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Decreased brain PME/PDE ratio in bipolar disorder: a preliminary ^{31}P magnetic resonance spectroscopy study

Xian-Feng Shi^{a,b}, Paul J Carlson^{a,b}, Young-Hoon Sung^{a,b}, Kristen K Fiedler^a, Lauren N Forrest^a, Tracy L Hellem^a, Rebekah S Huber^a, Seong-Eun Kim^c, Chun Zuo^{d,e}, Eun-Kee Jeong^{c,f}, Perry F Renshaw^{a,b,g}, and Douglas G Kondo^{a,b}

^aThe Brain Institute, University of Utah, Salt Lake City, UT

^bDepartment of Psychiatry, University of Utah, Salt Lake City, UT

^cDepartment of Radiology, University of Utah, Salt Lake City, UT

^dBrain Imaging Center, McLean Hospital, Harvard Medical School, Belmont, MA, USA

^eDepartment of Psychiatry, Harvard Medical School, Belmont, MA, USA

^fDepartment of Radiology, Korea University, Seoul, Korea

^gVISN 19 MIRECC, Salt Lake City Veterans Affairs Medical Center, Salt Lake City, UT, USA

Abstract

Objectives—The aim of the present study was to measure brain phosphorus-31 magnetic resonance spectroscopy (^{31}P MRS) metabolite levels and the creatine kinase reaction forward rate constant (k_f) in subjects with bipolar disorder (BD).

Methods—Subjects with bipolar euthymia ($n = 14$) or depression ($n = 11$) were recruited. Healthy comparison subjects (HC) ($n = 23$) were recruited and matched to subjects with BD on age, gender, and educational level. All studies were performed on a 3-Tesla clinical magnetic resonance imaging system using a $^{31}\text{P}/^1\text{H}$ double-tuned volume head coil. ^{31}P spectra were acquired without ^1H -decoupling using magnetization-transfer image-selected *in vivo* spectroscopy. Metabolite ratios from a brain region that includes the frontal lobe, corpus callosum, thalamus, and occipital lobe are expressed as a percentage of the total phosphorus (TP) signal. Brain pH was also investigated.

Results—Beta-nucleoside-triphosphate ($\beta\text{-NTP/TP}$) in subjects with bipolar depression was positively correlated with k_f ($p = 0.039$, $r^2 = 0.39$); similar correlations were not observed in bipolar euthymia or HC. In addition, no differences in k_f and brain pH were observed among the three diagnostic groups. A decrease in the ratio of phosphomonoesters to phosphodiester (PME/PDE) was observed in subjects with bipolar depression relative to HC ($p = 0.032$). We also

Corresponding author: Xian-Feng Shi, Ph.D., University of Utah Brain Institute, 383 Colorow Drive, Salt Lake City, UT 84108, USA, Fax: 801-585-5375, xianfeng.shi@hsc.utah.edu.

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observed a trend toward an inverse correlation in bipolar depression characterized by decreased phosphocreatine and increased depression severity.

Conclusions—In our sample, k_f was not altered in the euthymic or depressed mood state in BD. However, decreased PME/PDE in subjects with bipolar depression was consistent with differences in membrane turnover. These data provide preliminary support for alterations in phospholipid metabolism and mitochondrial function in bipolar depression.

Keywords

bipolar disorder; magnetization transfer; ^{31}P MRS

Recently, a number of reports have implicated abnormal brain energy metabolism in the patho-physiology of a number of psychiatric disorders, including bipolar disorder (BD) (1–5). This mitochondrial dysfunction might be reflected in alterations to the relative concentrations of phosphorous-bearing neurometabolites. These can only be measured *in vivo* through the use of phosphorus-31 magnetic resonance spectroscopy (^{31}P MRS) neuroimaging. ^{31}P MRS enables investigators to acquire regional measures of high-energy phosphorus compounds, including phosphocreatine (PCr), beta-nucleoside triphosphate [β -NTP; a proxy for adenosine triphosphate (ATP)], and inorganic phosphate (Pi), that are integral to brain bioenergetics.

