

Abnormal Brain Bioenergetics in First-Episode Psychosis

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Background: Converging evidence indicates impaired brain energy metabolism in schizophrenia and other psychotic disorders. Creatine kinase (CK) is pivotal in providing adenosine triphosphate in the cell and maintaining its levels when energy demand is increased. However, the activity of CK has not been investigated in patients with first-episode schizophrenia spectrum disorders. **Methods:** Using *in vivo* phosphorus magnetization transfer spectroscopy, we measured CK first-order forward rate constant (k_f) in the frontal lobe, in patients with first-episode psychosis (FEP; $n = 16$) and healthy controls ($n = 34$), at rest. **Results:** CK k_f was significantly reduced in FEP compared to healthy controls. There were no differences in other energy metabolism-related measures, including phosphocreatine (PCr) or ATP, between groups. We also found increase in glycerol-3-phosphorylcholine, a putative membrane breakdown product, in patients. **Conclusions:** The results of this study indicate that brain bioenergetic abnormalities are already present early in the course of schizophrenia spectrum disorders. Future research is needed to identify the relationship of reduced CK k_f with psychotic symptoms and to test treatment alternatives targeting this pathway. Increased glycerol-3-phosphorylcholine is consistent with earlier studies in medication-naïve patients and later studies in first-episode schizophrenia, and suggest enhanced synaptic pruning.

Key words: psychosis/phosphorus magnetic resonance spectroscopy/creatine kinase/mitochondria/energy metabolism/membrane phospholipids

Introduction

A growing body of evidence indicates abnormalities in energy metabolism and related mitochondrial functions in schizophrenia (SZ)¹: Specific mitochondrial DNA

variants and haplogroups are associated with SZ diagnosis²; mitochondrial density and structure, and expression and activity of oxidative phosphorylation components are altered, in several brain regions³⁻⁵; metabolic rate of glucose is reduced in the frontal lobe^{6,7}; lactate is increased in the cerebrospinal fluid^{8,9} and in the medial-prefrontal cortex,¹⁰ suggesting a shift from mitochondrial oxidation to glycolysis; ratio of oxidized to reduced nicotinamide adenine dinucleotide (NAD⁺/NADH) is reduced in the frontal lobe,¹¹ possibly due to reduced electron transport chain activity¹²; finally, abnormal mitochondrial function is also evident in the periphery, such as in platelets and lymphocytes.^{13,14} While the etiology of bioenergetic abnormalities is unclear, it is proposed that genetic susceptibility, aberrant dopaminergic activity, and exposure to factors such as substances may play a role.^{12,15} Different aspects of mitochondrial biology can be modified by agents that are investigational or readily available.¹⁶ For example, a recent meta-analysis found N-acetylcysteine, a precursor of the antioxidant glutathione, effective as an adjunct treatment in improving negative and total Positive and Negative Symptom Scale (PANSS) scores.¹⁷ However, currently, there are no routinely used pharmacological treatments targeting brain bioenergetics in SZ.

The brain is the most metabolically active organ and integrity of energy metabolism is required to sustain its fundamental activities, such as synaptic signaling and neuronal plasticity.¹⁸ In addition to generating ATP, mitochondria have other critical interacting functions, including regulation of reactive oxygen species and redox balance.¹⁹ It is also involved in the physiology of multiple neurotransmitter systems relevant for schizophrenia, including dopamine, glutamate, and serotonin.¹² Finally, healthy brain development is dependent on mitochondrial activity.²⁰

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Therefore, abnormalities in mitochondrial function and bioenergetics likely contribute to the pathophysiology of SZ spectrum and other psychotic disorders. However, more work is needed to identify which specific mechanisms are affected. Studying bioenergetic parameters in individuals experiencing a first episode of psychotic disorder provides an important window for this purpose, by eliminating the complex effects of chronic illness and long-term medication use.

