ORIGINAL ARTICLE

Brain Metabolic Alterations in Medication-Free Patients With Bipolar Disorder

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Background: Bipolar disorder (BD) has substantial morbidity and incompletely understood neurobiological underpinnings.

Objective: To investigate brain chemistry in medication-free individuals with BD.

Design: Two-dimensional proton echo-planar spectroscopic imaging (PEPSI) (32×32, 1-cm^3 voxel matrix) acquired axially through the cingulate gyrus was used to quantify regional brain chemistry.

Setting: The Center for Anxiety and Depression at the University of Washington in Seattle and the Bipolar Research Programs at McLean Hospital and the Massachusetts General Hospital in Boston.

Participants: Thirty-two medication-free outpatients with a diagnosis of BD type I (BDI) or BD type II (BDII), predominantly in a depressed or mixed-mood state, were compared with 26 age- and sex-matched healthy controls.

Main Outcome Measures: Tissue type (white and gray) and regional analyses were performed to evaluate distribution of lactate; glutamate, glutamine, and γ-aminobutyric acid (Glx); creatine and phosphocreatine (Cre); choline-containing compounds (Cho); *N*-acetyl aspartate; and *myo*-inositol. Chemical relationships for diagnosis and mood state were evaluated.

Results: Patients with BD exhibited elevated gray matter lactate ($P = .005$) and Glx ($P = .007$) levels; other gray and white matter chemical measures were not significantly different between diagnostic groups. Isolated regional chemical alterations were found. An inverse correlation between 17-item Hamilton Depression Rating Scale scores and white matter Cre levels was observed for BD patients.

Conclusions: Gray matter lactate and Glx elevations in medication-free BD patients suggest a shift in energy redox state from oxidative phosphorylation toward glycolysis. The possibility of mitochondrial alterations underlying these findings is discussed and may provide a theoretical framework for future targeted treatment interventions.

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BIPOLAR DISORDER (BD) IS A psychiatric disorder characterized by mood disturbances with recurrent episodes of mania, hypomania, and depression.[1] Bipolar disorder can be differentiated into clinical subtypes of BD type I (BDI), characterized by at least 1 episode of pure mania, and BD type II (BDII), characterized by less clinically severe hypomania and major depressive episodes.[2,3] There is an approximately 1% prevalence rate for BDI among the adult population; the prevalence rate may be somewhat higher for BDII.[4,5] Neurobiological mechanisms are thought to underlie BD, and there is evidence of a familial, and presumably genetic, predisposition for the disorder.[6]

Serendipitous observations of the mood-stabilizing effect of lithium salts and later certain anticonvulsants, such as valproic acid, have substantially improved the prognosis of patients with BD. Despite this progress, these medications are not universally effective, and many individuals are unable to tolerate treatment-related adverse effects.[6,7] In addition to incomplete clinical response, relapse and recurrence remain common clinical problems.[8,9] A recent survey of BD lifetime financial burden in the United States found associated costs to be up to $625 000 per patient, depending on treatment refractoriness and chronicity of symptoms.[10] Bipolar disorder continues to be associated with substantial morbidity and mortality, ranking worldwide among psychiatric disorders behind only unipolar depression and alcohol abuse for related disabilities and overall economic load of the illness.[11,12]

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Research conducted over several decades investigating biomarkers related to BD provides evidence for widespread alterations of multiple neurotransmitter or signal induction systems and physiological processes associated with the disorder, as reviewed by Manji and Lenox.[13] Increasingly, imaging techniques are being applied to better establish brain mechanisms underlying the etiology of BD. Positron emission tomography studies, used to characterize patterns of regional metabolism or cerebral blood flow in mood disorders, typically find regional or widely distributed abnormalities of reduced blood flow and metabolism in association with depressed mood states, including BD-related depression.[14-17] Magnetic resonance (MR) imaging studies, as reviewed by Strakowski et al,[18] variably find evidence of reduced regional gray matter volumes associated with BD.[16,19-23] Differing subcortical patterns of structural abnormalities may occur across the spectrum of affective disorders; for example, basal ganglia and amygdalar enlargement are observed in BD,[19,24] whereas volumes of these structures may be reduced in unipolar depression.[25,26] Similarly, neuroanatomic findings have been reported to distinguish BD clinical subtypes, such as the observation of lateral ventricular enlargement in BDI but not BDII patients.[27]

Magnetic resonance spectroscopy, an imaging modality related to MR imaging, provides an in vivo tissue-based measure of brain chemistry using clinical MR image scanners that is safe, noninvasive, and reproducible.[28,29] For neuropsychiatric applications at 1.5 T, as reviewed elsewhere,[30-32] hydrogen H1 (^{1}H) MR spectroscopy can detect and identify subtle alterations in brain chemical composition, despite normal-appearing structure, through quantifying the resonance frequency of certain ^{1}H-containing chemical compounds that include the combined resonances of creatine and phosphocreatine (Cre); choline-containing compounds (Cho); *N*-acetyl moieties predominately composed of *N*-acetyl aspartate (NAA); *myo*-inositol (mI), which also contains some contribution from scyllo-inositol at 1.5 T; the overlapping resonances of glutamine, glutamate, and γ-aminobutyric acid (Glx); and lactate. The development of rapid MR spectroscopy techniques, such as proton echo-planar spectroscopic imaging (PEPSI), allows fast, clinically feasible acquisition of 2-dimensional images of brain chemical composition.[33] Short-echo time (TE) measurements (eg, 20 milliseconds) can be acquired using PEPSI that provide a more accurate appraisal of brain chemical concentration, particularly as some disease states, such as autism, can alter tissue relaxation characteristics and substantially affect spectral quantification at longer TEs.[34]