BD is a severe brain disease affecting 1–4% of the population worldwide (6). Improved treatment for BD depends on the advancement of our understanding of its neurobiology. BD is associated with mitochondrial dysfunction (1, 7, 8) which affects the synthesis and regeneration of high-energy phosphates such as PCr and ATP in brain mitochondria. PCr serves as an energy reservoir in skeletal muscle and brain, whereas NTP [primarily ATP in the brain (9)] provides the energy for metabolic processes. For example, impaired oxidative phosphorylation might contribute to the alterations in brain PCr and NTP that have been reported in BD (7, 10).

A dynamic estimation of high-energy phosphate turnover provides an additional index for the assessment of brain bioenergetics, adding to the static evaluation acquired with ^{31}P MRS. Creatine kinase (CK) is the enzyme that catalyzes the reversible inter-conversion of PCr and NTP, and an alteration in the CK reaction rate might contribute to the etiology of mitochondrial dysfunction. The reaction is described by the following equation:

$\text{PCr}^{2-} + \text{NDP}^{-} + \text{H}^{+} \xrightleftharpoons{\text{CK}} \text{NTP}^{2-} + \text{Cr}$. NTP is hydrolyzed to release nucleoside diphosphate (NDP), Pi, and energy required to support brain activity. Thus, dysfunctional CK activity could result in altered NTP production (11–13). ^{31}P MRS measures of steady-state NTP lack sensitivity to detect compensation from upregulated CK activity and PCr consumption. Instead, measurements of the reaction rate constant (k_f) of NTP synthesis by CK might provide a more specific method for assessment of normal and pathological brain energy metabolism. Decreased k_f has been observed in animal models of brain diseases such as Huntington's disease, dementia, and diabetes mellitus (14). Additional evidence comes from post-mortem studies of individuals with a history of BD, which have consistently found reductions in CK in the frontal cortex (15, 16). However, Forester et al. (17) studied a

middle brain region and found no difference in k_f between geriatric subjects with BD and healthy comparison subjects (HC).

Cell membrane metabolites, including phosphomonoesters (PME) and phosphodiesteres (PDE), can also be evaluated using ^{31}P MRS. Brain mitochondrial dysfunction could also impact membrane metabolism as cell membrane turnover consumes approximately 10–15% of ATP production in the brain (18). Moreover, several ^{31}P MRS studies have reported altered PME (which consist of phosphocholine and phosphoethanolamine) and PDE (which include glycerophosphocholine, glycerophosphoethanolamine, and mobile phospholipids), suggesting abnormal membrane phospholipid metabolism in BD (19–22). PME are membrane precursors that play an important role in the synthesis of membrane lipids such as phosphatidylcholine and phosphatidylethanolamine, whereas PDE are the products of phospholipid breakdown. In particular, decreased membrane turnover has been associated with elevated PDE levels (23). A reduction in PME might also reflect altered membrane turnover rates. For example, Kato and colleagues (22) reported significantly altered frontal lobe PME in bipolar depression compared to euthymic subjects with BD. Similarly, Deicken et al. (24) found significantly lower PME and higher PDE in the bilateral frontal lobes of 12 unmedicated, euthymic patients with BD compared to HC.

BD reduces health-related quality of life in all phases of the disorder. However, there is an unmet therapeutic need for bipolar depression interventions (25), and understanding the changes in brain chemistry found in depressed individuals with BD would potentially provide an avenue for identification and evaluation of novel treatment strategies. In addition, intracellular pH is closely coupled with metabolic activity, and can be computed using the chemical shift difference between the Pi and PCr resonance peaks in the ^{31}P MRS spectrum.

Owing to the relatively low sensitivity of the ^{31}P nucleus, a comparatively large volume-of-interest (VOI) and signal averaging are required in order to achieve valid *in vivo* data. For this pilot study, a VOI encompassing the frontal lobe, corpus callosum, thalamus, and occipital lobe was selected in order to obtain an adequate signal. To measure k_f , multiple data acquisitions with progressively prolonged saturation of γNTP were required to retrieve the apparent relaxation time constant of PCr by fitting its signal intensity. The estimation of k_f was optimized by the resulting increase in the spectral signal-to-noise ratio.