Neurons largely rely on oxidative phosphorylation to produce adenosine triphosphate (ATP). ATP production in the mitochondria is coupled to the reversible creatine kinase (CK) reaction, which transfers the high energy phosphate (HEP) to creatine (Cr) to generate phosphocreatine (PCr). PCr is a smaller and more inert molecule and it diffuses throughout the cytosol to create a local HEP reserve, from which ATP is regenerated via reversal of the CK reaction. Thus, PCr/CK connects ATP production to the site of ATP utilization and provides a rapidly accessible HEP buffer to maintain stable ATP levels in the face of fluctuations in energy needs.^{21,22}

Phosphorus magnetic resonance spectroscopy (³¹P-MRS) allows examining brain HEP metabolism *in vivo*. Existing ³¹P-MRS studies in SZ measure only steady-state levels of ATP and PCr, and report inconsistent findings, with most studies reporting no change while others finding increases or decreases.²³ While discrepancies can be due to differences in sample characteristics and quantification methods, a major limitation of this approach is that steady-state levels of metabolites do not necessarily reflect the dynamics of their metabolism. Indeed, studies using stimulation paradigms that increase regional brain activity or model short- or long-term pathological conditions show that CK rate can significantly shift without any apparent change in ATP or PCr levels.^{24–28} This limitation can be overcome by phosphorus magnetization transfer magnetic resonance spectroscopy (³¹P-MT-MRS), which allows measuring CK rate and therefore gleaning into the exchange between HEP metabolites. In a series of recent ³¹P-MT-MRS studies, we reported that CK rate was reduced at rest in the frontal lobe in patients with chronic SZ and first-episode bipolar disorder (BD) with psychotic features.^{29,30} These results were consistent with another study from our group where individuals with BD with psychotic features failed to maintain ATP levels during increased regional brain activity.³¹

In this study, we investigated CK rate in the forward (ATP producing) direction (k_f) in the frontal lobe, in a well-characterized sample of patients with a first episode of schizophrenia spectrum disorders (first-episode psychosis; FEP), and matched healthy controls (HC), using ³¹P-MT-MRS. We hypothesized that, consistent with our previous studies, CK rate would be reduced in FEP. We also explored changes in the levels of phosphorus

metabolites involved in energy metabolism, as well as phospholipid membrane turnover.²³

Methods

Participants

The sample included 17 first-episode patients with a DSM-IV diagnosis of SZ ($n = 5$), schizoaffective disorder (SZA; $n = 5$), psychotic disorder—not otherwise specified (PSY-NOS; $n = 7$) and 50 healthy controls (HC). Patients were recruited from McLean OnTrack, an outpatient FEP treatment service at McLean Hospital.³² All except 5 patients (1 SZ, 1 SZA, and 3 PSY-NOS) were using psychiatric medications at the time of the scan. Medication information was missing for one participant. FEP at the time of entry to McLean OnTrack was defined as having an onset of overt psychotic symptoms within the past 1 year and no more than 1 lifetime psychiatric hospitalization. All patients were between the ages of 18 and 30, had no significant past or current neurological or medical disorders, intellectual disability, a history of head trauma with loss of consciousness, or history of ECT within the last 3 months. We also excluded individuals using supplements that affect HEP levels, such as creatine. The HC met the same criteria, and in addition, they had no personal or first-degree relative history of any psychiatric disorders. Three HC and 1 patient were removed from the initial sample because of low signal-noise ratio (SNR) in the MRS data (<15). In the remaining HC sample, when compared to patients, the proportion of females was significantly higher ($\chi^2 = 7.37$, $P = .007$) and age was non-significantly higher. Blind to the pattern of results, we removed the oldest female HC participants to attain a $P = .2$ as an arbitrary threshold in the chi-square test that compared sex ratios between groups. The final sample included 34 HC. Diagnostic groups were also matched for age (table 1).

This study was approved by the McLean Hospital IRB. All participants provided written informed consent. Diagnostic assessments were carried out using the Structured Clinical Interview for DSM-IV for patients and healthy controls. The PANSS, the Young Mania Rating Scale (YMRS), the Montgomery-Asberg Depression Rating Scale (MADRS), and Multnomah Community Ability Scale (MCAS) were used for clinical measures. The sociodemographic and clinical characteristics of participants are presented in table 1.

