

Original Article

Abnormal cellular energy and phospholipid metabolism in the left dorsolateral prefrontal cortex of medication-free individuals with bipolar disorder: an *in vivo* ¹H MRS study

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Objectives: While the pathophysiology of bipolar disorder (BD) remains to be elucidated, postmortem and neuroimaging studies have suggested that abnormalities in the dorsolateral prefrontal cortex (DLPFC) are implicated. We compared the levels of specific brain chemicals of interest measured with proton magnetic resonance spectroscopy (¹H MRS) in medication-free BD subjects and age- and gender-matched healthy controls. We hypothesized that BD subjects would present abnormal cellular metabolism within the DLPFC, as reflected by lower *N*-acetyl-aspartate (NAA) and creatine + phosphocreatine (Cr + PCr).

Methods: Thirty-two medication-free BD subjects (33.8 ± 10.2 years) and 32 matched controls (33.8 ± 9.0 years) underwent a short echo-time (TE = 30 ms) ¹H MRS. An 8-cm³ single voxel was placed in the left DLPFC, and individual concentrations of NAA, Cr + PCr, choline-containing compounds (GPC + PC), *myo*-inositol, and glutamate were obtained, using the water signal as an internal reference.

Results: BD subjects had lower Cr + PCr [$F_{(1,62)} = 5.85$; $p = 0.018$; one-way analysis of variance (ANOVA)] and lower GPC + PC [$F_{(1,62)} = 5.79$; $p = 0.019$; one-way ANOVA] levels in the left DLPFC. No significant differences were observed for other brain metabolites.

Conclusions: These findings provide further evidence that the pathophysiology of BD involves impairment in the DLPFC. Our findings can be interpreted as evidence for reduced cellular energy and phospholipid metabolism, consistent with the hypothesis of mitochondrial dysfunction in BD.

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Bipolar disorder (BD) is a prevalent and chronic major mental illness that is associated with high rates of disability and suicide (1). Despite strong

evidence that BD is associated with neurobiological changes, the molecular mechanisms underlying its pathophysiology remain largely undetermined (2). Postmortem studies demonstrate decreased neuronal and glial density in the dorsolateral prefrontal cortex (DLPFC) of BD subjects compared to healthy controls (3, 4). Since glial cells play a central role in providing energetic support for neurons (5), these findings suggest that energetic metabolism might be impaired in the DLPFC of BD subjects. The DLPFC regulates executive functions and organizes behavioral responses and strategies in learning new tasks (6). The role of DLPFC in the pathophysiology of BD is also supported by recent reports that BD patients perform more poorly on neuropsychological tests that assess prefrontal functioning than healthy subjects (7, 8). Further, anatomical and functional neuroimaging studies demonstrate decreased prefrontal cortical volume and abnormal prefrontal activation in BD (9).

Proton magnetic resonance spectroscopy (^1H MRS) allows the *in vivo* quantification of certain neurochemical compounds, which are involved in cellular energy and phospholipids metabolism (10–13). Using a short echo-time ^1H MRS, several brain metabolites, including *N*-acetyl-aspartate (NAA), creatine + phosphocreatine (Cr + PCr), choline-containing compounds (GPC + PC), *myo*-inositol (mI) and glutamate (Glu) can be detected (11, 13). While the exact function of NAA is still unclear, it has long been recognized as a marker of neuronal integrity (14). Phosphocreatine constitutes an intracellular energy buffering system that transports the energy generated in the mitochondria to the cytosol to maintain a constant concentration of ATP (15). The GPC + PC peak of the ^1H MRS detects mainly phosphocholine (PC) and glycerophosphocholine (GPC), which are products of membrane synthesis and breakdown, respectively. Acetylcholine and free choline comprise <5% of the GPC + PC peak signal at 3.2 ppm. *Myo*-inositol is a precursor of phosphatidylinositol, which is an important membrane component and a member of the phosphoinositide second-messenger system (13). Glutamate is the most abundant amino acid in the central nervous system and is associated with the modulation of long-term potentiation and memory consolidation (16). However, an excess of this neurotransmitter may lead to excitotoxic cell death (17).