In the present study, we aimed to examine individual ^{31}P brain metabolite ratios and k_f in subjects with BD. To our knowledge, there are no prior reports of the relationship between ^{31}P MRS high-energy phosphates and k_f in euthymic BD (17). We hypothesized that: (i) PCr/total phosphorus (TP) in BD would be reduced with respect to that measured in HC; and (ii) decreased k_f in BD would be observed when compared to HC. We also investigated the correlation in BD between PCr/TP and clinical ratings, including the Montgomery—Åsberg Depression Rating Scale (MADRS) (26). Our goals were to determine whether high-energy phosphates are associated with k_f ; if membrane-turnover-related metabolites such as PME and PDE are altered in BD; and the extent to which intracellular brain pH is altered in BD.

Patients and methods

Patients

Study participants were recruited through clinician referrals and Institutional Review Board (IRB)-approved advertisements. All subjects with BD were on a stable treatment regimen for at least four weeks prior to scanning. HC were matched on age, gender, handedness, and educational level. Inclusion criteria included age from 18 to 55 years; ability to provide informed consent; and absence of clinically significant cardiovascular, pulmonary, gastrointestinal, hepatic, renal, hematological, endocrine, or neurological disease as determined by medical history. Diagnoses were established via the administration of the Structured Clinical Interview for DSM-IV-TR Axis I Disorders, Research Version, Patient Edition (SCID-I/P) (27). Subjects in the sample met the criteria for bipolar I disorder; bipolar depressed subjects met the criteria for a current depressive episode of ≥ 2 weeks' duration and a MADRS score of ≥ 10 . Subjects with Young Mania Rating Scale scores of ≥ 12 and MADRS scores of <10 were classified as euthymic. HC had no psychiatric or substance use diagnosis as determined by the SCID-I/P, and were not taking psychotropic medication. In addition, subjects were excluded for any active medical illness or contraindication to scanning – e.g., ferromagnetic implants or a history of claustrophobia. The study protocol was approved by the University of Utah IRB, and written informed consent was obtained from all subjects with BD ($n = 25$; mean age 31.8 ± 8.6 years) and HC ($n = 23$; mean age 27.8 ± 5.6 years). The demographic and clinical characteristics of the study sample are presented in Table 1.

The creatine kinase forward reaction rate (k_f)

CK catalyzes the rapid regeneration of NTP from cytosolic PCr, thus providing an energy buffer to support brain metabolic activity. Downregulation of CK might underlie the reduction in high-energy phosphates observed in BD (16). However, CK resynthesizes NTP so rapidly that direct measurement of the reaction rates in tissue has not been possible using conventional methods. Thus, magnetization transfer (MT) techniques have been developed to measure the velocity of the CK reaction (28). Du et al. (29) applied MT in a study of schizophrenia and noted that prefrontal lobe k_f was decreased compared to HC. Forester et al. (17) applied MT to measure k_f , and found no significant difference in k_f comparing geriatric BD and HC in a central brain VOI. We utilized an MT image-selected *in vivo* spectroscopy pulse sequence (MT-ISIS) to measure k_f (28). Figure 1 displays representative raw and fitted ^{31}P MRS spectra, with and without γ -NTP suppression.

^{31}P MRS experiment

All scans were performed on a 3-Tesla (T) clinical magnetic resonance imaging (MRI) system (Trio-Tim, Siemens Medical Solutions, Erlangen, Germany) with Avanto gradients (40 mT/m strength and 150 T/m/s slew rate) using a $^{31}\text{P}/^1\text{H}$ dualtuned volume head coil (Clinical MR Solutions, LLC, Brookfield, WI, USA). ^{31}P spectra were acquired using the MT-ISIS pulse sequence (28) with VOI $11 \times 8 \times 3 \text{ cm}^3$, receiver bandwidth 2.5 kHz, and vector size 1,024. Proton decoupling was not implemented during data acquisition. To facilitate voxel placement, high-resolution T_1 -weighted images were acquired using a three-dimensional magnetization-prepared rapid gradient echo acquisition (MPRAGE) pulse