Magnetic Resonance Imaging and ³¹P-MT-MRS

All MRI methodological details are identical to those in our previous publication.³⁰ Briefly, the ³¹P-MT-MRS study-related acquisitions were conducted using a 4 Tesla whole-body imaging system (Unity/Inova; Varian NMR Instruments) using a specially designed half-helmet surface coil with dual tuned proton and phosphorus

Table 1. Demographic and Clinical Characteristics of the Final Sample

	Healthy Controls (<i>n</i> = 34)	FEP Patients (<i>n</i> = 16)
Age (range)	22.1 ± 2.9 (18–28)	21.9 ± 2.2 (19–26)
Sex (M/F) ^a	24/10	14/2
Education ^{a,b} (y)	5.2 ± 1.5	4.1 ± 1.1
PANSS		
Positive	-	11.1 ± 6.4
Negative	-	14.5 ± 7.7
General	-	25.6 ± 10.5
YMRS	-	3.9 ± 4.0
MADRS	-	11.0 ± 7.0
MCAS	-	40.6 ± 13.4
Antipsychotic users ^c	-	9 (60%)
CPZ ^c equivalent	-	214.3 ± 267.3
Lithium users ^c users (%)	-	4 (26%)
Valproate ^c users	-	2 (13%)
Substance use disorder—current ^d	-	2 (13%)

Note: Values are mean ± SD. PANSS, Positive and Negative Symptom Scale; YMRS, Young Mania Rating Scale, MADRS, Montgomery-Asberg Depression Rating Scale, and MCAS, Multnomah Community Ability Scale; FEP, first-episode psychosis.

^a*P* < .05.

^bRange is 3 (high school graduate), 4 (some college), 5 (2-year college graduate), 6 (4-year college graduate), 7 (some graduate or professional school), and 8 (completed graduate or professional school).

^cMedication information was missing for one patient.

^dDenotes meeting symptomatic criteria in the last month.

frequency channels placed on the forehead. A rapid 2-dimensional gradient-recalled echo image was initially used to acquire structural images in 3 dimensions. This permitted rapid determination of the position of the participant; the individual was repositioned if necessary. Next, global shimming of water signal was performed with a criteria of water linewidth < 24 Hz. Anatomical images were acquired with a multi-spin echo sequence in both sagittal and axial directions with a resolution of 0.94 mm. The surface coil with a single loop ³¹P coil of 7 cm diameter and butterfly shaped ¹H coil of 10 cm diameter was placed on the frontal lobe. The ³¹P MRS localization was achieved by the excitation profile of ³¹P coil (inlet of figure 1). Excitation profile was 90.5 cm³, calculated as $\pi r^2 * l = \pi * (3 \text{ cm})^2 * 3.2 \text{ cm} = 90.5 \text{ cm}^3$, where *r* (3 cm) and *l* (3.2 cm) were FWHM of the radius and penetration depth, respectively. The localized shimming was performed with a voxel of 6 × 6 × 4 cm³. The ³¹P-MT pulse sequence and experimental design have been described previously^{33,34}. A pulse train with varied RF pulse amplitudes according to the B₁-insensitive train to obliterate signal (BISTRO) scheme³⁵ was used to saturate selectively the resonance peak of γ -ATP for measurement of the CK reaction rate constant. Saturation

time was controlled by varying the cycling number of the BISTRO pulse train. A 200- μ s hard RF pulse was used to excite the phosphate spins and its flip angle (nominal 90°) was adjusted to achieve optimal NMR signal. TR = 14 seconds, Spectral width = 5 kHz, average for each saturation time = 32, acquisition time = 52 minutes. The pseudo first-order forward rate constant (k_f , (s⁻¹)), and the flux (*F*), of the CK reaction were calculated as in previous publications.³⁰ k_f was determined using progressive saturation on the γ -ATP resonance at 7 time points (0, 0.48, 1.89, 3.78, 6.61, 8.50, and 12.28 seconds) (figures 1 and 2).