Previous ^1H MRS studies have yielded inconsistent results concerning the metabolic profile of the DLPFC in adult individuals with BD. One study showed decreased NAA/Cr + PCr levels in the DLPFC of euthymic BD patients (18); however,

three other recent studies, which mostly involved medicated individuals, failed to replicate this finding (19–21). One study showed increased levels of glutamate + glutamine (Glx) in a small sample of acute manic patients (22), whereas other studies found no differences in NAA, Cr + PCr or GPC + PC levels between BD subjects and controls (19, 20). Studies conducted in pediatric BD subjects revealed decreased NAA/Cr + PCr (23) and decreased NAA levels (24), suggesting that neuronal dysfunction in DLPFC may occur early in the course of bipolar illness. It is possible that medication treatment may normalize NAA levels during the course of illness, or that this abnormality is present primarily in a subset of more severely ill individuals. Previous studies demonstrated that lithium treatment might increase brain NAA levels (25, 26) and decrease brain mI (27), as assessed using ^1H MRS.

The objective of this study was to investigate the neurochemistry of the DLPFC in a sizeable sample of medication-free BD subjects and matched healthy comparison subjects using the ^1H MRS technique. Previous studies assessing the DLPFC chemistry with ^1H MRS used small samples ($n = 8$ –20), and only one study excluded medicated subjects (18). Considering that mood stabilizers and antipsychotics alter the brain metabolites (25, 28), the present study was designed to exclude this important confounder. We hypothesized *a priori* that BD subjects would present abnormal cellular metabolism within the DLPFC, as reflected by lower NAA and lower Cr + PCr concentrations.

Methods

Subjects

BD and healthy control subjects were recruited from advertisements. All BD subjects were required to meet DSM-IV diagnostic criteria for bipolar disorder Type I or Type II as determined by psychiatrists using the Structured Clinical Interview for DSM-IV (SCID) (29). Exclusion criteria for all subjects were: age <18 years, current serious medical conditions, history of head trauma, organic mental disorders, and neurological disorders. Additional exclusion criteria for BD subjects were: use of any psychotropic medication during the 2 weeks immediately prior to the study (6 weeks if fluoxetine or depot drugs), and alcohol/substance abuse or dependence within the 6 months preceding study entry. We did not influence patients' medication status; all BD subjects were unmedicated before the study entry. Additional exclusion criteria for healthy subjects were: any DSM-IV Axis-I disorder, as assessed by

a psychiatrist using the SCID non-patient version, any history of alcohol/substance abuse or dependence, and history of any psychiatric or neurological disorders in any of their first-degree relatives. This study was approved by the Institutional Review Board (The University of Texas Health Science Center at San Antonio), and all subjects provided their signed informed consent before entering in the study.

Subject assessment

All subjects received a physical examination, and a medical and psychiatric history was taken. The diagnoses were established by trained psychiatrists using the SCID (29). The severity of manic and depressive symptoms was assessed using the Young Mania Rating Scale (YMRS) (30), and the Hamilton Depression Rating Scale (HAMD) (31), respectively. All subjects underwent a laboratory testing of liver, thyroid, kidney, electrolytes, blood count, cortisol, and β -hCG (females), as well as a screening for cannabis, cocaine, stimulants, opioids, benzodiazepines, hallucinogens, and alcohol. All subjects with abnormal laboratory tests, positive substance use, or pregnancy (females) were excluded from the study.

^1H MRS procedure

^1H MRS was carried out in a 1.5-T Philips Gyroscan Intera scanner (Philips Medical Systems, Bothell, WA, USA). Axial, sagittal and coronal T1-weighted localizer images were first obtained to verify patient positioning and determine voxel placement. Then, a $2 \times 2 \times 2$ cm (8 cm^3) voxel was placed in the left DLPFC (Fig. 1), using the superior frontal sulcus, the lateral fissure, and the genu of corpus callosum as anatomical landmarks (32). All ^1H MRS data were acquired using a point-resolved spectroscopy sequence (PRESS) with TE = 30 ms, TR = 3.0 s, bandwidth: 2 kHz, 4,096 complex data points. Water unsuppressed spectra were also acquired for absolute quantification of metabolites in units of mmol/kg wet weight (33). The quantification of the spectral metabolites NAA, glutamate, glutamine, mI, PCr + Cr, GPC + PC, taurine, alanine, aspartate, gamma-amino-butyric acid, glucose, and *N*-acetyl-aspartyl-glutamate, as well as lipid and macromolecule resonances (34), was done using the Linear Combination Model (LC Model) software (35), an operator-independent fitting routine (Fig. 2). Only the results of the more reliable metabolites (NAA, PCr + Cr, GPC + PC, *myo*-inositol and glutamate) were used in the analysis.