The purpose of this study was to characterize brain chemical composition in medication-free BD patients compared with age- and sex-matched healthy controls (HCs). To ensure adequate numbers of medication-free participants, a dual-site study was conducted between Boston, Mass, and Seattle, Wash. Medication-free BD patients were studied, because prior work[35-41] suggests secondary effects of mood stabilizers or antidepressants on MR spectroscopy–detectable chemicals and gray matter volume that could confound interpretation of findings. We evaluated diagnostic relationships for chemicals detectable by ^{1}H-MR spectroscopy, including Cho, Cre, mI, Glx, and NAA, to extend prior work that found metabolic alterations in mood disorders. The present investigation further sought to exploit the within-subject multiple voxel sampling acquired with PEPSI to evaluate whether lactate, a potential indicator of redox status but with a low signal-to-noise ratio in the resting state, was altered in BD. To assess tissue-type specificity for chemical findings, gray and white matter compartments were evaluated separately using established regression methods.[42-44] We report tissue-specific and regional brain chemical findings in BD patients vs HCs, relationships to mood state, and clinical subtypes of BDI and BDII.

METHODS

PARTICIPANTS

Participants were 32 adults with BD (14 men, 18 women; mean ± SD age, 30.3 ± 10.8 years) and 26 HCs (12 men, 14 women; mean ± SD age, 31.9 ± 7.7 years). Participants were recruited through the Bipolar Research Programs at McLean Hospital and the Massachusetts General Hospital (BD patients = 15; HCs = 16) or the University of Washington Center for Anxiety and Depression (BD patients = 17; HCs = 10). Written, informed consent, approved by either McLean Hospital and Massachusetts General Hospital, Harvard University, or the University of Washington Human Subjects Review Boards, was obtained from each participant before study enrollment. All participants tolerated the scanning procedure, but 3 BD patients (1 man, 2 women) and 2 HCs (2 men) had invalid studies due to technical acquisition problems or movement artifacts and were not included in the statistical analyses. Additionally, only long-echo MR spectroscopy data (lactate) were acquired for 1 BD patient. There were no significant differences in sex distribution ($\chi^2_1 = 0.11$, $P = .75$) or age ($t_{51} = 0.73$, $P = .47$) between diagnostic groups.

Psychiatric diagnosis was established through a *DSM-IV*–based structured psychiatric interview by skilled clinicians (D.L.D., C.D., A.L.S.). These clinicians also established diagnostic agreement between sites before study inception and consensus for individual subject inclusion throughout the study. The BD patients did not meet other *DSM-IV* Axis I diagnoses, including lifetime alcohol and/or other drug dependency or abuse within 6 months of study entrance. All participants were medication free for 8 weeks or longer before the study. Participants were outpatients seeking treatment and had not stopped treatment to participate in the study. Of the 29 BD patients with valid studies, 26 had never been treated with mood-stabilizing medications, and the 3 previously treated BD patients (2 taking lithium and 1 taking valproic acid) had not been taking mood stabilizers for at least 1 year. Twelve BD patients, including the 3 previously taking mood stabilizers, had been prescribed antidepressants in the past but had not been taking them for at least 8 weeks or longer before the study. No patients had previously been treated with antipsychotic medication. The results of urine toxicology screening obtained before the study were negative for psychoactive substances. Evidence of clinically significant medical problems through medical history, laboratory workup, including thyroid studies, and physical examination that evaluated for cerebral vascular disease, pulmonary disease, endocrine disorders, severe sensory or motor impairments (deaf or blind), documented head trauma, or metal implants was exclusionary for participation. Healthy controls had no current symptoms or medical history consistent with a *DSM-IV* Axis I diagnosis, and there was no history of clinically significant psychiatric disorders in their first-degree relatives.

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Subtype classification of BD patients into BDI (n=11; 5 men, 6 women; mean±SD age, 31.9±6.1 years) or BDII (n=17; 7 men, 10 women; mean±SD age, 28.9±10.5 years) was determined at study entrance by structured psychiatric interview. One participant was not classified and excluded from further analysis. Mood state before MR imaging or MR spectroscopy was assessed using the 17-item Hamilton Depression Rating Scale (HAM-D)[45] and the Young Mania Rating Scale (YMRS).[46] The mean±SD HAM-D score for the BD patients was 17.7±8.6 (BDI, 20.0±8.2; BDII, 16.1±9.0) compared with 1.2±1.1 for the HCs. The mean±SD YMRS score for the BD patients was 6.5±7.7 (BDI, 9.7±9.1; BDII, 4.6±6.4) compared with 0.3±0.5 for HCs. The BD patients had an average illness duration of 13.5±10.2 years (range, 0.5-33.0 years), with 13.6±18.1 depressive episodes and 7.3±7.6 hypomanic manic episodes.

MR IMAGING AND MR SPECTROSCOPY ACQUISITION

The PEPSI studies were performed using clinical 1.5-T SIGNA whole-body scanners (GE Medical Systems, Milwaukee, Wis), both equipped with version 5.8 Genesis operating software. Experimental receive-only linear birdcage coils, designed and built at the University of Washington, that provided approximately $\sqrt{2}$ enhanced signal-to-noise ratio and improved coil homogeneity over conventional quadrature head coils (Cecil Hayes, PhD, unpublished data, 1996) were used at both sites. The coil design incorporates a built-in head holder to minimize head motion during scanning.