31P-MRS Data Processing

The data processing was performed using FID-A.³⁶ The initial 3 FID points were truncated to remove any signal with broad spectral features. With the removal of the initial 3 FID points, the pre-acquisition delay of the ³¹P MRS acquisition was 700 μ s. A gaussian function of 0.1 second (10 Hz) was applied to smooth the spectra without significantly reducing the resolution. Metabolites quantification was performed by the LCModel with the basis of ATP, PCr, 2,3-diphosphoglycerate (DPG), glycerol-3-phosphorylcholine (GPC), glycerol-3-phosphorylethanolamine(GPE), membrane phospholipid (MP), phosphorylcholine (PC), phosphorylethanolamine (PE) and inorganic phosphate (Pi), simulated using VeSPA³⁷ with Gaussian lineshape with the parameters in Table 1 of ref.³⁸ (figure 3). The LCModel control parameters follow the previous strategies in ¹³C³⁹ and ³¹P MRS³⁸ quantifications.

Since we acquired spectra in the absence of saturation at approximately full relaxation, we were able to quantify the steady-state ratios of phosphate compounds, expressed using an internal reference of γ -ATP. The fitting of k_f and *T*₁ was performed in MATLAB, using the PCr signals output by LCModel. Brain pH was estimated on the basis of the chemical shift difference in ppm between inorganic phosphate and PCr.⁴⁰ In addition, magnesium ion (Mg²⁺) concentrations, deduced from the chemical shift difference in ppm between PCr and β -ATP,⁴¹ were also calculated.

Voxel Segmentation

The volume fractions of different tissue types (gray/white matter and cerebrospinal fluid) were calculated by segmenting T₁—weighted images of the same participants acquired on a Siemens 3T scanner; the percentage of different tissue types was extracted from the MRS voxel by incorporating voxel information (voxel position, size, orientation).

Statistical Approach

All analyses were carried out using SPSS (V.21). Two-sample *t*-tests and chi-square tests were used to compare