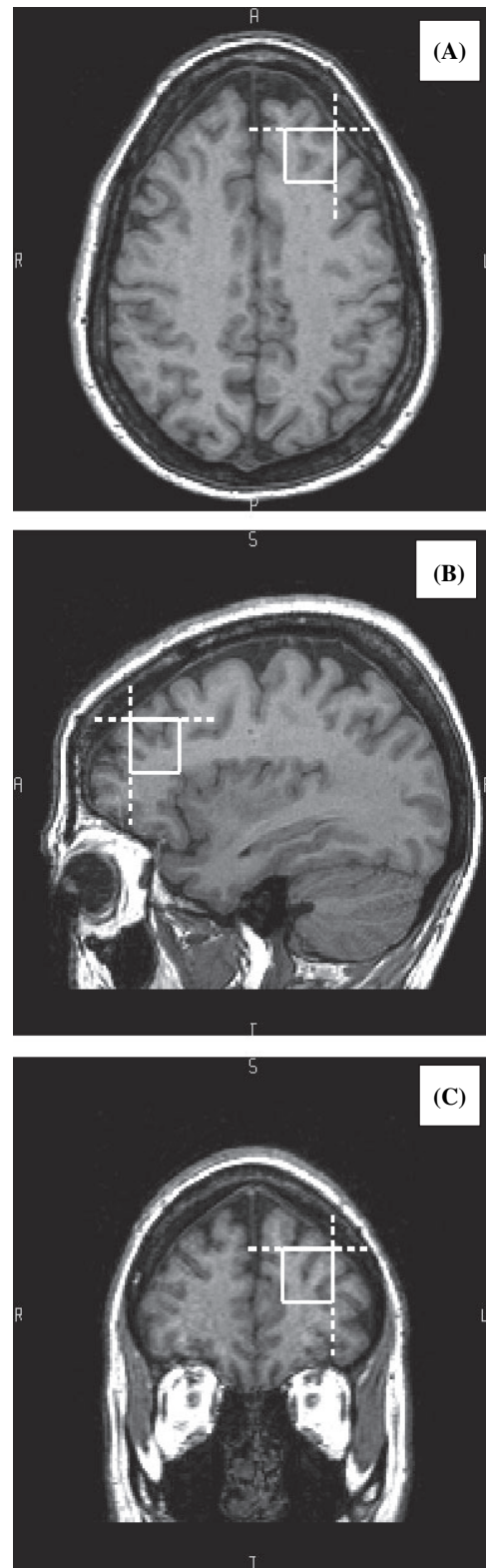


Fig. 1. Dorsolateral prefrontal cortex voxel placement. (A) = axial view; (B) = sagittal view; (C) = coronal view.

Statistical analysis

We performed multivariate analysis of covariance with subject age as the covariate. Following a

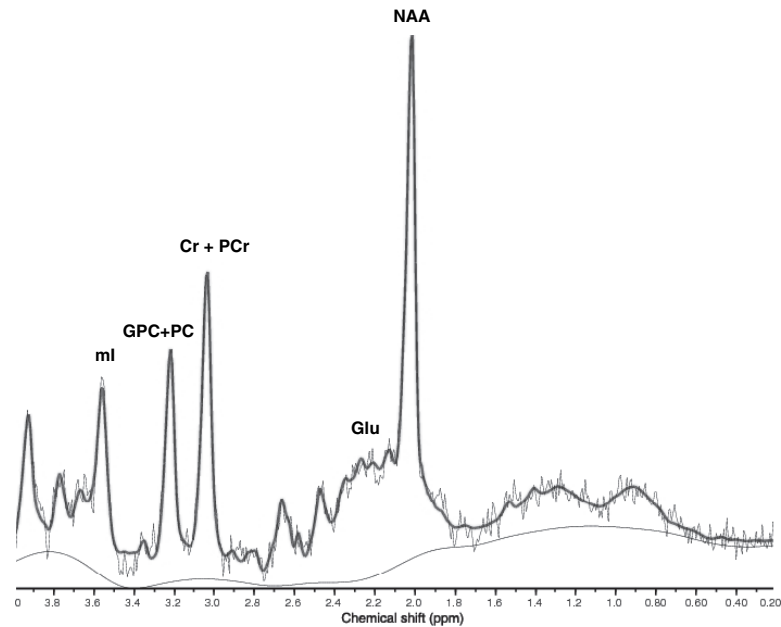


Fig. 2. Typical proton magnetic resonance spectroscopy spectrum. NAA = *N*-acetyl-aspartate; Cr + PCr = creatine plus phosphocreatine; GPC + PC = choline-containing compounds; ml = *myo*-inositol; Glu = glutamate.