sequence with the following parameters: repetition time (TR)/echo time (TE)/inversion time (TI) = 2,000/3.37/1,100 msec; flip angle = 8°; field-of-view = 256 × 192 × 144 mm³; 256 × 192 × 144 matrix size; 1 × 1 × 1 mm³ spatial resolution; bandwidth = 300 Hz/pixel. All metabolite levels were calculated using spectra without γ -NTP signal suppression. Spectra with MT off were acquired with the following parameters: TR 20.5 sec; time delay between excited radio frequency (RF) and analog-to-digital conversion 0.4 msec; average number 2. Spectra with MT on were collected using a different γ NTP saturation duration with different number of signal averages: eight ISIS phase cycles 0.25 sec and 0.5 sec; six 1.0 sec and 2.0 sec; and four 4.0 sec and 9.0 sec. Multiple Sinc RF pulses with bandwidth 75 Hz were used to saturate the γ -NTP signal. Additional detail can be found elsewhere (28). To ensure consistency in VOI localization, as shown in Figure 2, the inferior edge of the VOI in a sagittal plane was located parallel to the anterior commissure (AC)–posterior commissure (PC) line, which was identified on the midsagittal MPRAGE images, with the superior edge placed at the superior border of the corpus callosum. To effectively saturate the phosphorus signal from outside the VOI, six outer-volume saturation bands (not shown in Fig. 2) were placed 0.5 cm away from the VOI. In consideration of the low concentration of phosphate-containing metabolites in the brain, the limits of ³¹P coil sensitivity, and the large VOI, advanced active shimming of the magnetic field within the VOI was manually performed up to three times, to optimize the field homogeneity and achieve global water linewidths of less than 25 Hz. Board-certified faculty radiologists reviewed the anatomic images, to rule out the presence of intracranial abnormalities.

³¹P MRS data analysis

To minimize user bias in data processing, all time-domain free-induction decay (FID) signals were preprocessed using an automated MATLAB application (The MathWorks, Inc., Natick, MA, USA). Each FID was apodized with 10 Hz Gaussian line broadening. Subsequently, the FID signal was zero-filled from 1,024 points to 2,048 points to enhance apparent digital resolution, and then a forward Fourier transformation was implemented to obtain the spectrum in the frequency domain. In order to allow the spectrum to appear in the absorption mode, improve the convergence speed of the iterative optimization procedure, and reduce the chance of a curve-fitting error resulting from an initial biased estimate of those phase parameters in an iterative fitting method, zero- and first-order phase corrections were performed for all spectra. The signal intensity of each metabolite was obtained using the Advanced Method for Accurate, Robust and Efficient Spectral Fitting of MRS data with use of prior knowledge (AMARES) (30) fitting algorithm within the software application jMRUI 4.0 (31). AMARES is a time-domain curve-fitting approach, in which the zero-order phase and delay time are modeled parameters of the Lorentzian function. Metabolite concentrations were calculated as a percentage of the TP signal acquired from the VOI. Intracellular pH was computed using a modified Henderson–Hasselbalch relationship according to $\text{pH} = \text{pK}_{\text{Pi}} + \log[(\delta_{\text{Pi-H}} - \delta_{\text{H}})/(\delta_{\text{H}} - \delta_{\text{Pi}})]$, where δ_{H} is the observed chemical shift between Pi and PCr; $\delta_{\text{Pi-H}}$ (3.27 ppm) and δ_{Pi} (5.63 ppm) are the chemical shifts of Pi with/without protonation; and pK_{Pi} (6.75 ppm) is the logarithm of the acid–base balance between H_2PO_4^- and HPO_4^{2-} (32, 33).

Statistical analysis

Statistical analyses were performed using *R*-statistics software (34). One-way analysis of variance (ANOVA) was used to test for group differences in ^{31}P metabolite ratios. Subject age, gender, and white matter (WM) fraction within the VOI were included as covariates. Following ANOVA, post-hoc analyses employing the Tukey–Kramer honestly significant difference test were used to assess pairwise differences between group means, with the significance level set at $p = 0.05$. The k_f , PCr/TP, and PME/PDE were considered dependent variables, with subject group and mood state categorized as independent variables. Pearson correlation analyses were used to assess the relationship between ^{31}P metabolite ratios and the MADRS clinical ratings.

Brain structural image segmentation

To evaluate the influence of brain tissue components within the VOI, the MPRAGE images were segmented into gray matter (GM), WM, and cerebrospinal fluid (CSF) using FSL (FMRIB Software Library, Release 4.1; Oxford, UK). Dcm2nii software was used to convert MPRAGE images in Siemens DICOM format into NIFTI-formatted data (35). Brain tissue images were extracted by removing the outer skull and scalp surfaces using the brain extraction tool (36). Finally, the FAST/FIRST tool was utilized to calculate the segmented tissue percentage in the VOI (37, 38). Co-registration between the spectroscopic VOI and the segmented image was performed with a user-developed MATLAB program.