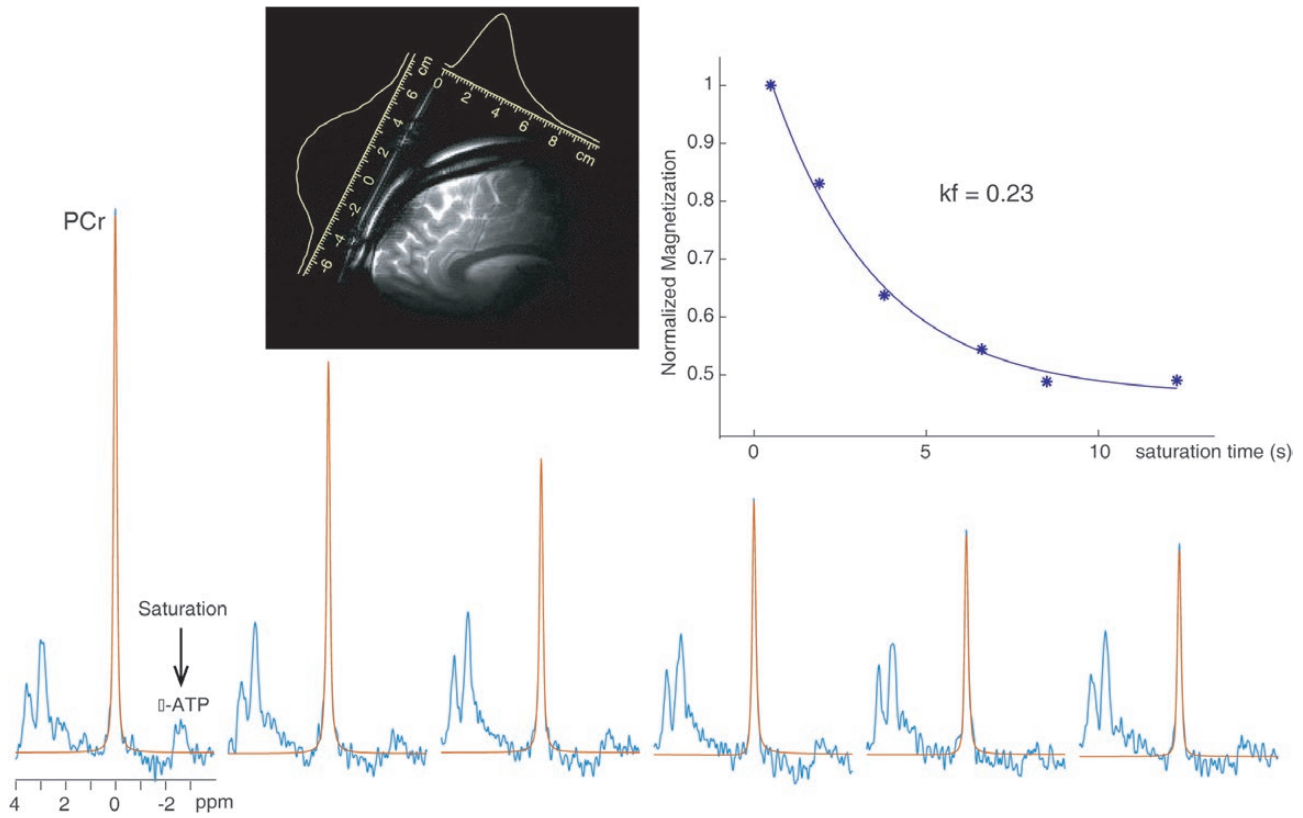


Fig. 1. Voxel position and representative phosphorus magnetization transfer magnetic resonance spectroscopy (^{31}P -MT-MRS) spectra depicting magnetization changes of phosphocreatine (PCr) (red line) with progressive saturation on γ -ATP resonance (saturation time varied from 0.48, 1.89, 3.78, 6.61, 8.50, and 12.28 s).

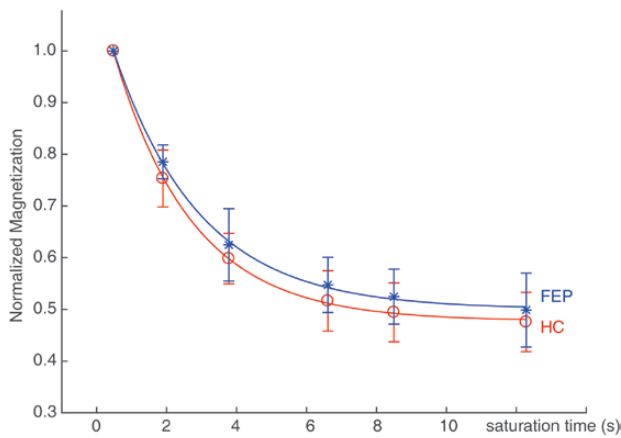


Fig. 2. Relative magnetization averages in the diagnostic groups during saturation at 0.48, 1.89, 3.78, 6.61, 8.50, and 12.28 s. Error bars are standard deviations.

demographic characteristics and linewidth as well as SNR of the PCr resonance for ^{31}P -MRS data (as a data quality measure) across groups.

Analysis of Primary Outcomes. Our primary outcome measure was CK k_f . We tested group differences in this dependent variable using a linear model with diagnosis as

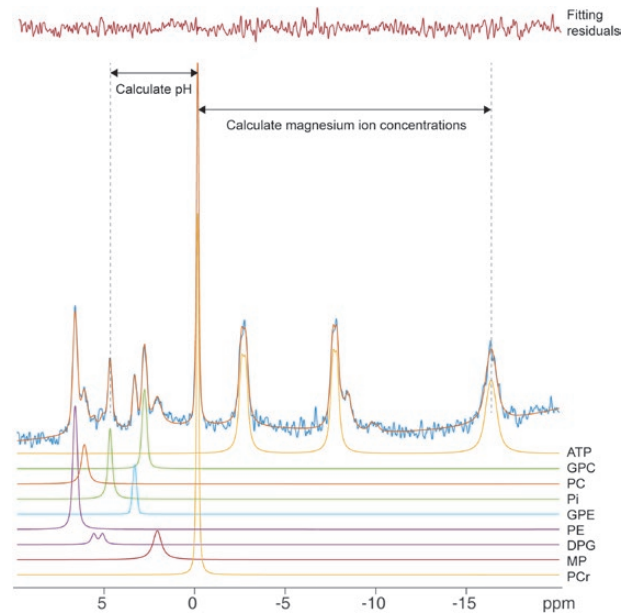


Fig. 3. Representative ^{31}P spectrum without saturation, and LCMoel fitting for the phosphorus metabolites. pH value and the concentration of magnesium ion were estimated by the chemical shift difference in ppm between inorganic phosphate (Pi) and phosphocreatine (PCr), and between PCr and β -ATP, respectively.

the predictor variable, controlling for the main effects of age and sex. A value of $P = .05$ was used as the threshold for statistical significance for our primary outcome.