statistically significant multivariate test we performed *post hoc* univariate analysis of covariance with age as the covariate to compare the groups (BD versus controls) on each metabolite. No further adjustment to reported p-values was made for analysis of multiple metabolites. On an exploratory basis, we also used analysis of covariance to examine the associations between the metabolite concentrations and clinical features including mood state, Axis-I comorbidities, BD subtype (BD Type I and BD Type II), and presence or absence of lifetime psychiatric hospitalization. We used partial correlations, adjusting for age to examine the associations between the metabolite concentrations and age at onset of illness, length of illness, HAMD and YMRS scores. All analyses were carried out using SPSS version 14.0.1 software (SPSS Inc., Chicago, IL, USA). The two-tailed significance criterion was set at $p < 0.05$ without adjustment for multiple comparisons.

Results

Demographic and clinical characteristics of the sample are displayed in Table 1. The BD sample consisted of 32 individuals [mean (SD) age = 33.8 ± 10.2 years; 21 females, 11 males; 17 depressed, 7 hypomanic, 1 mixed and 7 euthymic; 20 BD Type I, 12 BD Type II]. Seventeen BD subjects (53%) had a history of comorbid anxiety disorders, eight (25%) had a history of past

alcohol/substance abuse or dependence, and seven (22%) had no Axis-I comorbidity. The healthy comparison sample comprised 32 age- and gender-matched healthy volunteers [mean (SD) age = 33.8 ± 9.0 years; 22 females, 10 males]. BD and control subjects did not differ significantly in terms of age ($t = 0.0$; $df = 62$; $p = 1.0$; t -test), gender ($\chi^2 = 0.73$; $df = 1$; $p = 0.79$), or handedness ($\chi^2 = 3.15$; $df = 2$; $p = 0.2$). BD patients had lower educational status than controls (Mann-Whitney U -test, $Z = 3.8$, $p < 0.001$). Mean levels of all metabolites of BD and healthy subjects are displayed in Table 2. The multivariate analysis revealed that the BD and control subjects differed significantly on the profile of neuro-metabolites (Wilks' lambda = 0.73, $F = 4.3$, $df = 5,57$, $p = 0.002$). *Post hoc* univariate testing without correction for multiple metabolites showed that BD subjects had significantly lower Cr + PCr levels in the left DLPFC than control subjects [$F = 5.85$; $df = 1,62$; $p = 0.018$ (Fig. 3)]. BD subjects also had significantly lower GPC + PC levels than controls [$F = 5.79$; $df = 1,62$; $p = 0.019$ (Fig. 4)]. There was a non-significant trend for higher ml levels in the left DLPFC of BD subjects compared to controls ($F = 3.46$; $df = 1,61$; $p = 0.06$). There were no significant differences in NAA ($p = 0.93$) or Glu ($p = 0.21$) levels between patients and controls. In addition, there was no significant association between any of the brain metabolites and mood state, Axis-I comorbidities, BD subtype,

