196	200 ± 127	122 ± 102	101 ± 115	0.9 ± 0.5	1.1 ± 0.7	2.9 ± 1.1 [§]	5.1 ± 3.3 [¶]
22:4n-6	5176 ± 1438	4493 ± 1590	5860 ± 1744	5052 ± 1874	6.1 ± 1.7	6.6 ± 6.1 ^b	80 ± 24	88 ± 49 ^b
22:5n-6	988 ± 349	1199 ± 566 ^a	1210 ± 399	1317 ± 689	1.4 ± 0.9	1.8 ± 1.4 [†]	20 ± 11	28 ± 20
22:5n-3	319 ± 106	285 ± 89	349 ± 118	313 ± 106	0.5 ± 0.1	0.4 ± 0.3	8.2 ± 3.5	8.6 ± 4.4
22:6n-3 (DHA)	10232 ± 1848	8796 ± 2124	10940 ± 1996	9440 ± 2738	10 ± 7	8.1 ± 6.1	150 ± 54	189 ± 144
Total	91945 ± 18579	77445 ± 22569	93626 ± 18190	76956 ± 24317	98 ± 30	143 ± 40	2509 ± 560	2585 ± 1306
Total n-6	15444 ± 2362	13678 ± 3985	16490 ± 2939	14415 ± 4719	25 ± 9	30 ± 15 ^b	634 ± 159	707 ± 385
Total n-3	10797 ± 1867	9281 ± 2240	11412 ± 2047	9854 ± 2838	12 ± 8	9.6 ± 6.4	166 ± 57	210 ± 140
Total saturated	40860 ± 6364	34715 ± 9196	39809 ± 6766	33771 ± 10297	40 ± 17	63 ± 16 [*]	1194 ± 388	1174 ± 584
Total monounsaturated	24845 ± 10773	19771 ± 8114	25915 ± 8913	18916 ± 7415	21 ± 7	41 ± 17 ^{*,b}	516 ± 180	493 ± 256
n-6/n-3 ratio	1.5 ± 0.3	1.5 ± 0.1	1.5 ± 0.4	1.5 ± 0.2	2.1 ± 1.2	3.8 ± 1.9 ^a	4.1 ± 1.1	3.6 ± 0.9
AA/DHA ratio	0.77 ± 0.11	0.75 ± 0.04	0.73 ± 0.12	0.71 ± 0.05	0.58 ± 0.25	0.73 ± 0.32 ^a	3.2 ± 1.0	3.0 ± 0.7

Mean ± SD, n = 5 ([§]n = 4) for controls, n = 8 ([¶]n = 5, [†]n = 7) for BD patients

^{*} p < 0.05;

^a Mann-Whitney test was performed;

^b Aspin-Welch test was performed. All other results were obtained by a Student's *t*-test.

Table 5
Fatty acid concentrations in individual glycerophospholipids in frontal cortex from bipolar disorder and control subjects

Fatty acid	EtnGpl		ChoGpl		PtdIns		PtdSer	
	Control	BD	Control	BD	Control	BD	Control	BD
	nmol/g brain wet weight							
16:0	1509 ± 214	1302 ± 420	12362 ± 1418	11252 ± 2756	540 ± 455	207 ± 99 ^a	259 ± 72	140 ± 44 ^{**}
16:1n-7	136 ± 30	125 ± 40	217 ± 69	203 ± 91	21 ± 20	8.6 ± 4.9 ^b	13 ± 4	11 ± 5
18:0	6131 ± 664	5458 ± 1129	4027 ± 838	3965 ± 1290	1576 ± 502	1187 ± 386	6236 ± 2067	5557 ± 1822
18:1n-9	5003 ± 2976	3977 ± 2240	8318 ± 2327	7101 ± 2394	529 ± 393	248 ± 123 ^b	2786 ± 1916	2304 ± 1383
18:1n-7	2455 ± 1743	1922 ± 1114 ^a	1914 ± 406	1692 ± 407	178 ± 96	116 ± 52	358 ± 262	279 ± 147
18:2n-6	248 ± 49	238 ± 101	259 ± 47	259 ± 124 ^a	49 ± 32	33 ± 15 ^a	32 ± 16	33 ± 20
20:3n-6	600 ± 167	453 ± 223	261 ± 34	249 ± 122	122 ± 47	92 ± 44	142 ± 37	112 ± 36
20:4n-6 (AA)	3853 ± 457	3430 ± 788 ^a	1446 ± 181	1247 ± 361	1276 ± 232	970 ± 384	290 ± 87	256 ± 77
20:5n-3	60 ± 38	45 ± 39	97 ± 9	9.4 ± 3.2 ^a	4.4 ± 3.4	1.9 ± 0.6 ^b	7.7 ± 5.5	6.2 ± 4.5
22:4n-6	3894 ± 1133	3610 ± 1168	249 ± 43	224 ± 77	144 ± 60	105 ± 36 ^b	653 ± 169	602 ± 172
22:5n-6	591 ± 169	680 ± 310 ^a	65 ± 19	71 ± 40	22 ± 7	20 ± 8	327 ± 119	354 ± 153
22:5n-3	202 ± 64	189 ± 54	26 ± 9	25 ± 9	7.1 ± 2.2	6.8 ± 2.3	39 ± 12	40 ± 12
22:6n-3 (DHA)	6128 ± 993	5427 ± 1140	660 ± 125	508 ± 157	155 ± 36	115 ± 45	2640 ± 517	2194 ± 580
Total	30808 ± 6056	26854 ± 7327	29815 ± 5065	26805 ± 7043	4623 ± 1641	3109 ± 1103	13782 ± 4354	11887 ± 3884
Total n-6	9187 ± 1713	8411 ± 2276	2281 ± 233	2050 ± 609	1614 ± 329	1219 ± 457	1443 ± 346	1356 ± 387
Total n-3	6389 ± 1030	5661 ± 1192	696 ± 127	542 ± 162	166 ± 37	123 ± 47	2687 ± 528	2241 ± 588
Total saturated	7640 ± 783	6759 ± 1516	16389 ± 2240	15217 ± 3669	2116 ± 908	1394 ± 468	6495 ± 2090	5696 ± 1842
Total monounsaturated	7593 ± 4741	6023 ± 3346	10449 ± 2800	8996 ± 2858	727 ± 497	372 ± 179 ^b	3157 ± 2180	2593 ± 1531
Ratio of n-6/n-3	1.5 ± 0.4	1.5 ± 0.2	3.3 ± 0.5	3.8 ± 0.3	10 ± 2	10 ± 2	0.56 ± 0.18	0.61 ± 0.14 ^a
AA/DHA	0.64 ± 0.11	0.63 ± 0.06 ^a	2.2 ± 0.3	2.5 ± 0.2	8.5 ± 1.9	8.5 ± 2.2	0.11 ± 0.05	0.12 ± 0.03 ^a

Mean ± SD, n = 6 for controls, 8 for BD patient

* p<0.05;

** p<0.01;

^a Mann-Whitney test was performed;

^b Aspin-Welch test was performed. All other results were obtained by Student's t-test.