Secondary Analyses. We next carried out similar secondary analyses with parenchymal pH, metabolite ratios PCr/ γ -ATP, Pi/ γ -ATP, DPG/ γ -ATP, GPC/ γ -ATP, GPE/ γ -ATP, MP/ γ -ATP, PC/ γ -ATP, PE/ γ -ATP, and CK flux and Mg²⁺ concentration, as the dependent variables. Thresholds for the remaining secondary analyses were set at $P = .005$ (0.05/10), controlling for multiple tests using a Bonferroni correction. Age and sex effects were controlled for in secondary analyses too.

We also explored the association of our primary outcome variable CK k_f and other ³¹P-MRS measures, with age, sex, education, chlorpromazine equivalent of antipsychotic medication dose (CPZ), and PANSS, YMRS, MADRS and MCAS scores, using Pearson and Spearman correlations, where appropriate. $P = .0004$ (0.05/120; ie, 12 ³¹P-MT-MRS measures correlated with 10 demographic and clinical measures) was used as the threshold for significance, controlling for multiple tests using a Bonferroni correction. Furthermore, to examine if medication status or diagnosis were associated with k_f and other MRS measures, we compared all MRS measures, between medicated and unmedicated patients and between patients with SZ and other diagnoses, using non-parametric tests.

Results

Participant Characteristics

Level of education collected as ordinal categories in SCID was higher in HC ($P = .02$) (table 1). There were no other differences in documented participant characteristics between diagnostic groups.

Table 2. Results of ³¹P-MT-MRS Measurements in the Final Sample

Measure	HC ($n = 34$)	FEP ($n = 16$)	F	P
Rate constant of creatine kinase, s ⁻¹ (k_f)	0.26 ± 0.06	0.22 ± 0.05	4.47	.04^a
Flux of creatine kinase, $\mu\text{mol/g/min}$	50.03 ± 10.32	43.83 ± 12.00	3.52	.07
PCr/ γ -ATP	1.18 ± 0.16	1.19 ± 0.17	0.01	.95
Pi/ γ -ATP	0.41 ± 0.09	0.41 ± 0.10	0.35	.56
DPG/ γ -ATP	0.12 ± 0.05	0.11 ± 0.02	0.32	.56
MP/ γ -ATP	0.35 ± 0.10	0.33 ± 0.14	0.37	.55
GPC/ γ -ATP	0.52 ± 0.09	0.59 ± 0.10	9.69	.003^b
GPE/ γ -ATP	0.19 ± 0.06	0.21 ± 0.06	2.04	.16
PC/ γ -ATP	0.30 ± 0.09	0.30 ± 0.09	0.01	.92
PE/ γ -ATP	0.77 ± 0.14	0.83 ± 0.09	2.32	.13
Intracellular pH	7.02 ± 0.01	7.02 ± 0.01	0.46	.50
Magnesium ion concentration, mmol/L	0.16 ± 0.01	0.15 ± 0.01	1.52	.22

Note: Values are mean ± SD. ³¹P-MT-MRS, phosphorus magnetization transfer magnetic resonance spectroscopy; FEP, first-episode psychosis; HC, healthy controls.

^a $P < .05$.

^b $P < .005$ (Bonferroni corrected)

³¹P MRS Data Quality

The mean (SD) quality control measures of SNR and the linewidth of the PCr resonance did not differ for the first acquired spectra between HC and FEP (SNR: HC = 29.2 ± 6.4 and FEP = 27.1 ± 8.8, $P = .3$; Linewidth: HC = 5.5 ± 1.3 Hz and FEP = 6.2 ± 2.1 Hz, $P = .2$).