Abnormal energy and metabolism in bipolar disorder

Table 1. Demographic characteristics of bipolar subjects and healthy controls

	Bipolar subjects (n = 32)	Healthy controls (n = 32)	p-value
Age (years), mean (SD)	33.8 (10.2)	33.8 (9.0)	1.0
Age range (years)	20–54	20–60	
Gender (female/male)	21/11	22/10	0.79
Education history (SCID-I) (%)			
Grade 7–12 (without graduating)	1 (3.1)	1 (3.1)	
Graduated high school or equivalent	8 (25)	0	
Part college	13 (40.6)	10 (31.2)	<0.001
Graduated 2-year college	7 (21.8)	5 (15.6)	
Graduated 4-year college	3 (9.3)	4 (12.5)	
Part graduate/professional school	0	8 (25)	
Completed graduate/professional school	0	4 (12.5)	
Handedness (%)			
Right	32 (100)	29 (90.6)	0.2
Left	0	2 (6.3)	
Both	0	1 (3.1)	
BD subtype (Type I/Type II)	20/12	NA	
Mood state (%)			
Euthymic	7 (22)	NA	
Depressed	17 (53)	NA	
Hypomanic	7 (22)	NA	
Mixed	1 (3)	NA	
Axis-I comorbidity (%)			
Anxiety disorders	17 (53)	NA	
Alcohol/substance abuse or dependence	8 (25)	NA	
Length of illness (months), mean (SD)	119.7 (114.8)	NA	
Age at onset (years), mean (SD)	16.5 (6.8)	NA	
HAMD score, mean (SD)	16.0 (9.1)	NA	
YMRS score, mean (SD)	6.4 (6.3)	NA	
Lifetime hospitalization, yes (%)	12 (37)	NA	

BD = bipolar disorder; HAMD = Hamilton Depression Rating Scale; SCID-I = Structured Clinical Interview for DSM-IV – Axis I; YMRS = Young Mania Rating Scale; NA = not applicable.

Table 2. Concentrations of brain metabolites in bipolar disorder subjects and healthy controls

Metabolite	Bipolar subjects (n = 32)	Healthy controls (n = 32)	p-value ^a
NAA	6.61 (0.50)	6.60 (0.55)	0.93
Cr + PCr	4.84 (0.48)	5.15 (0.53)	0.018
GPC + PC	1.24 (0.20)	1.35 (0.13)	0.019
mI	3.99 (0.68)	3.68 (0.65)	0.06
Glu	5.36 (0.9)	5.66 (1.0)	0.21

Values are presented as mean (SD), mmol/kg wet weight.

^ap-values displayed are not adjusted for multiplicity due to the analysis of multiple metabolites. The unadjusted *post hoc* univariate p-values are shown following a significant multivariate test. Following Bonferroni correction, none of the p-values would remain statistically significant at the $p < 0.05$ level.

NAA = N-acetyl-aspartate; Cr + PCr = creatine plus phosphocreatine; GPC + PC = choline-containing compounds; mI = myo-inositol; Glu = glutamate.

or lifetime hospitalizations (all $p > 0.05$). Finally, mI levels were positively correlated with length of illness ($r = 0.42$; $p = 0.023$). There was no significant correlation between age at onset, HAMD or YMRS scores and any brain metabolite (all $p > 0.05$).

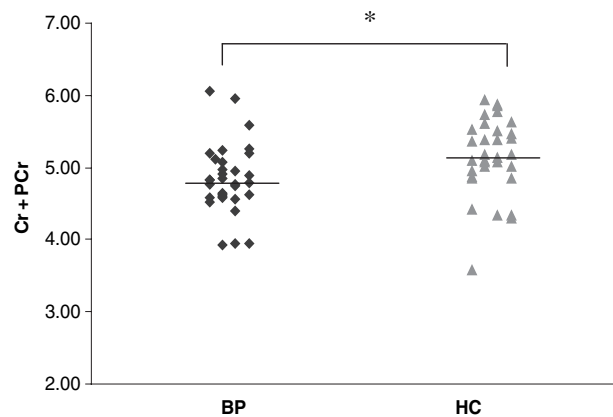


Fig. 3. Cr + PCr levels in bipolar subjects and healthy controls. Cr + PCr = creatine plus phosphocreatine; BP = bipolar subjects; HC = healthy controls. * $p = 0.018$.

Discussion

We found lower levels of Cr + PCr in the DLPFC of medication-free BD subjects compared to healthy volunteers. This finding is in line with previous phosphorus-31 magnetic resonance

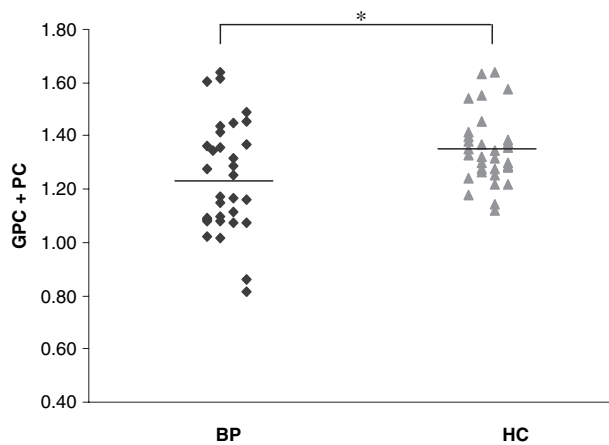
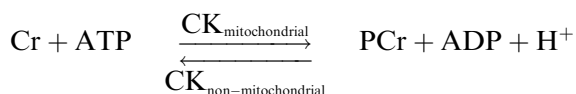