Results

MRS findings

MRS metabolite ratios in the bipolar depression, bipolar euthymia, and HC groups are summarized in Table 2. No significant difference in PCr/TP in BD was observed with respect to HC. In subjects with bipolar depression, β -NTP/TP was positively correlated with k_f ($p = 0.039$; $r^2 = 0.39$), as shown in Figure 3A. In addition, a trend ($p = 0.06$; $r^2 = 0.34$) was observed for an inverse correlation between PCr/TP and MADRS depression scores in the bipolar depression group (Fig. 3B). Contrary to our hypothesis, k_f in the BD group was not significantly lower compared to HC. However, subjects with bipolar depression were characterized by a decreased mean PME/PDE ratio compared to HC ($p = 0.032$) (Fig. 4A). There was no significant difference in PME/TP in the bipolar euthymia group relative to bipolar depression or HC (Fig. 4B). A trend toward increased PDE/TP was found in bipolar depression relative to HC ($p = 0.078$) (Fig. 4C). No significant differences in the other metabolite ratios were identified among the three subject groups.

VOI tissue segmentation

There was no significant difference in the mean VOI composition of GM, WM, and CSF among the three subject groups, following tissue segmentation of the reference images. Approximately 65% of the VOI was occupied by WM, as shown in Table 3. However, due to low image contrast around subcortical regions such as the thalamus, caudate, putamen, pallidum, and hippocampus, tissue misclassification can occur during segmentation of high-resolution T1-weighted images. In the present study, the thalamus, caudate, putamen,

pallidum, and hippocampus occupied 4.6%, 2.5%, 2.8%, 1.0%, and 0.3% of the total VOI, respectively. The summed volume among subcortical structures is 11.2%, and therefore, the contribution of misclassified voxels to tissue segmentation results is likely to be modest for these brain regions.

Discussion

The present study reports measures of k_f and ^{31}P MRS metabolite ratios in BD depressed and BD euthymic subjects and HC, using a magnetization transfer ^{31}P MRS technique. Our findings demonstrated a decreased PME/PDE ratio in subjects with bipolar depression compared to HC. Contrary to our hypothesis, we did not find a significant difference in k_f among our three subject groups.

Converging lines of evidence implicate mitochondrial dysfunction in the pathophysiology of BD. Our findings show a trend toward lower PCr/TP ($p = 0.06$) associated with higher MADRS scores in bipolar depression (see Fig. 3B). Prior studies have also reported decreased frontal lobe PCr in bipolar depression compared to HC (7, 10). However, in the present study we did not observe significant differences in PCr/TP among our three subject groups; this might have been due to our limited sample size.

We did not find a significant group difference in k_f . This is consistent with a recent report by Forester et al. (17), which found no difference in k_f in the thalamus, in geriatric subjects with bipolar depression and HC. Our sample size might have affected our ability to detect altered metabolite ratios among the three groups. Power analyses assuming a significance of 0.05 and a power of 0.80 revealed that the minimum detectable differences in group mean values are 10.1% and 11.3%, between the bipolar euthymia, bipolar depression, and HC groups, respectively. However, we found a significant correlation ($p = 0.039$) between $\beta\text{-NTP}$ and k_f , as shown in Figure 3A. No difference in $\beta\text{-NTP/TP}$ was found between the three subject groups. In bipolar depression, insufficient $\beta\text{-NTP}$ supplied by mitochondria can result in an increase in the conversion of PCr to $\beta\text{-NTP}$.

In subjects with bipolar euthymia, several investigators have reported decreased PME measured in multiple brain regions, when compared to HC (22, 24, 39–41). In the present study, a comparison of PME/TP in bipolar depressed and euthymic subjects found no significant difference. This differs from Kato et al. (22, 40), who reported elevated PME in bipolar depression compared to bipolar euthymia. This difference might have resulted from a different VOI in the two studies. In the reports by Kato and colleagues (22, 40), the frontal lobe was chosen for analysis. In addition, because most of the subjects in the present study had long histories of treatment, subjects' medication status was a potential confound. For example, Yildiz et al. (20) speculated that increased PME levels in bipolar depression vs. euthymia might be attributable to pharmacologic effects. Along those same lines, *in vivo* animal studies showed elevated PME in cat brain (42) and in rat brain (43) following both acute and chronic lithium treatment. *In vivo* studies have also noted increased PME following seven and 14 days of lithium administration (44). In light of these prior findings, it is not unexpected that there was no difference in PME/TP between our bipolar depressed and euthymic groups as few of the depressed BD subjects reported a history of lithium