Axial proton density and T2-weighted MR images were used for anatomic localization and tissue segmentation (TE, 13/91 milliseconds; repetition time [TR], 2000 milliseconds; field of view (FOV), 22 cm; matrix, 256×160; slice thickness, 3 mm). For PEPSI studies, anatomic volumes were acquired from an axial section centered on the anterior cingulate (**Figure 1**). The PEPSI pulse sequence has been described in detail elsewhere.[33,47] Two TEs and standard parameters were used for data acquisition (TE, 20/272 milliseconds; TR, 2000 milliseconds; spatial matrix, 32×32; nominal voxel size, 1 cm^3; FOV, 22 cm; slice thickness, 20 mm). Magnetic resonance spectroscopy data were acquired in scan blocks (20-millisecond chemical image: 4 numbers of excitation/[NEX]; 4-minute scan time; 272-millisecond chemical image: 8 NEX; 8-minute scan time; 20-millisecond water image: 2 NEX; 2-minute scan time), totaling approximately 20 minutes, including shimming and scan setup. A full echo was collected for 20-millisecond chemical data and a partial echo for 272-millisecond chemical data, the latter necessitating a magnitude calculation ($\sqrt{[real^2+imaginary^2]}$) for data reconstruction.[47] Unsuppressed short-echo water scans were also collected from the identical axial slice location (TE, 20 milliseconds; TR, 2000 milliseconds) for referencing both metabolite scans as previously described.[34]

MR SPECTROSCOPY ANALYSES

The PEPSI chemical images were analyzed using software developed at the University of Washington that used the LCModel package for spectral fitting[48] (Figure 1). Consistent with our prior work to optimize LCModel parameters[34] and to standardize measurements between sites, phantoms with known concentrations of brain chemicals were prepared at McLean Hospital and scanned at various acquisition parameters, and basis sets were created as detailed in the LCModel manual. All data sets were analyzed unbiased by user interaction on workstations (SGI Octane; Silicon Graphics Inc, Mountain View, Calif) running IRIX system 6.5.8.

To calculate chemical concentrations and relaxation estimates, LCModel water amplitudes were adjusted by water molarity estimates and attenuation constants were then multiplied by the partial volume tissue fraction. Chemical amplitude maps were adjusted for the differential relaxation between in vitro (phantoms) and in vivo estimates for Cre, Cho, and NAA, which served to scale chemical output similar to our previous work.[34] Metabolites were then multiplied by the inverted partial-volume–corrected water term to yield chemical concentration.[49]

Absolute T2 calculations from the short- and long-echo PEPSI data could not be accurately determined, because long-echo data were acquired as a partial echo, requiring a magnitude calculation for reconstruction. Thus, a relative measure of T2 relaxation (T2r) for each region was calculated from chemicals measured at both short and long TEs (Cre, Cho, and NAA), as previously described.[34]

Because short-echo data afford the least biased estimation of chemical concentration, long-echo data were used only for lactate measurements and T2 relaxation time calculations.[28,34,47] Chemical measures from each spectral array were determined from all valid voxels for each individual participant to achieve maximum signal-to-noise ratios. The number of valid voxels from the 32×32 spectra array acquired per subject was not significantly different comparing BD and HC groups for all chemical measurements ($t<0.86$ and $P>.38$ for all).

TISSUE SEGMENTATION

Dual-echo axial images were skull stripped and then segmented using multispectral analytic tools within an image manipulation and visualization package (Analyze; Mayo Clinic, Rochester, Minn). Specifically, sample regions of gray matter, white matter, and cerebrospinal fluid (CSF) (eg, intraventricular CSF) were manually selected by a single rater (I.K.L.) blinded to patient diagnosis. These pure exemplars, unbiased by partial volume, were used as starting points for gaussian classification. Following segmentation, a second script written in MATLAB, a computer language used for array computation and visualization, was used to offset the images to magnet center using MR image header coordinates and extract images within the PEPSI volume. Because PEPSI data sets are acquired at magnet center, aligning anatomic images in this way results in accurate registration.

The CSF and tissue maps were processed as previously detailed,[34,50] including summation of images within the PEPSI volume, spatial filtering, and array reduction to match the chemical array spatial resolution (32×32). On a voxel-by-voxel basis, CSF data were used for partial volume correction of metabolite quantification. Partial volume tissue estimates of the PEPSI slab were similar between groups (BD patients, 0.95±0.02; HCs, 0.96±0.01; $t_{50}=0.62$; $P=.54$). For regression analyses, white and gray matter maps were used. The percentage of gray matter comprising the axial slab studied using PEPSI was also similar between groups (BD patients, 52.96%±4.71%; HCs, 51.78%±4.98%; $t_{50}=.88$; $P=.38$). For each participant, using all valid spectroscopy voxels, regression analyses were performed comparing the fractional tissue volume (percentage of gray or white matter per voxel) to concentration and relaxation values. This approach, similar to that used by other investigators,[42-44] takes advantage of the large number of voxel samples obtained using spectroscopic imaging to calculate estimates of pure tissue neurochemistry (regression intercepts) and the relationship between tissue classes (regression slope). Characteristic chemical regression plots from an individual BD patient are shown in **Figure 2**.