CK k_f and Flux

Data from representative ³¹P-MT-MRS experiments are shown in figure 1 and the results are presented in table 2. The FEP group showed a statistically significant 15% reduction in CK k_f compared to HC. We also calculated the flux ($F = [\text{PCr}] \times k_f$) through this reaction. There was a 12% reduction in patients in this parameter, with a trend for statistical significance.

Other ³¹P-MRS Measures

GPC/ γ -ATP was elevated in patients compared to HC (table 2).

Additional Analyses

Relative ratios of gray matter (%GM), white matter (%WM) and cerebrospinal fluid (%CSF) were similar between groups: %GM (HC = 0.41 ± 0.3, FEP = 0.41 ± 0.4; $P = .93$), %WM (HC = 0.28 ± 0.2, FEP = 0.27 ± 0.3; $P = .08$), %CSF (HC = 0.28 ± 0.6, FEP = 0.26 ± 0.7; $P = .39$). When %GM/(%GM+%WM) was included as a covariate, CK k_f remained significantly different between groups ($F = 5.0$, $P = .03$). Flux was still not significantly different.

There were no significant correlations between MRS measures and demographics or scores of clinical scales.

No differences were found in the analyses that compared MRS measures between the patients with different medication status or diagnoses.

Discussion

In this study, we used ^{31}P -MT-MRS to measure CK enzyme rate and other phosphorus measures, in the frontal lobe of patients with FEP and HC. Our primary finding is a significant reduction in CK k_f in FEP. We also found increased GPC in patients.

CK k_f reduction in FEP is consistent with our previous MRS studies in chronic SZ and first-episode bipolar disorder with psychotic features,^{30,31,42} and confirms that this metabolic abnormality is common across psychotic disorders and presents early in the illness.⁴² It is also in agreement with post-mortem studies that found reduced CK activity and immunoreactivity⁴³ and reduced CK brain isoenzyme content in SZ⁴⁴ in SZ, and reduced mRNA expression in BD.⁴⁵ This abnormality is significant, because CK is pivotal in neuronal energy metabolism, and energy is required for critical functions, including maintaining Na^+ and Ca^{2+} gradients, neurotransmission (synthesis, release, and reuptake), intracellular signaling, and axonal or dendritic transport.⁴⁶ Brain development and neuroplasticity also depend on these processes, and have high energy requirements.^{47,48} In addition, studies examining CK specifically find that, its activity is correlated with brain activity^{24,33,49}; it is located in association with synaptic vesicles and synaptic plasma membranes⁵⁰; CK flux and expression are associated with specific stages in brain development^{51,52}; and CK knockout mice display abnormal hippocampal morphology and learning.⁵³ Therefore, taken together, it is plausible that reduced CK activity contribute to a range of brain abnormalities and cognitive outcomes, in psychotic disorders. Available evidence does not allow us to conclude if this reduction is a primary driving factor in the pathophysiology of the illness or secondary to other altered parameters in energy-related processes. However, given that CK activity is correlated with brain activity, coupled to oxidative phosphorylation,⁵⁴ and is inhibited by oxidative damage,²¹ reduced CK complements the bioenergetic state in SZ, which is characterized by impaired mitochondrial energy production, lower brain metabolic rate, and increased oxidative stress.^{1,55,56} Regardless of the direction of causality, given the role of PCr/CK system in maintaining ATP, reduced CK k_f in psychotic disorders indicates a susceptibility for failing to meet energy requirements. It is plausible that this susceptibility is unmasked with challenges to brain activity, eg, by use of substances that impair mitochondrial activity^{57,58} or substantial increases in energy demand within a short timeframe. We found evidence supporting the latter in a recent study, where patients with BD failed to maintain ATP levels in the occipital lobe during visual stimulation.³¹

CK flux and pH were not different from HC in FEP, although there was a trend for reduced flux in patients. These results are in contrast to the reductions

we previously reported in both measures in chronic SZ.³⁰ However, normal pH is consistent with the rest of the ^{31}P MRS studies in the literature, in SZ.¹⁰ Taken together, these findings suggest the possibility of progressive impairments in energy metabolism, with a shift to glycolysis later in the illness.¹⁵ This hypothesis can be tested in a large enough sample that includes first-episode patients and patients with a range of duration of illness.