treatment (as shown in Table 1) and one euthymic subject took only lithium as a treatment for BD. Importantly, the reports by Kato et al. (22, 40) examined the frontal lobe region, whereas the current study interrogated a larger brain VOI surrounding the corpus callosum. This difference in methodology might also have contributed to the discrepant findings.

We found that PDE/TP in bipolar depressed subjects demonstrated a trend ($p = 0.078$) toward an increase compared to HC. Moreover, we observed a significant difference in PME/PDE between bipolar depression and HC. Because PME include contributions from phospholipid membrane precursors, and PDE from membrane breakdown products, the PME/PDE ratio reflects cell membrane turnover. We found a significantly lower PME/PDE ($p = 0.032$) in BD depression vs. HC, a difference that was not present in the BD euthymia group. To the best of our knowledge, this is the first report of decreased PME/PDE in subjects with bipolar depression. Thus, these data add to the evidence for altered phospholipid membrane metabolism in BD. Further, the trend toward decreased PME/PDE in the BD euthymia group is consistent with a prior report by Deicken et al. (24), who found lower PME and higher PDE in unmedicated euthymic subjects with BD with respect to HC. Our data show a similar trend, of decreased PME/TP and increased PDE/TP in euthymic subjects with BD, but these metabolite ratio differences failed to reach statistical significance. The observation of no group difference between euthymic BD and HC might have resulted from the combination of our small sample size and the difference in VOI between the present study and that of Deicken et al. (24). In their recent review of neurobiological abnormalities in BD, Langan and McDonald (45) note that decreased frontal lobe PME might be an indicator of ‘stalled’ membrane synthesis. Because the conversion of PDE to PME is a catabolic, ATP-dependent process, inhibition of membrane synthesis might represent an indirect indicator of mitochondrial dysfunction.

In the mitochondrial dysfunction hypothesis of BD, a compensatory mechanism is thought to be the activation of glycolysis due to insufficient supplies of ATP. Impaired bioenergetic metabolism could lead to intracellular acidification resulting from an increased production of lactate, which is a byproduct of glycolysis. However, the present study found no between-group differences in intracellular brain pH. In a previous report, lower prefrontal pH in drug-free euthymic BD (19) was observed. One possibility is that decreased pH might only be present in focal brain regions, such as the basal ganglia (46) or frontal lobe (7, 19, 22, 40). In addition, medications might affect brain pH. For example, Jensen et al. (47) reported increased intracellular pH values in depressed subjects with BD who responded to treatment with triacetyluridine, compared to non-responders, an observation that suggests that triacetyluridine might improve mitochondrial function in bipolar depression by facilitating oxidative phosphorylation. Finally, interventions with positive studies in bipolar depression include omega-3 fatty acid (48) and uridine (49). It is therefore notable that omega-3 (50) and uridine (51) administration each have been reported to increase brain PME in humans, suggesting one potential mechanism of action for these novel treatments in BD.

A major limitation of the present study was its modest sample size, which in turn limits the generalizability of our findings. In addition, the relatively large VOI utilized was another limitation, and discrete brain regions should be investigated in future studies. The VOI included subcortical regions such as the thalamus, caudate, putamen, pallidum, and

hippocampus, and image contrast in these regions is relatively low compared to that in other brain regions. Therefore, tissue misclassification might occur during brain segmentation. To further improve the accuracy of image segmentation in subcortical regions, future studies should take this into account (52).

Conclusions

In the present report, phosphorus-bearing neurometabolite ratios and k_f were assessed using magnetization transfer and ^{31}P MRS methods to study bipolar depression, bipolar euthymia, and HC. We observed a significantly decreased PME/ PDE ratio in bipolar depression compared to HC, a finding that suggests altered phospholipid membrane metabolism in bipolar depression. By contrast, there was no difference in k_f among the three groups. In the bipolar depression group, we also observed a trend toward an inverse correlation characterized by decreased PCr/TP and increased MADRS depression severity; a positive correlation between $\beta\text{-NTP/TP}$ and k_f was also observed in these subjects. Our findings add new evidence for mitochondrial dysfunction in BD, particularly in bipolar depression. Future studies should include validation of these findings in a larger sample, narrowing the VOI to focal brain regions that have been implicated in BD, and measuring within-subject changes in brain chemistry between mood states in a longitudinal study.