REGION-OF-INTEREST ANALYSES

For region-of-interest (ROI) selection, the PEPSI voxel grid (32×32 matrix) was graphically overlaid onto the axial MR image data set for selecting anatomic loci: frontal white matter left and right, cingulate left and right, caudate left and right,

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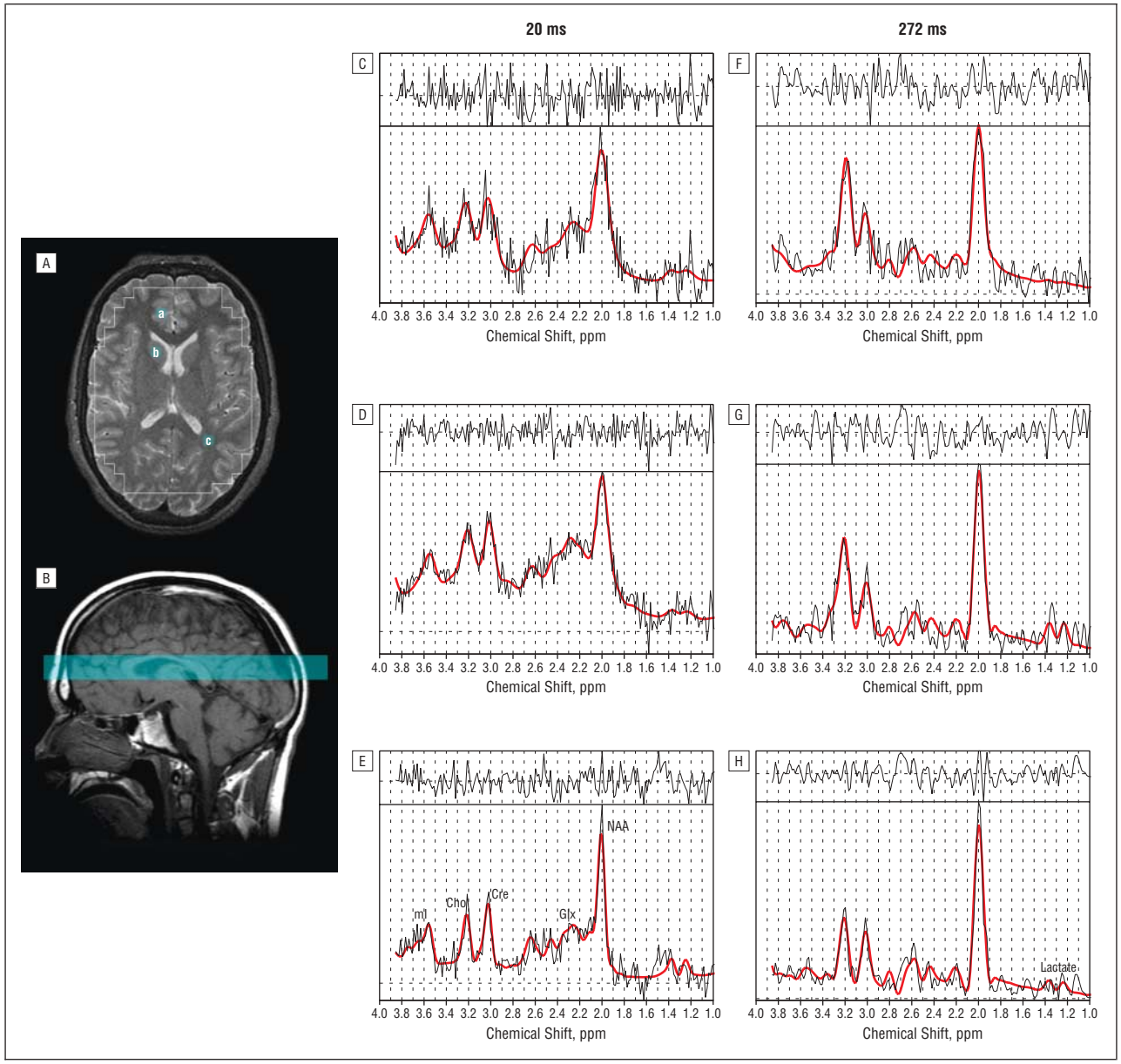

Figure 1. Central sagittal prescription image (B) with the proton echo-planar spectroscopic imaging (PEPSI) axial slab (20-mm thickness) overlaid in blue. The central PEPSI slab axial T2-weighted image (A) shows valid spectral voxels outlined in white; anatomic voxel locations for right anterior cingulate (a), right caudate nucleus (b), and left parietal white matter (c) are also identified. Short-echo (20-millisecond [ms]) (C-E) and long-echo (272-ms) (F-H) spectra corresponding to these voxel locations are shown; raw data are visible as the black fine line in the lower panel of each spectrum and the LCModel spectral fit is in red (baseline corrected), with the fit residual shown in the top panels. Metabolites measured at 20 ms: *myo*-inositol (mI); choline-containing compounds (Cho); creatine and phosphocreatine (Cre); glutamate, glutamine, and γ-aminobutyric acid (Glx); and *N*-acetyl aspartate (NAA). Metabolites measured at 272 ms: lactate.

putamen left and right, thalamus left and right, insula left and right, parietal white matter left and right, and midline occiput (**Figure 3**). A rater (S.D.F.) blinded to patient diagnosis recorded the voxel position for each ROI into a text template, and a MATLAB script was used to extract concentration data by region into a tabulated text file. For nuclei, the voxel chosen was centered on the anatomic target structure; if no corresponding voxel could be selected, a missing value was entered. In other larger anatomic regions, ROIs were averaged across contiguous voxels to maximize signal-to-noise ratio.