Fig. 4. GPC + PC levels in bipolar subjects and healthy controls. GPC + PC = choline-containing compounds; BP = bipolar subjects; HC = healthy controls. * $p = 0.019$.

spectroscopy (^{31}P MRS) studies that showed decreased PCr in the frontal lobe in bipolar depression (36, 37). Using ^1H MRS, Hamakawa et al. (38) reported that depressed BD patients had lower Cr + PCr in the left frontal lobe than euthymic BD patients, but no differences were found between BD patients and controls. Deicken et al. (39) recently showed that subjects with familial BD have lower Cr + PCr concentration in the right and left hippocampus than controls, suggesting that abnormal Cr + PCr levels may not be restricted to the prefrontal cortex in BD.

Under physiologic conditions, PCr is synthesized from Cr and the ATP generated in the mitochondria in a reaction catalyzed by the creatine kinase (CK) enzyme. This reaction is illustrated in the diagram below.



PCr is then transported to the cytosol acting as an energetic buffer to regenerate the ATP consumed by the cell. Cr + PCr is considered a marker of the cellular energy status (40), and decreased Cr + PCr concentrations reflect decreased energetic metabolism, possibly due to mitochondrial dysfunction. Therefore, our results are consistent with the hypothesis that BD subjects have abnormal energetic metabolism in the DLPFC. Whereas PCr is a major source of high-energy phosphates required for cellular homeostasis (41), Cr stabilizes mitochondrial CK in its octameric form, preventing the opening of the mitochondrial transition pore, an early event of apoptosis (42). Our finding is consistent with the hypothesis of mitochondrial dysfunction in BD (43) and raises the question of whether decreased

Cr + PCr is associated with the neuropathological changes observed in BD (44).

The fact that the Cr + PCr signal has long been used as an internal reference in ^1H MRS studies (45) resulted in a lack of investigation of possible abnormalities of Cr + PCr in BD. In addition, studies conducted in major depressive and schizophrenic patients demonstrated that the use of Cr + PCr as an internal reference might lead to a misinterpretation of the data (46, 47). In order to avoid this potential problem we analyzed only the individual concentrations of the neurometabolites.

We also demonstrated lower levels of GPC + PC in the left DLPFC in BD subjects compared to healthy subjects. Previous ^1H MRS studies found no differences in GPC + PC concentrations between BD subjects and controls in the DLPFC (18–22). Two studies that investigated other prefrontal regions found increased GPC + PC/Cr + PCr in the right cingulate cortex (48), and a trend for lower GPC + PC in the orbitofrontal gray matter (49). In fact, if Cr + PCr is truly lower in BD, the previous report of increased GPC + PC/Cr + PCr in the right cingulate cortex (48) could have been misinterpreted. Evidence of altered brain phospholipid metabolism in BD was also demonstrated with ^{31}P MRS (50). ^{31}P MRS studies have consistently demonstrated that BD subjects have significantly lower brain phosphomonoesters (PMEs) than normal controls (36, 51–54). Given that the membrane precursors PC and phosphoethanolamine (PE) are the main components of the PME peak (12), our finding supports the hypothesis that BD is associated with altered membrane phospholipid metabolism. Reduced GPC + PC signal indicates reduced cellular membrane phospholipids content or volume, which is consistent with postmortem studies showing decreased neuronal and glial cell density in the DLPFC of BD subjects (3, 4). Interestingly, Modica-Napolitano and Renshaw (55) revealed that PE and its metabolite ethanolamine inhibit mitochondrial electron transfer activity, suggesting that altered phospholipid metabolism might further affect mitochondrial functioning. Conversely (and perhaps more likely), mitochondrial dysfunction may fail to provide the energy required for normal membrane metabolism (56).