PCr/ γ -ATP was not different in patients compared to HC. This is consistent with most ^{31}P MRS studies in SZ that measure this ratio or separate metabolite levels, in varying samples and brain regions.²³ In FE SZ in the frontal lobe, out of 5 studies, only one reports PCr increase, and 2 report increase in ATP. We also did not find difference in PCr/ATP in our previous ^{31}P -MT-MRS study in chronic SZ, despite reduction in CK k_f in patients.³⁰ Enzyme rate constant (k) reflects intrinsic properties of the enzyme and is independent of concentrations of the metabolites. It is possible that a change in CK k_f could also be associated with a change in ATP and PCr but, as the literature summarized in the Introduction show, that is not necessarily expected.

We found significantly increased GPC in FEP. GPC and GPE are phosphodiesterases (PDE), which are believed to be freely mobile membrane phospholipid breakdown products.⁵⁹ Our results add to the body of evidence that shows increased PDE in the frontal lobe in early SZ.⁶⁰⁻⁶⁴ As we have recently summarized,²³ increased PDE along with reduced PME in the frontal lobe in medication-naïve patients is one of the earliest findings pertaining to the membrane phospholipids in SZ.⁶⁰ Increased PDE is associated with synaptic pruning in the context of brain maturation.⁶⁵ Therefore, this finding was interpreted as reflecting altered timing or abnormal enhancement of normal synaptic pruning in SZ.⁶⁰ A recent meta-analysis of ^{31}P MRS studies in SZ found elevated PDE in the temporal lobe, and elevated PME in the frontal lobe regions.⁶⁶ Our findings contradict with the result of this meta-analysis. Heterogeneity in the literature may be related to several factors, including clinical characteristics, such as illness duration and illness stage. Indeed, using the same methods, we found reduced PDE in chronic SZ,³⁰ suggesting that membrane metabolism may also vary during the course of the illness.

The present study has several limitations. First, our sample size is modest. Nevertheless, given the challenge of recruiting patients with FEP and the fact that there are no studies that have investigated CK activity in these patients, our study represents a significant contribution. Second, we cannot exclude the contribution of medications to the results. However, the literature is inconclusive in terms of the direction of antipsychotic effects on brain bioenergetics in general, and specifically on CK (see Yuksel et al³¹ for a brief summary). A substantial portion of the patient sample (5/16)

were medication-free, and the rest had been using medications for a short amount of time. CK k_f was similar between medication-free patients and those taking medications ($P = .51$). Furthermore, our analysis did not reveal any correlation of CPZ equivalent of antipsychotic dose with bioenergetic measures. Moreover, it is reassuring that the findings from the present study are consistent with those we previously found (k_f reduction) in first-episode BD patients,²⁹ who were using antipsychotics at a considerably lower dose. Besides, we have previously reported bioenergetic abnormalities in unmedicated individuals at high risk for developing psychotic disorders, showing a familial predisposition to these alterations outside of medication effects.⁶⁷ A third important limitation is that we did not quantify absolute concentrations of metabolites, because it is time-consuming and technically challenging. Instead, we used ATP as the internal reference because we were interested in biologically relevant ratios, such as PCr/ATP and Pi/ATP. Among the available ATP peaks, we picked γ -ATP, because β -ATP peak is broader and subject to more frequency offset, both of which can affect quantification accuracy. This is not ideal, because there is concern that ATP levels may be abnormal if HEP metabolism is impaired. However, the literature does not suggest that there is change in brain ATP in SZ and the pattern of our findings (only change in GPC/ATP) suggests no major abnormalities in ATP. In addition, γ -ATP concentration does not affect our primary finding, CK k_f , and other measures, including Mg²⁺ and pH.

In conclusion, using *in vivo* ³¹P-MT-MRS, we found reduction in the activity of a critical enzyme involved in brain energy metabolism, in FEP. Future studies are needed to identify the relation of reduced CK activity with symptoms and functional impairments in psychotic disorders, as well as treatment alternatives targeting this specific abnormality.

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