STATISTICAL ANALYSIS

Stepwise multivariate linear regression using SPSS statistical software version 11 (SPSS Inc, Chicago, Ill) assessed interrelationships between categorical variables of psychiatric diagnosis and clinical subtype diagnosis with continuous predictor variables of chemical concentrations (20 milliseconds for Cho, Cre, NAA, mI, and Glx; 272 milliseconds for lactate). Chemical T2r (Cho, Cre, and NAA) was separately modeled. Regional chemical composition by diagnosis was evaluated using independent *t* tests. For BD patients, mood state rating scales in relationship to chemical measures were evaluated using the Pearson *r*. Significance was fixed at a 2-tailed α of .05. Bonferroni correction for ROI analyses was applied by dividing the α level by number of regions assessed (adjusted $\alpha = .003$) and for mood assessment by number of gray-white chemical measures (adjusted $\alpha = .004$). Values are presented as mean ± SD. Regional and diagnostic subgroup analyses by mood were not performed because of power considerations.

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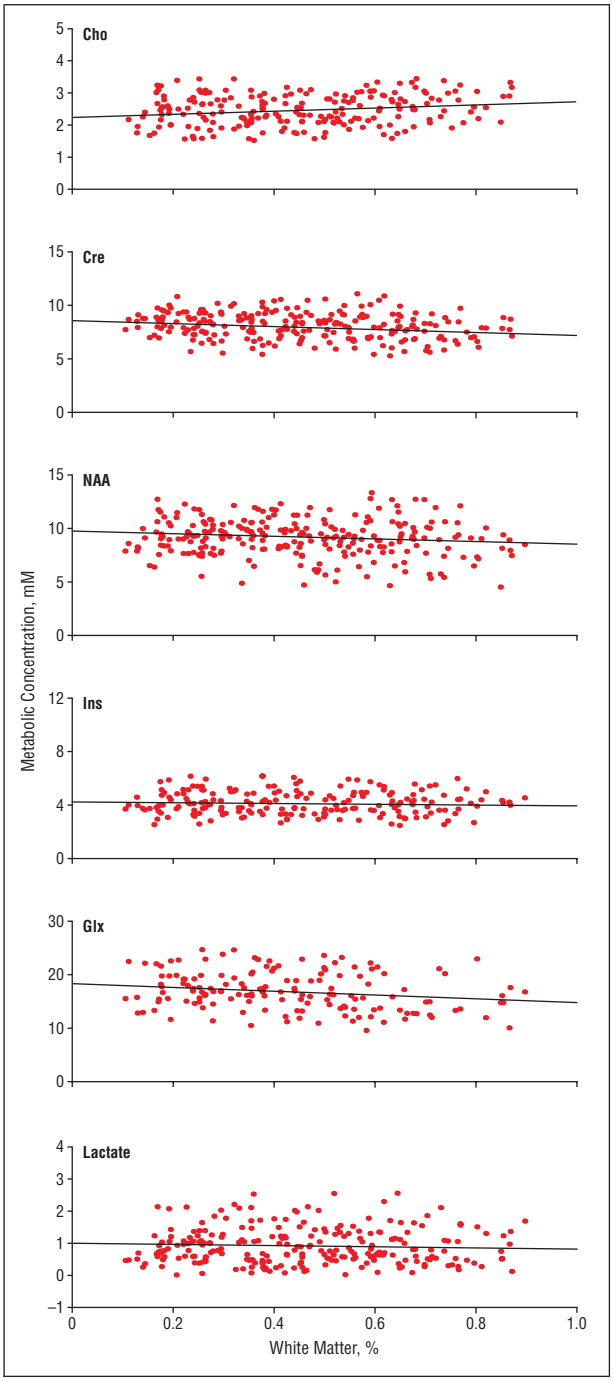

Figure 2. For an individual patient with bipolar disorder, metabolite concentration arrays are plotted relative to the percentage of white matter in each voxel. Regression lines, shown in red for each metabolite, illustrate chemical distribution by tissue type. Cho indicates choline-containing compounds; Cre, creatine and phosphocreatine; NAA, *N*-acetyl aspartate; Ins, inositol; and Glx, glutamate, glutamine, and γ-aminobutyric acid (Glx).

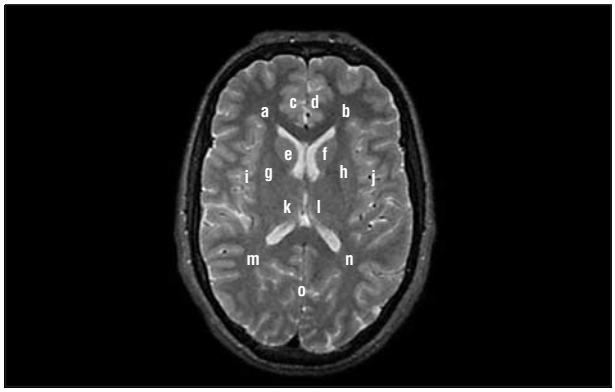

Figure 3. The central axial prescription image shows anatomic locations for regions-of-interest (ROI) selection. a and b indicate frontal white matter right and left; c and d, cingulate right and left; e and f, caudate right and left; g and h, putamen right and left; i and j, insula right and left; k and l, thalamus right and left; m and n, parietal white matter right and left; and o, occiput.