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Fig. 1.

Representative raw and fitted ^{31}P magnetic resonance spectrum with magnetization transfer on [bottom; repetition time (TR) = 9.6 sec] and magnetization transfer off (top; TR = 20.5 sec). GPC = glycerophosphocholine; GPE = glycerophosphoethanolamine; MT = magnetization transfer; ($\alpha/\beta/\gamma$)NTP = alpha/beta/gamma-nucleoside triphosphates; PCr = phosphocreatine; PME = phosphomonoesters; Pi = inorganic phosphate.

Fig. 2.
Volume-of-interest along axial, coronal, and sagittal plane.

Fig. 3. Regression plots of (A) beta-nucleoside triphosphate/total phosphate ($\beta\text{-NTP/TP}$) vs. the forward creatine kinase reaction rate ($p = 0.039$) and (B) phosphocreatine (PCr/TP) vs. Montgomery-Åsberg Depression Rating Scale (MADRS) score ($p = 0.06$) in subjects with bipolar depression.

Fig. 4.

Comparison of metabolite ratios among depressed (Dep) and euthymic (EU) subjects with bipolar disorder. (A) Significantly decreased phosphomonoester/phosphodiester (PME/PDE) metabolite levels in subjects with bipolar depression ($p = 0.032$) compared to healthy comparison subjects (HC). There was no significant difference between EU and Dep subjects. (B) No significant differences in PME level were noted among the three groups. (C) A trend toward an increased PDE was observed in Dep subjects with respect to HC subjects ($p = 0.078$).

Table 1

Demographic and clinical rating information of healthy comparison, bipolar euthymic, and bipolar depressed subjects

Group	ID #	Gender/age/duration of medication (years)	MADRS score	YMRS score	Medication (mg/day)
Healthy comparison					
•	n = 23				
•	Female (%) = 11 (48%)				
•	Ages = 27.8 ± 5.6 years				
Bipolar euthymia					
•	n = 14				
•	Female (%) = 7 (50%)				
•	Ages = 29.7 ± 9.0 years				
•	MADRS score = 5.3 ± 3.0				
•	YMRS score = 2.6 ± 2.3				
	1	F/23/no medications	8	2	No medications
	2	F/24/10	8	2	No medications
	3	M/29/10	6	4	VPA (2,000)/fluvoxamine (100)/trazodone (100)
	4	M/30/3	6	8	No medications
	5	F/22/5	4	0	LTG (200)/sertraline (100)/ARI (5)/clonazepam (1)
	6	M/24/5	8	5	VPA (NA)/Li (NA)/LTG (NA)/sertraline (NA)/bupropion (NA)/carbamazepine (NA)
	7	M/26/2	2	0	Li (900)
	8	F/39/17	0	0	Li (300)
	9	M/33/3	8	3	LTG (300)/desvenlafaxine (50)/amphetamine and dextroamphetamine (10)/clonazepam (1)/atenolol (20)/zolidem (10)
	10	F/30/no report	4	2	No medications
	11	M/34/8	8	2	LTG (100)
	12	F/19/1	0	5	Sertraline (NA)/duloxetine (NA)/ARI (NA)
	13	M/55/2	4	3	VPA (1,500)/QTP (400)/citalopram (40)/trazodone (50)/bupropion (150)
	14	F/29/6	8	1	Li (NA)/bupropion (NA)
Bipolar depression					
•	n = 11				
•	Female (%) = 6 (55%)				
•	Ages = 34.5 ± 7.5 years				
•	MADRS score = 22.5 ± 5.7				
•	YMRS score = 4.1 ± 2.7				
	1	M/32/1	28	4	Methylphenidate (5)/ARI (5)
	2	F/33/1	28	2	ARI (5)/bupropion (150)/clonazepam (0.5)/lisdexamfetamine (70)/benztropine (0.5)/levodopa (88)/LTG (300)
	3	F/20/2	18	5	Escitalopram (20)
	4	F/36/few weeks	22	4	Duloxetine (30)/ARI (5)
	5	M/38/1	24	2	Diazepam (10)/temazepam (30)/QTP (350)/citalopram (20)/baclofen (75)/carbamazepine (800)/cyclobenzaprine (10)/hydroxyzine (50)/lisinopril (20)
	6	M/39/no report	22	0	No medications
	7	M/44/1 month	24	5	LTG (100)
	8	F/33/2.5 weeks	22	4	Venlafaxine (37.5)