BD subjects showed a non-significant trend toward higher mI levels in the left DLPFC than controls. This trend is in line with our previous report that manic/mixed patients have higher left-to-right mI in the DLPFC (20). No differences in NAA or Glu levels between bipolars and controls were observed in the present study. Whereas no previous study investigated absolute Glu levels in the DLPFC in BD, 1 study that included untreated

adult individuals found decreased NAA/Cr + PCr in the DLPFC of BD (18), and subsequent adult studies failed to replicate this finding (19–22). Nonetheless, all negative studies had included patient samples that consisted mostly of medicated individuals, therefore allowing the possibility that negative results had been largely driven by possible medication effects. It is indeed possible that any regional abnormalities on NAA levels that are present and related to the illness may be altered by putative neuroprotective effects of mood stabilizers (25, 57). It is also important to note that several pediatric studies (23, 24, 58) found reductions in NAA levels in the DLPFC in bipolar individuals, suggesting the possibility that such abnormality may be more particular to certain illness subtypes, perhaps the most severe ones. This is also important when we consider our current sample, comprised of outpatients, without any currently psychotic subject, mostly with mild to moderate illness severity. Considering the prior adult findings in untreated subjects in the study by Winsberg et al. (18) and growing literature demonstrating such changes in pediatric BD, we cannot rule out the possibility that such change would be found in a sub-group of more severely ill untreated subjects.

In general, the levels of brain metabolites did not associate with the clinical presentation of BD. We found no significant associations between any metabolite and mood state, Axis-I comorbidities, BD subtype, lifetime hospitalization, age at onset, HAMD or YMRS scores. Since all subjects were recruited from the community, it is possible that we recruited a less severely ill sample compared to previous studies. We did find a positive correlation between mI levels and length of illness, suggesting that chronicity may lead to alterations of the phosphoinositide signaling system in BD (59). Using ^1H MRS, it has been demonstrated that 4 weeks of lithium treatment increase NAA (25, 26) and decrease mI concentrations (27). However, the long-term effects of chronic psychotropic use on brain chemistry have not been extensively investigated. One recent study showed that 3.6 months of lithium treatment decreased non-significantly gray matter Glx (glutamate + glutamine) and increased non-significantly gray matter mI in BD patients; no effects of 1.7 months of valproate treatment were observed (60). Therefore, even though we assessed only medication-free subjects in this study, the potential effects of previous medication exposure on brain metabolites cannot be absolutely ruled out.

Some limitations of the present study should be addressed. The single-voxel technique did not allow concomitant investigation of other brain regions

thought to be involved in BD. Biochemical examination of other implicated brain regions will be needed before an integrated and more complete understanding of BD pathophysiology will be possible. Another limitation is that differences in water and metabolite content due to different contributions of brain tissue and cerebrospinal fluid may be a potential source of error. To minimize this potential problem of partial volume effects, we guided the voxel placement using high-resolution T1-weighted images referred to standard anatomical landmarks. Although no association between mood state and metabolites concentration was found, the fact that patients were on various mood states might affect the results. Finally, the statistical analysis of multiple metabolites in relatively small samples presents a challenge for brain imaging researchers to balance the trade-off between preservation of statistical power and strong protection against Type I errors. The method employed here, unprotected univariate tests following a statistically significant multivariate test, is a relatively safe procedure, but it cannot absolutely hold the family-wise Type I error rate to $< 5\%$ in a strong sense. Our study also has important methodological strength. The recruitment of a large sample of medication-free BD subjects overcomes two important limitations affecting previous studies. The use of this sample avoids the known confounding effects of some psychotropic medications and provides sufficient statistical power to test the primary hypotheses. Further, the use of a short echo-time sequence with long repetition-time minimizes errors caused by the different relaxation times of water and brain metabolites. Finally, the use of individual metabolite concentrations excludes another potential confounder and improves the sensitivity in comparison to studies where metabolite concentrations were expressed as ratios.

In conclusion, the present study demonstrates lower Cr + PCr and GPC + PC levels in the left DLPFC of medication-free BD subjects compared to age and gender matched healthy individuals. These findings indicate that altered cellular energy and phospholipid metabolism may be involved in the pathophysiology of BD, and are consistent with the hypothesis of mitochondrial dysfunction (43). Prospective studies assessing the long-term effects of mood stabilizers on brain metabolites are warranted to further investigate the clinical relevance of the present findings.

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