RESULTS

Brain chemical concentrations by tissue type are given for each diagnostic group in **Table 1**. A significant model observed for gray matter chemical measures and diagnosis (BD patients vs HCs) ($F_{2,51}$=8.34, P=.001) was best predicted by elevated lactate (19.75% increase, β=.37, t=2.96, P=.005) and Glx levels (10.01% increase, β=.35, t=2.83, P=.007) in BD patients; other gray matter chemicals were nonsignificant within the model (β<.28, t≤1.07, P ≥.32 for all). The model for white matter chemicals and diagnosis did not reach statistical significance ($F_{1,51}$=3.61, P=.06). The models for diagnosis and gray matter T2r ($F_{1,51}$=1.06, P=.31) or white matter T2r ($F_{1,51}$=3.59, P=.07) also did not reach statistical significance. Similarly, the models for diagnosis and chemical regression slopes ($F_{1,51}$=3.12, P=.08) (Table 1) or T2r regression slopes ($F_{1,51}$=0.30, P=.59) did not reach statistical significance.

Brain chemical ROI relationships were assessed (Figure 3). Chemical alterations included increased Glx levels in the left cingulate (t_{39}=2.09, P=.04) and increased Cho levels in the left caudate (t_{37}=2.71, P=.01) in BD patients. The BD group also exhibited increased Cho levels in the right putamen (t_{50}=2.43, P=.02), increased NAA levels in the left putamen (t_{52}=3.57, P=.001), and increased Glx levels in the left insula (t_{51}=3.32, P=.002). After Bonferroni correction for the multiple regions compared (adjusted α=.003), only putamen and insula findings remained statistically significant.

The BD patients differentiated into BDI and BDII clinical subtypes were further evaluated for gray and white matter chemical differences (**Table 2**). Comparing BDI patients and HCs, a significant model for gray matter chemicals ($F_{1,33}$=12.24, P=.001) was predicted by elevated lactate levels in the BDI subgroup (31.45% increase, β=.53, t=3.50, P=.001); other gray matter chemicals did not significantly contribute to the model (β<.19, t≤1.25, P≥.22 for all). No significant model for white matter chemicals and BDI vs HC diagnosis was found ($F_{1,33}$=0.59, P=.74). There were also no statistically significant models for gray or white matter T2r and diagnosis (F<1.23, P>.27 for all). Modeling chemical regression slopes, a significant model ($F_{1,33}$=6.39, P=.02) was predicted by an increased lactate white-gray regression slope in BDI patients (0.06±0.50 vs –0.35±0.51, β=.41, t=2.53, P=.02).

Comparing BDII patients and HCs, a statistically significant model for gray matter chemicals ($F_{2,40}$=7.13, P=.002) was predicted by elevated Glx levels (13.97% increase, β=.47, t=3.29, P=.002) and trend elevated lactate levels (11.3% increase, β=.24, t=1.70, P=.10) in the

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Table 1. Brain Chemical Concentrations by Tissue Type*

	Cho	Cre	NAA	mI	Glx	Lactate
Gray matter chemical concentration, mM						
Bipolar	2.52 (0.66)	9.41 (0.74)	10.60 (1.26)	4.59 (0.98)	17.80 (2.51)†	0.97 (0.24)†
Control	2.30 (0.26)	9.11 (0.73)	10.18 (0.43)	4.36 (0.50)	16.18 (1.85)	0.81 (0.16)
White matter chemical concentration, mM						
Bipolar	2.59 (0.35)	7.98 (0.84)	9.76 (0.89)	4.50 (0.62)	15.69 (2.60)	0.97 (0.25)
Control	2.49 (0.28)	7.58 (0.63)	9.77 (0.44)	4.25 (0.75)	14.51 (1.99)	0.91 (0.23)
White-gray matter chemical regression slope						
Bipolar	−0.10 (1.67)	2.26 (1.07)	1.31 (2.53)	−0.08 (1.45)	2.89 (2.79)	−0.21 (0.60)
Control	−0.39 (0.52)	2.13 (1.13)	0.35 (0.87)	−0.14 (1.03)	2.24 (2.46)	−0.35 (0.52)

Abbreviations: Cho, choline-containing compounds; Cre, creatine and phosphocreatine; Glx, glutamate, glutamine, and γ-aminobutyric acid; mI, *myo*-inositol; NAA, *N*-acetyl aspartate.
*Values are given as mean (SD).
†$P<.01$ vs control.

Table 2. Gray and White Matter Chemical Differences*

	Cho	Cre	NAA	mI	Glx	Lactate
Gray matter chemical concentration, mM						
Bipolar I	2.29 (0.32)	9.27 (0.69)	10.30 (0.60)	4.39 (0.80)	17.00 (2.19)	1.06 (0.24)†
Bipolar II	2.66 (0.79)	9.47 (0.80)	10.80 (1.55)	4.73 (1.09)	18.44 (2.56)†	0.90 (0.22)‡
Controls	2.30 (0.26)	9.11 (0.73)	10.18 (0.43)	4.36 (0.50)	16.18 (1.85)	0.81 (0.16)
White matter chemical concentration, mM						
Bipolar I	2.55 (0.37)	7.71 (0.49)	9.57 (0.88)	4.30 (0.62)	14.95 (2.59)	0.88 (0.33)
Bipolar II	2.61 (0.36)	8.16 (0.99)	9.84 (0.93)	4.68 (0.55)	16.20 (2.63)§	1.02 (0.19)
Controls	2.49 (0.28)	7.58 (0.63)	9.77 (0.44)	4.25 (0.75)	14.51 (1.99)	0.91 (0.23)

Abbreviations: Cho, choline-containing compounds; Cre, creatine and phosphocreatine; Glx, glutamate, glutamine, and γ-aminobutyric acid; mI, *myo*-inositol; NAA, *N*-acetyl aspartate.
*Values are given as mean (SD).
†$P<.01$ vs control.
‡$P=.10$ vs control.
§$P<.05$ vs control.