Group	ID #	Gender/age/duration of medication (years)	MADRS score	YMRS score	Medication (mg/day)
	9	M/42/5	14	4	VPA (1,000)
	10	F/23/1	14	4	LTG (200)
	11	F/40/5	32	11	QTP (400)/Li (900)

ARI = aripiprazole; F = female; Li = lithium; LTG = lamotrigine; M = male; MADRS = Montgomery-Åsberg Depression Rating Scale; NA = Not Available; QTP = quetiapine; VPA = valproic acid; YMRS = Young Mania Rating Scale.

Table 2

Mean ratios of metabolite compounds over total phosphate, k_f , and their standard deviations

	Healthy comparison (n = 23)	Bipolar euthymia (n = 14)	Bipolar depression (n = 11)	p-value ^a	p-value ^b		
					EU versus HC	DEP versus HC	EU versus DEP
PCr/TP	0.150 ± 0.011	0.149 ± 0.013	0.149 ± 0.008	0.988	0.997	0.987	0.997
β-NTP/TP	0.089 ± 0.013	0.087 ± 0.015	0.083 ± 0.016	0.482	0.856	0.452	0.790
Pi/TP	0.057 ± 0.012	0.056 ± 0.010	0.056 ± 0.012	0.984	0.982	0.998	0.995
PDE/TP	0.268 ± 0.033	0.272 ± 0.025	0.293 ± 0.033	0.090	0.911	0.078	0.226
PME/TP	0.165 ± 0.015	0.155 ± 0.024	0.153 ± 0.015	0.103	0.247	0.139	0.915
PME/PDE	0.628 ± 0.104	0.577 ± 0.103	0.530 ± 0.096	0.036	0.316	0.032	0.496
pH	7.042 ± 0.017	7.035 ± 0.018	7.038 ± 0.019	0.494	0.475	0.804	0.911
Mg ²⁺ (μmol/L)	0.156 ± 0.021	0.162 ± 0.034	0.153 ± 0.022	0.689	0.787	0.953	0.690
k_f (sec ⁻¹)	0.324 ± 0.110	0.342 ± 0.099	0.332 ± 0.103	0.884	0.873	0.979	0.969

Bold values represent significantly different p-value. The total phosphorus concentration (TP) was used for standardization. DEP = bipolar depression; EU = bipolar euthymia; HC = healthy comparison subjects; k_f = creatine kinase reaction rate; Mg²⁺ = magnesium; PCr = phosphocreatine; PDE = phosphodiester; Pi = inorganic phosphate; PME = phosphomonoester; β-NTP = beta-nucleoside triphosphate.

^a p-value from the one-way analysis of variance model followed by

^b the Tukey–Kramer honestly significant difference post-hoc test.

Table 3

Percentage of mean brain tissues within the volume-of-interest among healthy comparison, bipolar euthymic, and bipolar depression groups

	Healthy comparison	Bipolar euthymia	Bipolar depression
Gray matter	26.8 ± 2.5	26.4 ± 3.8	26.1 ± 1.3
White matter	65.4 ± 2.8	64.8 ± 3.5	65.9 ± 3.3
Cerebrospinal fluid	7.8 ± 2.9	8.7 ± 3.9	8.0 ± 2.4
Thalamus	4.7 ± 0.8	4.7 ± 0.6	4.3 ± 0.7
Caudate	2.6 ± 0.3	2.3 ± 0.4	2.5 ± 0.4
Putamen	3.0 ± 0.5	2.8 ± 0.6	2.6 ± 0.2
Pallidum	1.0 ± 0.2	1.0 ± 0.2	0.9 ± 0.1
Hippocampus	0.3 ± 0.3	0.3 ± 0.2	0.2 ± 0.2

Data are expressed as mean value ± standard deviation.