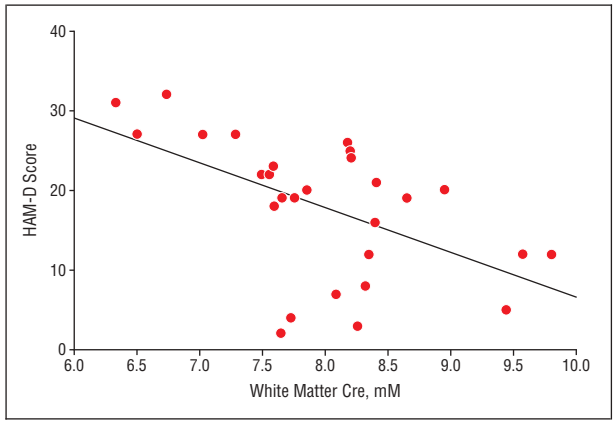

Figure 4. The relationship between white matter concentrations of creatine and phosphocreatine (Cre) and the Hamilton Depression Rating Scale (HAM-D) scores is shown, demonstrating an inverse relationship.

BDII subgroup; other gray matter chemicals did not contribute to the model ($\beta<.11$, $t\leq0.69$, $P>.49$ for all). A significant model for BDII compared with HCs for white matter chemicals ($F_{1,40}=5.47$, $P=.03$) was predicted by elevated Glx levels in the BDII subgroup (11.6% increase, $\beta=.35$, $t=2.34$, $P=.03$); other white matter chemicals did not contribute to the model ($\beta<.23$, $t<1.39$, $P>.17$ for all). Additionally, significant models observed for gray matter T2r ($F_{1,40}=5.27$, $P=.03$) were predicted by shortened Cho T2r (decreased 15.1% from HCs, $\beta=.35$, $t=2.30$, $P=.03$) and white matter T2r ($F_{1,40}=4.13$, $P=.05$) predicted by shortened Cho T_{2r} (decreased 9.9% from HCs, $\beta=.31$, $t=2.03$, $P=.05$) in BDII patients. The model for white-gray regression slope and BDII vs HC diagnosis did not reach statistical significance ($F_{1,40}=3.21$, $P=.08$).

Clinical mood state of BD patients at time of scanning was evaluated in relationship to averaged gray-white matter chemical measures using the Pearson *r*. An inverse correlation with HAM-D scores was found for white matter Cre ($r_{28}=-0.55$, $P=.003$); no significant interactions were observed between HAM-D scores and other gray or white matter chemicals ($r<.33$, $P>.09$). The YMRS scores were inversely correlated with mI levels both in gray matter ($r_{28}=-0.46$, $P=.01$) and white matter ($r_{28}=-0.42$, $P=.03$); no significant relationships were observed for other gray or white matter chemicals ($r<.36$, $P\geq.06$). After Bonferroni correction for the number of gray and white matter chemical measures (adjusted $\alpha=.004$), a significant interaction between white matter Cre and HAM-D scores remained (**Figure 4**).

COMMENT

Medication-free BD patients demonstrated elevated lactate and Glx levels in gray matter compared with the HC

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group; other gray-white matter chemical concentrations, chemical T2r measures, and chemical regression slopes between white and gray matter were not significantly different between groups. The BDI patients demonstrated elevated gray matter lactate and altered lactate tissue distribution that was shifted toward gray matter compared with HCs; the BDII patients exhibited elevated Glx and trend elevated lactate levels in gray matter, as well as elevated white matter Glx levels. The BDII patients additionally demonstrated shortening of Cho T2r in both gray and white matter. Chemical differences for BDI and BDII patients may reflect intrinsic diagnostic differences or illness severity, because BDI patients had higher HAM-D and YMRS scores. A significant inverse correlation also was found for white matter Cre and HAM-D scores in BD patients. We believe that this is the first report of elevated gray matter lactate levels in BD. Several recent single-voxel ^{1}H-MR spectroscopy studies of BD also have reported elevated Glx levels.[51,52] Lactate levels in those studies were not reported because of characteristics of the acquisition parameters and signal-to-noise ratio considerations.

Although our line-fitting analyses fit the separate components of Glx, we did not report individual values because of the substantial overlap of these multiplets at 1.5 T. Thus, we cannot identify what component of Glx was elevated in BD patients. However, findings of decreased Glx measured in the anterior cingulate cortex of medicated major depressive disorder patients have been suggested to primarily reflect lowered glutamate levels.[53] Future investigation using higher-field scanners may improve deconvolution of the Glx spectral peaks and better address limitations of spectral resolution at 1.5 T.

Other brain chemicals and T2r measures in both gray and white matter were not significantly different between diagnostic groups. White-gray regression slopes for chemical distributions between tissues are compatible with prior work[42-44] and did not differentiate diagnostic groups, although BDI patients demonstrated an increased slope for lactate distribution between white and gray matter. Regional findings, although somewhat overlapping with the anatomic distribution found in previous work,[38,53-56] should be interpreted conservatively. Further studies would be necessary to confirm regional Glx and NAA elevations observed in this BD sample.

Investigations of mood disorders using single-voxel ^{1}H-MR spectroscopy have largely tested hypotheses of Cho alterations wherein both regional elevations[39,56,57] and decreased Cho levels[38,40,58] have been reported in association with depressed mood state. In our study, tissue-specific Cho levels were not found to be altered in relationship to either BD diagnosis or mood state. We also did not find evidence of tissue-specific or regional NAA reductions that have been reported in association with BD and perhaps more specifically with psychosis.[52,59-61] Variable findings reported in the literature may reflect differences in clinical populations, medication status, brain regions studied, or MR spectroscopy acquisition parameters that, in the setting of disease state–related T2 changes, as found for Cho in this study, could substantially influence chemical concentration estimates.

In this study, mood-related findings of an inverse correlation for white matter Cre with HAM-D scores are comparable with prior work that found decreased phosphocreatine/Cre levels in association with depression.[62,63] In this sample, the predominantly depressed or mixed-mood state of the BD patients limits any relationship being identified between manic symptoms and chemical alterations. A growing MR spectroscopy literature supports the presence of brain metabolic alterations in BD with variable relationships to mood state. Initial MR spectroscopy studies of BD used phosphorus P31 (^{31}P)–labeled MR spectroscopy to evaluate brain energy and phospholipid metabolism.[37] A meta-analysis of 8 ^{31}P-MR spectroscopy studies provides support for state-specific alterations of phospholipid membranes and high-energy phosphates in BD that primarily reflect increased phosphomonoester levels and decreased phosphocreatinine levels in the depressed state.[54] Decreased phosphomonoester levels also have been observed in euthymic BDI patients, although not BDII patients, compared with HCs.[62] More generally, findings of decreased β and total nucleotide triphosphate (primarily composed of adenosine triphosphate) provide evidence for abnormal brain energy metabolism in mood disorders.[55,64] Because brain intracellular pH determined by ^{31}P-MR spectroscopy is found to be decreased in medication-free BD patients in both manic and depressed mood states, as well as in euthymic BD patients stabilized with lithium treatment, metabolic aberrations may be intrinsic to the underlying pathophysiology of BD.[65,66] Findings of decreased frontal lobe intracellular pH in BD patients,[65] consistent with lactate acidosis, are postulated by those investigators to reflect underlying mitochondrial dysfunction.[66]

It is possible that lactate elevations observed in this study could reflect nonspecific manifestations of state-dependent anxiety, although participants in our study did not report anxiety or any particular discomfort with being in the scanner. Despite substantial baseline anxiety, we also do not typically observe elevated baseline lactate levels in patients with panic disorder about to undergo a lactate infusion designed to induce panic.[47,50,67,68] However, the diagnostic specificity of lactate and Glx findings remains uncertain. In a study using essentially the same methods, we observed no alterations in either lactate or Glx in young children with autism.[34] On the other hand, elevated lactate levels have also recently been found in association with first-break psychosis.[69]

Our findings of elevated gray matter lactate levels in the BD patients suggest a shift in redox state from oxidative toward glycolytic energy utilization. Although its role in brain metabolism and homeostasis remains unresolved,[70,71] earlier considerations of lactate being solely the by-product of anaerobic metabolism have been substantially modified by more recent work suggesting an important role for lactate in brain bioenergetics.[72-74] Normal neuronal activation requires that some fraction of the energy expended comes from glycolysis. During rapid neuronal firing, coupled to increased extracellular glutamate levels, approximately one third of energy expenditure is estimated to be contributed via glycolysis and increased lactate production.[75] It is conceivable that in-

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creased or more sustained rapid neuronal firing, possibly reflecting neuroexcitatory effects of increased extracellular glutamate, with a resultant increased fraction of energy utilization via glycolysis, could explain the elevated gray matter lactate levels observed in BD patients. Because hypoperfusion is among the more consistent brain metabolic alterations found in BD,[14,15,17] this factor could also contribute to, or account for, a shift in redox state toward glycolysis. Alternatively, or in conjunction with these considerations, recent in vivo evidence supports an association between elevated brain lactate and Glx levels and cyanide-induced inhibition of mitochondrial oxidative metabolism; mechanisms underlying brain glutamate increases are postulated to reflect alterations in the release and reuptake of glutamate that are dependent on intact oxidative metabolism.[76]

Elevated brain lactate is considered to be a sensitive marker for mitochondrial compromise,[77] but severe clinical manifestations (eg, MELAS [mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke] syndrome) also are variably associated with reduced NAA levels.[78] Although we did not find evidence for reduced NAA in BD patients, this might reflect the lesser severity of mitochondrial compromise speculated to account for lactate and Glx level elevations observed in our sample. The spectrum of clinical and metabolic manifestations of mitochondrial dysfunction may reflect variable loading of mitochondrial DNA polymorphisms, some possibly linked to BD,[79] that exhibit differing threshold response to oxidative stress.[80] Phospholipid membrane alterations found in association with BD may also interfere with mitochondrial function, just as mitochondrial dysfunction can alter phospholipid metabolism.[81-84]

Considerations regarding the possible role of mitochondrial compromise in BD have important treatment implications; for example, evidence of the possible neuroprotective properties of lithium,[35,36,41] also observed under conditions of elevated glutamate levels or ischemia, are hypothesized, in part, to be mediated through bcl-2 elevation, a neuroprotective protein that also has mitochondrial membrane-stabilizing effects.[85] Although the demonstration of causal relationships remains challenging, the present work suggests subtle alterations of energy metabolism in BD.

To summarize, gray matter lactate and Glx level elevations in medication-free BD patients suggest altered cellular energy metabolism. These chemical alterations, suggestive of a redox shift toward glycolysis, may reflect a generalized pattern of compromised mitochondrial metabolism in BD.

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